

CONSEQUENCES OF HYBRIDIZATION IN *VICIA SATIVA* AGGREGATE

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1. INTRODUCTION

THE *V. sativa* aggregate contains three different chromosome numbers: $2n = 10$, $2n = 12$ and $2n = 14$, and in each group karyotypic variation has been reported (Mettin and Hanelt, 1973; Hollings and Stace, 1974). In natural populations of *V. sativa*, plants having different chromosome numbers or karyotypes have been found side by side (Ladizinsky, 1978a). Despite morphological and chromosomal differences, the various entities are cross compatible and the F_1 hybrids are partially fertile. The behaviour of such F_1 hybrids and the resultant F_2 populations has been studied (Mettin and Hanelt, 1973; Yamamoto, 1966, 1968, 1974, 1980; Ladizinsky, 1978b). This paper describes the cytology and fertility of F_2 populations derived from crosses between *V. sativa* lines differing in chromosome numbers and karyotypes.

2. MATERIALS AND METHODS

The material for this study was obtained from interkaryotypic hybrids between lines from natural populations (Ladizinsky, 1978b). The accession numbers of these crosses, chromosome numbers and karyotype are shown in fig. 1.

3. RESULTS

F_2 populations of six cross combinations between parents differing in their chromosome numbers or karyotypes were studied. The results obtained are summarized below.

$2n = 10A \times 2n = 10B$: The F_1 hybrid was characterized by multivalent association in meiosis, indicating two reciprocal translocations between the parental lines. Pollen fertility in the F_1 hybrid was 10 and 13 per cent of the ovules developed into seeds (table 2). Of the thirty-six F_2 seeds 31 had $2n = 10$ and 5 had $2n = 11$. This is in contrast to 150 F_2 plants, all with $2n = 10$, resulting from the F_1 hybrid between two other karyotypically different $2n = 10$ *V. sativa* (Mettin and Hanelt, 1973).

Conspicuous karyotypic variation was found among the 36 F_2 plants. This variation was determined according to the number of the satellited chromosomes and the occurrence of two $2n = 10A$ chromosomes, the metacentric and the submetacentric chromosomes, Nos. V and II, respectively (fig. 1). While one or two metacentric chromosomes were found in the F_2 plants, seven plants had three submetacentric chromosomes, 26 had three satellited chromosomes and four had four satellited chromosomes in

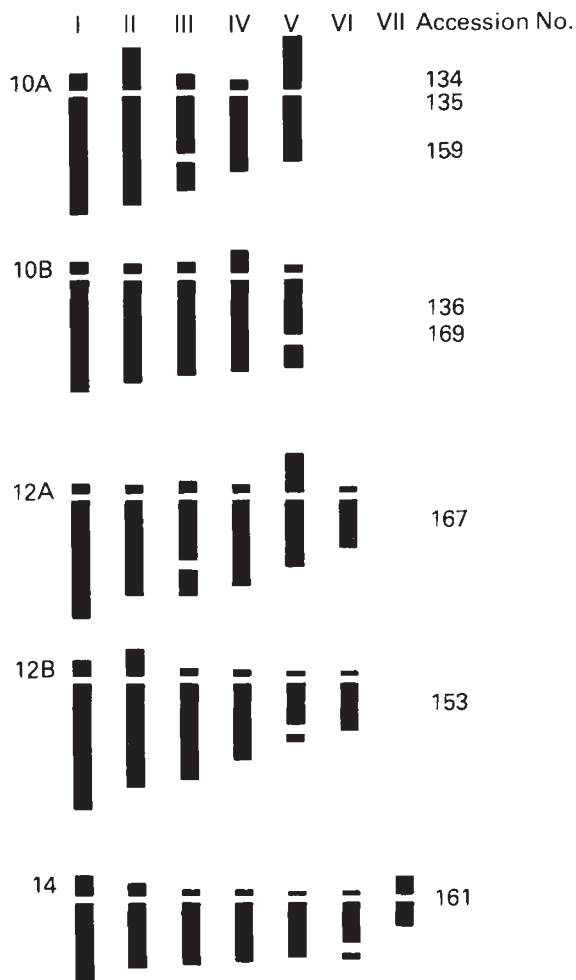


FIG. 1.—Idiograms and accession numbers of five karyotypes of *V. sativa* used in this study.

their karyotype. The difference in chromosome numbers or karyotypes could not be detected by morphology or plant development.

Chromosome association in meiosis was studied in eighteen plants. Only two plants, both with $2n = 10$, had chromosomes regularly paired in bivalents. In six plants, two trivalents, two quadrivalents or pentavalents were observed. This association pattern was similar to that found in the F_1 . In the remaining plants only one multivalent, trivalent or quadrivalent, per cell was recorded; but the frequency of these multivalents varied considerably among the plants. Generally, the fertility of the F_2 plants was considerably improved over the F_1 . In one plant with regular meiosis, pollen fertility was 93 and 70 per cent of the ovules developed to seeds. These values are similar to those of the parental lines. In another plant with regular chromosomal pairing, 68 per cent of the pollen grains were stainable and 47 per cent of the ovules developed to seeds. The karyotype of this plant contained two satellited chromosomes, two submetacentric

chromosomes but only one metacentric. This chromosome was involved in a heteromorphic bivalent that was detected in all cells. In plants exhibiting multivalent association pollen fertility varied from 21.0-52.8 per cent with a corresponding percentage of seed set.

$2n = 10A \times 2n = 12A$: The F_1 hybrids of this combination had irregular chromosome pairing in meiosis. In metaphase I about 60-70 per cent of the chromosomes paired in bivalents, while the rest were left unpaired or involved in multivalent configurations. Consequently, the fertility of this hybrid was relatively low (table 1).

Three chromosome numbers: $2n = 10$, $2n = 11$ and $2n = 12$ were found in the 59 F_2 plants (table 2). Fifty of the 59 plants reached maturity. Of twenty F_2 plants examined, two with $2n = 10$ and three with $2n = 12$, had regular meiosis and only bivalents were observed in metaphase I. Other plants with an even chromosome number had univalents and multivalents, and chromosome association was similar to that of the F_1 . The fertility of the F_2 plants with regular chromosome pairing was high. Considerable variation in pollen fertility was found in the nonregular chromosome plants (table 3), which had a corresponding variation in seed set.

$2n = 10A \times 2n = 12B$: The F_2 of this hybrid differed from the previous F_2 in not having any $2n = 10$ plants and only a few $2n = 11$ plants (table 3). Among the $2n = 12$, only 4 plants had regular bivalent formation in metaphase I and their fertility was high.

$2n = 10 \times 2n = 14$: In this combination, hybrids involving both $2n = 10A$ and $2n = 10B$ were studied (table 2). Chromosome associations in metaphase I was characterized by relatively large number of univalents and frequent multivalent association. Pollen fertility was low (5.5-12.2 per cent) and only 6 per cent of the flowers produced pods.

Of the 60 seeds collected from sixteen $2n = 10A \times 2n = 14$ F_1 hybrids, 58 germinated. The chromosome number in the F_2 plants varied from 10-15. In two plants, partial polyploidy was found, with $2n = 19$ and 22 (table 2). Of the twenty $2n = 10B \times 2n = 14$ F_2 seeds collected, 16 germinated, with a range of chromosome numbers from $2n = 11$ to $2n = 14$. Two plants were complete polyploids $2n = 24$.

The F_2 of the $2n = 10 \times 2n = 14$ plants were planted in the greenhouse and transferred to the field after growth of 4-5 leaves. Altogether twenty-nine plants of this combination died in the field before reaching flowering. No correlation was found between mortality and specific chromosome number, karyotype or specific hybrid combination.

Chromosome association in meiosis was studied in 20 plants of this combination. Chromosomes regularly paired in bivalents in the two $2n = 10$ and three $2n = 14$ plants. In other plants, various patterns of chromosome associations were noticed. The fertility of the F_2 plants was in accordance with chromosome number and meiotic behaviour (table 4). Seed yield was generally low, even in the meiotically stable plants.

$2n = 12A \times 2n = 14$: F_1 hybrids of this combination also exhibited irregular chromosome pairing and low fertility (table 1). Thirty seeds collected from the F_1 hybrids were sown, but only twenty-three germinated. The majority of the F_2 had $2n = 14$, one plant was polyploid $2n = 26$, but no $2n = 12$ plants were found. The majority of the F_2 plants died in the field and only seven reached flowering. These exhibited chromosomal instability and were sterile or only partially fertile.

TABLE 1
Metaphase I chromosome pairing and fertility of hybrids between five karyotypes of Vicia sativa

Hybrid combination	2n	No. cells	I	Mean chromosome associations per cell			V-VII	% Pollen fertility	% Pods formed from flowers	% Ovules developed to seeds
2n = 10A × 2n = 10B (159 × 136)	10	20	0.45	Rod II 2.40 Ring II 0.90	III 0.15	IV 0.55		9.8	46.3	13.5
2n = 10A × 2n = 12A (134 × 169)	11	18	0.11	1.78	1.62	0.72	0.11	4.3	57.8	33.5
(159 × 167)		36	0.30	1.68	1.60	0.70	0.22	13.8	59.0	18.3
(159 × 169)		20		1.85	1.65	0.75	0.50	15.8	41.3	14.2
2n = 10A × 2n = 12B (134 × 153)	11	52	0.82	2.32	1.41	0.54	0.16	12.0	49.0	13.2
2n = 10A × 2n = 14 (135 × 161)	12	40	2.32	2.75	0.44	0.45	0.10	8.3	10.2	11.3
(134 × 161)		26	3.61	2.92	0.65	0.22	0.07	12.2	8.1	13.8
(159 × 161)		24	3.40	2.51	0.72	0.30	0.06	13.1	12.8	14.1
2n = 10B × 2n = 14 (136 × 161)	12	72	3.40	2.98	0.14	0.43	0.12	6.2	6.4	21.5
2n = 12A × 2n = 14 (169 × 161)	13	77	4.50	2.46	0.93	0.44	0.08	6.3	19.6	12.1

TABLE 3

Frequency distribution of % pollen fertility in F_2 derivatives from $2n = 10A \times 2n = 12A$ hybrids

	0-20	21-40	41-60	61-80	80-100	Total no. plants
$2n = 10$			1	2	2	5
$2n = 11$	3	14	10			27
$2n = 12$		3	6	3	6	18
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TABLE 4

Frequency distribution of % pollen fertility in F_2 derivatives from $2n = 10 \times 2n = 14$ hybrids

	0-20	21-40	41-60	61-80	81-100	Total no. plants
$2n = 10$			1	1	2	4
$2n = 11$	1		1			2
$2n = 12$	2	3	5	2	1	13
$2n = 13$	1	3				4
$2n = 14$			2	3	2	7
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4. TRANSMISSION RATE OF PARENTAL CHROMOSOMES TO THE F_2 PLANTS

Some of the chromosomes in the *V. sativa* aggregate can be identified easily (fig. 1). Four such chromosomes Nos. II, III and V of $2n = 10A$ and No. VII of $2n = 14$ were examined for transmission rate to the F_2 .

The frequency distribution of each of the four chromosomes in the F_2 is shown in fig. 2. Absence of any of these chromosomes in the gametes of the F_1 hybrids, did not produce gamete mortality. Even the zygote, seed, and seedling formed by these gametes could tolerate the absence of various chromosomes. However, in most F_2 plants examined each of the four chromosomes was represented by one or two homologues, and rarely by three or four homologues. This high number can be explained by nondisjunction at anaphase II or in pollen mitosis.

The average transmission rate for each of the four chromosomes throughout the various F_2 populations was 1.30, 1.06, 1.50 and 1.14 for

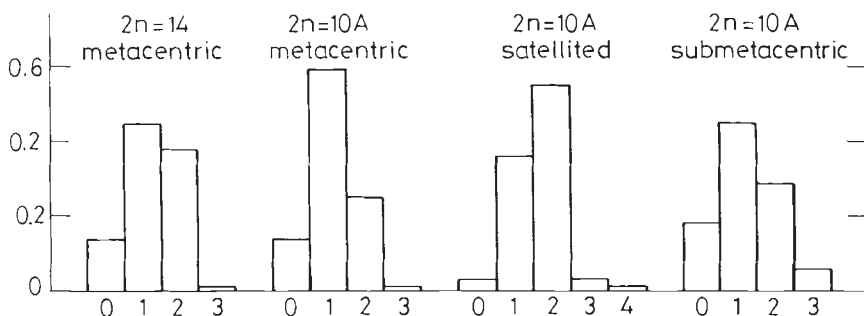


FIG. 2.—The proportion of different number of homologues per plant of 4 detectable chromosomes among F_2 progeny of *V. sativa* interkaryotypic hybrids.

the $2n = 14$ metacentric, and chromosomes Nos. II, II and V of $2n = 10A$, respectively. The $2n = 14$ and $2n = 10A$ metacentrics are partially homologous and capable of forming heteromorphic bivalents or trivalents when two $2n = 10A$ and a $2n = 14$ metacentric are found in the same p.m.c. These two chromosomes have similar transmission rate despite the marked difference in their size. A similar comparison between the transmission rate of the $2n = 10A$ and either $2n = 14$ or $2n = 12B$ satellited chromosomes, which differ markedly in size but at least partially have the same function, could not be made due to the occasional difficulty in identifying the $2n = 14$ and $2n = 12B$ satellites in the F_2 .

5. DISCUSSION

The cytogenetic affinities between the various karyotypes and the possible karyotype evolution of the *V. sativa* aggregate have been considered previously (Ladizinsky, 1978*b*). The performance of the F_2 populations further support these affinities. As demonstrated by Yamamoto (1966, 1968, 1980), Nerson (1970), Mettin and Hanelt (1973), and this study, these F_2 populations contain plants with even chromosome numbers, stable karyotypes, and reasonable fertility. In this respect, there was little difference between F_2 populations derived from karyotypically different parents. However, the proportion of chromosomally stable and reasonably fertile F_2 populations was relatively low, about 15 per cent. Most of the F_2 plants were karyotypically unstable since they possessed only one homologue of a particular chromosome or chromosomes, or sometimes three or four homologues of the same chromosome. About one third of the F_2 plants had odd chromosome numbers. However, due to the partial fertility of these F_2 plants, stable populations could be obtained in later generations.

The remarkable karyotypic variation in the F_2 indicated that viable gametes produced in the F_1 hybrids could tolerate various chromosome combinations both in number and constitution. In the interkaryotypic hybrids of *V. sativa*, each chromosome was present in a single homologue. If this chromosome was randomly transmitted to the viable gametes it could be detected in the F_2 at a frequency of about 1.0. Of the four chromosomes studied, three were transmitted at a high rate and one of them, the $2n = 10A$ satellited chromosome, was transmitted at a remarkable high rate of 1.5. The various transmission rates depend apparently not only upon the genetic quality of the various chromosomes but also upon the genetic background. This is why different transmission rates of the same chromosome were found in different hybrid combinations. Also the chromosome number in the F_2 apparently depends upon the genetic background. According to the karyotypic drawings, it can be concluded that the F_2 of a $2n = 10 \times 2n = 12$ hybrid examined by Nerson (1970) was derived from parents having karyotypes similar to the *A* types in this study. Chromosome counts in 15 F_2 plants did not reveal any $2n = 10$ plants, but did show the presence of $2n = 13$ and $2n = 14$ which were not found in the F_2 $2n = 10A \times 2n = 12A$ examined in this study.

The karyotypes and fertility of the F_2 populations indicate that gene flow between *V. sativa* plants with different chromosome numbers or karyotypes is possible but slow. This process may be significant in natural

populations, where mixed stands of plants differing in chromosome number or karyotype are found (Ladizinsky, 1978a). *V. sativa* is typically self pollinated, but it is reasonable to assume that some out crossing does occur. The immediate consequence of hybridization in the *V. sativa* aggregate is a few poorly adapted or partially fertile progeny, but restoration of fertility occurs in the F_2 . Such hybridization could thus contribute significantly to the evolution of the aggregate by forming new stable chromosomal and genetic recombinants which provide the aggregate with greater ecological amplitude, and wider adaptive radiation.

6. REFERENCES

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