MENDELIAN INHERITANCE OF CHROMOSOME SHAPE IN MATTHIOLA¹

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Received March 9, 1927

INTRODUCTION

A variety of stock (*Matthiola incana* R. Br.) known as Snowflake has been shown by FROST (1919) to produce frequent mutants of at least eight distinct kinds. These mutant forms are genetically inconstant, part of the progeny always being normals. These forms have been determined (FROST and MANN 1924, and papers in preparation) to be trisomic. Within the variety Snowflake, all plants cytologically examined, whether normal or with one or more extra chromosomes, have had strikingly long first-metaphase chromosomes in their pollen mother cells.

Miss ALLEN (1924) figured chromosomes for several pure single varieties of *Matthiola*, showing them to be much shorter than those found in Snowflake. The four pure single races used in the hybridization studies described below likewise all had very short chromosomes at and immediately before the first metaphase. The contrast in chromosome shape between Snowflake and these pure single varieties was very striking.

These short-chromosome varieties were crossed with Snowflake trisomics. The F_1 hybrids had short chromosomes, about like those of the pure single parents. It had seemed possible that the first-metaphase bivalents of these hybrids might each consist of a short and a long element, representing the respective parental types, but no suggestion of such a situation was observed. The short form was dominant in the group as a whole; each bivalent was composed of two similar members, whose source could not be determined. When F_2 hybrids were examined, it was at once evident that genetic segregation for chromosome shape had oc-

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² Except as noted below, the cytological evidence presented in this paper has been secured by Mrs. LESLEY (Division of Genetics, UNIVERSITY OF CALIFORNIA), who has prepared all the figures. The breeding work has been done and the evidence on somatic characters secured by FROST (CITRUS EXPERIMENT STATION), who also made some of the tabulated determinations of chromosome shape, including the earlier ones made for the short-chromosome races and their F_1 hybrids.

curred. Every plant clearly belonged to one of the P_1 types, and the F_2 ratio indicated monohybrid segregation.

The pollen mother cells were studied in smears stained in Belling's iron-aceto-carmin. The roots were fixed in chrom-acetic-urea and stained in iron-haematoxylin. The material for the special examination of tetrads was all collected in May, 1926. Some rather marked variations in the apparent bulk of the meiotic chromosomes in the figures are to be attributed to variable swelling in acetic acid. This must probably be attributed to differences in the condition of the chromatin, since differences quite as great are often found in the same smear.

Since the work on chromosome shape has been incidental to study of trisomy and tetrasomy, the F_1 and F_2 data represent mainly plants with extra chromosomes, and the F_2 plants, with the exception of 6 of the 19 normals, were progeny of trisomic F_1 individuals. It is probable that the ratio for chromosome shape was not affected greatly if at all by trisomy, however, for the following reasons: (1) The extra chromosome of the F_1 plants was presumably derived in most if not all cases from the variety (Snowflake) that carried the recessive gene for chromosome shape; (2) in at least three of the trisomic types (Large, Slender and Small), the extra chromosome is a fragment which usually segregates at meiosis, in the pollen mother cells, independently of the normal pair from which it was derived; (3) the various trisomic F_1 parental types gave the following results: Small parent, 20 progeny, ratio 14:6; Slender parents, 12 progeny, ratio 9:3; Smooth parents, 12 progeny, ratio 9:3; other parents (five types), 18 progeny, ratio 13:5.

THE PARENTAL RACES AND THEIR F_1 HYBRIDS

Snowflake is a glabrous, white-flowered, double-throwing variety, medium early in blooming and of rather open growth habit. Two of the pure single races were grown from seed kindly furnished by Miss EDITH R. SAUNDERS. Both of these are glabrous, rather small, and compact in habit; one (series 133) has white flowers and is very early, while the other (series 34) has deep-red flowers and is medium early. The other two races were obtained in California; both are pubescent, of open growth habit, and (except for the earlier form of series 39) late for annual stocks; series 38 is a purple-flowered form from mixed commercial seed, and series 39 is a pale-red race from a Riverside flower garden.

Miss SAUNDERS (1911, 1924) has identified two dominant genes, C and R , which are necessary for the development of sap color, and two additional dominant genes, H and K , which, *together with C and R* , are neces-

sary for the development of pubescence; there is very close linkage between *Rr* and *Hh*. Series 38 and series 39 (sap-colored pubescent) therefore are *CCRRHHKK*. Since Snowflake (white glabrous) gives a monohybrid F_2 ratio for sap color in these crosses, it must lack either *C* or *R*, but not both; since it gives a dihybrid F_2 ratio for pubescence (about 9 colored pubescent: 3 colored glabrous: 4 white glabrous) when crossed with series 34 (colored glabrous), and a monohybrid ratio (about 3 colored pubescent: 1 white glabrous) with series 38 and 39, it must contain *KK*; it must therefore be either *ccRRHHKK*, *CCrrhhKK* or *CCrrHHKK*. Series 34 (colored glabrous) must be either *CCRRHHkk* or *CCRRhhKK*.³ Snowflake carries the dominant gene *B* for purple (Miss SAUNDERS 1911, 1924; TSCHERMAK 1912), and also a recessive gene for intense flower color (evidently *p*; Miss SAUNDERS 1911), which is linked with the genes for single and double flowers. Light purple and deep purple (series 38) are *BBPP* and *BBpp*, pale red (series 39) is *bbPP*, and deep red (series 34) seems to be *bbpp* plus further intensification.

Series 133 gave glabrous white single F_1 hybrids; the other three pure single races gave pubescent purple single F_1 hybrids. No clearly genetic variation not to be ascribed to extra chromosomes was observed within any F_1 hybrid family, or within any of the small families grown from selfing of the types used in crossing. With series 39, several types differing in earliness and leaf size were used in crossing, but these appeared to be homozygous for all characters observed. With series 38, a light purple (*BP*) and a deep purple (*Bp*), both homozygous, were crossed with Snowflake, but the deep purple is represented only by one F_1 plant in the data for chromosome shape.

The variety Snowflake differs strikingly from all of the other parental varieties in the shape of the paired chromosomes at the first metaphase in pollen mother cells. Figures 1a and 1b, compared with figure 2a, show this difference clearly. An attempt was made to choose lateral metaphase figures in which the chromosomes were at their maximum elongation. If one were to compare such a figure from Snowflake with the most contracted figures seen in the pollen mother cells of any of the pure single varieties which entered into the crosses, the contrast would be far greater. In the examination of aceto-carmin smears for chromosome shape every plant can be classified with certainty, since while each type varies considerably, the majority of the pollen mother cells from the pure single varieties have exceedingly short chromosomes, and none has chromosomes

³ The F_2 ratios (approximating 9:3:4) show that, if series 34 is *CCRRhhKK*, Snowflake must be *ccRRHHKK*.

as long as the longest in Snowflake. Snowflake pollen mother cells, on the other hand, characteristically have elongated chromosomes, and none of them has the short dumb-bell shape typical of the pure single races. These statements apply equally to the short-chromosome and long-chromosome types, respectively, among the hybrids. On account of the great length of the chromosomes in Snowflake pollen mother cells at first metaphase, it is impracticable to make counts from polar views in that variety, but this can often be done in the pure single varieties studied, since their chromosomes often approach the familiar dumb-bell shape. At later stages—first anaphase, second metaphase and second anaphase—the differences in shape are far less striking, although the chromosomes usually seem to be somewhat more compact in the pure single varieties than in Snowflake (figures 1c to 1e and 2b to 2d). Comparison of chromosome size at these stages and in somatic cells (root tips; figures 1f and 2e) shows that the actual amount of chromatin present is practically the same in the two parental types in spite of the very considerable differences in shape during the first metaphase. This is in accord with the fact that the pollen mother cells are of about the same size in all types examined.

That the short type of chromosome is dominant in the F_1 is shown by table 1, and by figure 3a, which, like figures 1a, 1b and 2a, was selected

LEGEND FOR FIGURES 1-4

The chromosomes were drawn at a magnification of 4000 diameters. Drawings reduced one sixth in reproduction.

FIGURE 1.—(a) Chromosomes of a pure single variety (series 34, plant 25.5-4), at first metaphase, showing contracted or short form. Of the 62 F_2 plants examined for chromosome shape, 33 were hybrids of series 34 with Snowflake.

(b) Same for another pure single variety (series 38, plant 25.6-1), except that chromosomes have been separated for clearness.

(c) First anaphase of plant 25.6-1.

(d) Polar view of one second-metaphase group of same.

(e) Lateral view of one second-anaphase figure of same.

(f) Somatic metaphase from root-tip cell of plant 25.5-4.

FIGURE 2.—(a) Lateral first metaphase of Snowflake variety. Chromosomes have been separated for clearness. Note great elongation as compared with figures 1a and 1b.

(b) First anaphase of same.

(c) Polar view of one second-metaphase group of same.

(d) Lateral view of second-anaphase group of same.

(e) Somatic metaphase from root tip of same.

FIGURE 3.—(a) Early first anaphase from an F_1 plant.

FIGURE 4.—(a) Lateral first metaphase of an F_2 plant with short chromosomes (25.21-30; Large Smooth, double trisomic type.)

(b) Same of an F_2 plant with long chromosomes (25.19-51; Narrow trisomic type). The unpaired extra chromosome is not comparable with the one at the extreme left in figure 4a, because the extra chromosome of Narrow is typically larger than that of Large.

to show the chromosomes at the greatest extension which was seen during the first metaphase in pollen mother cells.

Non-disjunction, fragmentation and non-reduction are always found in smears of pollen mother cells from Snowflake plants with 7 pairs of chromosomes. Of 675 pollen mother cells from one greenhouse plant examined after cytokinesis, 27 had formed dyads and 14 triads, and 15 had given rise to four microspores and one microcyte—abnormalities totaling 8.3 percent of the number of mother cells. On the other hand, the pure single varieties used in the crosses show extremely little abnormality of reduction or of tetrad formation. Rarely, in the short-chromosome races, one pair of chromosomes separates either before or after the rest at first metaphase and as a result one occasionally sees a chromosome which has been left out of a nucleus following the first division. A greenhouse plant of series 34, comparable with the Snowflake plant mentioned, showed, in the groups from 308 pollen mother cells, only 2 microcytes and no dyads or triads—a proportion of abnormality of only 0.6 percent. This great contrast in amount of abnormality appears to be typical. In the F_1 plants, in which also the chromosomes have the greatly contracted form characteristic of the pure single varieties, cytological abnormalities are similarly rare. This may mean that the contracted condition of the chromosomes in the pure single varieties and in the F_1 is associated with closer pairing, or "stronger affinity," than that which characterizes the long-chromosome Snowflake variety. The closeness of pairing and normality of reduction in the F_1 further indicate that the chromosomes of these varieties of *Matthiola* are strictly homologous.

Miss ALLEN (1924) compared double-throwing and pure single strains of *Matthiola incana* and found the cytology to be identical in all forms investigated. She found "no evidence of any irregularities in distribution such as is described so frequently among *Oenothera* hybrids." From her figures and the text it appears that both the eversporting (sulphur-white) and the pure single varieties studied had the short or dumbbell-shaped chromosomes which we have found to be characteristic of our pure single varieties. In this and in the regularity of its chromosome distribution the sulphur-white variety differs from Snowflake. It is therefore evident that the elongated type of chromosome is not necessarily associated with the "double-throwing" genic constitution.

Since such differences in chromosome shape may be found in different varieties of the same species of plants it would be impracticable to use chromosome length in pollen mother cells as an indication of chromosome size in a species, unless all varieties had been ascertained to be alike in

chromosome extension. It would obviously be better to use chromosome volume in comparative studies of this type.

Table 1 shows the numbers of plants examined for chromosome shape in the pure single races and in the hybrids. It should be added that many Snowflake plants have been examined cytologically, including normals and plants with one, two and three extra chromosomes, and no individual with short chromosomes has been found. With the pure single races, three generations are represented among the 8 plants examined; with series 38, two P_1 color types are represented by F_1 hybrid progeny examined; with series 39, one P_1 habit type is represented by F_1 plants examined, and two types in the other two cases. Aside from Snowflake and part of its F_2 hybrids, we have not found long chromosomes in any *Matthiola* plants.

TABLE 1

Inheritance of first-metaphase chromosome shape. Numbers of plants with short and with long chromosomes, in four pure single races (short) and in hybrids of these with Snowflake (long).

GENERATION	CHROMOSOMES SHORT					CHROMOSOMES LONG				
	133	34	38	39	All	133	34	38	39	All
P_1 series*:										
P_1 type (pure single)	2	2	1	3	8	0	0	0	0	0
Hybrid F_1	3	7	4	4	18	0	0	0	0	0
Hybrid F_2	..	23	1	21	45	..	10	3	4	17

* These are the pure single races employed in crossing. In all the crosses, one P_1 race was Snowflake (long chromosomes), trisomic forms of this variety being used as parents.

THE F_2 HYBRIDS

The crosses involving series 34 give the expected segregation and recombination of color and pubescence characters in F_2 , producing purple and red pubescent and purple and red glabrous, both with several grades of color intensity, and white glabrous; the compact habit of series 34 also reappears, apparently as a simple recessive character. The crosses of series 38 (light-purple form) give purple pubescent and white glabrous, and those of series 39 give purple pubescent, red pubescent, and white glabrous; in both cases the recessive intense form pp (deep purple or light red, as contrasted with the light purple or pale red produced when P is present) appears. F_1 hybrids from a late form of series 39 produce in F_2 a late type with large leaves, which is very distinct under some conditions and may be a simple recessive.

Clearly the hybrids of Snowflake with these pure single races show in F_2 the typical Mendelian segregation for color and pubescence which

Miss SAUNDERS (1924) and TSCHERMAK (1912) have so extensively demonstrated for this species. Part of the F_2 families, as expected, also include the recessive double-flowered type; since, however, the doubles have no stamens, only singles could be used in the determination of chromosome shape.

Only about one-fourth of the 62 F_2 plants classified for chromosome shape in table 1 were chosen, in connection with the present study, to represent various somatic segregation types; the rest were chosen primarily either for special study of trisomy and tetrasomy among the hybrids, or simply for determination of chromosome number to check the classification of mutant types. On account of the method of selection of plants for the determination of chromosome shape, the ratios for the various other segregating characters have no significance.

As tables 1 to 3 show, all plants examined for chromosome shape in the F_2 had either long (elongated) chromosomes like Snowflake or short (contracted) ones like the pure single varieties used in the crosses. Here, as in the parental varieties, there has been no case of questionable classification. The long-chromosome character appears to be a simple recessive, appearing in only 17 out of the 62 plants examined. The data for chromosome shape were accumulated during a period of several months, and they were never far from a 3:1 ratio after 12 plants had been examined.

Figures 4a and 4b show chromosome-shape differences between an F_2 plant with short and one with long chromosomes at first metaphase. Figure 4a shows the very compact condition which is usual in short-chromosome plants but which is never approached in plants with long chromosomes. The chromosomes in figure 4b are not exceptionally long for F_2 long-chromosome plants.

In the F_2 as in the parent varieties and the F_1 , reduction was relatively normal in pollen mother cells from plants with short chromosomes even when odd chromosomes were present in addition to the normal seven pairs, whereas abnormalities of reduction similar to those found in Snowflake were always found in the pollen mother cells of the segregants with long chromosomes.⁴ It seems probable that the elongation of the chromosomes is dependent upon a single gene and that normality of reduction is either directly or indirectly controlled by it also. Possibly the elongated form entails mechanical difficulties in the conjunction and disjunction of the chromosomes. It may also favor fragmentation.

⁴ Since the F_2 data on abnormal cytokinesis were mainly obtained from trisomics, detailed presentation is reserved for a general report on the cytology of these forms.

Snowflake plants with the normal complement of chromosomes are remarkable for their abundant production of trisomic progeny (FROST 1919). Although we have no adequate records from short-chromosome races or their F_1 hybrids for comparison, it seems highly probable that these will produce much smaller percentages of chromosomal mutants. In view of the extensive and thorough study of *Matthiola* by Miss SAUNDERS (1924 and papers there cited) and by TSCHERMAK (1912), it seems improbable that these workers would have failed to record variant forms so striking and so abundant as these mutants have proved with Snowflake. DE VRIES (1906, p. 338), however, describes one form suggestive of chromosomal mutation; the "stiff and erect appearance" of these variants (called "generals" in Erfurt) suggests our Large trisomic, although the other characters mentioned, especially the "more luxurious growth" hardly favor this identification.

Since the gene for long appears to be exceptional in the species, it seems likely that it arose from its "short" allelomorph by mutation. It is therefore appropriate to use the symbol l for long and L for short.

The two chromosome types in F_2 were well distributed among the various trisomic and segregant types (tables 2 and 3). The recombinations of characters seem to demonstrate that gametic recombination for chromosome shape has repeatedly occurred.⁵ The F_1 hybrids derived from series 34 and 39 (red corolla) received the gene for purple from the Snowflake parent only. Therefore, the genes B (purple) and l (long) entered these hybrids from the same parent, and the one red long ($bbll$) F_2 plant of table 3 indicates separation of B and l in gametogenesis. In a similar way, the ten white shorts (rr or cc plus Ll or LL) indicate that L separated from a gene for color (presumably Miss Saunders's R or C). All the colored longs ($BBll$, $Bbll$ or $bbll$, with R and C) also indicate separation of L from R or C . Two of the three intense shorts ($ppLl$ or $ppLL$) from series 39 indicate separation of P and L ; the third is doubtful, since this plant was an extreme (tetrasomic) Slender and doubtless had *two* extra chromosomes (fragments) derived from the intensity-carrying (PP) pair of Snowflake. It is therefore evident that the L and l genes recombine with Bb , Pp and Rr or Cc .

⁵ Complications due to trisomy may invalidate this conclusion in an occasional case. For example, a plant of the constitution $\frac{ICR}{LCR}$ would be colored short, but the addition of an extra chromosome carrying l but not the color genes would give colored long, providing ll is dominant over L .

Doubleness, a recessive character for which all Snowflake singles are heterozygous, is not represented in the data for chromosome shape, because doubles lack stamens. In F_2 progenies segregating for doubleness,

TABLE 2

Numbers of F_2 plants, with short and with long first-metaphase chromosomes, belonging to various trisomic and tetrasomic types.

	CRENATE	DARK	LARGE	NARROW	SLENDER	SMALL	SMOOTH	LARGE SMOOTH	NORMAL	ALL TYPES
Chromosomes short	4	2	2*	1	5†	8†	6†	1	16	45
Chromosomes long	3	0	0	1	3	4†	3	0	3	17

* Both tetrasomics. † Including one tetrasomic in each case.

however, all single longs indicate separation of l from a gene for double. These progenies gave the ratio 26 short: 8 long, while the all-single progenies, derived from F_1 plants homozygous for single, gave the ratio 19:8. L and l therefore recombine freely with the single-double allelomorphs.

TABLE 3

*Relation of first-metaphase chromosome shape to other segregating characters in F_2 . Numbers of plants with the characters indicated.***

SOMATIC CHARACTERS	CHROMOSOMES SHORT				CHROMOSOMES LONG			
P_1 series:	34	38	39*	All	34	38*	39	All
Pubescent purple	8	1	10 (2)†	19	7	3 (1)	3	13
Pubescent red	3		8 (1)	11	1			1
Glabrous purple	3			3				
Glabrous red	1			1				
Glabrous white	7		3	10	2		1	3
Totals	23†	1	21 (3)	45†	10	3 (1)	4	17

* The F_2 hybrids from series 38 and 39 which showed the recessive intense color (pp) derived from Snowflake are indicated by the numbers in parenthesis.

† One of these intense shorts was extreme (presumably tetrasomic) Slender.

‡ Including one feeble tetrasomic, on which no open flowers were observed.

** The following data, for normal F_2 plants, progeny of normal F_1 plants heterozygous for red, have been obtained too late for inclusion in table 3: purple, 11 short:4 long (homozygous purple, 7:3); red, 7:5. In addition, 5 miscellaneous F_2 plants were all short. The F_2 totals are therefore now: 68 short:26 long.

In the case of the F_2 plants descended from the later form of series 39, no recombination for lateness was demonstrated; the 5 early (dominant) and 7 late plants examined all had short chromosomes. This absence of the double recessive form following a "repulsion" cross (late short $\times$ early long) suggests linkage, but several times as many late plants would be

necessary to give this absence much statistical significance. Of the 14 F_2 descendants of series 34 whose chromosomes were examined last (late in 1926), 13 had been classified for the recessive compactness derived from that parental race; 4 of these were compact, but none were long, and therefore no recombination was demonstrated.

Table 3, with the late data added in the last footnote, includes 46 purples and 25 reds derived from F_1 parents heterozygous for red. There is no significant indication of linkage between *Bb* and *Ll*, for the ratios are: purple, 32 short:14 long; red, 19:6. The purples (ratio 7:3) listed as homozygous were determined by examination of the seed (embryo) color in their capsules.

SUMMARY

1. One horticultural variety of *Matthiola incana* R. Br has chromosomes which are remarkably long at the late prophase and the metaphase of the first meiotic division in the pollen mother cells. Other races have much shorter first-metaphase chromosomes.

2. The long-chromosome condition is recessive in F_1 hybrids and has reappeared in F_2 in about one-fourth of the individuals. It therefore seems to be due to a single gene, which may be denoted by *l*.

3. Normal plants of the long-chromosome variety produce several percent of trisomic progeny, of at least eight kinds. In three of these mutant types the extra chromosome is clearly a fragment of a normal chromosome.

4. Abnormal chromosome behavior has frequently been observed in the pollen mother cells of the long-chromosome race. In the short-chromosome races, on the other hand, reduction and tetrad formation are almost always normal. This is also true of the F_1 hybrids between long and short, and of the F_2 plants with short chromosomes, but the F_2 segregants with long chromosomes show much the same amount and type of abnormality of reduction as the long-chromosome variety. Evidently the long-chromosome gene in some way favors abnormalities of meiosis—possibly as a direct result of the length and attenuation of the chromosomes.

5. Three genes for color characters, which other workers have found to exhibit typical Mendelian behavior in hybrids, have given evidence of recombination with the genes for chromosome shape. The genes for chromosome shape appear not to be linked with those for single *versus* double flowers.

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