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Hybrid Dysgenesis in *Drosophila melanogaster*: A Possible Explanation in Terms of Spatial Organization of Chromosomes

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Abstract

Male recombination and female sterility, two aspects of hybrid dysgenesis in *D. melanogaster*, have been studied in crosses between a locally collected wild population and laboratory strains. Dysgenesis occurs in the F_1 hybrid of such crosses only if the wild type is used as male parent and the laboratory strain as female, suggesting an interaction between genotype and cytoplasm. However the results from further crosses are difficult to interpret in terms of a conventional genotype–cytoplasm model, and suggest that for dysgenesis to occur it is necessary that the wild-type chromosomes be contributed by the male parent. Furthermore, receipt of any of the three major wild-type chromosomes in crosses to laboratory females is sufficient to cause hybrid dysgenesis.

A model in terms of spatial organization of chromosomes is put forward to explain these results. It is postulated that (1) normal nuclear functioning requires a definite spatial organization of chromosomes, which is presumably achieved by chromosome–membrane associations, (2) chromosomes are inherited from the female parent with spatial ordering preserved, i.e. membranes and associated chromosomes are handed on directly from the female parent, (3) spatial ordering is not necessarily preserved in male gametes, and paternally derived chromosomes carry information enabling them to become correctly organized within the zygote nucleus, and (4) hybrid dysgenesis results when the chromosome(s) from the male of one strain lack the information to become correctly organized in the nucleus of a second strain. The model seems to explain all aspects of the results, and offers the possibility that the present system may yield information on the genetics of membrane development and other aspects of spatial organization in the normal nucleus.

Introduction

Over the past few years there have been numerous independent reports of abnormal behaviour in crosses involving strains of *Drosophila melanogaster*. Male recombination, meiotic drive, and an abnormally high rate of production of visible and lethal mutations and chromosome abnormalities have all been found at various times, often in association with each other (Minamori 1969; Hiraizumi *et al.* 1973; Kidwell *et al.* 1973; Sved 1974; Voelker 1974; Waddle and Oster 1974; Yamaguchi and Mukai 1974; Woodruff and Thompson 1975). The mutation and male recombination phenomena found in crosses involving Australian strains collected from the Hunter Valley district of New South Wales (Sved 1973, 1974) seem typical of the findings in strains collected from other countries (U.S.A., Japan, U.K.).

Important light has been thrown on these phenomena by Kidwell and Kidwell (1975*a*) who found male and female sterility in the progeny of a wide range of crosses between field and laboratory strains of *D. melanogaster*. Furthermore, they found that this sterility, as well as male recombination, occurred non-reciprocally, i.e. when one strain was used as the female parent and the other strain as male parent, but not vice versa. There is evidence (M. G. Kidwell, J. F. Kidwell and J. A. Sved, unpublished data) that sterility and many of the previously mentioned phenomena

are different manifestations of what may be suitably termed 'hybrid dysgenesis' in *D. melanogaster*. These authors have in addition shown that hybrid dysgenesis is found not only in crosses between field and laboratory strains but also in an unexpectedly high frequency of crosses between laboratory strains.

Various crosses have been made to study the inheritance of male recombination [Voelker 1974; Waddle and Oster 1974; Slatko and Hiraizumi 1975; see also Minamori and Sugimoto (1973) and earlier references for genetical studies on possibly related phenomena]. These have shown that the inheritance is of a complex nature, apparently sometimes involving chromosomal transmission and sometimes cytoplasmic transmission. Therefore 'transmissible factors' which, like virus particles, can become incorporated into chromosomes have been proposed to explain the inheritance. It is the purpose of the present paper to report the results of crossing programs which show that despite the complexity there are some recognizable patterns in the inheritance of hybrid dysgenesis. An alternative to the transmissible factor will be put forward to explain these results. This hypothesis is in terms of spatial organization, or membrane association, of chromosomes.

Materials and Methods

Strains Used

The following strains were used.

(1) Wild type—a stock made by pooling collections from six wineries of the Hunter Valley district of New South Wales. The collections were made early in 1974, and the stock has been kept in bottles since then.

(2) *al cn bw*—the stock used to measure male recombination throughout the present experiments. The markers, *al* (0), *cn* (57·5) and *bw* (104·5) cover most of chromosome II. The stock was newly synthesized from laboratory strains of *al* and *cn bw*.

(3) *cn, e*—a laboratory stock used for female sterility tests, containing *cn* on chromosome II and *e* on chromosome III.

(4) *Cy/Pm*—contains In(2L)Cy + In(2R)Cy/In(2LR)bw^{V1} (Lindsley and Grell 1968). This stock is also marked with Ubx(TM2)/Pr on chromosome III.

Procedures for Measuring Male Recombination

All tests were carried out using three *al cn bw* virgin females together with a single *al cn bw*/+ + + male, generally aged between 24 and 48 h. Progeny counts were made at around 11 and 14 days. All experiments were carried out at 25±2°C.

The mean percentage of recombinant products is used as the measure of male recombination. As discussed by Kidwell and Kidwell (1975*b*), the exclusive use of this statistic may have shortcomings if clusters of recombinant products are found. However, since large clusters were rarely observed in the present experiments, the use of other possible statistics (percentage of males showing recombination, and minimum recombination percentage) does not seem warranted.

Tests for Female Sterility

Single non-virgin females generally aged between 24 and 48 h were placed with two males in a tube and allowed to lay for 5 days. At this time all tubes containing dead females were excluded regardless of the presence of larvae. Amongst the remaining tubes the absence of even a single larva at this time or after a further 2 days was taken to indicate sterility. Large numbers of eggs, apparently infertile, were present in the majority of cases where the female was classified as sterile.

Results

Inheritance of Male Recombination

Kidwell and Kidwell (1975*a*) found that male recombination, like infertility, occurred when the laboratory strain was used as female parent and the wild type as

male parent of the hybrid, but not vice versa. The same rules have subsequently been found to apply in crosses involving the Hunter Valley wild-type flies (Table 1). The overall recombination percentage in *al cn bw*/+ + + males is between 1 and 2% when the wild type is used as male parent, and nearly zero in the reciprocal cross. There is a significant difference in the ratio *al cn bw* : + + + in the two crosses ($\chi^2 = 44{\cdot}6$, $P < 0{\cdot}001$), a difference indicative of meiotic drive (cf. Hiraizumi *et al.* 1973).

Table 1. Results from backcross of males heterozygous for second chromosome markers *al*, *cn* and *bw*

+ Parent of heterozygote	Progeny						Recombination (%)
	al cn bw	+ + +	*al* + +	*al cn* +	+ *cn bw*	+ + *bw*	
Male	1722	2291	22	16	11	16	1·62
Female	1117	2060	0	0	0	1	0·03

The most obvious explanation for results such as the above would seem to be an interaction between genotype and cytoplasm. A second-backcross program was set up to check on the validity of this explanation. The program involved constructing *al cn bw*/+ + + triple heterozygotes by an intercross and backcross procedure, in which the *al cn bw* parent was used as both male and female parent in both crosses as shown in Table 2.

Table 2. Recombination percentage from *al cn bw*/+ + + males in second-backcross test

The *al cn bw* chromosome is designated as *m* in the table. The maternally derived chromosome is written first in the genotype, and the female genotype is written first in crosses. All genotypes expected to contain the cytoplasm from the *al cn bw* strain are marked with an asterisk

	Series No.			
	1	2	3	4
Parental cross	**m*/*m* × +/+	**m*/*m* × +/+	+/+ × **m*/*m*	+/+ × **m*/*m*
Backcross	**m*/*m* × **m*/+	**m*/+ × **m*/*m*	**m*/*m* × +/*m*	+/*m* × **m*/*m*
Hybrid genotype	**m*/+	*+/*m*	**m*/+	+/*m*
Recombinants	84	40	142	8
Total progeny	6961	8902	7461	5947
Recombination (%)	1·21	0·45	1·86	0·13

The results of Table 2 are not in good agreement with a simple genotype–cytoplasm model. In particular the relatively low level of recombination in series 2 is not predicted by such a model. A possibility which these results cannot exclude is the involvement of the X chromosomes, since the wild-type genotype contributes one half of the genetic material to the X chromosome of series 2, but none to series 1 and 3. The X chromosome genotype, however, cannot explain reciprocal differences in female sterility.

A second set of double backcrosses was carried out to check these findings. A different laboratory stock, *Cy*/*Pm*, was substituted for the *al cn bw* stock in the first cross (Table 3). This was done to reduce the possibility of undetected recombination in the hybrid female genotype in series 2 and 4. The results from this experiment show a striking difference between series 1 and 3 on one hand, and series 2 and 4 on the other, a trend which is also evident in Table 2. Once again it is

possible to explain these results by suitable assumptions regarding cytoplasm, X-chromosome, and chromosome II. However an alternative and in some senses more simple explanation can be put forward to explain both these trends and the initial non-reciprocal observation. It may be postulated that in the present series of crosses male recombination occurs only if the + chromosome comes from the male rather than the female parent. This is of course logically equivalent to asserting that recombination only occurs if the *al cn bw* chromosome comes from the female parent, but the reason for focussing on the + chromosome will be made clear later. The model which will be put forward in the following section suggests a mechanism whereby the properties of a chromosome could be affected by its parental origin.

Table 3. Results from second-backcross test in which *Cy/Pm* replaces *al cn bw* in the parental cross

The *al cn bw* chromosome is designated by *m*, and the female genotype is given first in all cases. Cytoplasm from the *al cn bw* stock is marked * and that from the Cy/Pm stock is marked †

	Series No.			
	1	2	3	4
Parental cross	†*Cy/Pm* × +/+	†*Cy/Pm* × +/+	+/+ × †*Cy/Pm*	+/+ × †*Cy/Pm*
Backcross	**m/m* × †*Cy*/+	†*Cy*/+ × **m/m*	**m/m* × +/*Cy*	+/*Cy* × **m/m*
Hybrid genotype	**m*/+	†+/*m*	**m*/+	+/*m*
Recombinants	74	7	74	5
Total progeny	4945	5278	4959	4081
Recombination (%)	1·50	0·13	1·49	0·12

One further result which will be mentioned in this section concerns a test which was carried out to see whether male recombination could be eliminated by continued backcrossing. Males of the genotype *al cn bw*/+ + + were backcrossed to females of the *al cn bw* stock for 23 generations, and the incidence of male recombination was recorded in each generation. A drop in the frequency of recombination occurred in the first few generations, but over the last 20 generations there was only a slight and non-significant decline. This suggests that any factors affecting recombination on chromosome II are in fact true genetic factors, since they cannot be diluted out by continued backcrossing.

Inheritance of Female Sterility

In addition to the male recombination studies in hybrids between the *al cn bw* and wild-type strains, male and female fertilty were also investigated. Approximately 15–20% of hybrid females were found to be sterile when the *al cn bw* stock was used as female parent, and fertility was essentially normal in the reciprocal cross. On the other hand, unlike the findings of Kidwell and Kidwell (1975*a*), male fertility appeared to be normal in both crosses.

Female sterility was further studied in the same second-backcross programs as used for studying male recombination. Percentage sterility of *al cn bw*/+ + + females is shown in Table 4. The results indicate that the occurrence of male recombination and female sterility are closely correlated. In particular the results can also be explained under the assumption that it is the direction of the cross which produces the hybrid that is of importance.

The frequency of complete sterility in these crosses was somewhat lower than convenient for further experimentation. However, in a survey of laboratory stocks a stock marked with *cn* on chromosome II and *e* on chromosome III was found to give about 70–80% sterile female offspring in crosses to wild-type males. The double-backcross procedure was repeated with this stock and the results are given in Table 5. Once again there is an excess of steriles in series 1 and 3, although the comparatively high value for series 2, in line with the observations on male recombination in Table 2, is noted and will be discussed later.

Table 4. Percentage of sterile females produced by backcross tests

Series 1–4 are numbered as in Tables 2 and 3. Experiment 1 refers to females produced by the crossing program of Table 2, and experiment 2 refers to females produced as in Table 3

	Series No.			
	1	2	3	4
Sterile/total				
Experiment 1	5/20	0/20	3/20	0/20
Experiment 2	7/81	1/113	12/89	1/111
Overall	12/101	1/133	15/109	1/131
Percentage sterile	12	1	14	1

Table 5. Percentage of sterile females produced by backcross test involving *cn, e* laboratory stock

The percentage sterile value is obtained by averaging the percentage steriles for the four classes *cn*, *e*, *cn*, +, +, *e* and +, +

	Series No.			
	1	2	3	4
Parental cross	*cn, e* × +	*cn, e* × +	+ × *cn, e*	+ × *cn, e*
Backcross	*cn, e* × *cn, e*/+	*cn, e*/+ × *cn, e*	*cn, e* × +/*cn, e*	+/*cn, e* × *cn, e*
Total females tested	207	103	231	61
Percentage sterile	59	33	85	0

Table 6. Chromosome(s) received from the wild type through the male line and the percentage female sterility

	+ Chromosomes							
	X, II, III	X, II	X, III	X	II, III	II	III	None
No. of females tested	96	39	46	50	35	41	47	84
Percentage sterile	93	98	91	60	70	82	78	4

The use of the *cn, e* stock in this test provided important additional evidence, particularly for series 1 and 3. The use of the hybrid as male parent in the backcross means that it is possible to infer individually the wild-type chromosomes received from the male, assuming that the amount of male recombination is relatively low and can be ignored. Furthermore, it can readily be seen that series 1 and series 3 differ in that the X-chromosome received from the male in series 1 is from the *cn, e* stock, whilst the equivalent X-chromosome in series 3 is of wild-type origin. Thus the results from series 1 and series 3 can be summarized in a table giving presence or absence of each of the three major wild-type chromosomes. The results (Table 6)

show a striking difference between the class which receives no wild-type chromosomes and those classes which receive one or more. Apparently receipt of any of the three major wild-type chromosomes from the male is sufficient to produce hybrid dysgenesis in the progeny of this cross.

The Model

Spatial Organization of Chromosomes

Early studies on the role of chromosomes, dating from the work of Sutton (1903), envisaged chromosomes as free-floating entities within the nucleus. There is now a considerable amount of evidence against this view and in favour of the more orderly view that chromosomes occupy characteristic positions in the nucleus, both at the resting stage and in division. It is this 'spatial organization' of chromosomes, and the manner in which it arises, which is the basis of the model of the present paper.

It is usually envisaged that spatial organization of chromosomes is maintained by associations with the nuclear membrane. The evidence for the association of chromosomes, or chromatin, and membranes is extremely diverse, and is considered only briefly here (see Comings 1968; King 1970, Ch. 5; DuPraw 1970, Ch. 11; Ashley and Wagenaar 1974; Franke and Scheer 1974; Zentgraf *et al.* 1975). Associations with the nuclear membrane have been seen in early mitotic preparations (Vanderlyn 1948) and in early meiotic preparations (Pusa 1963; Gillies 1972). In salivary gland nuclei of *Drosophila*, Holmquist and Steffensen (1973) have demonstrated a three-dimensional organization of the polytene chromosomes which is attributed to connections to the nuclear membrane. Similarly, in a variety of non-dividing cells multiple attachments to the nuclear membrane have been reported (e.g. DuPraw 1965), whilst Davies and Small (1968) have reported characteristic configurations of membrane-associated chromatin. Membrane-associated DNA has been isolated by Franke *et al.* (1973) and Zentgraf *et al.* (1975) and shown to be richer in repetitive sequences than is bulk nuclear DNA.

Notwithstanding the volume of evidence relating to chromosome–membrane connections, there are still several conceptual difficulties associated with the notion that spatial organization of chromosomes is maintained by such connections. The first such difficulty relates to the ability of most organisms to tolerate a wide variety of chromosome additions and rearrangements. This implies that the connection to the nuclear membrane must be of a particularly versatile kind. It should be noted, however, that the existence of an additional chromosome often seems to be associated with instability both of chromosome number and of control of cell division (Koller 1964). It is not inconceivable that chromosome division and cell division could be physically associated through membrane connections, much as in the bacterial model of Jacob *et al.* (1963) in which case the direct association of chromosome abnormalities and mis-division could readily be rationalized.

A second difficulty concerns the apparent breakdown of the nuclear membrane in mitosis and its subsequent reconstitution. The evidence cited above leaves open the possibility that spatial organization is lost each time the nuclear membrane breaks down, and is then re-established in the following interphase. The model put forward below, however, requires that there is not a randomization of maternally- and paternally-derived chromosomes during mitotic divisions, at least during divisions early in development. It is, in fact, unclear to what extent the breakdown of the

nuclear membrane is complete, particularly around the chromosomes (Comings and Okada 1970), so that it is by no means certain that all interchromosomal contact is lost during mitosis.

A third problem concerns the random disjunction of non-homologous chromosomes in meiosis, which is readily understood if chromosomes are free-floating. It is possible that under the spatial organization hypothesis some sort of randomizing mechanism is needed to ensure independent disjunction of maternally- and paternally-derived non-homologous chromosomes. However, examples have been found in which the disjunction of non-homologous chromosomes is non-random, a phenomenon labelled as 'genetical affinity' by Wallace (1953).

The Origin of Spatial Organization

Perhaps the greatest conceptual difficulty which must be dealt with when considering spatial organization of chromosomes is the manner in which it originally arises. All of the evidence cited above refers to observations on cells in adult stages. This leaves unanswered the question of how this organization of chromosomes in adult tissues originates. Alternative possibilities are that it arises during development, perhaps as a gradual process, or that it reflects an organization which exists as early as the zygote stage. The latter possibility is the one which will be considered here.

There seems to be little direct evidence on the possibility of spatial organization in the earliest developmental stages, whether in *Drosophila* or in any other organism. Observations on the chromosome configuration during the early divisions in *D. melanogaster* have been made by Sonnenblick (1950) and King (1970, see summary). It appears that the maternally- and paternally-derived chromosome sets may be distinct during most of the first cleavage division, coming together only at telophase. Thus the merging of chromosomes comes at a stage when the nuclear membrane is ostensibly not present, making it very difficult to observe directly any establishment of spatial organization in the formation of the zygote nucleus.

The notion that spatial organization of chromosomes originates at the zygote stage still leaves unanswered questions about the mode of origin. In particular there seem to be, broadly speaking, two alternative classes of explanation:

(1) The spatial organization is not really lost between generations, but is directly handed on in the gametes. Spatial organization in the zygote could then be achieved by chromosomes merging in a spatially oriented manner (Sved 1966).

(2) The spatial organization could be newly produced in each generation if the chromosomes carry (genetical) information allowing them to become oriented in the newly constituted zygote.

The observations of Costello (1970) lend indirect support to the first hypothesis. Costello found that the ordered arrangement of chromosomes in the first cleavage division of the flatworm *Polychoerus carmelinis* could be accounted for in terms of two identical linear arrangements of chromosomes with only minor permutations of order in one. This he interpreted as arising from identical, or near-identical, organization of chromosomes in the male and female gametes. Opposed to this view, however, are the more direct observations of Taylor (1964) on chromosome order in grasshopper sperm. Autoradiographic studies were made on a species, *Romalea microptera*, in which the chromosomes occur in a linear order in the sperm. The

differentially labelling X chromosome was found to occupy a variable position in the line of chromosomes, an observation which argues strongly against the hypothesis of a direct transmission of spatial ordering in the male gamete. The contrasting results in the flatworm and grasshopper could of course reflect different modes of organization in the two organisms [for a more complete discussion of chromosome organization in sperm, see DuPraw (1965, Ch. 17)].

Principally because of Taylor's (1964) evidence, the model which is preferred is one in which spatial organization is not handed on directly in the sperm. This fits in better with the evidence (Table 6) that chromosomes coming from the sperm are individually able to produce effects in the zygote. However the model which will be considered is essentially a compromise between the two classes of explanation suggested above. It is postulated that the paternally derived chromosomes carry information allowing them to become oriented in the zygote, but that the organization of maternally derived chromosomes is handed on directly. This means that the nuclear membrane of the egg nucleus essentially becomes the nuclear membrane of the zygote, that the maternally derived chromosomes are already associated with this, and that the paternally derived chromosomes must establish an orientation and attachment. It is unfortunately difficult to give a physical representation of the suggested fertilization process since, as described earlier, the merging of maternal and paternal chromosomes occurs at a stage which is difficult to visualize, a stage when the nuclear membrane is diffuse. The model envisages that the information enabling the paternally derived chromosomes to become oriented is true genetic information. which is replicated normally. This is suggested by the persistence of male recombination in the long-term backcross experiment described previously.

Spatial Misorganization

The model thus far has been concerned with the mechanisms for spatial organization of chromosomes in normal cells. The second aspect of the model concerns the reasons for the breakdown of these mechanisms in the abnormal hybrids. It is envisaged that different modes of spatial organizations have evolved in strains which have been reproductively isolated for many years. Thus in some cases the chromosome contributed by the male parent will lack the information necessary to become correctly oriented within the nucleus of a different strain. This is described as spatial misorganization, and it is assumed that spatial misorganization leads to hybrid dysgenesis and its various manifestations.

Mechanisms can readily be suggested, although these are of course speculative, whereby spatial misorganization could lead to male recombination, infertility, and mutation. Mitotic recombination might be expected if spatial organization is normally required for ensuring non-contact of homologous chromosomes. Infertility might be a result of meiotic breakdown, possibly through difficulties of chromosome pairing etc. It is a little more difficult to account for mutation. Preliminary experiments (Sved, unpublished data) have indicated that chromosome breakage associated with mitotic recombination cannot account for the increased mutation rate in dysgenic hybrids. However, a somewhat different explanation can be suggested in terms of mistakes in DNA replication. Although early suggestions that DNA synthesis is associated with the nuclear membrane now seem to have been discounted (Comings and Okada 1973), this does not rule out the possibility

that DNA replication is restricted to particular sites in the nucleus. Spatial organization might thus be important in the process of chromosome duplication.

Discussion

The experiments reported in this paper have dealt with genetic recombination in the male and with female sterility. The model proposed to explain the results is in terms of chromosomes, and even more specifically in terms of spatial organization in the nucleus. It may at first sight seem arbitrary and in contravention of Ockam's razor that the model should be at such a different level from the observations. It is not easy to counter such criticism, since clearly the model can in no sense be deduced from the observations. However, it is felt that the model brings together a sufficiently wide variety of observations to make it merit serious consideration.

The principal reason for introducing the model is the observation that the inheritance of hybrid dysgenesis seems explicable if it is postulated that the properties of a chromosome are different depending on whether it is maternally- or paternally-derived. The model in essence postulates that these properties are spatial properties of the chromosome. In a variety of other studies it has been demonstrated that the properties of a chromosome are dependent on its parental origin, and it is instructive to consider these, plus possible explanations which have been suggested in some cases.

(1) The meiotic mutant *pal* in *D. melanogaster* leads to either complete or partial loss of paternally derived chromosomes in some progeny (Baker 1975). An analysis of the mosaic progeny (in which there has been a partial loss) suggests that the loss of some of the chromosomes may come later than the first cleavage division, and therefore that the maternally- and paternally-derived chromosomes are in some way distinguishable after merging.

(2) Delayed expression of paternally contributed isozymes has been demonstrated in interspecific hybrids, chicken–quail (Castro-Sierra and Ohno 1968) and various trout hybrids (Hitzeroth *et al.* 1968; Yamauchi and Goldberg 1974). Models have been put forward in terms of specific recognition of alien genes by some component of the egg cytoplasm.

(3) Johnson (1975) has shown that the T^{hp} mutation, an allele at the *T* locus in mouse, produces different phenotypes depending on whether it is contributed by the female or male parent. A possible interpretation in terms of earlier expression of maternally derived genes has also been put forward in this case, supported by the fact that the more extreme phenotype occurs when the gene is contributed by the female. It is somewhat striking that the *t* series of alleles at this locus also shows sterility and meiotic drive. Furthermore, a locus controlling hydrid sterility, Hst-1, maps within 6 map units of the *T* locus (Forejt and Ivanyi 1975). It should be noted that the H-2 locus, known to be concerned with surface membrane properties, lies 15 map units from the *T* locus (Snell and Stimfling 1966).

(4) In marsupials, X-chromosome inactivation occurs in females, but unlike the random inactivation in mammalian X-inactivation it is always the paternally derived chromosome which is inactivated. Cooper (1971) has put forward a model to explain this in terms of a transposable controlling element.

(5) In coccid insects, the paternally derived chromosome set plays almost no role in the morphogenesis of male offspring (Brown and Nur 1964). In many

cases the paternally derived chromosome set is not passed on to the offspring, but in some species, as in the marsupial X-chromosome example, the inactive chromosome(s) can be passed on in the sperm, and thereby become re-activated in the next generation.

(6) A case of permanent hybridity in frogs, which in some ways is similar to the coccid insect example, has been reported by Tunner (1974). The hybrid status is maintained by backcrossing of hybrid females to males of one pure species, combined with selective loss during oogenesis in the hybrid female of the paternally derived chromosome set.

(7) The extensive studies reported by Laven (1967) on incompatibility of inter-strain crosses in the *Culex pipiens* complex of mosquitoes, in addition to studies by Smith-White and Woodhill (1954) in the *Aedes scutellaris* complex, fall into a slightly different category than the above-mentioned studies, but are nevertheless very relevant to hypotheses on hybrid dysgenesis. In the *Culex* complex at least 17 different crossing types appear to exist, exhibiting a mixture of uni-directional and bi-directional incompatibility between pairs. This incompatibility is attributable to the fact that the chromosomes from the male do not contribute to morphogenesis even though the sperm apparently penetrates the egg in most cases. The most striking aspect of the phenomenon is that the maternal incompatibility properties appear to be non-chromosomally inherited. Laven (1967) speculates that this could be attributable to a maternally inherited RNA fraction.

None of the above studies can definitely be related to the present study on hybrid dysgenesis. However, all could conceivably involve similar phenomena, and in no case can the involvement of chromosome–membrane associations be definitely excluded.

It should be noted that in the present study neither the finding of male recombination nor of female sterility provides direct evidence that the maternally- and paternally-derived chromosomes retain their identity throughout morphogenesis. Both phenomena could formally be explained by postulating that the observed effects at the adult stage are attributable to some early disturbance in morphogenesis induced by the paternally derived chromosome(s), but that the distinction between maternal and paternal chromosomes is lost during later development. However the difference in transmission ratios of the *al cn bw* and + + + chromosomes depending on parental origin (Table 1) provides some direct evidence that paternal and maternal chromosomes may retain their identity until spermatogenesis. Such retention of identity is demonstrated more convincingly by several of the above-mentioned studies.

It is worthwhile to consider briefly possible alternatives to the spatial organization model. As shown in this paper, and as found by other authors, the inheritance of male recombination is of sufficient complexity that no simple interaction of genotype and/or cytoplasm can be invoked for its existence. The results can formally be explained by invoking 'transmissible factors', or viruses, which can become associated with any chromosome or be transmitted through the cytoplasm. Reciprocal hybrid dysgenesis, such as found by Kidwell and Kidwell (1975*b*), would then demand the existence of more than one such non-interacting factor. A similar argument has been put forward by Laven (1967) to discount the possibility of viruses as

an explanation of the mosquito incompatibility. There is also the question of how such viruses could be responsible for diverse effects such as infertility, mutation, male recombination etc. In this respect, since spatial organization could account for all of the above, the spatial organization hypothesis seems closer to the level of the phenomena which have to be explained.

The idea of spatial involvement is supported a little more directly by the effect of chromosome III on male recombination in chromosome II. The male recombination results given in Table 3 include a mixture of results from individuals which received the wild-type chromosome III from the male parent and those which received the laboratory stock chromosome (marked with *Ubx*) from the male parent. The overall recombination percentages in chromosome II for these two classes are 1·87% and 1·08% respectively, and are highly significantly different, showing that the hybrid genotype for chromosome III enhances male recombination in chromosome II. Under the spatial organization model this could be attributed to spatial interference. This result is analogous to the inter-chromosomal effects in female recombination found by Schulz and Redfield (1951), effects which were explained by Oksala (1958) and King (1970, Fig. V-12) in terms of spatial models.

Any direct demonstration of the involvement of spatial misorganization of chromosomes in hybrid dysgenesis would seem unlikely at the present time. As discussed earlier, even to demonstrate the existence of spatial organization would not be easy. To demonstrate differences in spatial organization between normal and abnormal hybrids would seem corrrespondingly more difficult. It is by no means certain that any physical differences between normal and abnormal organization would be large.

A prediction regarding infectivity, albeit a negative prediction, can be made under the spatial organization hypothesis. It seems unlikely under this hypothesis, although perhaps not inconceivable, that dysgenesis could be directly transferred between individuals. On the other hand, under the transmissible factor hypothesis infectivity ought to be possible and has in fact been achieved in the *D. paulistorum* complex (see Ehrman and Daniels 1975). Waddle and Oster (1974) attempted, without success, to demonstrate infectivity in their studies of male recombination in *D. malanogaster*, although injection was apparently not tried.

One apparently anomolous aspect of the results was the finding of significant levels of male recombination (Table 2) and female sterility (Table 5) in crosses where the wild-type chromosome came from the female rather than from the male. However, in these cases the female parent is itself showing hybrid dysgenesis. To explain these results, therefore, it is necessary only to postulate that hybrid dysgenesis may be directly inherited, at least to a limited extent, from mother to offspring. This seems quite consistent with the model, since it is postulated that chromosomes and associated membranes are inherited directly from the female parent, so that any misorganization might also be directly transmitted.

While the spatial organization model has been used as a plausible explanation for reciprocal differences in hybrid dysgenesis, some qualification seems necessary. The model envisages that, within each strain, the organization is such that paternally derived chromosomes contain the information to become correctly co-ordinated within the nucleus. Thus if the chromosome set of one strain lacks the necessary information to become correctly co-ordinated within the nucleus of a second strain, it

would seem the simplest prediction that the chromosomes of the second strain ought also to lack the corresponding information in crosses to females of the first strain. Why this is not so, or at least why the two sorts of spatial misorganization should have such different consequences, remains unexplained at present. Complete reciprocal differences, however, are not a universal feature of hybrid dysgenesis. The crosses involving the ID strain made by Kidwell and Kidwell (1975*a*) show male recombination in both directions, although somewhat more strongly in one direction.

It would be of interest to know whether the spatial organization model might explain instances of hybrid sterility other than those of the present study. It is surprising that female fertility seems to be affected more than male fertility in the present study, the reverse of what is commonly found (Dobzhansky 1970, p. 333). However, this does not necessarily mean that the model is inapplicable to the study of male sterility. In particular it seems possible that the results of Dobzhansky (1974) for *D. pseudoobscura* might be explicable in similar terms. However, the example of *D. paulistorum* mentioned previously, where direct infectivity has been demonstrated, shows that the model is unlikely to be universally applicable.

The experiments reported in this paper have dealt mostly with the effects of changing the male contribution to the hybrid. The effect of modifying the female contribution has not been investigated in detail. Experiments investigating the female contribution offer more scope for extending the study in the long term. If the model put forward has any validity, it means that the study of the female component is really the study of membranes and of chromosome–membrane associations. This offers the possibility that the present system could be exploited to investigate aspects of the genetics of membrane development, as well as other aspects of spatial organization in the normal nucleus.

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