Mitotic recombination in *Musca domestica* L. and its influence on mosaicism, gynandromorphism and recombination in males

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SUMMARY

In the housefly, mosaics appear spontaneously but rarely. Sexual mosaics or gynandromorphs also appear in strains in which sex determination is based on autosomal sex factors. Rare cases of recombination in the male have been reported by some authors. In field and laboratory populations, mitotic plates with figures indicating exchange of chromatid segments are regularly observed in tissues of individuals of both sexes and at all stages of development. All these anomalies are interpreted as outward manifestation of the same phenomenon: mitotic recombination. The cytological basis of mitotic recombination, its relative frequency, its influence on linkage and genetic variability are discussed.

1. INTRODUCTION

Somatic crossing over was suggested by Serebrovsky (1925) to explain somatic mosaicism in heterozygous individuals of the domestic fowl, and Stern (1936) investigated it in *Drosophila melanogaster*. Following the observation by Hinton (1970) of recombination in males of *Drosophila ananassae*, a number of authors (e.g. Hiraizumi *et al.* 1973; Slatko & Hiraizumi, 1973; 1975) have demonstrated that it can occur also in particular genetic stocks of *D. melanogaster*. Becker (1976), Woodruff & Thompson (1977) have discussed the mechanism, postulating that it occurs in a different way from crossing over in the females.

Milani (1967) discussed the rare cases of recombinants among the progeny of heterozygous males of *Musca domestica* L. and the appearance of mosaics and gynandromorphs, pointing out variations in the distribution of the two phenomena, both in presence and frequency, in different populations and families.

Different sex-determining mechanisms have been described in the housefly (Milani, Rubini & Franco, 1967; Rubini, Franco & Vanossi Este, 1972). Some strains, called 'standard', have an $XX–XY$ mechanism, the Y chromosome being always associated with maleness. Other strains, called 'atypical' (females and males XX), have autosomal dominant sex factors either for maleness (M) or for femaleness (F) and maleness (M). Sex-chromosomes and autosomal sex factors can be variously combined, to all appearance without harmful effects on viability and fertility, both in laboratory strains and in field populations (Rubini, van Heemert & Franco, 1977). Flies carrying F are always females, even in the presence of more

than one M or Y chromosome or their combinations (Rubini & Franco, 1968). In the absence of F, the M factor causes holandric inheritance of its linked genes, making possible a parallel analysis of mosaicism and gynandromorphism.

In a study of housefly mosaics obtained by irradiating larvae heterozygous for two genes in repulsion, Nöthiger & Dübendorfer (1971) have interpreted cellular clones homozygous for one of the markers as resulting from somatic crossing over during mitosis.

Examination of mosaics and gynandromorphs, in conjunction with information on recombination in the male, and direct observation of chromatid exchange during mitotic division at different stages of development has permitted us to form the hypothesis that these phenomena result from a single event being expressed at different levels: mitotic recombination. This term is preferred to somatic crossing over, as proposed by Becker (1976) for *Drosophila*.

2. MATERIALS AND METHODS

The observations were made on *Musca domestica* L. (complement $2n = 12$). Mosaics were recognized during examination of specimens supposed to be heterozygous for recessive genes in different combinations. The genes involved are: ali curve (*ac*, autosome I), wings curled upward; aristapedia (*ar*, autosome II), aristae substituted by tarsal segments; antennapedia (*Atp*, autosome II), antennae turned into legs; carmine (*cm*, autosome II), compound eyes dark-ruby/semi-transparent; brown body (*bwb*, autosome III), basic colour of the body brown, white[3] (*w*[3], autosome III), eye colour pale greyish-white, autonomous; apterous (*apt*, autosome V), wings and halteres extremely reduced, calyptrae absent; tarsi fusi (*tf*, unlocalized), distal tarsal segments fused, wings crumpled and/or with bubbles.

Gynandromorphs were recognized during examination of specimens heterozygous for the male determining factor M (autosome III) either in repulsion with *bwb* or alone.

Macrophotographs were made with a Leitz Aristophot camera on Kodak Ektachrome film (printed in Cibachrome) or on Ilford Pan F film.

Cytological analysis was carried out on adult males and females of various origins, i.e. (I) freshly collected in the field; (II) obtained in the laboratory; (*a*) from strains of recent and long colonization; (*b*) from crosses made during research on formal genetics. Gonads were dissected from individuals of different ages injected with a 0·01 % colchicine solution for 10 min.

Developing eggs (12–14 h), larvae (4–5 days), pupae (2–3 days) and adults (2 days) of the 17 years old laboratory strain WHO/IN/*Musca domestica*/1 (WHO in the following) were cytologically examined without colchicine.

Adults' gonads, larval and pupal cephalic ganglia and imaginal discs were dissected in Ringer solution and then placed in hypotonic solution (0·8 % sodium citrate). After 5–15 min, depending on the size of the organ, the swollen tissues were transferred into the stain (2 % aceto-lactic-orcein) for 10 min at room temperature or for several days at 4 °C in a refrigerator. All these tissues were

squashed in aceto-lactic solution (9:8:1 mixture of glacial acetic acid, 88 % lactic acid and distilled water).

Embryonic tissues from developing eggs were quickly dissociated in a drop of distilled water directly on the slide, stained for 10 min and then squashed in the stain (2 % acetolactic-orcein).

Chromosomal observations were made under phase contrast directly after squashing from temporary preparations. The five pairs of autosomes were identified on the basis of arm length ratios and centromere positions, following Perje's (1948) numeration (1...5). Photographs have been made with a Leitz-Orthoplan plus Orthomat photo microscope on Agfa-Ortho 25 professional or Ilford Pan F 135 film.

3. MOSAICISM AND GYNADROMORPHISM

(i) *Morphological observations*

Mosaics which, according to the theory of Nöthiger & Dübendorfer (1971), can be interpreted as a result of somatic crossing over, appear spontaneously but rarely, a few per 10^4 individuals. They are most easily recognized when mutations lead to an overall somatic effect such as a change in body colour. Otherwise they may be observed only as bilateral mosaics or as mosaics within the particular organ affected by the mutation.

Plates 1 and 2 illustrate examples of recently observed somatic mosaics and gynandromorphs; other examples have been reported by Franco, Lanna & Milani (1962), Franco & Rubini (1966) and Milani (1967).

The most frequent cases are bilateral mosaics for body colour (Plate 2*a*), eye colour (Plate 1*c*; 2*b*), the form of the antennae (Plate 1*c*), wings (Plate 1*a, c*; 2*a*) and feet (Plate 1*b*). Less frequent, or possibly more difficult to recognize, are spotted mosaics which, while easily seen when the eye colour is involved (Plate 2*c*), are less obvious when other small portions of the body are affected. The rarest are antero-posterior mosaics.

In addition to mosaics for somatic mutations, sexual mosaics or gynandromorphs also appear. They are either bilateral (Plate 2*d, f*), or antero-posterior (Franco & Rubini, 1966; Milani, 1967). In the examples reported, the two kinds of mosaic (phenotypic and sexual) always coincide and seem therefore to be due to the same event.

The authors' observations confirm those of Milani (1967) that gynandromorphs are relatively frequent in strains in which sex determination is based on autosomal sex factors (strains MIII first named TYII; 'atypical strains', Milani *et al.* 1967). It is true in particular of those in which sex determination is based on the male factor M localized on autosome III (Rubini & Franco, 1972).

No case of gynandromorphism has been recorded in strains known with certainty to be 'standard' (♀♀ XX; ♂♂ XY), which is consistent with the fact that the X and Y chromosomes never pair during mitosis, so that exchange of chromatid segments could not involve the sex determinants on the sex-chromosomes.

(ii) *Cytological interpretation*

Stages of mitotic recombination are illustrated in scheme 1, using the example of housefly autosome III. The scheme demonstrates how mosaicism for body colour and gynandromorphism can be two aspects of the same phenomenon, when the gene for masculinity M and the autosomal marker bwb are inherited in repulsion. The result of such recombination is evident in the individuals presented in Plate 2*d–f*, which are simultaneously bwb/bwb^+ and gynandromorphic, with zones of the body either mutant female or normal male.

Depending on its position, mitotic recombination in cells of an individual heterozygous for M ($\male XX\ M/m$) may produce two distinct cellular clones, one homozygous M/M and one homozygous m/m. The genic combination $XX\ M/M$ is normal in males of 'atypical' strains while the combination $XX\ m/m$, characterizes the female sex (Milani *et al.* 1967; Rubini & Franco, 1972).

The relatively high frequency of bilateral mosaics and gynandromorphs seems to indicate that mitotic recombination occurs easily also during the first mitotic division of the zygote. As in *D. melanogaster* (Sonnenblick, 1950), this first division seems normally to be directed perpendicularly to the sagittal plane of the egg, and more rarely in other orientations which give rise to antero-posterior mosaics and gynandromorphs. Recombination in later divisions leads to mosaics and gynandromorphs in more restricted portions of the body (Plate 2*c, e*).
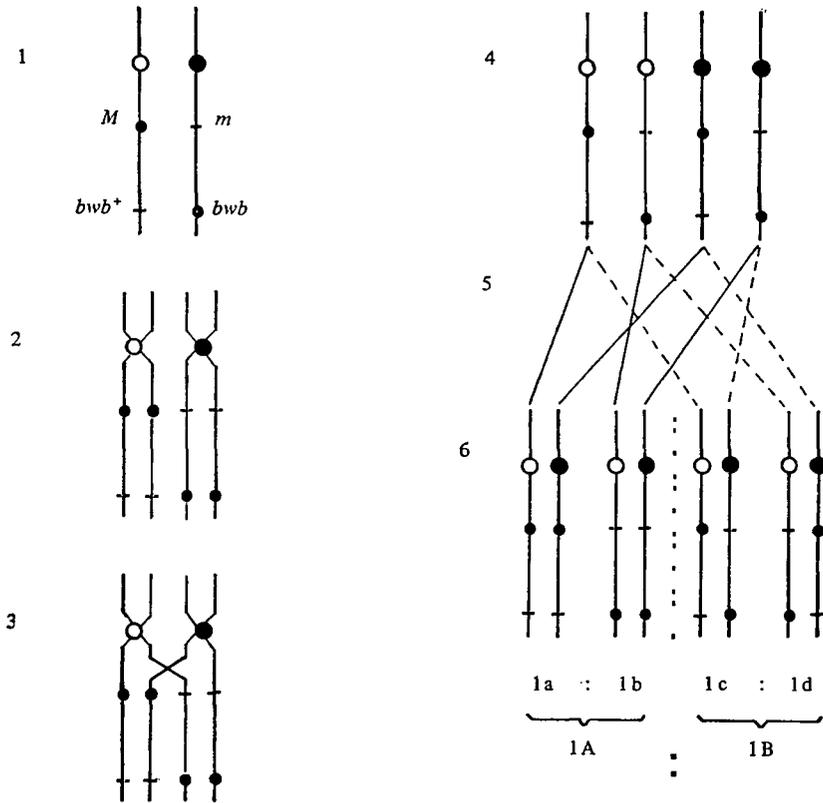
4. CHROMOSOMAL OBSERVATIONS

During a routine cytological examination of the testes of an adult male, a chromosomal figure was observed which could be interpreted as an exchange of chromatid segments between homologous chromosomes at mitosis. Such an event in gonadal cells prior to meiosis would cause the formation of cellular clones with associations of genes differing from the original and the appearance of unexpected recombinants in the progeny of a heterozygous male.

This first chance observation of exchange of chromatid segments in gonadal mitosis suggested that mitotic recombination, proposed as an explanation for the origin of mosaics and gynandromorphs, might well occur in adult tissues but would pass unobserved because of the total absence of phenotypic effect in the adult individual. A systematic search has therefore been made for similar chromosome configurations.

Observations were made on gonads of adults pretreated with colchicine for a few minutes before dissection. In order to determine whether any exchanges might have been produced by this substance, long ago known for its effect on meiotic chiasmata (Levan, 1939), squashes prepared without it have also been examined. For this purpose preparations were made of embryonic, larval, pupal and adults' tissues.

Exchange of chromatid segments appears to be more common in female gonads (one in every 10–15 gonads examined) than in male gonads (one in every 50).

Scheme 1. *Interpretation of the mechanism of mitotic recombination which can lead to the formation of cellular clones with genic associations different from those in the initial cell*

The example refers to autosome III of a male housefly ($\male$ M bwb^+/m bwb) and explains the origin of mosaics and gynandromorphs presented in figure 2 and discussed in the text.

(1) Genic constitution of two homologous chromosomes in the original cell ($\bigcirc$, centromere of paternal origin; $\bullet$, centromere of maternal origin).

(2) Prophase and metaphase normal; each chromosome divided into two chromatids, centromeres not divided.

(3) Prophase and metaphase when an exchange occurs between two chromatids, one of paternal, the other of maternal origin.

(4) Duplication and separation of the centromeres at the start of anaphase and the genic constitution of the chromosomes after exchange.

(5–6) Segregation of the centromeres in anaphase and telophase. In each daughter-cell there should be one centromere of paternal and one of maternal origin.

The solid lines lead to segregation 1A, which results in the formation of two genetically different cells (1a, 1b), each differing from the original cell. The cell 1a gives rise to male cells homozygous for M and for bwb^+ ($\male$ M bwb^+/M bwb^+); cell 1b gives rise to female cells homozygous for m and for bwb ($\female$ m bwb/m bwb). The broken lines lead to segregation 1B, which result in the formation of cells (1c, 1d) with combinations of genes identical to the original cell.

Since the possibility of creating type A or B is the same, only 50% of cases of mitotic recombination lead to the appearance of mosaics or gynandromorphs.

This could well result from the fact that in a good preparation of an ovary it is usually possible to examine some tens of mitotic divisions either in germaria or in follicular cells, while in a good preparation of a testis rarely more than 7 or 8 mitotic gonial divisions are observable at any one time.

More than 60 mitotic exchanges were found: about 20 in adult gonadal tissue treated with colchicine (Plate 3) and as many in adult tissue without colchicine (Plate 4), 5 in embryonic material, 4 in cephalic ganglia and imaginal discs of larvae and 3 in pupal tissues (Plate 5). In one case (Plate 3*f*), and possibly in others (Plates 4*d*, 5*d*, *f*), more than one exchange were found to coincide in the same mitotic division. These multiple events could explain the appearance of mosaics such as those shown in Plates 1(*c*) and 2(*a*) in which the mosaicism involved genes localized on different chromosomes. The observed exchanges seem to involve all 5 autosomes but are more easily recognized in the longer ones, I, II and the long arm of III. This is partly because it is difficult to distinguish true exchanges from simple overlapping in the short arms of chromosome III and the small chromosomes IV and V.

It has not been possible to recognize differences between individuals injected with colchicine and those without. The absence of any observable difference could be due to the fact that mitotic recombination produces a clearly recognizable result only in plates in a particular state of contraction, i.e. when the chromosomes, no longer closely paired in prophase (Rubini, 1965), start to separate with the 4 chromatids well in evidence (prometaphase) (Plates 3*a–c*; 4*a*; 5*c, d, f*). After further contraction (metaphase) the two homologous chromosomes finally separate but usually remain parallel and only rarely is it still possible to recognize an exchange at this stage (Plate 3*h*). Colchicine treatment permits the observation of a larger number of metaphase plates but, by increasing chromosome contraction, it could prevent the observation of exchanges which might otherwise have been visible. Moreover, in squash preparations only a very few of the hundreds of thousands of mitoses that occur during development are in perfect condition for observation.

These considerations lead us to believe that the frequency of mitotic recombination in the housefly might be much greater than it would appear from: (1) the number of manifest mosaics plus gynandromorphs; (2) the number of exchanges observed in the chromosomes; (3) the number deduced from the appearance of recombinants in the progeny of males.

5. CONCLUSION AND DISCUSSION

Examples of recombination in male houseflies described and discussed by Milani (1967) may be interpreted as mitotic recombination during spermatogonial multiplication. However, while cytologically observed exchanges seem relatively frequent, examples of recombination in the progeny of males are extremely rare (Milani, 1956; Milani & Travaglino, 1957; Sullivan, 1961) or absent (Hiroyoshi, 1961; Tsukamoto, 1964).
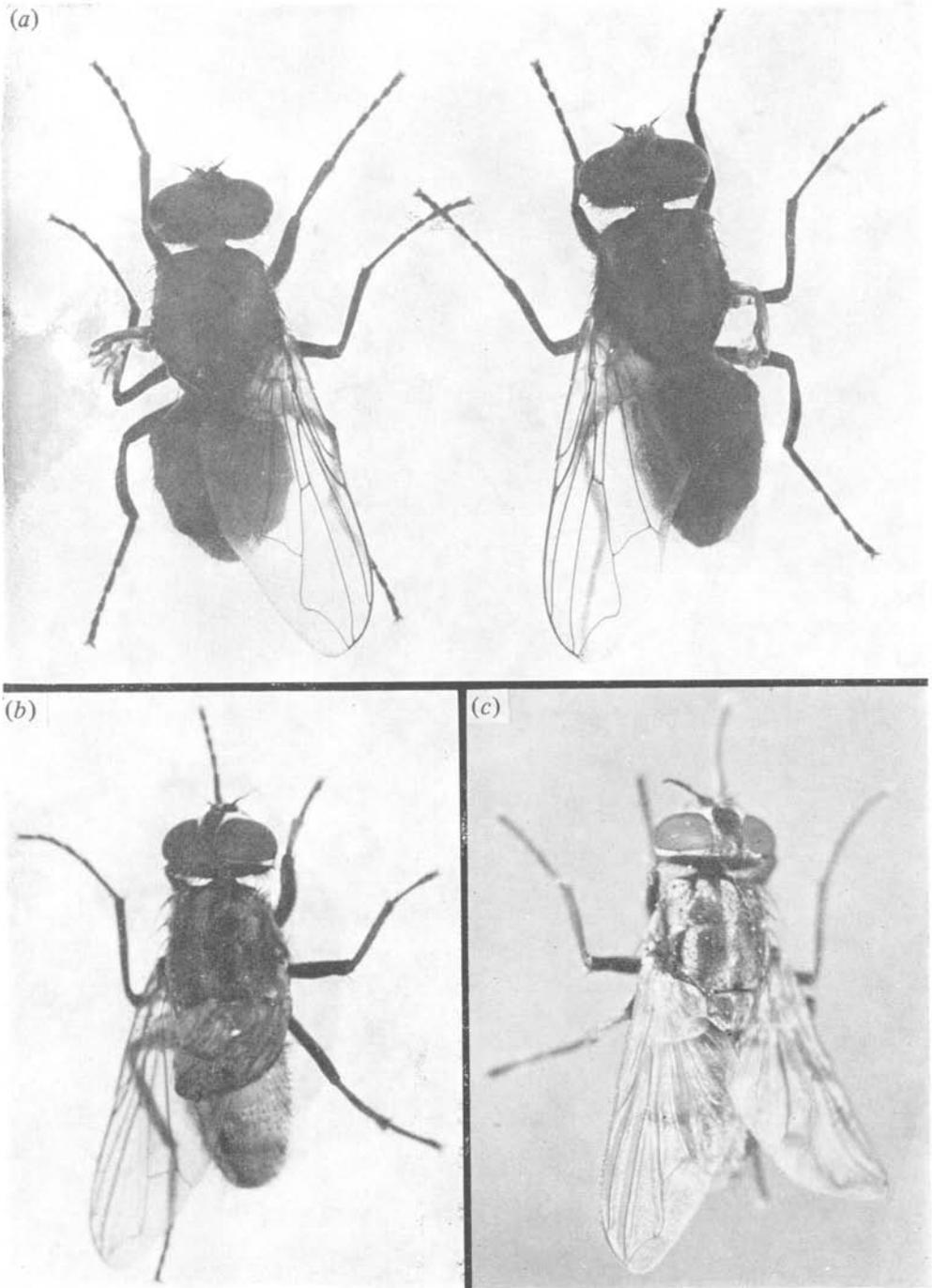
PLATE 1

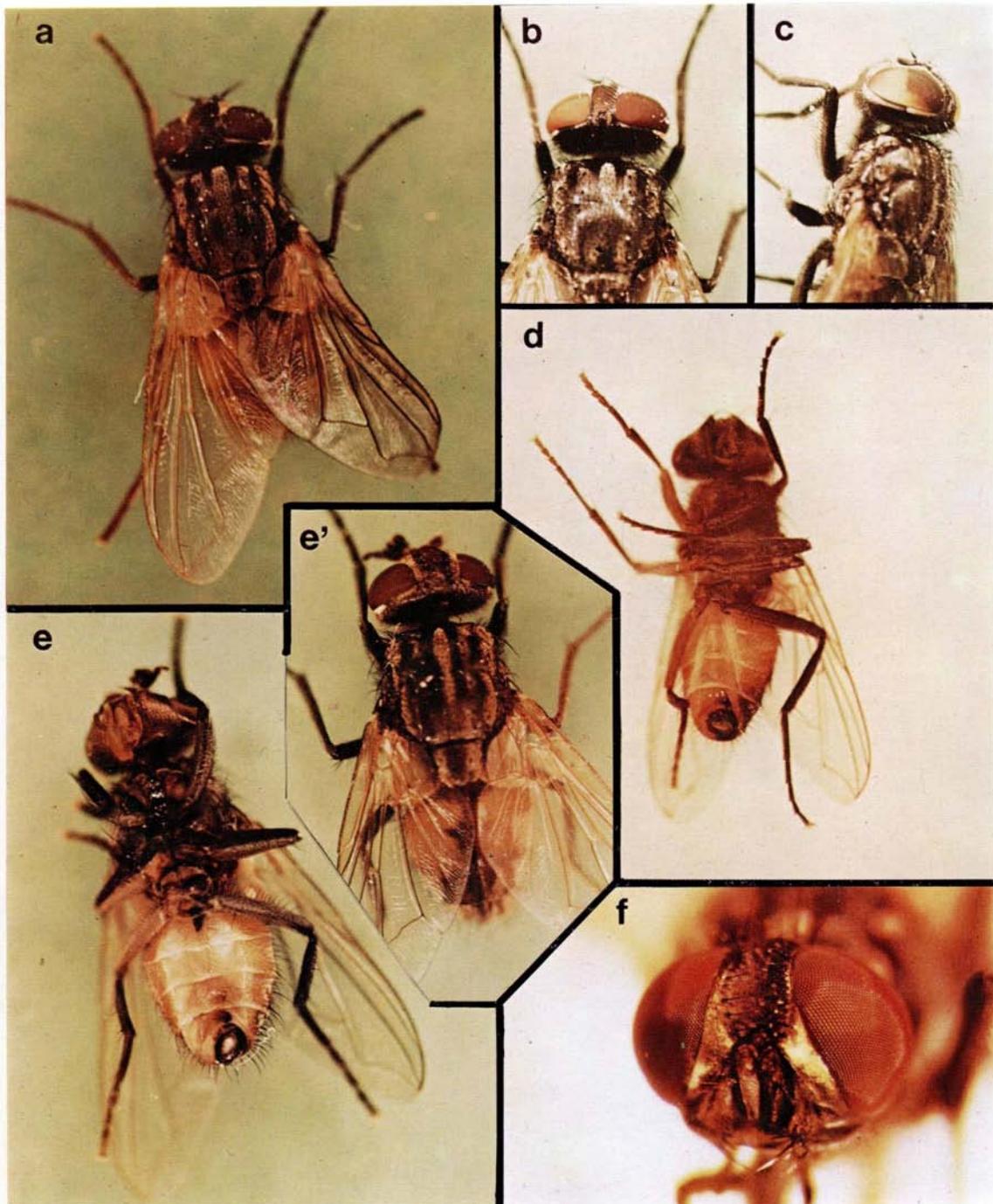Bilateral mosaics of *Musca domestica* L. (L = left and R = right of the specimen in dorsal view).

(a) L *apt*/R *apt⁺* and L *apt⁺*/R *apt*.
(b) L *tf⁺*/R *tf*.
(c) L *ac⁺*; *ar cm*/R *ac*; *ar⁺ cm⁺*.

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Mosaics and gynandromorphs of *Musca domestica* L. (L = left and R = right of the specimen in dorsal view).

(a) Bilateral mosaic: L *bwb*; *ac⁺*/R *bwb⁺*; *ac*.

(a) Bilateral mosaic: L bwb; ac^+/R bwb^+; ac.

(b) Bilateral mosaic, head: L w^3/R w^{3+}.

(c) Spot mosaic, L eye: $1/2\ w^3/1/2\ w^{3+}$.

(d) Bilateral mosaic and gynandromorph: L ♂ bwb^+/R ♀ bwb.

(e, e′) Complex mosaic and gynandromorph, ventral (e) and dorsal (e′) view:

	L	R
Head	♀ bwb; atp	♀ bwb; atp
Prothorax	♂ bwb^+	♂ bwb^+
Mesothorax	♂ bwb^+	♀ bwb
Metathorax	♂ bwb^+	♀ bwb
Abdomen	♂ bwb^+	♀ bwb

(f) Bilateral mosaic and gynandromorph, head: L ♂ bwb^+/R ♀ bwb.

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PLATE 2

PLATE 3

Exchange of chromatid segments in gonadal mitosis of colchicine injected specimens*:

(a) Two days old ♀, F₂ from field-collected flies (Piemonte, Italy), autosome I involved.
(b) As in (a), autosome II involved.
(c) Ovary in a field-collected ♂* (Toscana, Italy), unknown age, autosome III involved.
(d) Three days old ♀ from an hybrid laboratory strain, autosome III involved.
(e) Two days old ♀, F₁₂ from field-collected flies (Valle d'Aosta, Italy), autosome IV involved.
(f) Twelve days old ♂ from an hybrid laboratory strain, autosomes I and II involved.
(g) As in (e), autosome IV involved.
(h) As in (e), autosome IV or V involved.

* As explained in the Introduction, the presence of autosomal sex determinants allows the appearance of XX, XY, YY flies of both sexes which are usually normal; very seldom there are flies showing different degrees of intersexuality (for example ♂ with ovaries, as in (c)).
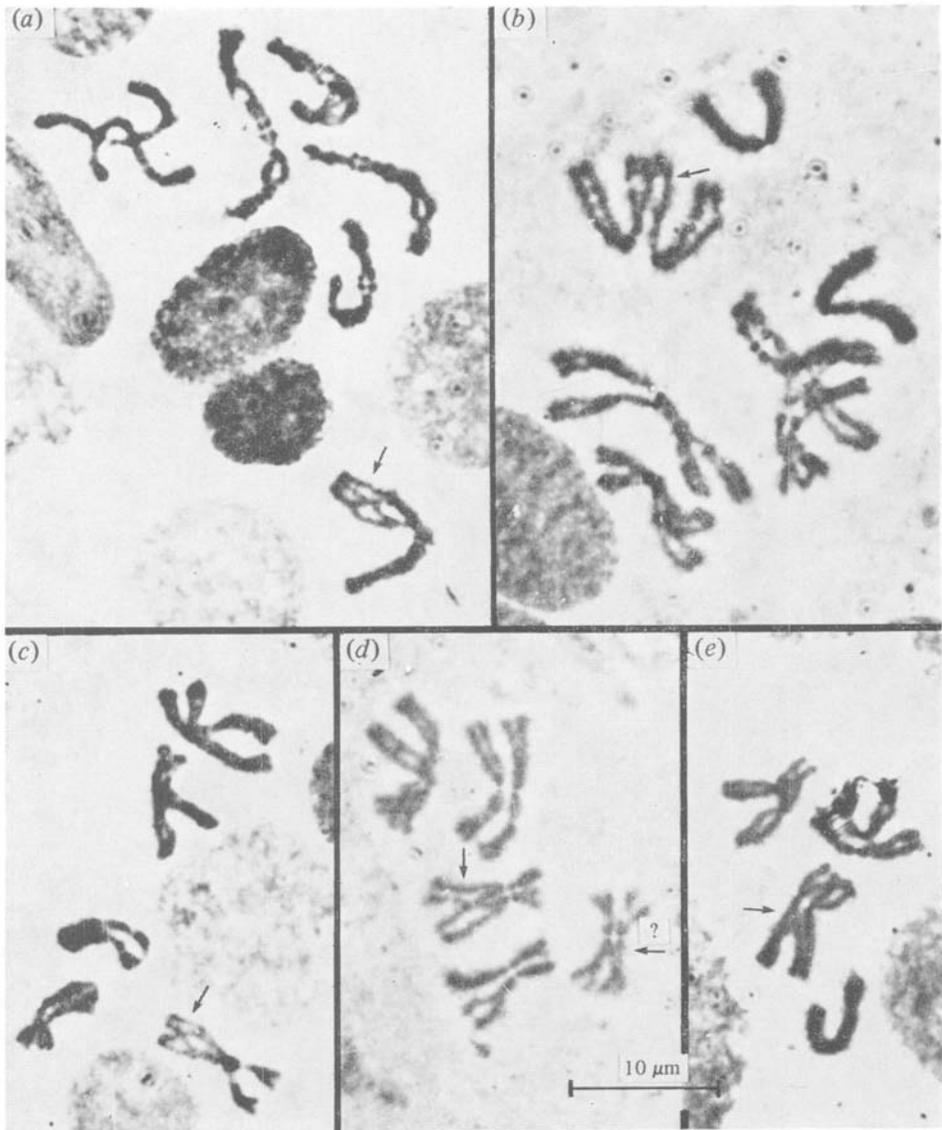
PLATE 4

Exchange of chromatid segments in gonadal mitosis of two days old ♀♀ of WHO strain without colchicine injection.

Autosomes involved: (a) I; (b) II; (c) II; (d) III, may be V also; (e) II.

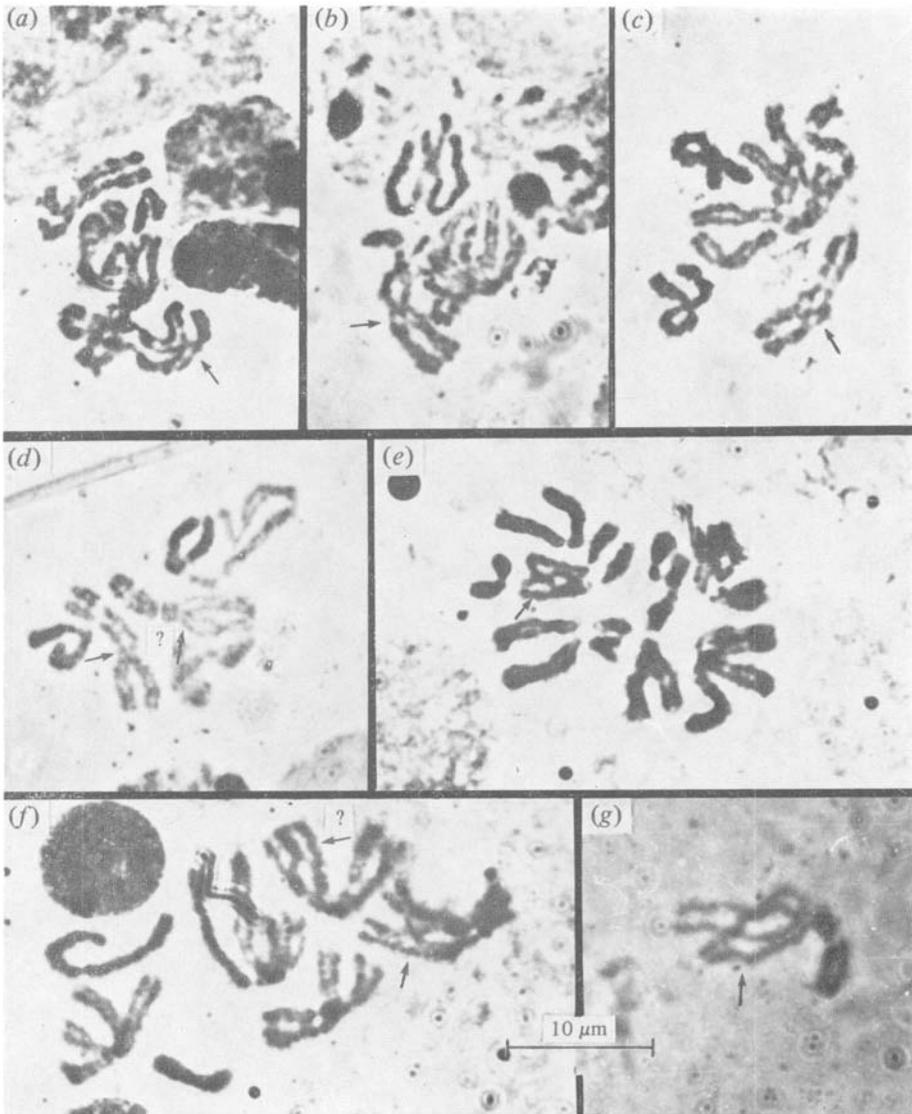P. G. RUBINI, M. VECCHI AND M. G. FRANCO

PLATE 5

Exchange of chromatid segments in embryonic, larval and pupal mitosis from specimens of WHO strain, without colchicine injection:

(a) Embryonic mitosis, autosome I involved.
(b) ♀ embryonic mitosis, autosome I or II involved.
(c) As in (b), autosome II involved.
(d) As in (b), autosome II and perhaps I involved.
(e) Cephalic ganglia of ♂ larva, autosome IV involved.
(f) Imaginal discs of ♂ larva, autosome III and perhaps I involved.
(g) Single autosome II from mitotic division of nervous tissue of ♂ pupa.

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This discrepancy could be due to: (*a*) faulty design of crosses; (*b*) an intrinsic characteristic of the phenomenon; (*c*) genetic differences between strains.

(*a*) *Faulty design of crosses.* Back-crosses of males hetereozygous for various marker genes are normally made only in the first stages of investigating a new mutation. When it comes to actual mapping, back-crosses are made to the heterozygous female, simply because recombination in the male is not expected. Opportunities for observing recombination in the male are therefore limited.

(*b*) *An intrinsic characteristic of the phenomenon.* Mitotic recombination seems often to be restricted to the pericentric region. This is known from two lines of evidence: (*i*) direct observation of its closeness to the centromere in chromosomes which are only slightly contracted (Plates 3*a*, *b*; 4*e*; 5*b–d*) whereas (perhaps due to a mechanism comparable to terminalization of the chiasmata at meiosis) it is found in intermediate regions of the arms of more contracted chromosomes; (*ii*) the observation that in mosaics involving the sex determinant *M III* and the mutation *bwb* (scheme 1; Plate 2*d–f*) the associations *M bwb*⁺ and *m bwb* are always preserved, although it can be demonstrated that the two genes, localized on autosome III, are at more than 50 crossover units apart (Rubini & Franco, 1972). Mitotic exchange in a preferential pericentric position would therefore permit us to recognize, in male progeny, only recombinants between genes localized on the opposite arms of chromosomes, irrespective of their map distance calculated from recombination frequency during female meiosis. This localization is comparable to the preferential position of recombination reported in males of *D. melanogaster*, as quoted and discussed by Becker (1976).

(*c*) *Genetic differences between strains.* This hypothesis is supported by indirect evidence as discussed by Milani (1967), who suggests that discrepancies could be due to the fact that the various authors have observed different strains. The hypothesis of genetic variation has neither been proven nor rejected, but it contrasts with our report of mitotic recombination being recognized in individuals belonging to lines of very different geographical origin and history. For instance it has been observed in specimens belonging to highly selected lines, maintained for more than 17 years in the laboratory, as well as in individuals freshly collected in the field from geographically different zones.

No information is available about the age of the males in which recombination has been detected. However, on the basis of evidence that exchange of chromatid segments occurs during mitotic division in individuals of various ages, one might expect an accumulation of clonal recombinant tissue and hence its higher frequency in older rather than younger males.

The fact that, according to our hypothesis, gynanders can appear only in individuals from populations in which autosomal sex-determinants are present, leads to the conclusion that it must be restricted to certain genetic lines. This has always been the case where checked in our studies of crosses. As regards gynandromorphs reported in the literature, we assume that autosomal sex-determining factors were present in the strains used in those studies, even if they were not recognized. Differences between strains are then explained not by a gene that

induces gynandromorphism, but by the presence or absence of the autosomal sex-determinant *M* which, by analogy with the present report of mitotic recombination in autosome III, is essential for the appearance of mosaics of sexual characters.

These considerations do not exclude the possibility that the ease with which mitotic recombination occurs is controlled by genetic mechanisms. Close somatic pairing at mitosis might be the condition under which radiations, as used by Nöthiger & Dübendorfer (1971), can cause breakage and reunion of the chromatids; this has been proposed by a number of authors for spontaneous and induced mitotic recombination in *Drosophila* (Voelker, 1974; Becker, 1976; Yamaguchi, 1976). It seems improbable, however, that radiations can cause a completely new phenomenon. It is more probable, as in the case of mutations, that mitotic recombination merely increases in frequency after radiation.

The frequency of this event does not appear to be substantially different in male and female gonial cells. The mapping of gene distances in the housefly, based on the frequency of recombination in female meiosis, might be affected to some extent by mitotic recombination in gonadal female tissues, since it is impossible among the offspring to distinguish the recombinants due to meiotic recombination from those due to mitotic recombination. However, the preferentially pericentric localization of mitotic exchanges limits the significance of mitotic recombination on accurate mapping work, distance between genes localized on the same chromosome arm not being affected.

The phenomenon of mitotic gonadal recombination may well be of marked significance in increasing the genetic variability in this species, which is one in which many characters have been shown to be polymorphic (Milani, 1967; Hiroyoshi, 1977; Bryant, 1977).

It is remarkable that after the first observation in 1972 (Rubini, unpublished) of a chromosomal figure showing mitotic exchange, the identification of similar figures has been repeated with remarkable regularity and frequency when these have been specifically looked for. Yet a retrospective examination of more than 2000 photographs of mitotic plates taken in the period between 1962 and 1972, has not brought to light any figures which can be interpreted as demonstrating mitotic recombination. This may be due to the fact that in early studies when we were not looking for it, mitotic figures with unusual chromosomal configurations were discarded because they were unclear. It is also possible that the frequency of the phenomenon is increasing.

This is so in the opinion of the authors who got the impression that mosaics and examples of recombination in the male have been generally more frequent in recent years. In this context it is interesting to note that, in *Drosophila*, reports of recombination in the male also have become more frequent from 1971 onward (Woodruff & Thompson, 1977). Bearing in mind the possibility of inducing 'somatic crossing over' by radiations (Nöthiger & Dübendorfer, 1971), it may be suggested that all these phenomena are the result of breakage and reunion of the chromosomes and that such breakage is particularly easy in positions close to the centromere.

These considerations lead us to the not unreasonable hypothesis that the increase of this phenomenon in recent years may be due to an increase in chemical or physical mutagenic factors in the environment.

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