Structure and Behavior of Chromosomes of Two Varieties of *Hesperis matronalis* L.

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Received September 27, 1972

During the author's stay at the University of Manitoba, Winnipeg, Canada, seeds of two varieties of *Hesperis matronalis* of the family Cruciferae were obtained by the author. Of those, one variety with small purple flowers was widely cultivated in Winnipeg as a garden plant. Another variety with white and comparatively large flowers was collected from Rumania. The two varieties were grown in the experimental field of the University of Manitoba to explore the potentiality of them as suitable oil yielding plants.

Hesperis matronalis provides with materials of considerable cytological interest as indicated by the presence of cytotypes in relation to chromosome number (Jaretsky 1928, Manton 1932, Easterly 1963). It was thought worthwhile to find out whether the two varieties of *H. matronalis* collected from different areas possessed same or different chromosome number as well as their karyotypic differences, if any. Both the factors taken together may justify the adaptability of these two varieties to two distinctly different geographical regions. The present work was, therefore, undertaken to work out the detailed karyotypes of the two varieties of *H. matronalis*.

Materials and methods

Seeds of two varieties of *Hesperis matronalis* L. var. I (small purple flowered) and var. II (large white flowered) were maintained in the Department of Plant Science, the University of Manitoba, Canada. Root-tips were pretreated in half saturated aqueous solution of paradichlorobenzene for 2 hours at 8°C followed by fixation in acetic-alcohol (1:2) for 45 minutes. The materials were then hydrolysed and stained in aceto-orcein. Flower buds were studied by aceto-carmin squash technique. Drawings were made at a table magnification of approximately $\times 1800$.

Observations

Two varieties of *H. matronalis*—purple flowered and white flowered—possess $2n=14$ and 24 chromosomes respectively (Figs. 1 and 14). Chromosomes are long with mostly median and a few subterminal primary constrictions. Variation in the somatic chromosome complements has been observed in the white flowered

Figs. 1-13. *Hesperis matronalis* var. I. 1, normal somatic metaphase ($2n=14$). 2A, idiogram. 2B, *Hesperis matronalis* var. I. Somatic metaphase ($2n=14$). 3-13, meiotic stages (vide text, for details).

variety. As chromosome morphology of the two varieties reveals much similarity, the different chromosome types are described on a comparative basis. The chromosome types are:

Type A—Unusually long chromosome with nearly submedian primary constriction and a secondary constriction placed at the distal end of the long arm. Primary constriction region of this chromosome is well exaggerated and shows centromeric granules in most of the cells. Besides, this being the longest pair, much longer than all the other types, can be easily identified.

Type B—Long chromosome, shorter than type A, with two constrictions, primary and secondary, one median in position and the other submedian in position, placed very near the former.

Type C—Long chromosomes with nearly median primary constrictions.

Type D—Comparatively medium-sized chromosome, with two constrictions, primary and secondary, placed at the two distal ends.

Type E—Medium-sized chromosome with submedian primary constriction placed at one end and a very small satellite located at the end of the short arm.

Type F—Represents medium-sized chromosomes with nearly median primary constrictions.

Type G—Medium-sized chromosomes with subterminal primary constrictions.

1. *Hesperis matronalis* L. var. I (small purple flowered)

$$(2n=14=2L^s + 2L + 2M^s + 8M) = 2A + 2C + 2E + 2F + 6G$$

Chromosome size ranges from 5.4 μ to 11.8 μ .

Meiosis is irregular. Metaphase I shows seven bivalents (Figs. 3–4). One bivalent is comparatively larger than the rest. Diakinesis reveals seven bivalents, one of which is very large with three chiasmata (Fig. 5). The large bivalent is very easily identifiable in diakinesis. This large bivalent is probably formed by the very long chromosome pair observed in the somatic cells. Thus this large pair can be very easily identified in meiosis and mitosis as well. One bivalent is found attached with the nucleolus. One faintly stained extra-chromosomal chromatin body is observed in many PMCs during metaphase I, anaphase I and telophase I (Figs. 6–8). Chromatin bridges with fragments are observed in many PMCs in anaphase I (Figs. 9–10). One PMC. shows a bridge and four fragments (Fig. 11). Laggards are observed in some of the PMCs (Fig. 12). Faintly stained extra-chromosomal chromatin body is present in PMCs. with or without fragments (Figs. 6–13). Of the PMCs. analysed, 66% show abnormal behaviour and 34% are normal (Table 1).

2. *Hesperis matronalis* L. var. II—(large white flowered)

$$(2n=24 = 4L^s + 4L + 4M^s + 12M) \\ = 2A + 2B + 4C + 2D + 2E + 8F + 4G$$

Size difference of the chromosomes varies from 5.1 to 11.0 μ . Four pairs of chromosomes are characterized by secondary constrictions or satellites (Figs. 14 and 15).

In addition to the normal karyotype, several somatic nuclei with abnormal chromosome complements are on record, $2n=23$, 25 and 28 (Figs. 16–18). Of these, $2n=25$ complement has been observed in a number of somatic cells.

Figs. 14-17. *Hesperis matronalis* var. II. 14, normal somatic metaphase ($2n=24$). 15A, idiogram table of the two varieties of *Hesperis*. 15B, *H. matronalis* var. II. Normal somatic metaphase ($2n=24$). 15C, variation metaphase ($2n=23$). 16-17. Variation somatic metaphases showing $2n=23$ and 25 chromosomes.

Table 1. PMC analysis in *Hesperis matronalis* var. I

	Dia- kinesis	Meta- phase I	Ana- phase I	Telo- phase I	Total PMCs	Irregular PMCs (%)
No. of normal PMCs	14	20	—	—	34	
No. of PMCs with 1 faintly stained chromatin body	—	16	8	6	30	
No. of PMCs with 1 fragment + 1 faint chromatin body	—	—	—	7	7	
PMCs with 1 bridge + 1 fragment	—	—	9	—	9	
PMCs with 1 bridge + 1 fragment + 1 faint chromatin body	—	—	6	—	6	
PMCs with 1 bridge + 4 fragments + 1 faint chromatin body	—	—	2	—	2	
PMCs with 2 laggards + 1 faint chromatin body	—	—	12	—	12	
					100	66%

Figs. 18-24. *Hesperis matronalis* var. II. 18, variation somatic metaphase ($2n=28$). 19-24, meiotic stages (for details, vide text).

Twelve bivalents are observed at metaphase I (Fig. 19). In some of the PMCs eleven bivalents and three univalents are noticed (Fig. 20). Two ring bivalents are recorded in most of the PMCs. Metaphase II shows twelve chromosomes in one pole and thirteen chromosomes in the other pole (Fig. 21). Thus some of the PMCs with eleven bivalents and three univalents in metaphase I or separation of twelve-thirteen chromosomes in metaphase II confirm the somatic variation number ($2n=25$) as observed in a number of cases (Fig. 17). Slight meiotic irregularities, namely lagging and late separation of bivalents, are found in a few mother cells (Figs. 22–24).

Discussion

The genus *Hesperis* is remarkable in the family Cruciferae having very long chromosomes. A few other genera like *Matthiola*, *Iberis* etc. are also characterized by long chromosomes.

The previous literature in *Hesperis matronalis* shows the presence of different chromosome numbers, viz. $n=8$, 12, 14 and 16 (vide Table 2). Manton (1932)

Table 2. Previous and present records of chromosome number in *Hesperis*

Species	Previous record			Present record	
	2n	n	Author	2n	n
<i>Hesperis matronalis</i> L. var. I	—	—	—	14	7
<i>H. matronalis</i> L. var. II	—	—	—	24	12
<i>H. matronalis</i> L.	24	—	Manton (1932) Love and Love (1956)		
<i>H. matronalis</i> L. ssp. <i>matronalis</i> var. <i>matronalis</i>	24	—	Dvorak (1964b)		
<i>H. matronalis</i> L. ssp. <i>matronalis</i> var. <i>nivea</i> (Baumg) Priessm.	24	—	Dvorak (1964b)		
<i>H. pychonotricha</i> Borb. and Deg.	14	—	Dvorak (1964a)		
<i>H. bicuspidata</i> Willd.	14	—	Manton (1932)		
<i>H. steveniana</i> DC.	14	—	Manton (1932)		
<i>H. sylvestris</i> Crantz.	14	—	Dvorak (1964b)		
	26	—	Manton (1932)		
<i>H. tristis</i> L.	14	—	Manton (1932)		
<i>H. alba</i> Mill.	24	—	Dvorak (1964b)		
<i>H. runcinata</i> W. and K.	24	—	Manton (1932)		
<i>H. lutea</i> Max.	28	—	Manton (1932)		

reported $n=12$ chromosomes in this species and criticized the earlier report of $n=14$ in the same species by Jaretsky (1928). A pair of very long metacentric chromosomes was observed by Manton (*l.c.*) which, she suggested, was possibly recognized by Jaretsky (*l.c.*) as four different chromosomes instead of two. It was further suggested that $n=12$ was a tetraploid number with $x=6$ as the base which

had been derived by aneuploid loss of a chromosome from a form with $n=7$ chromosomes.

The present record of $2n=24$ chromosomes in one variety of *H. matronalis* including a pair of very long chromosomes with median centromeres is the first report in this species. In other species of *Hesperis*, $n=7, 12, 13$ and 14 chromosomes have been reported by the previous workers (vide Table 2).

The two varieties of *Hesperis matronalis* studied here, which differ markedly in phenotype—one characterized by small and purple flowers, whereas the other with white and large flowers—differ in the number and morphology of their chromosomes. Purple flowered variety has been found to possess $2n=14$ and the white flowered variety, $2n=24$ chromosomes respectively. In one respect the two varieties show identity i.e. karyotype is graded in both revealing long to medium-sized chromosomes. But a detailed comparison of their karyotypes exhibits much difference. Chromosome type B having primary and secondary constrictions placed

Table 3. Morphology, variations and length of chromosomes

Species	Normal no. (2n)	Chromosome morphology		Variation no. (2n)	Chromosome length	
		Size difference in diploid complement (2n)	Types of chromosome in diploid complement (2n)		Total length in haploid complement (μ)	Length per chromosome (μ)
<i>Hesperis matronalis</i> var. I	14	* $2L_1^s + 2L + 2M^s + 8M$	$2A + 2C + 2E + 2F + 6G$	—	52.3	3.7
<i>H. matronalis</i> var. II	24	$4L^s + 4L + 4M^s + 12M$	$2A + 2B + 4C + 2D + 2E + 8F + 4G$	23, 25, 28	90.2	3.8

* L—Long; M—Medium-sized;

L_1^s —Very long with secondary constriction;

L^s —Long with secondary constriction;

M^s —Medium-sized with secondary constriction.

very close to each other at the middle and the type D having primary and secondary constrictions at the two distal ends are present only in the variety with $2n=24$ chromosomes. In addition, the high numbered variety possesses more long chromosomes and chromosomes with mostly median constrictions whereas the low numbered variety, except one very long metacentric chromosome pair, is characterized by comparatively medium-sized chromosomes with mostly subterminal constrictions. Thus the two varieties have distinctly different karyotypes—mostly symmetrical in one and asymmetrical in the other.

In the variety with $2n=14$ chromosomes, high meiotic irregularities (66% of the PMCs) have been noticed which may indicate that its two sets of chromosomes are not identical. Irregularities include chromatin bridges, fragments, laggards and extra-chromosomal chromatin bodies.

The other variety ($2n=24$) reveals a good deal of variation in the somatic tissue, the altered nuclei showing $2n=23, 25$ and 28 chromosomes in a large number of cases (Table 3). In view of perfect seed-setting and mostly regular meiosis, this

inconstancy in somatic chromosome complements has possibly no role in speciation in this variety. This phenomenon has been observed in a large number of genera reproducing asexually where this abnormal behaviour has been suggested as a means of speciation in them (vide Sharma 1956).

These two varieties, which differ in their phenotypes markedly, exhibit distinct karyotypic differences regarding number and structure of their chromosomes. Their cytological data taken in conjunction with their morphological differences, therefore, suggest the separation of the two varieties as two distinct species.

Summary

Two oil-yielding varieties of *Hesperis matronalis* L. of Cruciferae have been cytologically studied. Their chromosome numbers are—1) *Hesperis matronalis* L. var. I $2n=14$; 2) *H. matronalis* L. var. II $2n=24$. Detailed karyotype analysis in the two varieties shows much difference in the structure of their chromosomes. Meiosis has been worked out in both the varieties, which is mostly regular in one and irregular in the other. On the basis of cytological data and marked phenotypic differences, separation of the two varieties as two distinct species has been suggested.

Acknowledgement

The author is grateful to the National Research Council of Canada for the award of a post-doctorate fellowship.

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