CYTOLOGICAL STUDIES IN THE AEGILOPS

AND IN CERTAIN RUST FUNGI

by

ROBERT CAMERON McGINNIS

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ABSTRACT

PART I

A number of intergeneric and intrageneric F_1 hybrids involving species of *Triticum* and *Aegilops* were studied cytologically at metaphase I of meiosis to determine the genomes present in the hexaploid *Ae. juvenalis* (Thell.) Eig. The amount and kind of pairing exhibited was employed in establishing genome relationships. It was concluded that *Ae. juvenalis* has the D genome of *Ae. squarrosa* L. only slightly altered from the D of *T. aestivum* L. In addition there was evidence that the C^u genome of *Ae. umbellulata* Zhuk. is also present in *Ae. juvenalis*. The third genome was not determined. It was shown that *Ae. juvenalis* carries one reciprocal translocation and that one of the chromosomes involved is probably located in the D genome.

The genomes of *Ae. variabilis* Eig (C^uS^v) appeared to be more closely related to each other than their genome symbols would indicate. In all crosses involving *Ae. variabilis* a higher pairing frequency was observed than when other tetraploid species were used.

PART II

Germinating sporidia of five species of the Uredinales were examined cytologically at mitosis to determine their chromosome numbers. Studies at metaphase showed a haploid number of six chromosomes for

Puccinia graminis Pers. and three for *P. coronata* Corda. Less clearly defined were the chromosomes of *P. minussensis* Thum., *P. helianthi* Schw. and *Melampsora lini* (Pers.) Lev., but their haploid numbers appeared to be n = 3, 6-8, and 4, respectively.

Prophase chromosomes of *P. graminis* were observed to be united to form a continuous chain. At metaphase, what were apparently residual terminal attractions were observed, the six chromosomes appearing as three loose pairs.

Evidence for the existence of two polyploid series in the Uredinales with basic chromosome numbers of three and two or four is presented and discussed.

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PART I

GENOME ANALYSIS OF *AEGILOPS JUVENALIS*

INTRODUCTION

The genus *Aegilops* has long proven to be of interest from a cytological, genetical and phylogenetical standpoint since it is in the same sub-tribe -- Triticinae -- as *Triticum*. It has contributed the D and possibly the B genome of common wheat and has a similar polyploid series to *Triticum* with species of n = 7, 14 and 21 chromosomes. Twenty-two species are known in which five genomes have been identified. Each of these genomes is considered an entity homologous or homoeologous with the chromosomes of a particular diploid species. Relationships are based largely on the amount and kind of pairing found at meiosis in interspecific and intergeneric F_1 hybrids.

Genome formulae are known for 20 of the *Aegilops* species. Only in the species *Ae. juvenalis* (Thell.) Eig and *Ae. sharonensis* Eig is this knowledge lacking. The present study was conducted on the hexaploid *Ae. juvenalis* in an attempt to determine its genomic constitution.

REVIEW OF LITERATURE

A complete review of the literature on genome analyses in the Aegilops is too extensive to be presented here in its entirety. Only the literature pertinent to the species used in this study will be cited.

Cytological data on Ae. juvenalis are almost lacking. On a morphological and taxonomical basis this species has been placed in the section Pachystachys by previous investigators (6, 32). This section also includes Ae. squarrosa which is now known to have the D genome (18, 26, 27).

In the hybrids of Ae. juvenalis x Agropyron intermedium, Favorsky (7) observed 12-14 pairs, while in the cross Ae. variabilis x A. intermedium he found 11-14 pairs. This indicates that these two Aegilops species have a similar relationship to A. intermedium. Kostoff (24) points out that some of these bivalents probably result from autosyndetic pairing within the Agropyron parent and possibly also from the Aegilops, but that it is not unlikely that part of the pairing is the result of allosyndesis.

Ae. triuncialis has been investigated very extensively. In 1929 Kihara (14) reported 1-7 bivalents (most frequently 4 or 5) in the F_1 hybrid between Ae. triuncialis and T. dicoccum. When crossed with T. durum, 0-6 bivalents were noted. Percival (30) in the cross Ae. triuncialis x T. durum observed 1-6 bivalents, but in crosses with other tetraploid wheat species, only 1-3 bivalents. In the intergeneric cross Ae. triuncialis x T. aestivum, Kihara (14) reported a range of 0-5 bivalents with most commonly 2 or 3. For

the same cross Percival (30) observed 1-5 pairs while Aase (1) found 0-3. These workers concluded that there was no appreciable homology between the chromosomes of *Ae. triuncialis* and species of *Triticum*. In 1940 Kihara (17) proposed the genomes CC^u for *Ae. triuncialis* based on the pairing observed in a number of interspecific crosses. Additional evidence was later presented by Kihara and Kondo (22) when they crossed *Ae. triuncialis* with the synthetic amphiploid *Ae. caudata* (C) x *Ae. umbellulata* (C^u) and observed a high frequency of normal pairing.

Ae. ovata has also been studied extensively in combination with many different wheat species (1, 3, 4, 5, 8, 11, 12, 14, 15, 21, 23, 29, 30, 31). In general for all crosses concerned, the number of bivalents varied from 0 to 7 per microsporocyte with 0 or 1 bivalent being most frequent. The conclusion reached was that no homology exists between the chromosomes of *Ae. ovata* and those of the *Triticum* species studied. In 1949, Kihara (20) summarized the genome analyses of most of the *Aegilops* species and presented the formula C^uM^o for *Ae. ovata*. This formula is based largely on earlier studies of interspecific *Aegilops* hybrids by Kihara and Lilienfeld (23).

Ae. variabilis has not been studied as extensively as the above two species. In 1947, Kihara (19) presented evidence from interspecific crosses showing that *Ae. variabilis* has the genomes C^uS^v. The C^u genome was purported to have come from *Ae. umbellulata* as did the C^u of *Ae. triuncialis* and *Ae. ovata*. The S^v genome was believed to have been derived originally from the S of *Ae. speltoides* but altered somewhat during evolutionary processes.

Several workers have reported on the meiotic behaviour in crosses involving *Ae. ventricosa* and wheat species (10, 19, 20, 23, 25, 30, 34, 37). These investigations have shown that *Ae. ventricosa* does not carry the A or B genomes of *Triticum* but that one set of chromosomes is closely related to the D genome of *T. aestivum*. Kihara (20) has proposed the genome formula DM^V for *Ae. ventricosa* based mainly on the pairing in crosses with *Ae. cylindrica*, *Ae. ovata* and *T. aestivum* (9, 14, 20, 23, 30, 34).

That the direction in which the cross is made has no effect on the amount of pairing in the hybrid was proven quite conclusively by Kihara (14). However, Kihara's study indicated that there were seasonal variations in the number of bivalents formed. Even earlier, Bleier (4) suggested that environment might influence pairing but had no experimental proof to support this idea. Summarizing the work of previous investigations, Thompson (38) pointed out that caution must be exercised in drawing inferences from pairing respecting homologies since changes in pairing frequencies can occur as a result of external conditions. More recently Sears (33) has demonstrated that the maximum pairing of which a hybrid is capable may not be expressed in certain seasons, leading to variations in pairing from year to year.

MATERIALS AND METHODS

Parent Species

Three species of *Triticum* and five of *Aegilops* were used for making crosses. These parental species are listed below with their chromosome numbers and genome formulae.

	2n Chrom. No.	Genomes
Triticum aestivum L. var. Redman	42	ABD*
Triticum durum Desf. var. Carleton	28	AB
Triticum dicoccum Schrank var. Khapli	28	AB
Ae. juvenalis (Thell.) Eig [*Aegilops turcomanica* Roshev.]	42	?
Aegilops variabilis Eig	28	C^uS^v
Aegilops triuncialis L.	28	C^uC
Aegilops ovata L.	28	C^uM^o
Aegilops ventricosa Tausch	28	DM^v

F_1 Hybrids

A total of 13 interspecific and intergeneric hybrids were obtained from crosses involving the above parent species. These were as follows:

Ae. juvenalis x *T. aestivum*
" " x *T. durum*
" " x *Ae. variabilis*
" " x *Ae. ovata*

---

* It should be noted that throughout this study the symbol D first named by Kihara (13) is used to designate the third genome of *T. aestivum*. The symbol C is commonly used synonymously by many workers but in the strict sense can be applied only to certain of the *Aegilops* species.

Ae. juvenalis x *Ae. ventricosa*

T. aestivum x *Ae. variabilis*

" " x *Ae. ovata*

" " x *Ae. triuncialis*

" " x *T. durum*

" " x *T. dicoccum*

T. dicoccum x *Ae. ovata*

Ae. triuncialis x *T. dicoccum*

T. dicoccum x *T. durum*

Cytological Techniques

Whole spikes of parents and hybrids were collected and fixed in Carnoy's solution A for cytological examination of pollen mother cells. Fixed material was stored in the refrigerator until cytological examination could be made.

Cytological investigations were conducted on all parent species and F_1 hybrids. Slides were prepared of pollen mother cells at metaphase I of meiosis using the aceto-carmine smear method described by Smith (36). Wherever possible, chromosome counts were made on different florets on a spike as well as on different spikes. An attempt was made to study at least 200 pollen mother cells of each hybrid. In cases where material was limited, all available cells were counted. A record was kept of the number of univalents, bivalents and multiple associations for each cell. In addition, attention was paid to the type of pairing exhibited -- whether the bivalents were open or closed.

Smear preparations were kept semi-permanent up to three

months by ringing the cover-slip with a mixture of gum-mastic and paraffin. Slides were then made permanent by the tertiary-butyl alcohol method (36).

Cytological examinations were made on a Leitz Ortholux microscope at a magnification of 600 diameters. Photomicrographs were taken with a Leitz MAKAM camera on Eastman's Contrast Process Panchromatic film at 400 diameters.

RESULTS

All parent species studied with the exception of *Ae. juvenalis* showed almost completely normal chromosome pairing at metaphase I of meiosis (Figs. 1, 2, 4, 5). In *Ae. juvenalis*, however, a reciprocal translocation was present, resulting in a chain (rarely a ring) of four in about 15% of the pollen mother cells examined (Fig. 3). The translocation would be manifested in the F_1 hybrids as an open bivalent or multivalent depending upon the chromosome homology between the species in the cross. *Ae. ventricosa* exhibited a tendency toward asynapsis in a low percentage of cells where up to four univalents were present.

Pairing in the three intrageneric *Triticum* crosses proved to be as expected. In the cross *T. aestivum* x *T. dicoccum*, there were usually $14^{II}7^{I}$ at metaphase I with occasionally $13^{II}9^{I}$ or $12^{II}11^{I}$. Similar pairing was observed in *T. aestivum* x *T. durum* hybrids. In hybrids of *T. dicoccum* x *T. durum*, most commonly 14^{II} occurred (Fig. 6). A low frequency of cells had up to four univalents. These results are in accordance with numerous workers (1, 2, 16, 39, 40).

The data concerning the pairing in the remaining 10 interspecific and intergeneric F_1 hybrids involving *Aegilops* and *Triticum* are presented in Tables I to X. In these tables the average number of bivalent associations is based on the premise of Kihara (13) that a trivalent is equivalent to one association and a quadrivalent to two associations. In Figs. 7-12, typical metaphase I plates for certain of these hybrids can be seen.

In order to make a direct comparison of the pairing frequencies observed in the different hybrids, the pertinent data from Tables I to X are summarized in Table XI. Standard errors of mean number of bivalents for each hybrid are also given in Table XI. In Table XII the t values for differences between means are presented.

TABLE I

Chromosome Associations in the hybrid *Ae. juvenalis* x *T. aestivum*

	Number of				Number of Cells	Percentage of Cells
	Univalents	Bivalents	Trivalents	Quadrivalents		
	34	4			3	1.13
	32	5			8	3.01
	30	6			19	7.14
	28	7			28	10.53
	26	8			17	6.39
	24	9			14	5.26
	22	10			5	1.88
	20	11			1	0.38
	33	3	1		4	1.50
	31	4	1		6	2.26
	29	5	1		15	5.64
	27	6	1		24	9.02
	25	7	1		23	8.65
	23	8	1		18	6.77
	21	9	1		8	3.01
	19	10	1		5	1.88
	17	11	1		2	0.75
	28	4	2		3	1.13
	26	5	2		13	4.89
	24	6	2		12	4.51
	22	7	2		14	5.26
	20	8	2		4	1.50
	16	10	2		1	0.38
	23	5	3		1	0.38
	21	6	3		1	0.38
	28	5		1	2	0.75
	26	6		1	4	1.50
	24	7		1	7	2.63
	22	8		1	1	0.38
	25	5	1	1	1	0.38
	23	6	1	1	1	0.38
	21	7	1	1	1	0.38
Total	6884	1799	208	17	266	100.03
Average per cell	25.88	6.76	0.78	0.06		

Average number of bivalent associations* = 7.66

Type of pairing - frequently up to 4 closed bivalents

* In this and succeeding tables:
trivalent = 1 bivalent association
quadrivalent = 2 bivalent associations

TABLE II

Chromosome Associations in the hybrid *Ae. juvenalis* x *T. durum*

	Number of				Number	Percentage
	Univalents	Bivalents	Trivalents	Quadrivalents	of Cells	of Cells
	35	0			9	3.41
	33	1			12	4.54
	31	2			20	7.58
	29	3			50	18.94
	27	4			50	18.94
	25	5			37	14.02
	23	6			19	7.20
	21	7			1	0.38
	19	8			1	0.38
	32	0	1		2	0.76
	30	1	1		9	3.41
	28	2	1		6	2.27
	26	3	1		18	6.82
	24	4	1		17	6.44
	22	5	1		4	1.51
	20	6	1		2	0.76
	25	2	2		3	1.14
	23	3	2		3	1.14
	25	3		1	1	0.38
Total	7208	909	70	1	264	100.02
Average per cell	27.30	3.44	0.27	0.004		

Average number of bivalent associations = 3.72

Type of pairing - all open bivalents

TABLE III

Chromosome Associations in the hybrid _Ae. juvenalis_ x _Ae. variabilis_

Number of				Number of Cells	Percentage of Cells
Univalents	Bivalents	Trivalents	Quadrivalents		
21	7			1	0.48
19	8			3	1.45
17	9			10	4.83
15	10			11	5.31
13	11			15	7.25
11	12			7	3.38
9	13			4	1.93
7	14			1	0.48
3	16			1	0.48
20	6	1		1	0.48
18	7	1		2	0.97
16	8	1		17	8.27
14	9	1		24	11.59
12	10	1		16	7.73
10	11	1		9	4.35
8	12	1		9	4.35
6	13	1		1	0.48
4	14	1		1	0.48
17	6	2		3	1.45
15	7	2		4	1.93
13	8	2		15	7.25
11	9	2		13	6.28
9	10	2		12	5.80
7	11	2		1	0.48
5	12	2		2	0.97
10	8	3		4	1.93
6	10	3		1	0.48
13	9		1	2	0.97
11	10		1	3	1.45
9	11		1	1	0.48
7	12		1	1	0.48
16	6	1	1	2	0.97
12	8	1	1	1	0.48
10	9	1	1	1	0.48
8	10	1	1	1	0.48
4	12	1	1	1	0.48
15	5	2	1	1	0.48
11	7	2	1	1	0.48
9	8	2	1	1	0.48
7	9	2	1	1	0.48
5	10	2	1	1	0.48
8	7	3	1	1	0.48
Total 2595	1982	215	19	207	99.95
Average per cell 12.48	9.53	1.03	0.09		

Average number of bivalent associations = 10.74

Type of pairing - usually 3 and occasionally up to 5 closed bivalents

TABLE IV

Chromosome Associations in the hybrid *Ae. juvenalis* x *Ae. ovata*

Number of Univalents	Bivalents	Trivalents	Quadrivalents	Number of Cells	Percentage of Cells
23	6			1	0.49
21	7			13	6.37
19	8			15	7.35
17	9			20	9.80
15	10			12	5.88
13	11			3	1.47
11	12			1	0.49
22	5	1		2	0.98
20	6	1		9	4.41
18	7	1		20	9.80
16	8	1		29	14.22
14	9	1		8	3.92
21	4	2		1	0.49
19	5	2		8	3.92
17	6	2		10	4.90
15	7	2		16	7.84
13	8	2		8	3.92
11	9	2		4	1.96
16	5	3		3	1.47
14	6	3		1	0.49
12	7	3		2	0.98
10	8	3		1	0.49
15	4	4		2	0.98
13	5	4		1	0.49
21	5		1	1	0.49
19	6		1	3	1.47
17	7		1	1	0.49
15	8		1	2	0.98
20	4	1	1	1	0.49
16	6	1	1	2	0.98
14	7	1	1	2	0.98
17	4	2	1	1	0.49
15	5	2	1	1	0.49
Total 3418	1527	204	14	204	99.97
Average per Cell 16.76	7.49	1.00	0.07		

Average number of bivalent associations = 8.63

Type of pairing - usually 2-3 and occasionally 4 closed bivalents per cell

TABLE V

Chromosome Associations in the hybrid *Ae. juvenalis* x *Ae. ventricosa*

Number of				Number	Percentage
Univalents	Bivalents	Trivalents	Quadrivalents	of Cells	of Cells
31	2			2	3.13
29	3			7	10.94
27	4			3	4.69
25	5			6	9.37
23	6			6	9.37
21	7			6	9.37
19	8			5	7.81
17	9			2	3.13
28	2	1		1	1.56
26	3	1		2	3.13
24	4	1		4	6.25
22	5	1		3	4.69
20	6	1		2	3.13
18	7	1		3	4.69
16	8	1		2	3.13
23	3	2		2	3.13
19	5	2		1	1.56
17	6	2		2	3.13
23	4		1	2	3.13
19	6		1	1	1.56
17	7		1	1	1.56
20	4	1	1	1	1.56
Total 1458	339	28	5	64	100.02
Average per cell 22.78	5.30	0.44	0.08		

Average number of bivalent associations = 5.90

Type of pairing - frequently 2-3 closed bivalents per cell

TABLE VI

Chromosome Associations in the hybrid *T. aestivum* x *Ae. ovata*

Number of Univalents	Number of Bivalents	Number of Trivalents	Number of Cells	Percentage of Cells
33	1		6	7.59
31	2		24	30.38
29	3		22	27.85
27	4		9	11.39
25	5		6	7.59
30	1	1	3	3.80
28	2	1	5	6.33
26	3	1	2	2.53
24	4	1	2	2.53
Total 2303	213	12	79	99.99
Average per cell 29.15	2.70	0.15		

Average number of bivalent associations = 2.85

Type of pairing - all open bivalents

TABLE VII

Chromosome Associations in the hybrid *T. dicoccum* x *Ae. ovata*

Number of Univalents	Number of Bivalents	Number of Cells	Percentage of Cells
28	0	8	8.25
26	1	53	54.64
24	2	28	28.87
22	3	4	4.12
20	4	3	3.09
18	5	1	1.03
Total 2440	138	97	100.00
Average per cell 25.16	1.42		

Average number of bivalent associations = 1.42

Type of pairing - all open bivalents

TABLE VIII

Chromosome Associations in the hybrid T. aestivum x Ae. variabilis

Number of			Number	Percentage
Univalents	Bivalents	Trivalents	of Cells	of Cells
31	2		2	0.53
29	3		6	1.58
27	4		31	8.16
25	5		39	10.26
23	6		71	18.68
21	7		60	15.79
19	8		32	8.42
17	9		11	2.89
15	10		4	1.05
13	11		1	0.26
30	1	1	1	0.26
26	3	1	4	1.05
24	4	1	13	3.42
22	5	1	30	7.89
20	6	1	27	7.11
18	7	1	20	5.26
16	8	1	6	1.58
14	9	1	3	0.79
23	3	2	2	0.53
21	4	2	4	1.05
19	5	2	9	2.37
17	6	2	2	0.53
16	5	3	1	0.26
14	6	3	1	0.26
Total 8318	2275	144	380	99.98
Average per cell 21.89	5.99	0.38		

Average number of bivalent associations = 6.37

Type of pairing - occasionally one closed bivalent per cell

TABLE IX

Chromosome Associations in the hybrid *T. aestivum* x *Ae. triuncialis*

Number of				Number of Cells	Percentage of Cells
Univalents	Bivalents	Trivalents	Quadrivalents		
33	1			2	0.81
31	2			1	0.40
29	3			23	9.31
27	4			46	18.62
25	5			42	17.00
23	6			47	19.03
21	7			23	9.31
19	8			13	5.26
17	9			3	1.21
15	10			1	0.40
11	12			1	0.40
26	3	1		6	2.43
24	4	1		10	4.05
22	5	1		8	3.24
20	6	1		6	2.43
18	7	1		4	1.62
21	4	2		1	0.40
25	3		1	3	1.21
23	4		1	2	0.81
21	5		1	1	0.40
19	6		1	1	0.40
15	8		1	1	0.40
24	2	1	1	1	0.40
22	3	1	1	1	0.40
Total 5951	1270	38	10	247	99.94
Average per cell 24.09	5.14	0.15	0.04		

Average number of bivalent associations = 5.37

Type of pairing - rarely 1 closed bivalent per cell

TABLE X

Chromosome Associations in the hybrid *Ae. triuncialis* x *T. dicoccum*

Number of			Number	Percentage
Univalents	Bivalents	Trivalents	of Cells	of Cells
22	3		2	2.67
20	4		9	12.00
18	5		19	25.33
16	6		12	16.00
14	7		8	10.67
12	8		4	5.33
10	9		3	4.00
8	10		1	1.33
19	3	1	1	1.33
17	4	1	5	6.67
15	5	1	7	9.33
13	6	1	2	2.67
11	7	1	2	2.67
Total 1213	418	17	75	100.00
Average per cell 16.17	5.57	0.23		

Average number of bivalent associations = 5.80

Type of pairing - all open bivalents

TABLE XI

Summary of Data on Pairing Frequencies in the F_1 Hybrids

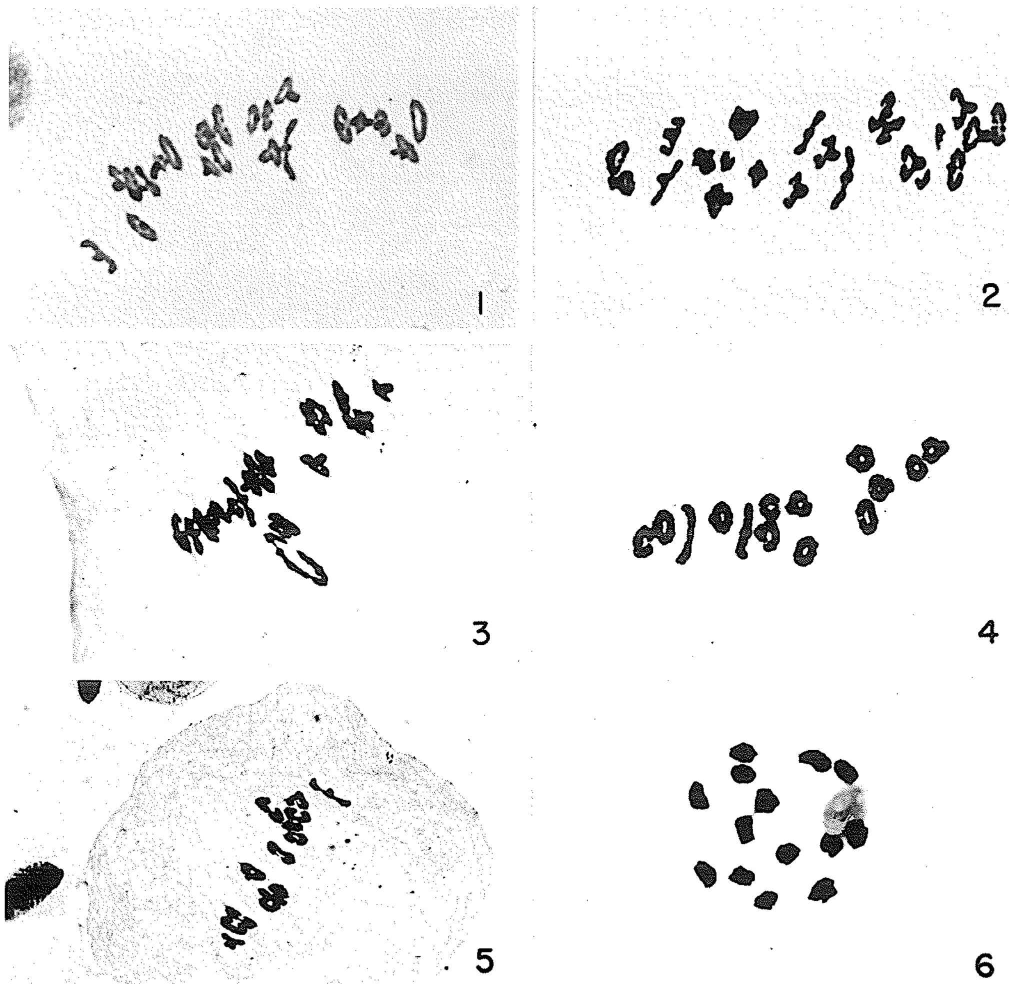
Hybrid	Number of Pollen Mother Cells with Bivalent Associations* 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Mean Bivalent Association	Standard Error of Mean	Total Number of Cells
Ae. juvenalis x T. aestivum					7	14	37	67	58	55	19	6	3					7.6692	∓.0963	266
Ae. juvenalis x T. durum	9	14	29	56	71	58	23	3	1									3.7159	∓.0938	264
Ae. juvenalis x Ae. variabilis								2	8	34	50	52	34	18	6	2	1	10.7633	∓.1075	207
Ae. juvenalis x Ae. ovata							4	32	54	71	34	8	1					8.6225	∓.0797	204
Ae. juvenalis x Ae. ventricosa			2	8	5	12	11	10	11	5								5.8906	∓.2392	64
T. aestivum x Ae. ovata		6	27	27	11	8												2.8481	∓.1215	79
T. dicoccum x Ae. ovata	8	53	28	4	3	1												1.4227	∓.0908	97
T. aestivum x Ae. variabilis			3	6	35	54	105	96	55	18	7	1						6.3658	∓.0772	380
T. aestivum x Ae. triuncialis		2	1	23	52	56	59	30	18	3	2		1					5.3765	∓.1023	247
Ae. triuncialis x T. dicoccum				2	10	24	19	10	6	3	1							5.8000	∓.1665	75

* Trivalent = 1 bivalent association
Quadrivalent = 2 bivalent associations

TABLE XII - t Values for Differences of Mean Number of Bivalents in Pollen Mother Cells of F_1 hybrids.

Hybrid \ Hybrid	Ae. juvenalis x T. aestivum	Ae. juvenalis x T. durum	Ae. juvenalis x Ae. variabilis	Ae. juvenalis x Ae. ovata	Ae. juvenalis x Ae. ventricosa	T. aestivum x Ae. ovata	T. dicoccum x Ae. ovata	T. aestivum x Ae. variabilis	T. aestivum x Ae. triuncialis	Ae. triuncialis x T. dicoccum
Ae. juvenalis x T. aestivum										
Ae. juvenalis x T. durum	29.3950									
Ae. juvenalis x Ae. variabilis	21.4321	49.3823								
Ae. juvenalis x Ae. ovata	7.6259	39.8612	15.9983							
Ae. juvenalis x Ae. ventricosa	6.8984	8.4551	18.5833	10.8380						
T. aestivum x Ae. ovata	31.0872	5.6518	48.7795	39.7385	11.3417					
T. dicoccum x Ae. ovata	47.1865	17.5630	66.3753	59.5266	17.4662	9.3963				
T. aestivum x Ae. variabilis	10.5587	21.8100	33.2258	20.3460	1.8910	24.4342	41.4825			
T. aestivum x Ae. triuncialis	16.3190	11.9648	36.3034	25.0415	1.9766	15.9191	28.9132	7.7219		
Ae. triuncialis x T. dicoccum	9.7157	10.9030	25.0388	15.2897	0.3109	14.3186	23.0785	3.0826	2.1671	

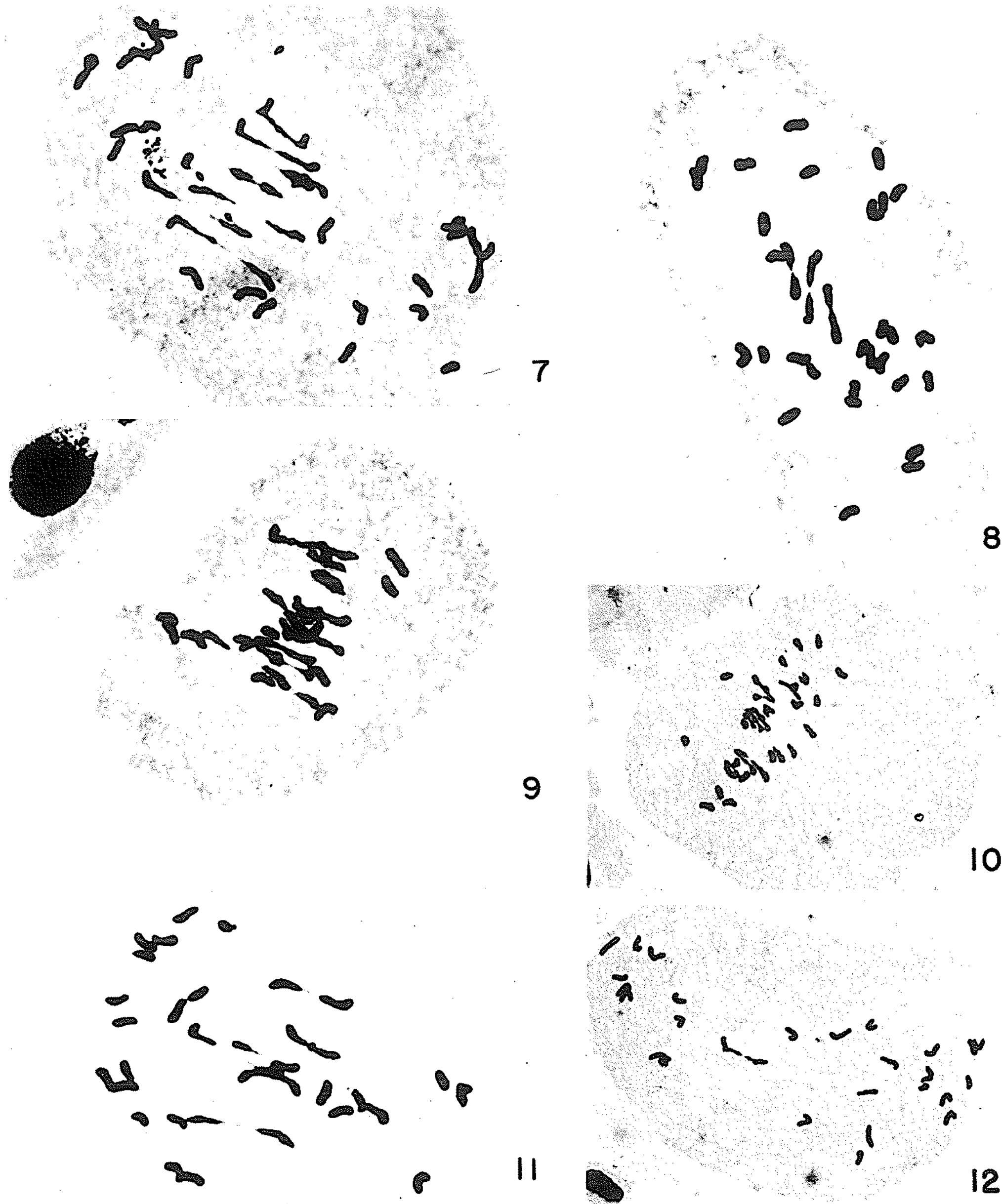
t.05 at 150 D.F. = 1.976 t.01 at 150 D.F. = 2.609



Pairing at metaphase I in parents and F_1 hybrids.

Fig. 1. T. aestivum. 21^{II}
Fig. 2. Ae. juvenalis. 21^{II}
Fig. 3. Ae. juvenalis. $19^{II}R^{IV}$
Fig. 4. T. durum. 14^{II}
Fig. 5. Ae. triuncialis. 14^{II}
Fig. 6. T. dicoccum x T. durum. 14^{II} (Late diakinesis).

Fig. 5 at 400X; all others at 600X.



Pairing at metaphase I in F_1 hybrids.

Fig. 7. Ae. juvenalis x T. aestivum. $6^{II}C^{III}27^{I}$.
Fig. 8. Ae. juvenalis x T. durum. $3^{II}29^{I}$.
Fig. 9. Ae. juvenalis x Ae. variabilis. $9^{II}2C^{III}11^{I}$.
Fig. 10. Ae. juvenalis x Ae. ovata. $5^{II}3C^{III}16^{I}$.
Fig. 11. T. aestivum x Ae. variabilis. $6^{II}23^{I}$.
Fig. 12. T. dicoccum x Ae. ovata. $1^{II}26^{I}$.

Figs. 10 and 12 at 400X; all others at 600X.

DISCUSSION AND CONCLUSIONS

Significance of Pairing

Since there is apparently considerable homoeology between a great number of species within the Triticinae, it is difficult to determine accurately the nature of the pairing observed in interspecific and intergeneric hybrids. Even within a haploid where normally no synaptic mates are present, usually some pairing is observed. This may be accounted for by inter- or intragenomic homoeology. O'Mara (28) points out "the possibility also exists that some seemingly homologous association between non-homologous chromosomes may actually be an expression of a tendency to random and irrelevant pairing which manifests itself when a true homology can not be realized." Thus it seems that pairing can be intergenomic (either autosyndetic or allosyndetic), intragenomic or irrelevant.

There seems little doubt that where closed bivalents are regularly observed, the pairing is much more discrete and therefore more apt to be between chromosomes of a fairly close relationship. Furthermore, where one or more chiasmata can be clearly seen, this should be indicative of at least partial homology. Of lesser significance probably, is the occurrence of stretched open bivalents where the chiasmata cannot be seen. There appears to be some evidence in this study to indicate that chromosomes can unite end-to-end merely by strong terminal attractions (Fig. 11). At metaphase the united regions stretch to form a tapered end, typical of an open bivalent but with no actual chiasma visible. Such cases can hardly be considered as true bivalents but could be a type of secondary

pairing which may or may not be an indication of segmental homology or gene specificity.

As a basis for the genome homologies to be established from this study, the following tenets should apply:

1. Where closed bivalents occur regularly, true homology must exist.
2. Up to three or four open bivalents may be accounted for through autosyndetic or intragenomic pairing.
3. In hybrids involving *Ae. juvenalis*, one pair may occur as a result of the reciprocal translocation present in the *juvenalis* parent. This pair could be homologous to one or two chromosomes of the opposite parent and hence may or may not be a true indication of intergenomic homology.
4. Where seven or more pairs occur in a high frequency of cells some or all chromosomes of a genome are likely to be homologous.

Pairing in the Hybrids Under Study

In the cross *Ae. juvenalis* x *T. aestivum* (Table I), the range in bivalents per cell was from 4 to 12 with an average of 7.67 as summarized in Table XI. In these cells there were frequently up to 4 closed bivalents. From these data it is concluded that the above species have one genome in common. Since pairing between 7 chromosomes of the two constituent species was not always complete and regular, it must be assumed that some chromosomal changes have occurred within each species during their evolution.

These changes must prevent complete compatibility in pairing at the present time.

On the basis of the *juvenalis-aestivum* cross alone, it can be hypothesized that *Ae. juvenalis* has either the A, B or D genome in its chromosome complement, but the actual genome involved cannot be determined. However, when *Ae. juvenalis* was crossed with *T. durum* which has only the A and B genomes, the average bivalent frequency was 3.72 per cell (Table II) and the range was 0 to 8 (Table XI). The amount of pairing observed in this hybrid can be easily accounted for on the basis of non-homologous association. These two species, therefore, do not have a homologous genome. Consequently, the pairing observed in the *Ae. juvenalis* x *T. aestivum* hybrid must have been between chromosomes of the D genome and this genome must be common to both species. This cytological study, therefore, supports the morphological evidence used by previous investigators to place *juvenalis* in the same section as *squarrosa* which has the D genome.

In the cross *Ae. juvenalis* x *Ae. variabilis* (Table III) there was an average of 10.74 bivalents per cell. Of these, usually 3 and occasionally 4 or 5 were closed bivalents, signifying considerable allosyndetic pairing. The genomes in *Ae. variabilis* have been previously identified as C^uS^v. From the amount and kind of pairing in the above hybrid, it is concluded that *Ae. juvenalis* also carries one of these genomes.

Ae. ovata has the genomes C^uM^o. Studies of *Ae. juvenalis* x *Ae. ovata* (Table IV) showed an average of 8.63 pairs per cell with

usually 2 or 3 and occasionally 4 closed bivalents per cell. These data are again indicative of a single genome common to the two species.

The genome homologies observed in the above two crosses can be explained in two ways. Since the C^u genome is common to both *Ae. ovata* and *Ae. variabilis*, it could also be the one in common with *Ae. juvenalis*. Such a hypothesis would satisfy the pairing behaviour observed. On the other hand should *Ae. juvenalis* contain S^v and M^o, the pairing in the hybrids could be equally as well reconciled. On the basis of morphology, however, the former explanation would seem more valid. Furthermore, the probability of choosing two species out of the 22 available for crossing which would reveal the S^v and M^o, is very low. It therefore seems more likely that *Ae. juvenalis* contains the C^u genome.

Pairing in *Ae. juvenalis* x *Ae. ventricosa* (Table V) proved to be the most erratic and least conclusive in the entire study. An average of 5.90 bivalents per cell was observed with frequently 2 or 3 closed ones. Kihara (20) has suggested that *Ae. ventricosa* carries the D genome, although earlier Kihara and Lilienfeld (23) concluded that the set is not intact. The present investigation indicates that the D genome of *Ae. ventricosa* is not completely homologous with that of *Ae. juvenalis* since a relatively low number of bivalents was observed. The presence of 2 or 3 closed bivalents per cell, however, is probably evidence of a close relationship between certain of the chromosomes of the D genome. In this regard, Sears (34) has already demonstrated that it is not homologous to the D of *T. aestivum* but that it has considerable

homoeology with it.

In the hybrids *T. aestivum* x *Ae. ovata* and *T. dicoccum* x *Ae. ovata*, only 2.85 and 1.42 pairs per cell, respectively, were observed (Tables VI and VII). All were open bivalents indicating no real homology between species. The larger number of pairs in the *aestivum-ovata* cross probably resulted from the 7 chromosomes of the D genome being available for pairing. These chromosomes were absent in the *dicoccum-ovata* cross.

Somewhat less easily explained is the high average frequency of bivalents (6.37) in the *T. aestivum* x *Ae. variabilis* hybrid (Table VIII). Since these parents reportedly do not have any genomes in common the pairing must be attributed to autosyndesis particularly since only rarely was a closed bivalent observed. Furthermore, since the *aestivum-ovata* hybrid gave such a low number of bivalents, the pairing in the *aestivum-variabilis* cross most likely resulted mainly from autosyndesis within or between the *variabilis* genomes. Supporting such a hypothesis is the high number of bivalents observed in *Ae. juvenalis* x *Ae. variabilis* (10.74) as compared with the number found in *Ae. juvenalis* x *Ae. ovata* (8.63). These data would suggest that at least four *variabilis* chromosomes have an affinity for forming two pairs when their normal homologues are absent. In addition, it raises some doubt as to the discreteness of the genomes as formulated by Kihara. Quite possibly the C^u and S^v genomes are more closely related than their symbols would indicate.

The intergeneric crosses *T. aestivum* x *Ae. triuncialis* and *Ae. triuncialis* x *T. dicoccum* proved to be in somewhat the same

category as the *aestivum-variabilis* cross in regard to pairing behaviour. These hybrids had 5.37 and 5.80 bivalents per cell, respectively (Tables IX and X). Only rarely in the *aestivum-triuncialis* hybrid and never in the *triuncialis-dicoccum* cross was a closed bivalent observed. Since *Ae. triuncialis* has the genomes C^uC, the C^u supposedly having been modified from the C, the pairing in these hybrids may be largely attributed to homoeology between the *triuncialis* chromosomes. Sears (35), however, points out that the cytological and morphological data show no closer relationship of C to C^u than C to the M of *Ae. comosa* and concluded that Kihara's formulae tend to overemphasize the closeness of this relationship. The present study indicates that some homoeology exists between certain chromosomes of *Ae. triuncialis*. In both crosses studied the average pairing was almost the same. If the number of *Triticum* chromosomes present had a major influence on the number of bivalents formed, a marked reduction in pairs would have been noted in the *triuncialis-dicoccum* hybrid. Such a reduction was not observed; in fact a slight increase was noted.

The Effect of the Reciprocal Translocation

Of interest in this study is the effect that the reciprocal translocation in *Ae. juvenalis* has on the pairing in progeny involving this species. Presumably in hybrids where no homology exists between genomes, such a translocation would be manifested as an open bivalent. In crosses where a chromosome homologous to one of the translocated chromosomes is present, a trivalent would frequently result, and

where both homologues are present, either a quadrivalent or two open bivalents would be observed.

There appears to be some evidence from the data in this study to indicate that at least one chromosome involved in the translocation is located in the D genome. In the hybrid *Ae. juvenalis* x *T. aestivum*, 7.66 bivalents per cell were observed while in the *Ae. juvenalis* x *T. durum* cross, there were 3.72 bivalents per cell. This represents a difference of only 4 bivalents per cell, between the two crosses, a relatively small difference when the amount of genome homology involved in the two crosses is considered. In the first case, there is considerable homology of the D genomes while in the latter, no homology exists. However, if one of the translocated chromosomes is located in the D genome, it would partially account for this discrepancy. In the cross with *T. durum*, one pair would be present as a result of the translocation whereas in the cross with *T. aestivum*, its presence would not be revealed except as a multivalent. On this basis, the real difference between the two crosses with respect to bivalent formation would be 5, which is much closer to the expected. Additional evidence that one of the translocated chromosomes is in the D genome, comes from a measure of the multivalents formed in each cross. The *juvenalis-aestivum* cross had an average of 0.84 multivalents per cell (Table I) while in the *juvenalis-durum* hybrid, only 0.27 multivalents were present (Table II)

This hypothesis also helps to explain the higher frequency of bivalents observed in the hybrids *Ae. juvenalis* x *Ae. ovata* and *Ae. juvenalis* x *Ae. variabilis* than in *Ae. juvenalis* x *T. aestivum*.

In the former hybrids, one extra pair from the translocation would usually be present since neither of the tetraploid *Aegilops* species have the D genome. Of course such a hypothesis also precludes the possibility that either of the translocated chromosomes belongs to the C^u genome.

Future Investigations

On the basis of the available data from this study, it is probable that *Ae. juvenalis* has the genome formula C^uD -. The third genome remains undiscovered. In order to confirm the proposed genomes, and to determine the third genome, a number of other interspecific *Aegilops* hybrids should be studied cytologically. If the crosses are possible, it would be desirable to examine hybrids of *Ae. juvenalis* with a number of diploid species -- *Ae. speltoides* (S genome), *Ae. squarrosa* (D), *Ae. caudata* (C), *Ae. umbellulata* (C^u) and *Ae. comosa* (M). Also of interest would be the cross with tetraploid *Ae. crassa* (DJ). The information obtained from these crosses should aid in definitely establishing the three genomes of *Ae. juvenalis*.

PART II

CYTOLOGICAL STUDIES OF CHROMOSOMES OF RUST FUNGI

INTRODUCTION

Many cytological investigations of the rust fungi have been reported over the past 70 years. However, because of the extremely small size of the chromosomes in these organisms, only limited researches dealing with chromosome number and behaviour have been made, and in most cases the authors have been in doubt as to the accuracy of their counts. A knowledge of chromosome numbers in different species is basic to a wider understanding of rust genetics and phylogeny. It should also prove of value to the taxonomist as an aid in proper classification.

REVIEW OF LITERATURE

Literature pertinent to the chromosome numbers for members of the Uredinales is quite limited and is lacking entirely for the species concerned in this study. As early as 1898 attempts were made to count chromosomes in this group of organisms. In that year Juel (9), although unable to make a definite count, was convinced that there were more than two chromosomes at nuclear fusion in *Coleosporium campanulae*. A few years later Holden and Harper (8) observed six to eight chromosomes in the first division nucleus of *C. sonchi-arvensis*. More recently, observations made by Ashworth (2) disclosed a haploid number of eight to ten haploid chromosomes in *C. tussilaginis*, while Olive (11) reported eight pairs in meiotic studies on *C. vernoniae*.

A similar number has been observed in species of other genera. In Cronartium ribicola Colley (4) reported eight haploid chromosomes while Berliner and Olive (3) counted eight pairs in four species of Gymnosporangium.

Chromosome numbers have been reported for only two species of Puccinia. Allen (1) suggested a probable haploid number of five for P. malvacearum. Later, however, Savile (12) found four to be the haploid number in this species as well as in P. sorghi. In species of other genera, a count of four haploid chromosomes was reported in Uromyces fabae, U. hyperici and Melampsora bigelowii (12).

MATERIALS AND METHODS

The following species were available for cytological investigation, the host on which they were collected being in parentheses:

Puccinia graminis Pers.* (Agropyron trachycaulum (Link) Malte)
P. coronata Corda f. sp. secalis Peturs. (Agropyron repens (L.) Beauv.)
P. minussensis Thum. (Lactuca pulchella (Pursh) DC.)
P. helianthi Schw. (Wild Helianthus tuberosus L.)
Melampsora lini (Pers.) Lev. (Linum usitatissimum L.)

Studies were made on sporidia (basidiospores) and are therefore concerned with chromosomes in the haploid phase of these species since meiosis occurs in the promycelia of the teliospores

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* Unfortunately the variety and race were not identified on differential hosts. However, since race 15B of P. graminis var. tritici was far more prevalent than any other race in the area where the collection was made, it was probably the organism concerned in this study.

just prior to sporidial formation.

Cytological material was obtained in the following manner: Short sections of telia-bearing straws or leaves were soaked in water and pressed firmly against a wet blotting paper in the top of a Petri dish. A thin microscope slide was placed on water-soaked blotting paper in the bottom section of the Petri dish to collect the sporidia as they were discharged from the germinating teliospores. The Petri dish was then placed in a cool chamber (55-60°F) over night. Under optimum conditions several thousand sporidia, at varying stages of germination, were present on a single slide.

Thin-walled sporidia were found to be well suited to smear preparations since they could be easily flattened. Furthermore, they adhered closely to the slide, making it unnecessary to coat the slide with an adhesive. No appreciable loss of spores occurred during the fixing process.

Fixing was accomplished by immersing the slides with the sporidia, in either Carnoy's A (6:3:1) solution or acetic-alcohol (1:3). The two fixatives appeared equally suitable since staining properties were similar. Good smears were sometimes obtained simply by staining the fresh, unfixed material. The time of fixing was varied from a few hours to several days. So far as could be determined, the time did not appear to be critical; a short fixing time of 4 hours was equally as effective as a number of days.

Best staining was obtained with aceto-orcein (5), although aceto-carmine and aceto-lacmoid were tested. Of the three acetic stains, only orcein did not stain the cytoplasm deeply. Aceto-carmine

and aceto-lacmoid frequently gave no differentiation of nucleus and cytoplasm, staining all cell components a uniformly dark color. Aceto-lacmoid, in addition, seemed to contract the cytoplasm usually toward the germ tube end of the cell leaving a large clear space at the opposite end. Aceto-orcein, therefore, was adopted for this study.

A smear technique patterned after Smith's (14) was employed in staining slides. After a drop or two of stain had been placed on the slide, a No. 1 cover glass (22 x 40 mm.) was added. Excess stain was soaked up with a blotter, and the slide was then carefully heated to near boiling several times. After heating, considerable pressure was applied to the cover glass to flatten the sporidia and to press out the excess stain. To prevent drying, the cover glass was ringed with a temporary seal of paraffin-gum mastic. The prepared slides were stored in a refrigerator and remained in good condition for periods of two months or more. It was frequently noted that greater contrast between the chromosomes and cytoplasm resulted after a few days storage at cold temperatures.

Attempts to make the slides permanent by the tertiary-butyl alcohol method described by Smith (14) did not prove very satisfactory. Frequently critical cells were lost and it was often difficult to remove sufficient Canada balsam to regain the extreme thinness of the original preparation.

Cytological examinations were made with oil immersion usually at a magnification of 1350X, although occasionally a magnification of 2800X gave clearer chromosome differentiation. Photomicrographs were taken on Eastman's Contrast Process Panchromatic

film with a Leitz MAKAM camera.

RESULTS

Because sporidia were discharged on each slide over a period of about 16 hours, it was possible to study sporidia at different stages of development from freshly discharged, ungerminated ones, to those having germ tubes up to 100 u in length. It was observed that normally the basidiospore nucleus divided before germination of the spore (Fig. 1). This behaviour is in accordance with the findings of Allen (1) and Savile (12) on several different rust species. In a low percentage of cells of P. graminis, germination was precocious and preceded nuclear division (Fig. 4). In contrast to this behaviour, it was observed that in P. coronata usually four nuclei and sometimes up to eight were present before germination occurred. This phenomenon might be explained on the basis of the sporidial discharge taking place under temperature and moisture conditions conducive for nuclear division but unfavorable for cell germination.

Chromosome Studies of Puccinia graminis

From an examination of the chromosomes during the first nuclear division a phenomenon not heretofore reported to the best of the writer's knowledge was observed. At late prophase the chromosomes appeared to be attached in such a manner as to form a continuous chain or "spireme" (Figs. 2 and 3). During the course of this study five such cells were observed, leaving little doubt that this chromosome association, if not the normal condition, must

occur frequently. It is believed that these cells were at late prophase. A random arrangement of the "spireme" throughout the cell is shown in Fig. 2. Presumably no polar forces were yet active. A slightly later prophase stage is shown in Fig. 3 where the forces of division have caused some orientation of the chromosomes to form a spiral arrangement. Limits of individual chromosomes could not be seen, the overall length of the configuration being about 14 u.

Six sporidia at metaphase had deeply-stained chromosomes which were sufficiently distinct and spread so that a reliable chromosome count could be made (Fig. 4). On the basis of the evidence obtained from these cells,it is concluded that *Puccinia graminis* has a haploid number of six chromosomes. A study of several other sporidia at the same stage of division gave no contradictory evidence, although a definite count of six could not be established in these cases. All six chromosomes appeared to be of uniform size at metaphase, but slight variations were noted between different cells. Measurements showed the chromosomes to be from 0.8 u to 1.4 u in length and about 0.3 u to 0.5 u in width.

It is surprising that not more nuclei were observed at the metaphase or early anaphase stages of division. Although no actual count was made, it is estimated that only about one cell in 5,000 to 10,000 cells was at metaphase or early anaphase. Probably this stage of division is of very short duration, perhaps lasting not more than a few seconds. The low frequency of metaphases indicates the necessity of studying a large number of spores.

In Fig. 4 it can be seen that the six chromosomes actually

appear as three associations of two chromosomes. Although focusing showed the chromosomes to be clearly separated, residual terminal attractions appeared to be present. Similar configurations were observed in two other cells at the same stage of mitosis. Such pairing could be taken as further evidence that the prophase chromosomes normally form a chain. The presence of the three loose pairs would signify greater terminal attractions between specific pairs of chromosomes. Thus, under the forces set up during division, the more weakly paired ends of the chromosomes would break away first in the "spireme", resulting in three semi-united pairs.

Three cells were observed at late metaphase. The chromosomes had split longitudinally, remaining joined only at the medial centromeres. All six chromosomes were completely separated with no apparent pairing attractions which indicated that anaphase movement would progress in the conventional manner observed in higher plants. Unfortunately it was impossible to get well-spread anaphase preparations. Therefore, chromosome behaviour could not be studied in detail at this stage. The chromosomes at early anaphase were much reduced in size from metaphase (Fig. 5). Although a definite count could not be established at this stage, there appeared to be twelve chromosomes.

<u>Chromosome Studies of Puccinia coronata</u>

So far as could be determined, mitosis is normal in this rust. Thousands of cells were examined but in only 24 of them could chromosome counts be made at metaphase. Three chromosomes were observed in all of these cells (Fig. 6). In addition, counts were

made at anaphase in 21 cells where the three chromosomes had split and the six daughter chromosomes were seen to be migrating to the poles (Fig. 7). Other stages of mitosis were not studied in detail. It is concluded from these observations that the haploid number of chromosomes is three in P. coronata.

The chromosomes are extremely small. At metaphase they measured 0.6 u to 0.75 u in length, and about 0.4 u in width, while at anaphase they were of the order of 0.4 u to 0.5 u in each dimension.

Chromosome Studies of Puccinia minussensis and P. helianthi

In general, studies of these species proved unsatisfactory and no definite conclusions could be drawn. The teliospores of both species discharged relatively few sporidia during the usual period allotted. Furthermore, there was considerable contamination from such fungi as Alternaria, and Sporobolomyces.

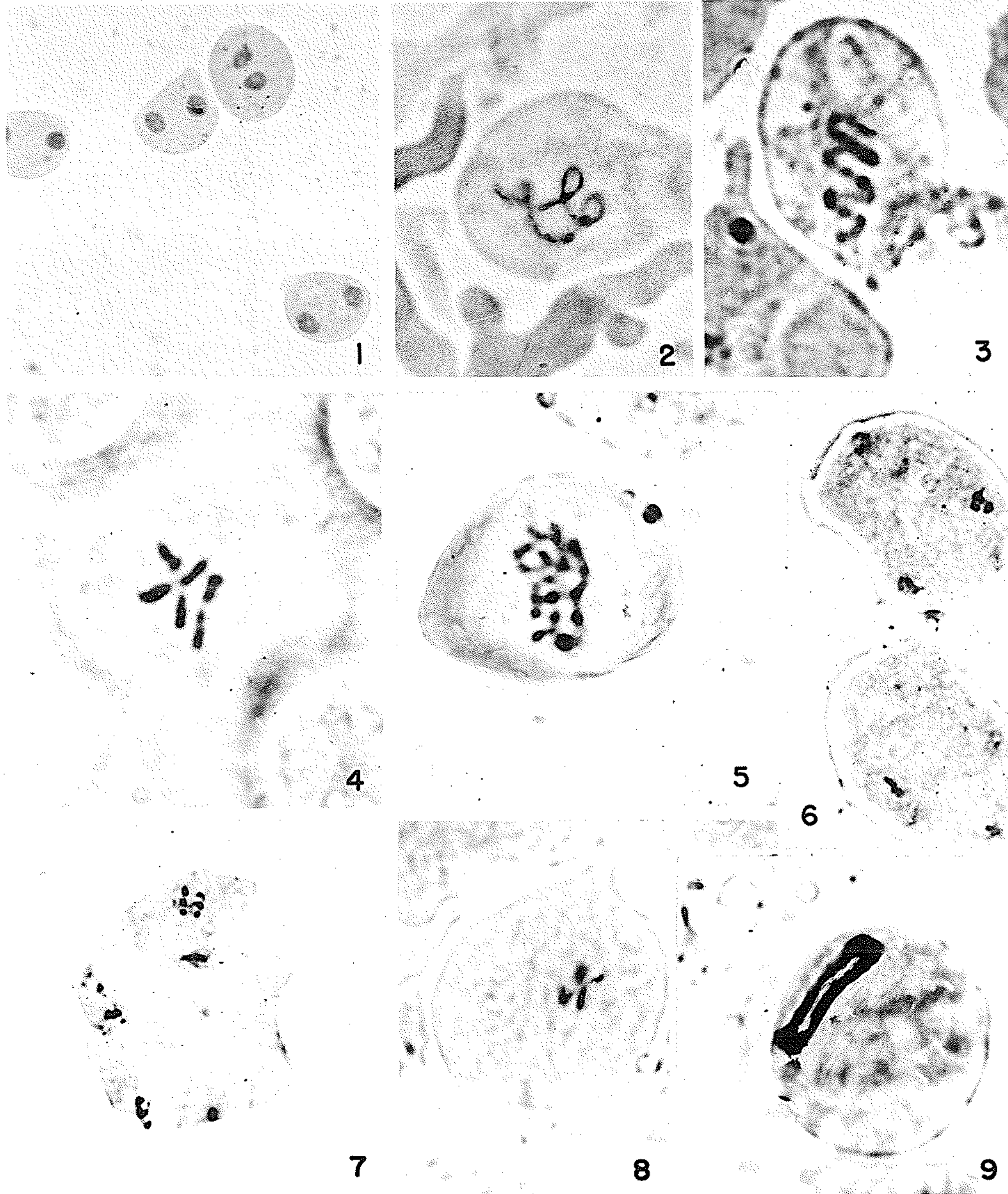
So far as could be determined, mitosis is normal in both rusts. Metaphase chromosomes appeared to be of the same general size and shape as those described above. They did not, however, spread sufficiently to allow an accurate count. On the basis of several observations, the haploid complement appeared to be n = 3 for P. minussensis and n = 6 to 8 for P. helianthi.

Chromosome Studies of Melampsora lini

Somewhat more reliable results were obtained with M. lini than with the two last mentioned species. The sporidia were discharged copiously with little or no contamination. Unfortunately

though, the chromosomes were faintly stained in most cells. In a number of cells there appeared to be four chromosomes (Fig. 8) but in no case could this be concluded definitely.

A number of divisions appeared to be atypical in that at anaphase the nuclear components formed a large ring of chromatin material while stretching to opposite poles (Fig. 9). In these cases, no chromosome individuality could be seen.



Mitosis in sporidia of rust fungi.

Fig. 1. Ungerminated sporidia of Puccinia helianthi with two nuclei in each cell. 850X.

Figs. 2-5. Mitosis in Puccinia graminis. Fig. 2. Continuous chain of chromosomes at prophase. 3150X. Fig. 3. Later prophase; chromosomes still united but oriented at plate in spiral arrangement. 3150X. Fig. 4. Six chromosomes at metaphase. 3150X. Fig. 5. Early anaphase showing probably 12 chromosomes. 3300X.

Figs. 6 and 7. Metaphase and anaphase in Puccinia coronata showing 3 and 6 chromosomes, respectively. 1500X.

Figs. 8 and 9. Metaphase and anaphase, respectively, in Melampsora lini showing probably 4 chromosomes at metaphase and a ring formation at anaphase. Fig. 8 at 2200X; Fig. 9 at 3000X.

DISCUSSION AND CONCLUSIONS

The fact that *P. coronata* has n = 3 chromosomes while *P. graminis* has n = 6 chromosomes suggests that a polyploid series might exist in the genus *Puccinia*. Supporting such a hypothesis is the unusual mitotic behaviour in *P. graminis* where three semi-united pairs were observed at metaphase. Three, being an odd number, would likely represent the basic chromosome number for the genus. On this basis, *P. coronata* would be a true diploid and *P. graminis* either an auto- or allotetraploid. Furthermore it is possible that *P. coronata* is a constituent species of *P. graminis* in which case the two species would have at least one genome in common. Studies of meiotic chromosome pairing in the promycelium of the F_1 hybrid between the two species -- if such a cross can be accomplished -- would reveal the existence of such chromosome homology.

Although inconclusive, the haploid numbers suggested from the studies on *P. minussensis* (n = 3) and *P. helianthi* (n = 6-8) also support the hypothesis of polyploidy in *Puccinia*. *P. minussensis* would be a diploid and *P. helianthi* a tetraploid. Possibly these species contain the same genomes as *P. coronata* and *P. graminis*, respectively, but have undergone chromosomal changes (inversions, deletions, translocations) of sufficient magnitude to give rise to a new host range.

The proposed number of four chromosomes for *M. lini* is in accordance with the work of Savile (12) on *M. bigelowii*. Since eight chromosomes were reported for species in a number of genera there is an indication of a second polyploid series in the Uredinales.

The basic number for this series could be either four or two. Although at present there is no evidence to support the idea of two as a basic number, such a possibility cannot be disregarded. Thus the species with n = 4 chromosomes might be either diploid or tetraploid while the species with n = 8 chromosomes might be tetraploid or octoploid. Of interest is the fact that if two is the basic number for this series and three for the second polyploid series, any chromosome constitution known at present or to be found in the future could be reconciled on the basis of autoploidy or alloploidy within or between the two series.

Besides the polyploidy speculation, this study has uncovered a perplexing situation with regard to the chromosome associations in P. graminis. The unusual spireme-like union of chromosomes at mitosis is very difficult to interpret and explain. Certainly in the light of present day cytological knowledge no completely satisfactory explanation can be advanced. The possibility of actual chiasmatic union of chromosomes can be almost excluded since crossing-over -- reported rarely in mitotic divisions in higher plants -- could hardly involve all the chromosomes of the haploid rust organism. A second hypothesis might be that chromosomes in this organism form a true spireme. Early cytologists believed that a continuous thread of chromosomes or spireme was formed prior to chromosome differentiation in prophase. More recently this theory has been considered untenable (6). It is not proposed that the old conception be revived, but it is suggested that this study presents some evidence to support the existence of a spireme stage in mitotic

prophase of this organism.

A third, and possibly the most valid hypothesis, would be that of a chromosome union effected by end-to-end attractions of the chromosomes. Although not usually associated with mitosis, terminal attractions have been observed in higher plants, particularly haploids, during meiosis, where up to five chromosomes have been found to be associated in this manner (10, 13). Several workers (7, 13, 15) have postulated that the attraction is due to genic or segmental homology between otherwise non-homologous chromosomes, and is an indication of a lower basic chromosome number for a species. In some respects, meiotic haploid chromosomes are like mitotic chromosomes since ordinarily there is no synapsis in either case. Consequently, it could be postulated that in P. graminis, forces of attraction occur between segments of different chromosomes resulting in a closely connected chain. Furthermore, if there is attraction between homologous or homoeologous segments of chromosomes it might be indicative of polyploidy in this organism.

A final consideration is that of the effect polyploidy in a fungus might have on pathogenicity and host range. It has been observed frequently in higher plants that polyploids are better adapted to more varied conditions than the constituent species. The possibility for a similar behaviour occurring in the lower organisms cannot be discounted.

The questions raised by this study indicate the need for further cytological studies of the rust fungi. To date, research in this field has served to raise as many questions as it has answered.

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