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Non-disjunction and heterochromatin in the meiosis of males of Drosophila melanogaster

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Citogenetica. — *Non-disjunction and heterochromatin in the meiosis of males of* Drosophila melanogaster [(*)]. Nota [(**)] di Rosella Carotti, Antonio De Marco, Michele E. Grandolfo e Laura Toti, presentata dal Socio G. Montalenti.

RIASSUNTO. — Sono stati incrociati maschi, con inversioni di differente lunghezza e differenti punti di rottura sulla X, in presenza ed in assenza dell'inversione SM5 sul secondo cromosoma, al fine di verificare se esiste o meno appaiamento non omologo nel maschio e se c'è qualche rapporto tra eterocromatina e non disgiunzione. I risultati ottenuti dimostrano che non si manifesta appaiamento non omologo tra il cromosoma X e i cromosomi II; che c'è una certa relazione tra l'eterocromatina dell'X, e soprattutto i suoi segmenti hA e hB, e la non disgiunzione di XY; che tra le eccezioni si recupera costantemente una maggiore percentuale di maschi rispetto alle femmine.

There are numerous differences between the male meiosis and the female meiosis of *Drosophila melanogaster*. Phenomena that are present in the female—such as non-homologous pairing, interchromosomal effect and distributive pairing—are not detectable or absent in the male. Consequently, non-disjunction must be explained differently according as to whether the female or the male germ-line is considered.

We have considered it opportune to analyse some of the phenomena that may be associated with non-disjunction in the male; in fact, although it seems important to have a more thorough knowledge of the subject, little information exists about it [1].

We thought that heterochromatin could play an important role in the process of male pairing and disjunction; this theory seemed to us to be supported by the observation that one stock, lacking most of the heterochromatin of the X-chromosome in In (I) $sc^4 sc^8$, yields a high percentage of non-disjunction for the X and Y chromosomes.

In particular, we wished to approach the following problems:

a) to check whether, in the male of *Drosophila m.*, there really exists no non-homologous pairing, as the mechanism capable of explaining the non-disjunction; *b*) to find out to what extent the heterochromatin of the X-chromosome may play a part in pairing and in disjunction of the X-chromosomes. It is to be noted that certain data that we have obtained, and are in course of publication, on In(I) $sc^4 sc^8$ males, with and without SM5 inversion on the 2nd chromosome, favour both the importance of heterochromatin in chromosome non-disjunction and the importance of non-homologous pairing in the carrier males of both inversions.

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MATERIALS AND METHODS

The following experiments were planned and analysed:

Exp. I) Crosses were effected between normal females and males carrying in the X–chromosome inversions having different break-points in the euchromatin and in the heterochromatin, while the structures of the remaining chromosomes were normal.

For the control cross, $yw/y^{+}Y$; $^{+}/_{+}$; $^{+}/_{+}$; $^{+}/_{+}$ males and y/y ; $^{+}/_{+}$; $^{+}/_{+}$; $^{+}/_{+}$ females were used.

With this group of experiments it may be calculated whether, and to what extent, there is any variation in the percentage of non-disjunction of the sex chromosomes when, in the X–chromosome, there are inversions that concern the heterochromatin to a different extent.

Exp. II) Crosses were effected between normal females and males carrying in the X–chromosome the same inversions as in the previous experiment, but with the addition, in the second chromosome, of the multiple inversions SM5. The second chromosomes conveniently marked are thus: the one with the inversion by Cy, the other by Sp. Hence, thanks to the markers present in the second chromosomes, it is possible to show whether there is preferential segregation of one of them compared with the exceptional gametes nullo–XY and XY.

In the control cross, $yw/y^{+}Y$; Cy/Sp ; $^{+}/_{+}$; $^{+}/_{+}$ males and y/y $^{+}/_{+}$; $^{+}/_{+}$; $^{+}/_{+}$ females were used.

With this second group of experiments it is possible to determine the value of the non-disjunction of the sex chromosomes in males that at the same time carry an inversion in the X chromosome and an inversion in the second chromosome, and to verify whether this value has increased or not, compared with the first experiment and the control. Moreover, it must be noted that in both experiments the Y–chromosome of the male is always marked by y^{+} and the X–chromosome of the female by *yw*; in this way it is possible to recognise among the progeny individuals that are non-disjunctional for the sex chromosomes, since the male exceptions deriving from a nullo–XY gamete do not show the y^{+} phenotype, and the female exceptions, deriving from an XY gamete, show the y^{+} phenotype. Moreover, the X–chromosome of the male is in all cases distinguishable from that of the female, since it always carries specific markers. It is to be noted that consideration has not been given to the exceptions obtained in clusters of individuals among the progeny of a single male; in fact, they have generally been attributed to exchange occurring during gonial mitosis.

The inversions used, with the respective break-points (indicated in Genetic Variations of *Drosophila m.* by Dan Lindsley and R. H. Greel, 67) [5] are listed in Table I.

TABLE I

Nature of X-inversions used, with the respective break-points.

INVERSION	BREAKPOINTS	BREAK RIGHT
In(I)sc^{8}, $y^{31d}\,sc^{8}\,w^{a}$	1B2–3; 20B–D1	*h*A
In(I)*lv*231 , *yl*(I)*v*231	1C–D; 19–20	*h*B (?)
In(I)EN , *y bb*	1A; 20; 20B–C	*h*B
In(I)rst^{3} , $y\ rst^{3}$ *car bb*	3C3–4; 20B	*h*C
In(I)y^{4} , y^{4}	1A8–B1; 18A3–4	euchrom.
In(I)*dl*–49 , $y\ fa^{n}$	4D7–E1; 11F2–4	euchrom.
In(I)sc^{4} , $y\ sc^{4}$	1B3–4; 19F–20C1	euchrom. *h*D
In(I)65 , *yf*	1C; 10B	euchrom.

The total number of crosses, for each stock carrying a specific inversion, is in the region of a hundred. Each male, about 5 days old, was crossed in a single vial with 3 virgin females, at least 3 days old; after 5 days, males and females were transferred to fresh vials for 5 more days. The counts of the progeny were made on the 16th and 20th days. The cultures were kept at 24° C.

RESULTS AND CONCLUSIONS

The results obtained from experiments I and II are summarised in Tables II and III respectively. In the cases in which the differences between the various stocks and the control seemed more marked, we compared the different probabilities encountered with the probabilities of the normal case, supposing the difference found to be not significant. Applying the theory of large samplings, we obtained the variable standardised statistics z (indicated in Table II and Table III), which were compared with z_a , z_b , z_c, respectively corresponding to the levels of significance of 0.01, 0.05, 0.10, where

$$z = \frac{|\mathrm{Pi} - \mathrm{Po}|}{\sigma|\mathrm{Pi} - \mathrm{Po}|} \quad ; \qquad \mathrm{P} = \text{Non-disjunction frequencies}$$

$$\mathrm{N} = \text{Progeny}$$

$$\sigma_{\mathrm{Pi}-\mathrm{Po}} = \mathrm{PQ}\left(\frac{1}{\mathrm{No}} + \frac{1}{\mathrm{Ni}}\right) \quad ; \quad \mathrm{P} = \frac{\mathrm{No\,Po} + \mathrm{Ni\,Pi}}{\mathrm{No} + \mathrm{Ni}}$$

(for $i = 1, \cdots, 8, 1^1, \cdots, 8^1$). As is shown in the Tables, some of the differences are significant only for the more extreme values, compared with the control.

TABLE II

Progeny and non-disjunction frequencies from the crosses :

♂♂ In(I)/y^+Y ; $^+/_+$; $^+/_+$; $^+/_+$ × ♀♀ *yw*/*yw* ; $^+/_+$; $^+/_+$; $^+/_+$ ($X_0/N_0 = P_0$).

INVERSION	Exception	Progeny	Non-disjunction frequencies	z	Level of significance
	X	N	P	z	*ls*
Control	$X_0 = 10$	$N_0 = 12{,}916$	$P_0 = 0.000774$	—	—
In(I)*sc*^{8}	$X_1 = 15$	$N_1 = 14{,}450$	$P_1 = 0.001038$	$z_1 \doteq 0.91$	*ns* (*)
In(I)*lv*231 . . .	$X_2 = 19$	$N_2 = 14{,}271$	$P_2 = 0.001331$	$z_2 = 1.56$	*ls* 10 %
In(I)EN . . .	$X_3 = 10$	$N_3 = 14{,}169$	$P_3 = 0.000706$	$z_3 = 0.21$	*ns* (*)
In(I)*rst*^{3}	$X_4 = 5$	$N_4 = 12{,}202$	$P_4 = 0.000410$	$z_4 = 0.95$	*ns* (*)
In(I)*y*^{4}	$X_5 = 9$	$N_5 = 12{,}395$	$P_5 = 0.000726$	$z_5 = 0.14$	*ns* (*)
In(I)*dl*-49 . . .	$X_6 = 5$	$N_6 = 14{,}594$	$P_6 = 0.000343$	$z_6 = 1.27$	*ns* (*)
In(I)*sc*^{4}	$X_7 = 2$	$N_7 = 13{,}235$	$P_7 = 0.000151$	$z_7 = 2.14$	*ls* 5 %
In(I)65	$X_8 = 2$	$N_8 = 13{,}243$	$P_8 = 0.000151$	$z_8 = 2.14$	*ls* 5 %

TABLE III

Progeny and non-disjunction frequencies from the crosses :

♂♂ In(I)/y^+Y ; Cy/Sp ; $^+/_+$; $^+/_+$ × ♀♀ *yw*/*yw* ; $^+/_+$; $^+/_+$; $^+/_+$ ($X_0/N_0 = P_0$).

INVERSION	Exception	Progeny	Non-disjunction frequencies	z'	Level of significance
	X′	N′	P′	z'	*ls*
Control	$X'_0 = 8$	$N'_0 = 11{,}419$	$P'_0 = 0.000700$	—	—
In(I)*sc*^{8}	$X'_1 = 12$	$N'_1 = 9{,}717$	$P'_1 = 0.001230$	$z'_1 = 1.09$	*ns* (*)
In(I)*lv*231 . . .	$X'_2 = 13$	$N'_2 = 14{,}271$	$P'_2 = 0.000911$	$z'_2 = 0.58$	*ns* (*)
In(I)EN . . .	$X'_3 = 14$	$N'_3 = 10{,}591$	$P'_3 = 0.001322$	$z'_3 = 1.72$	*ls* 5 %
In(I)*rst*^{3}	$X'_4 = 11$	$N'_4 = 12{,}199$	$P'_4 = 0.000902$	$z'_4 = 0.54$	*ns* (*)
In(I)*y*^{4}	$X'_5 = 6$	$N'_5 = 12{,}824$	$P'_5 = 0.000468$	$z'_5 = 0.75$	*ns* (*)
In(I)*dl*-49 . . .	$X'_6 = 10$	$N'_6 = 12{,}852$	$P'_6 = 0.000768$	$z'_6 = 0.12$	*ns* (*)
In(I)*sc*^{4}	$X'_7 = 5$	$N'_7 = 12{,}848$	$P'_7 = 0.000389$	$z'_7 = 1.28$	*ls* 10 %
In(I)65	$X'_8 = 3$	$N'_8 = 12{,}746$	$P'_8 = 0.000235$	$z'_8 = 1.92$	*ls* 5 %

(*) = non-significant.

From the data taken as a whole, it is found that there is no preferential segregation of the second chromosomes compared with the exceptional gametes and that the values of XY non-disjunction do not vary significantly when the SM5 inversion is present (Exp. II) or absent (Exp. I) at least in the cases where non-disjunction occurs at very low frequency; that there is a slight increase in non-disjunction when the inversions involve the heterochromatin, especially in the regions nearer the centromere (*h*A and *h*B); that there is a slight reduction in non-disjunction when the inversions are entirely and extensively euchromatic.

TABLE IV

Exceptional classes nullo–XY *and* XY (*absolute frequencies and in brackets percentage of the two classes in each experiment*).

INVERSION	With SM5		Without SM5	
	Exc. nullo XY	Exc. X+Y	Exc. nullo XY	Exc. X+Y
Control	0.00061 (87.5%)	0.00009 (12.5%)	0.00035 (50%)	0.00035 (50%)
In(I)sc^8	0.00083 (79.9%)	0.00021 (20.1%)	0.00092 (75%)	0.00030 (25%)
In(I)*lv*231	0.00075 (58%)	0.00055 (42%)	0.00056 (61.5%)	0.00035 (38.5%)
In(I)EN	0.00049 (70%)	0.00021 (30%)	0.00113 (85%)	0.00018 (15%)
In(I)rst^3	0.00032 (75%)	0.00008 (25%)	0.00057 (63%)	0.00032 (37%)
In(I)y^4	0.00048 (66.6%)	0.00024 (33.4%)	0.00031 (66%)	0.00015 (34%)
In(I)*dl*–49	0.00033 (100%)	— —	0.00038 (50%)	0.00038 (50%)
In(I)sc^4	0.000149 (100%)	— —	0.00023 (76%)	0.00015 (24%)
In(I)65	0.00075 (50%)	0.00075 (50%)	0.00007 (35%)	0.00015 (65%)

Although these observations must be corroborated by a greater number of data, it may nevertheless be considered that the heterocromatin segments *h*A and *h*B are essential for the recognition and pairing of the X and Y chromosomes. In fact, when these segments are lacking, as in In(I) sc^4sc^8, intense alteration of the above processes occurs, and hence a considerable increase in non-disjunction. When, on the other hand, these regions are still present but are led by the inversion far from the centromere, they are still capable of causing pairing, but with certain difficulty and alteration, as is shown by the slight increase in the non-disjunction. We can therefore stress that, for a good pairing and normal disjunction of the XY chromosomes, what is important is not only the presence of the heterochromatic regions nearest to the centromere but perhaps also their succession along the chromosome (perhaps there exists a polarity of pairing as revealed by a study of meiotic mutants, in the female germ line). Less clear are the results obtained when the inversion possesses both break-points in the euchromatin. As a working hypothesis it may be supposed that the reversed polarity of the euchromatic portions of the X–chromosome makes the pairing with the Y–chromosome more stable (perhaps because it permits pairing with the long arm Y^L of Y?).

The last fact o be explained is the difference found in the recovery of exceptional males compared with the exceptional females. The results obtained for the various inversions are shown in Table IV. These differences may be attributed either to a preferential expulsion in the male of nullo–XY gametes compared with X+Y gametes (perhaps because the XY chromosomes not paired at meiosis arelost) [3] or by assuming a selective mechanism at the female level, so that the nullo–XY gametes will preferentially fertilise the ovum if compared with the X+Y gametes [2–4].

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