

## INTRODUCTION

Previously, the artificial construction P[ArB] was inserted into various sites of chromosomes 2 and 3 of *Drosophila melanogaster*, and the resulting mutations were shown to alter cell division [1–3]. A great number of polyploid metaphases were detected in neuroblasts of homozygotes for *v27* (61F) and *v40* (78D). The *v27* mutation was shown to be an allele of the *Klp61* gene, while *v40* appeared to alter an unknown gene [3]. The *v40* mutation is not completely lethal. Therefore, homozygotes for *v40* can be used to study the alterations of cell division and to compare effects induced by the mutations in somatic and germ tissues.

The objective of this work was to analyze in detail alterations of cell division resulting from the *v40* mutation.

## MATERIALS AND METHODS

Flies with the genotype *yw*; *v40/CyO*, *y<sup>+</sup>*, and *yw*; *v40* were studied. A balancer strain carrying an insert of the *P* element and the *y<sup>+</sup>* gene was described previously [1].

To obtain preparations of metaphase chromosomes, ganglia were isolated from third-instar larvae, incubated in a hypotonic solution (1% sodium citrate) for

The frequency distribution of metaphases differing in chromosome number (bivalents) in females and males is presented in Table 1. The results demonstrated that the distribution had peaks that corresponded to even numbers of all chromosomes in females and the X chromosome, autosomes, and chromosome IV in males. This can be caused by an increase in cell ploidy or nondisjunction of homologous chromosomes, which can result from tight somatic pairing or formation of the unipolar spindle. The distribution pattern differed from the one expected for sister-chromatid nondisjunction, because the abnormality must result in an odd number of metaphase chromosomes in daughter cells.

The absence of chromosome loss was also characteristic of the *v40* mutation. Since no metaphases with the single X chromosome or single chromosome IV were detected in females, the role of chromatid loss also seemed insignificant. Apparently, female cells carrying the single X chromosome must be viable, because such cells are known to be so in gynandromorphs. Viability of flies carrying single chromosome IV implies that cells with single chromosome IV would be detected if they appeared as a result of chromosome nondisjunction. The same is also true for the Y chromosome. Although one Y0 metaphase was detected, this can be considered the exception rather than the rule, because the Y chromosome was in excess in 72 other metaphases.

Thus, due to the low frequency of chromosome loss during cell division, the distribution pattern cannot be explained by nondisjunction of chromatids or homologous chromosomes. A possible explanation can involve either the increase of cell ploidy when replication occurs but the spindle remains unformed or the formation of the unipolar spindle.

Frequency distributions of polyploid metaphases differing in number of the X chromosomes and autosomes and number of the X chromosomes and chromosomes IV in females are shown in Figs. 1*a* and 1*b*. If the polyploidy can be explained by complete chromosome nondisjunction, metaphase classes 2X, 4A; 4X, 8A; and 8X, 16A (Fig. 1*a*) and 2X, 2IV; 4X, 4IV; and 8X, 8IV (Fig. 2*b*) are expected to be most numerous, i.e., ploidy must be  $2^n$ ,  $n = 1, 2, 3 \dots$ , for each chromosome. The observed distributions significantly differed from the expected one. This is the evidence against complete nondisjunction. The probability of detecting a metaphase with four X chromosomes is  $27/523 = 0.052$  for the distribution in Fig. 1*a*. The probability of detecting a metaphase with six autosomes is  $36/523 = 0.069$  for this distribution. If nondisjunction of the X chromosomes and autosomes is independent, the probability of detecting a metaphase with four X chromosomes and six autosomes must be the product of the above probabilities:  $0.052 \times 0.069 = 0.0036$ . However, the actual probability of detecting such a metaphase, calculated from the distributions shown in Fig. 1*a*, was  $10/523 = 0.019$ , i.e., five times higher than expected.

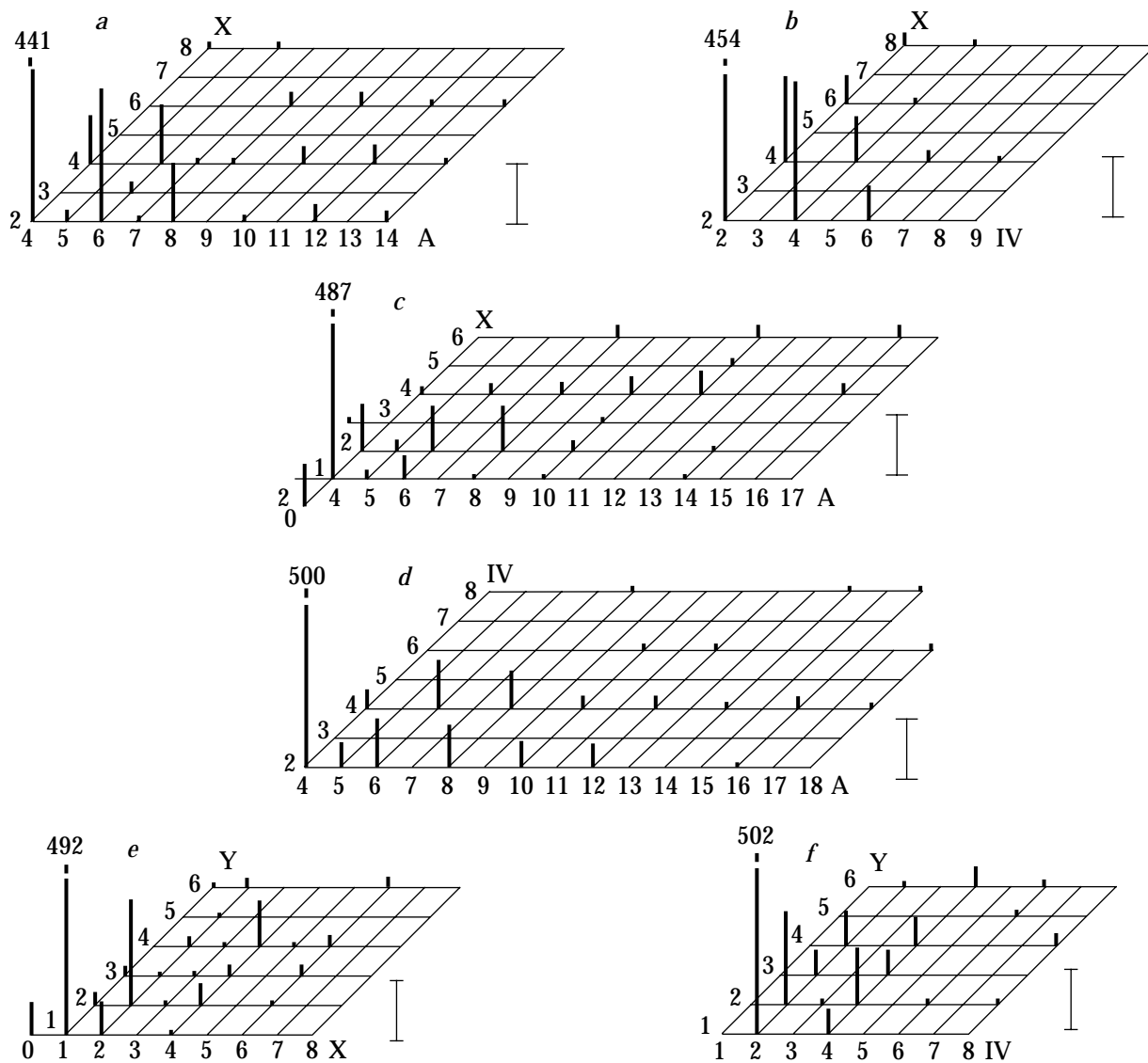
**Table 1.** Distribution of metaphases differing in chromosome number\*

Chromosome number							
	X	A	IV	X	Y	A	IV
0				8	1		
1				596	503		
2	484		477	31	28	1	527
3	1			2	8	1	
4	27	509	35	14	13	501	26
5		2		1	1	4	
6	8	36	10	7	5	16	3
7		2					
8	3	13	1	1	1	14	4
9							
10		6				7	
11							
12		6				7	
13							
14		4				2	
15							
16		1				4	
17							
18		2				2	
19							
20		1					
21							
22							
23							
24							
25							
26		1				1	

\* X and Y, the X and Y chromosomes; A, autosomes; and IV, chromosome IV.

Frequency distribution of polyploid metaphases differing in chromosome number in males is shown in Figs. 1*c* (X–autosomes), 1*d* (IV–autosomes), 1*e* (X–Y), and 1*f* (Y–IV). Probabilities calculated for these distributions as described above are given in Table 2. The association of nondisjunction of different chromosomes is obvious in all cases. This is also evidence that the chromosome number increased due to increased cell ploidy or formation of a unipolar spindle in homozygotes for the *v40* mutation.

If the latter is the actual reason, then the distribution (Table 1) can deviate from the expected one when some chromosomes (each consisting of two chromatids) are not attached to the spindle. In this case, the appearance



**Fig. 1.** Frequency distribution of metaphases differing in number of chromosomes (bivalents) (a); X and chromosome IV in females (b); X and autosomes in males (c); chromosome IV and autosomes in males (d); Y and X chromosomes in males (e); Y and chromosome IV in males (f). The column height corresponding to ten nuclei is indicated at the right.

of new classes can be expected. For the X chromosome, a new class  $8X - 2X = 6X$  would appear in addition to classes  $2X$ ,  $4X$ , and  $8X$ . The distribution (Fig. 1a) would have additional peaks  $(4X - iX; 8A - jA)$ , where  $i$  and  $j$  are even integers,  $i < 4$  and  $j < 8$ . Thus, additional

peaks  $(4X; 4A)$ ,  $(4X; 6A)$ ,  $(4X; 8A)$ ,  $(2X; 8A)$ , and  $(2X; 6A)$  can be expected. All but one such peak were actually detected; peak  $(4X; 8A)$  was absent, possibly, because only a limited number of chromosomes can be attached to the spindle. Likewise, the distribution

**Table 2.** Nonrandom chromosome disjunction

Distribution	Probability of detecting the given chromosome number	Product of probabilities	Observed probability
Fig. 1b	$P(X = 4) = 0.052; P(IV = 4) = 0.067$	0.0035	0.017
Fig. 2a	$P(X = 2) = 0.052; P(A = 6) = 0.027$	0.0014	0.016
Fig. 2b	$P(IV = 4) = 0.045; P(A = 6) = 0.029$	0.0013	0.014
Fig. 3a	$P(X = 2) = 0.055; P(Y = 2) = 0.050$	0.0028	0.034
Fig. 3b	$P(IV = 4) = 0.046; P(Y = 2) = 0.050$	0.0023	0.018

(Fig. 2*b*) is expected to have peaks (2X; 4IV), (4X; 2IV), and (4X; 4IV); the distribution (Fig. 2*c*) would have peaks (2X; 4A), (2X; 6A), and (2X; 8A); and the distribution (Fig. 2*d*), peaks (2IV; 8A), (2IV; 6A), (4IV; 8A), (4IV; 6A), and (4IV; 4A). All these peaks were detected.

Table 1 shows that the rule of even peak numbers can be applied to the Y chromosome only to a certain extent. This might indicate that the Y chromosome has a specific spindle attachment. A deviation from this rule was also detected in other chromosomes (Table 1). Metaphases with an odd chromosome number are characterized in Table 3. Those with odd numbers of two or more chromosomes were very rare. This suggests that these metaphases result from rare chromatid nondisjunction. In several cases, e.g., (7A; 4X; 4IV), chromatid nondisjunction appeared to occur after polyploidization, because the reverse order of events would have resulted in even chromosome numbers.

Since the above analysis indicated that the *v40* mutation caused formation of the unipolar spindle, anaphases of homozygotes for *v40* were studied. Anaphases with the unipolar spindle were actually

**Table 3.** Metaphases with an odd chromosome number (bivalents)

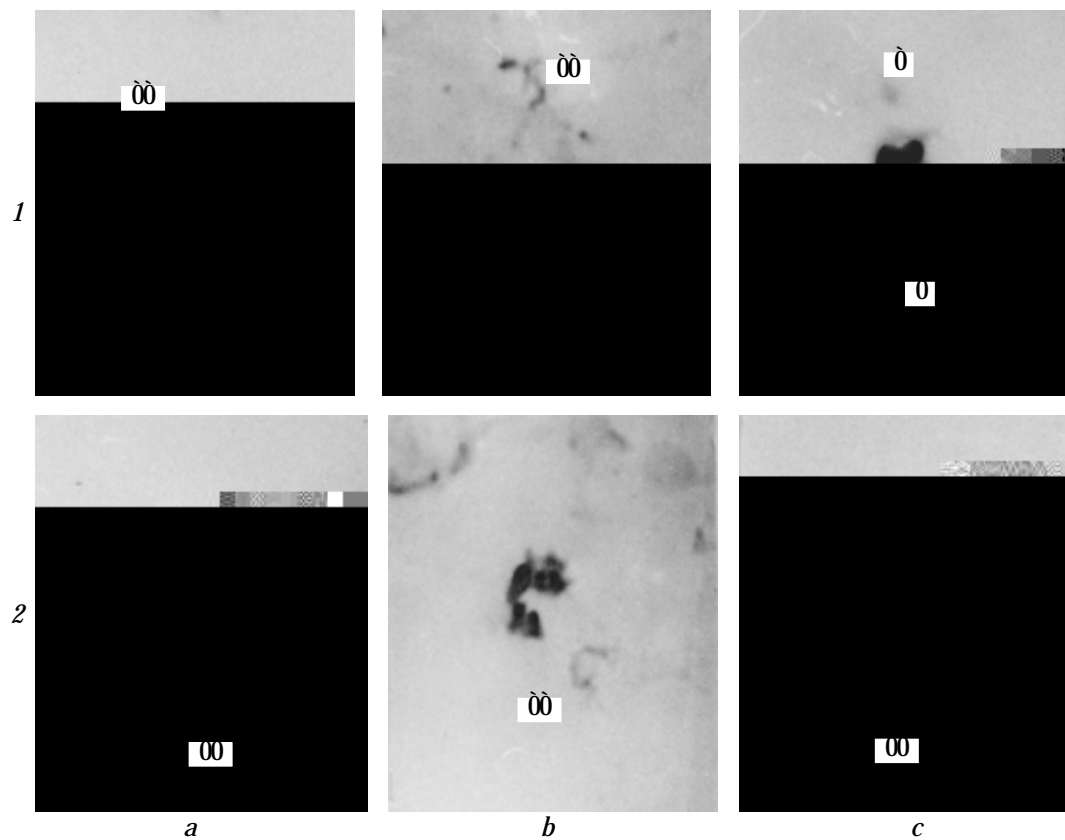
A	X	IV	A	Y	X	IV
7	2	2	5	1	1	2
6	3	2	4	1	3	2
7	4	4	10	4	3	4
5	2	2	16	6	3	4
5	2	2	10	2	5	6
			10	3	2	2
			8	2	3	2
			8	6	3	4
			12	4	3	4
			8	1	3	2
			5	1	1	2
			3	2	1	2
			2	3	3	2
			12	5	4	4

detected in neuroblasts of homozygotes for *v40*. An example of these anaphases are presented in Fig. 2*a*.

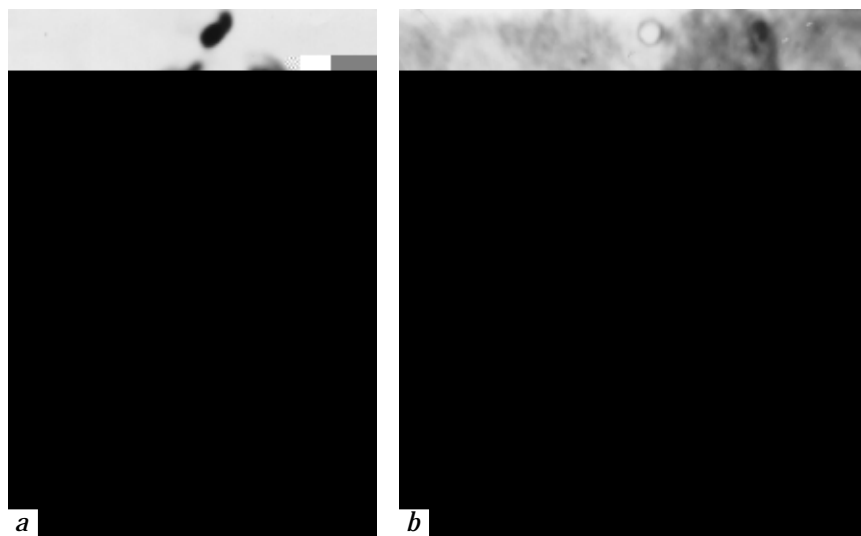
We tested whether anaphases with the unipolar spindle, which are revealed in homozygotes for the *v40* muta-



**Fig. 2.** Abnormalities of neuroblast division in homozygotes for *v40*: (a) unipolar anaphase, (b) circular mitotic figure similar to that characteristic of the *mgr* and *polo* mutations, and (c) chromosome breaks (indicated with arrows).



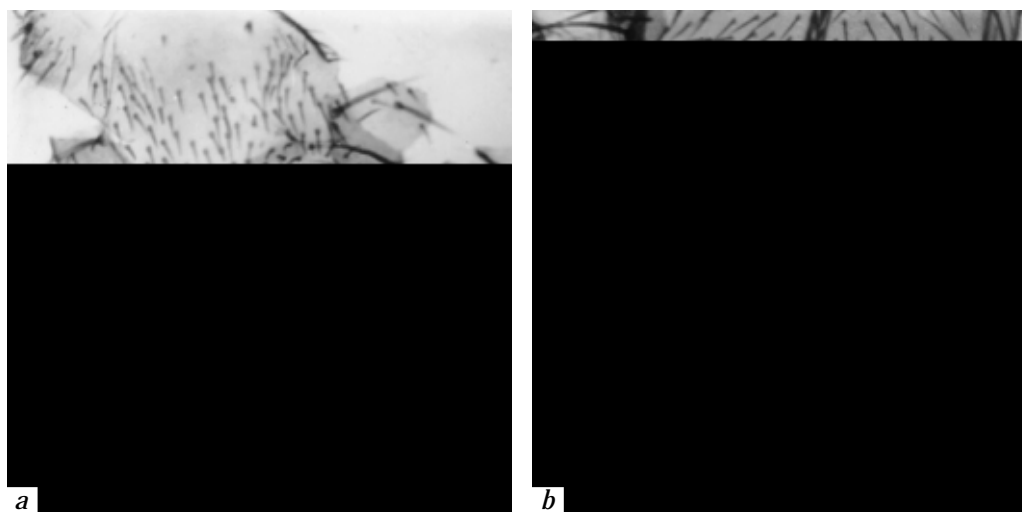
**Fig. 3.** Centriole (c) disjunction in (1) heterozygotes and (2) homozygotes for the *v40* mutation. Centriole disjunction occurred during the prometaphase in heterozygotes (1c) but was not observed during the prometaphase (2b) and metaphase (2c) in homozygotes.



**Fig. 4.** Chromosome abnormalities in germ cells of homozygotes for *v40*: polyploid nuclei in a germarium (a) and premeiotic cyst of a testicle (b) are indicated with arrows. Phase contrast microscopy.

tion, represented an artifact determined by the method used to obtain cell preparations. The number of anaphases with one or two poles was analyzed in ganglia of larvae homo- and heterozygous for *v40* (Table 4). The results showed that anaphases with the unipolar spindle

occur at a very low frequency in heterozygotes and actually represent artifacts caused by the loss of a pole during preparation. The frequency of such anaphases in homozygotes is many times higher and cannot be explained by such artifacts.



**Fig. 5.** Cuticle of (a) homozygote and (b) heterozygote for *v40*. One of scutellar bristles is absent from the homozygote.

Mutations *mgr* (*merry-go-round*) 86E3-20 and *polo* 3-46 result in polyploidy of *D. melanogaster* neuroblasts because of the formation of a defective spindle [6, 7]. Circular mitotic figures (CMF), i.e., circles of chromosomes with their centromeres and chromatid arms pointing inward and outward, respectively, are characteristic of these mutations [6, 7]. Since such structures disappear after colchicine treatment, they are considered to result from the formation of the unipolar spindle. A structure similar to CMF caused by the *mgr* and *polo* mutations was also detected in neuroblasts of larvae homozygous for the *v40* mutation (Fig. 2b).

Broken metaphase chromosomes were also sometimes observed in neuroblasts of larvae homozygous for the *v40* mutation (Fig. 2c).

To study centriole disjunction in homo- and heterozygotes for the *v40* mutation, preparations were stained according to Nokkala [5]. The method was developed in order to study spermatogenesis. Our results showed that it can also be used in the case of neuroblasts.

Centrioles were located opposite to the chromocenter in interphase nuclei of heterozygotes (Fig. 3, upper panels) and reached the poles during prometaphase–metaphase. However, centriole disjunction was disturbed in *v40* homozygotes (Fig. 3, lower panels).

Studies on the centrosome disjunction in *v40* homozygotes performed with the use of anti-centrosome monoclonal antibodies confirmed these results (C. Sunkel, personal communication).

Previously, the *v40* insert was shown to be a lethal causing death of larvae and pupae [3]. However, mass rearing allowed several adult homozygous for this mutation to be obtained (E.S. Belyaeva, personal communication). This provided a possibility to study the

alterations of cell division in female and male germ lines.

Females homozygous for *v40* had degenerated ovaries containing germariums. No later stages of oocyte development were detected. A premeiotic polyploid metaphase resulting from chromosome nondisjunction

**Table 4.** Number of bi- and unipolar anaphases in homo- and heterozygotes for *v40*

<i>v40</i>	2 poles	1 pole
Heterozygotes	59	1
Homozygotes	132	55

**Table 5.** Phenotypes of homozygotes for mutations affecting cell division\*

Gene	Phenotype
<i>asp</i> <sup>1</sup> 96A20	Abnormal wings, rough eyes
<i>dally</i>	Abnormal eyes, antennas, wings, and genitals
<i>dia</i> 38E	<i>dia</i> <sup>9</sup> / <i>dia</i> <sup>9</sup> —rough eyes
<i>esg</i> 35D1-2	Poorly differentiated abdomen
<i>rod</i> 100B5-D1	Rough eyes, rare abdominal bristles, cut wings
<i>stg</i> 99A5-6	Small eyes, abnormal bristle pattern on the body
<i>cdc</i> 31E	Defective abdomen at 25°C
<i>tw</i> s 85E7-F16	Noncoordinated movements, additional sensory structures, rough eyes
<i>polo</i> 3-46	<i>polo</i> <sup>1</sup> / <i>polo</i> <sup>2</sup> —abnormal tergites and sternites
<i>mgr</i> 86E3-20	Abnormal cuticle, rough eyes
<i>dal</i> 31A-32F	Altered tergite position

\* Data obtained via Internet, from the FLYBASE database.



**Fig. 6.** Highly condensed chromosomes (chromosome bows) in ganglia of *v40* homozygotes.

during primary oocyte divisions in a germarium is shown in Fig. 4a. Likewise, adult males displayed almost complete degeneration of internal testicular structures. Cytological analysis revealed polyploid nuclei appearing during primary spermatocyte divisions in testicles of homozygous larvae (Fig. 4b).

As other mutations affecting cell division, *v40* was associated with abnormal scutellar bristles in homozygotes (Fig. 5). This phenotype was observed for several generations after the mutation had appeared, but was not detected in later generations, possibly, because of accumulation of modifiers.

### DISCUSSION

The *v40* insertional mutation was revealed by the presence of polyploid metaphases with highly condensed chromosomes (Fig. 6). Considering the compact chromosome structure, we propose that this mutation be termed *chromosome bows* (*chb<sup>v40</sup>*).

Comparative analysis showed that the *chb<sup>v40</sup>* mutation determining abnormal scutellar bristles is similar to other mutations affecting cell division in phenotypic expression (Table 5).

A great number of polyploid metaphases in homozygotes allowed a single hypothesis to be selected from several assumptions considering the nature of the primary mitotic defect caused by mutation. The frequency distribution of nuclei differing in chromosome number is consistent only with the hypothesis that states the unipolar spindle is formed in mutants. This conclusion was confirmed by direct observation of abnormal anaphases.

Previously, CMF were shown to be characteristic of the *mgr* and *polo* mutations. This alteration was assumed to result from the inactivation of one of the centrosomes during mitosis in mutants [6, 7]. Material of the centrosomes identified with labeled antibodies Bx63 displayed no specific regular organization in pre-blastodermal embryos homozygous for the *polo* mutation [7].

Our experiments showed that centriole disjunction is blocked in *v40* homozygotes.

The results obtained indicate that *chb<sup>v40</sup>*, as the *mgr* and *polo* genes, encodes a centrosome protein.

Polyploid cells resulting from abnormal mitotic division of primary oocytes and spermatocytes were revealed in female and male germ lines. Thus, the *chb<sup>v40</sup>* mutation affects similarly both somatic and germ cells.

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