and behaviour, strongly suggest that the extra chromosome is another Y. So the exceptional mouse carried an XYY sex-chromosome constitution.

To date, there is no evidence for fertility in XYY mice. About the present individual we have no record. However, our observations are in good agreement with those of Rathenberg and Muller[5] who also reported apparently normal-sized testes with considerable amounts of spermatozoa in their XYY mouse, in which fertility was also not tested. The mice of Cacheiro and Generoso[6] had very small numbers of sperms. The first case reported by Cattanach and Pollard[3] was sterile, with small testes and a few sperms only. Thus a great deal of variation has been noted with regard to testis size and sperm production in XYY mice. Interestingly, no XYY mouse has so far been reported to exhibit aspermia (an XO/XYY mosaic mouse of course showed aspermia[4]). So the possible occurrence of fertile XYY individuals cannot be ruled out. In radiation experiments, reduction of the sperm population to a level below 10% of normal is supposed to lead to sterility[9]. But in our case the epididymal sperm population was only reduced to 63% of the normal level. It is therefore not obvious that our individual was sterile.

The sex-chromosomes in the primary spermatocytes in our case exhibited all possible types of association (table). 2:1 ratio of types 2–3 association in the present individual suggests randomness of the pairing tendency of X and Y, which is in accordance with the findings of Rathenberg and Muller[5] but contradicts an earlier report[4]. Further evidence of equivalent pairing is provided by sex trivalent formation. The absence of certain association types in some earlier findings[3,4] may be due to the fact that the observations were based on limited data.

Incidences of different types of association involving X, Y and extra (E) chromosomes. 100 cells scored.

Association types	No. observed	Figure
1. XYE trivalent	15	2
2. XY bivalent and E univalent, or XE bivalent and Y univalent	55	3
3. X univalent and YE bivalent	27	4
4. X, Y, E univalents	3	-

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The differential banding pattern produced by Actinomycin-D/Acridine-Orange counterstaining in metaphase chromosomes of *Drosophila melanogaster*

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Summary. A longitudinal differentiation is found in fixed metaphase chromosomes of *Drosophila melanogaster* after treatment with Actinomycin-D and subsequent Acridine-Orange staining. We postulate that our findings are strictly related to the presence of AT-rich DNA in specific chromosomal areas of this species.

A number of counterstaining procedures which produce longitudinal differentiation in mammalian metaphase chromosomes are known[1-6]. These methods involve the coupled use of compounds which bind and, in some cases, emit secondary fluorescence when interacting with specific DNA base sequences. 2 main points are invoked for explaining metaphase banding patterns after counterstaining: 1. The binding competition between the compounds used. 2. The transfer of electronic energy from one compound to the other[6].

With the exception of the data reported by Lin et al.[7], however, counterstaining has thus far been carried out to increase the fluorescence contrast of the producible, standard banding pattern induced in metaphase chromosomes by fluorochromes such as Quinacrine, 33258 Hoechst, DAPI, DIPI, Chromomycin A_3, Olivomycin and so on, which are known for showing enhanced secondary fluorescence when interacting with adenine-thymine (AT) or guanine-cytosine (GC)-rich polynucleotide sequences[1,2,4,6]. Acridine-Orange (AO), on the contrary, is a planar molecule which shows the same fluorescence response as a consequence of binding any DNA base composition[8]. As the fluorometric properties of AO are identical when this fluorochrome interacts with both AT or GC-rich DNA, a uniform green secondary fluorescence is shown when this dye stains native, double-stranded DNA in fixed chromosomes[8].

Actinomycin-D (AMD), on the other hand, is a compound which is known to be a guanine binder agent[9]. Its interaction at GC sites in untreated fixed chromosomes would thus possibly affect both binding and fluorescence emission of AO according to the greater or smaller amount of such GC sites present in specific chromosome areas[7].

In this paper we attempt to contribute to the reports of fluorescent bandings as tools for investigating eukaryote chromosme structure, by describing and discussing in terms of DNA base composition the findings obtained in metaphase chromosomes of *Drosophila melanogaster* after AMD/AO counterstaining procedures.

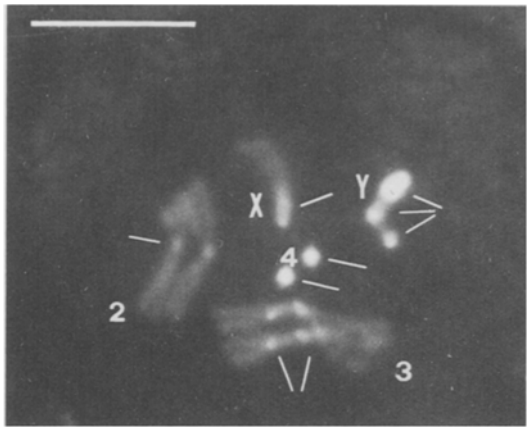
Material and methods. Standard cytological preparations are obtained from third instar larvae of *Drosophila melano-*

gaster (Oregon R) according to Gatti et al.[10]. Fixed chromosomes are then treated as follows: incubation in 200 µg/ml AMD (Sigma) solution, dissolved in Sorensen phosphate buffer M/15, pH 6.81 for 30 min at 21 ± 2 °C. After washing in tap water (3 rinses), preparations are stained with AO according to Bobrow[11]. Control chromosomes are those stained with AO without any post-fixation treatment.

Observations under fluorescence microscopy are effected with a 20 EB Dialux Leitz Microscope equipped with a 100 Z/50 W mercury lamp, exciter and barrier filters (3 mm BG 12 and K 515 respectively) and a Leitz 100/1.32/0.60 NPT Fluotar oil-immersion objective.

Results and discussion. AO staining of untreated (control) preparations shows a relatively uniform bright-green fluorescence in the chromosomes of the species studied. AMD preincubated cytological preparations of *D. melanogaster,* on the contrary, reveal metaphase longitudinal differentiation when the chromosomes are stained with AO. We wish to stress, in this connection, the finding of 2 classes of fluorescence intensity responses when AO staining follows AMD incubation in the chromosomes of *D. melanogaster:* a) bright-green fluorescence, unchanged as compared to that found in control chromosomes, and b) pale-green fluorescence, reduced as compared to that of control chromosomes. The first fluorescence class (bright-green) is localized at the heterochromatic areas, while all euchromatin shows the second class (pale-green) of fluorescence response (figure).

Given the above-mentioned interaction characteristics of AMD and AO with DNA, we believe that the finding of bright (unchanged) or pale (reduced) AO fluorescence after AMD treatment is directly related to DNA base composition of specific chromosome regions. An extreme exemple of this would be that of DNA containing only AT base pairs: such a polynucleotide sequence would not be able to bind even one molecule of AMD and would thus show a standard, unaffected interaction with AO. By contrast, chromosome areas containing polynucleotide sequences with high amounts of guanine residues would reveal reduced AO fluorescence due to the binding competition of previously bound AMD molecules. On the other hand, the significant overlap existing between DNA-bound AO emission and DNA-bound AMD absorption spectra[7] also makes it possible for fluorescence energy transfer to occur from AO to AMD, as suggested by Sahar and Latt[6]. This would induce a fluorescence intensity decrease in the regions in which the 2 compounds are present in close proximity.



Metaphase chromosomes of *Drosophila melanogaster* after AMD/AO counterstaining. Note the bright fluorescence localized at the heterochromatic areas (see arrows) while all euchromatin shows pale fluorescence. The bar represents 10 µm.

Chromosomal regions which show a pale-green fluorescence would thus contain a high amount of GC base pairs. This would allow AMD binding which, consequently, would produce reduced AO fluorescence intensity, as compared to that shown by control chromosomes and by those zones which reveal unaltered, bright AO fluorescence after AMD counterstaining since they contain a small amount of GC interspersion. On these bases, all chromosome regions which show bright AO staining after AMD incubation should contain AT-rich DNA. This hypothesis is supported by both cytological and biochemical data regarding the DNA of this species. Unvaried AO fluorescence in pretreated AMD preparations, in fact, is present in chromosome areas which also show positive reaction with Quinacrine and/or 33258 Hoechst (for comparison see Gatti et al.[10]), thus indicating they are AT-rich[12,13]. Moreover, molecular in situ hybridization experiments show that, among other AT-rich satellite DNAs, the 1.672 g/cc satellite is also localized in the same chromosomal areas which reveal bright-green fluorescence after AMD incubation in this species. This is noteworthy because the 1.672 g/cc satellite contains almost exclusively AT base pairs[14,15]. Also the regions which show bright fluorescence after AMD/AO counterstaining are the same ones which show resistance to photo-oxidation[16], a treatment known for destroying guanine residues[16,17]. This would further indicate that these areas should contain AT-rich polynucleotide sequences with a small amount of interspersed guanine.

Lastly, on the bases of the data reported by Lin et al.[7], we wish to note that the fluorescence contrast between bright and nonbright regions after AMD/AO procedure seems to be more pronounced in *D. melanogaster* than in human chromosomes. As the guanine interspersion amount present in specific chromosome areas is fundamental in order to make chromosome sites available for AMD binding and thus for AO fluorescence alteration, the comparison between our data and those obtained by Lin et al.[7] would indicate that no human chromosome region is present which contains the very low amount of guanine found at some heterochromatic areas of *D. melanogaster.*

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