HYBRID DYSGENESIS IN *DROSOPHILA MELANOGASTER*: A SYNDROME OF ABERRANT TRAITS INCLUDING MUTATION, STERILITY AND MALE RECOMBINATION[1]

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Manuscript received October 13, 1976
Revised copy received April 26, 1977

ABSTRACT

A syndrome of associated aberrant traits is described in *Drosophila melanogaster*. Six of these traits, mutation, sterility, male recombination, transmission ratio distortion, chromosomal aberrations and local increases in female recombination, have previously been reported. A seventh trait, nondisjunction, is described for the first time. All of the traits we have examined are found nonreciprocally in F_1 hybrids. We present evidence that at least four of the traits are not found in nonhybrids. Therefore we have proposed the name *hybrid dysgenesis* to describe this syndrome.—A partition of tested strains into two types, designated P and M, was made according to the paternal or maternal contribution required to produce hybrid dysgenesis. This classification seems to hold for crosses of strains from within the United States and Australia, as well as for crosses between strains from the two countries. Strains collected recently from natural populations are typically of the P type and those having a long laboratory history are generally of the M type. However, a group of six strains collected from the wild in the 1960's are unambiguously divided equally between the P and M types. The dichotomy of this latter group raises interesting questions concerning possible implications for speciation.—Temperature often has a critical effect on the manifestation of hybrid dysgenesis. High F_1 developmental temperatures tend to increase the expression of sterility, sometimes to extreme levels. Conversely, low developmental temperatures tend to inhibit the expression of some dysgenic traits.—There are potentially important practical implications of hybrid dysgenesis for laboratory experimentation. The results suggest that care should be exercised in planning experiments involving strain crosses.

A new wave of interest in mutator phenomena in Drosophila has been rising steadily during the last decade. In addition to high spontaneous mutation rates (*e.g.*, SVED 1973) many investigators have observed such phenomena as

[1] This work was supported by National Science Foundation research grant GB-43820, Public Health Service research grant GM-20103 and by the Australia Research Grants Commission.

male recombination, male and female sterility, transmission ratio distortion and chromosomal aberrations (*e.g*, HIRAIZUMI *et al.* 1973; KIDWELL, KIDWELL and NEI 1973; KIDWELL and KIDWELL 1975a; MINAMORI 1969; PICARD 1976; SVED 1976b; VOELKER 1974; WADDLE and OSTER 1974; WOODRUFF and THOMPSON 1975; YAMAGUCHI 1976; YAMAGUCHI and MUKAI 1974). It is not clear whether or not earlier reports of mutator phenomena such as those of IVES (1950) and GREEN (summary, 1973) involve different mechanisms from those described more recently. The etiology of the recently described phenomena is highly complex. Evidence for a chromosomal association has been reported in several of the above studies, but it has proved difficult to localize the causal factor(s) to a single chromosome.

The purpose of this paper is to provide a synthesis of some of these varied phenomena. We show that a number of aberrant traits have several characteristics in common. In particular, they are properties of hybrids and occur nonreciprocally. In view of the hybrid and evidently dysgenic nature of most of the associated traits, to be documented below, we have proposed *hybrid dysgenesis* as a suitable name for this syndrome (SVED 1976b; KIDWELL and KIDWELL 1976). Hybrid dysgenesis has been defined (KIDWELL and KIDWELL 1976) as "a syndrome of correlated genetic traits that is spontaneously induced in hybrids between certain mutually interacting strains, usually in one direction only."

MATERIALS AND METHODS

The original research reported in this paper was carried out at Brown University (by M.G.K. and J.F.K.) and at the University of Sydney (by J.A.S.) The wild-type strains used are described in Table 1. Details of the marker stocks are given by LINDSLEY and GRELL (1968). Standard Drosophila techniques and media were employed. Temperatures were held at 25 ± 1° except where otherwise stated.

CHARACTERISTICS OF HYBRID DYSGENESIS

In this section we introduce the nonreciprocal aspects of hybrid dysgenesis, followed by a description of the individual dysgenic traits. Finally, we describe experiments which indicate the specific hybrid nature of the phenomena.

Nonreciprocality

A striking characteristic of most interacting strain crosses observed in our laboratories is the large differences in the frequencies of dysgenic traits between the progeny of reciprocal strain crosses. In order to accommodate this nonreciprocality, it is convenient to designate strains as either P or M, according to whether they produce dysgenesis when used as either paternal (P) or maternal (M) parent. Most strains we have tested appear to be unambiguously of either the P or M type, with the possible exception of Oregon-R-C which will be discussed later. Strains of the same type have similar general properties with respect to dysgenic potential, but may differ in the intensity of interaction. The cross which produces dysgenesis (*i.e.*, M strain female × P strain male) is designated

TABLE 1

Description of wild-type Drosophila melanogaster *strains used*

Strain		Collection information			Year entered our laboratory
		Year	Place	Collector	
Brown laboratory					
Ames I*	AE	1938	Ames, Iowa	J. W. Gowen	1961
Canton-S	CA		Canton, Ohio		1970
Ceres†	CE	1967	Ceres, N.Y.	A. Chabora	1973
Cranston	CR	1964	Cranston, R.I.	M.G.K.	1964
Harwich	HA	1967	Harwich, Mass.	M.L. Tracey, Jr.	1967
Nettlebed	NE		Gt. Britain		1964
Oregon-R-C*	OR (B)	1925	Roseburg, Ore.	D.E. Lancefield	1961
Ottawa	OT	1962	Ottawa, Canada	J.F.K.	1962
Oxford†	OX	1967	Oxford, N.C.	A. Chabora	1973
Painesville†	PA	1967	Painesville, Ohio	A. Chabora	1973
Princeton*	PR	1929	Princeton, N.J.	J.W. Gowen	1961
Weymouth g	WG	1974	Providence, R.I.	M.G.K.	1974
Weymouth i	WI	1974	Providence, R.I.	M.G.K.	1974
Winnepesaukee	WE	1974	Moultonboro, N.H.	M.G.K.	1974
Sydney laboratory					
Hunter Valley	HV	1974	Hunter Valley, N.S.W.	I. Franklin	1974
Oregon-R-C	OR (S)	1925	Roseburg, Ore.	D.E. Lancefield	1975

* GOWEN and JOHNSON 1946.
† O'BRIEN and MACINTYRE 1969.

the A cross. The reciprocal cross, which yields normal progeny, is designated the B cross.

Occasionally B crosses do exhibit dysgenic traits at frequencies greater than nontrival levels, but significantly lower than the A cross frequencies. For example, when the Cranston strain was crossed with the second chromosome marker strain *al cl b c sp*[2] in reciprocal combinations, cross A males had 0.83 percent minimum male recombination compared with 0.07 percent in cross B. In contrast, from the same crosses, F_1 A sterility was 46.0 percent in males and 57.8 percent in females compared to F_1 B sterility of 0 percent in both sexes.

Many other examples of reciprocal differences in dysgenic traits are well documented. KIDWELL and KIDWELL (1975a) first described nonreciprocality in association with male recombination and sterility in a number of strain crosses.

Confirmation with respect to one or the other of these two traits in other strain crosses was provided by WOODRUFF and THOMPSON (1975); SVED (1976b); and IVES (personal communication). The demonstration of nonreciprocality has been extended to distortion of transmission frequencies (KIDWELL and KIDWELL 1976), recessive *X*-linked lethal mutation (KIDWELL, KIDWELL and IVES 1977), local increases in female recombination (KIDWELL 1977) and nondisjunction (see below).

The most compelling general hypothesis to explain nonreciprocality is that hybrid dysgenesis is dependent on cytoplasm-chromosome interactions between different strains. An alternative hypothesis of a suppressor *X* effect is tenable with respect to dysgenic traits induced in male progeny but is untenable for female traits because female progeny of reciprocal crosses are expected to have identical *X*-chromosome complements. It is highly unlikely that dysgenesis in the two sexes is dependent on different mechanisms.

Description of dysgenic traits

In this section we describe in more detail the properties of individual dysgenic traits. We refer interchangeably to the systems studied in our two laboratories, which were independently discovered and subsequently shown by joint testing to be similar in most respects. We refer also to systems studied in other laboratories which have not been specifically tested for what we consider to be two of the critical characteristics of hybrid dysgenesis, namely nonreciprocality and hybrid manifestation. However, on the basis of the apparent similarity of these systems with our own and in the absence of any evidence of major differences, we tentatively include them as additional manifestations of the hybrid dysgenesis syndrome.

1. *Male recombination*: Nontrivial frequencies of male recombination have been reported many times in both major autosomes and their occurrence is clearly related to genetic properties of the parental strains (HIRAIZUMI 1971; VOELKER 1974; WADDLE and OSTER 1974; SVED 1974; WOODRUFF and THOMPSON 1975; KIDWELL and KIDWELL 1975a; YAMAGUCHI 1976). Strains associated with male recombination tend to manifest it in both chromosomes II and III, but the magnitude of the frequencies in the two chromosomes seems to be a property of each specific strain combination. Selection for high and low male recombination (KIDWELL and KIDWELL 1976) resulted in significant responses in both directions. A detailed summary of male recombination characteristics is provided by WOODRUFF and THOMPSON (1977).

2. *Sterility*: Sterility is defined as the complete absence of progeny in an individual after the opportunity to mate with at least two members of the opposite sex from a fully fertile strain. High frequencies of both male and female sterility have been found in some inter-strain crosses (KIDWELL and KIDWELL 1975a), though in contradistinction to HALDANE's (1922) rule, female sterility tends to exceed male sterility. Dysgenic F_1 females have been observed to accept and mate with males of both parental strains, although there were significant differences in mating speed between dysgenic and nondysgenic females of the

same genotype (*i.e.*, females arising from reciprocal crosses). In some crosses females produced eggs, while in others there was a complete absence of egg-laying. Additional data on F_1 A female sterility are provided in later sections of this paper.

3. *Mutation*: In both the first and second chromosomes of hybrids (Type A in our classification) SLATKO and HIRAIZUMI (1973) observed lethal mutation rates up to ten times above normal, associated with the presence of second chromosomes from the Texas T-007 strain. KIDWELL, KIDWELL and IVES (1977) have also reported a 5–10 fold increase in *X*-linked lethal mutations in hybrids involving male parents from both the Cranston and South Amherst populations. These experiments indicate that the strain source of the monitored *X* chromosome was not critical to the production of high mutation rates. There was no evidence for nonrandom location of induced lethals within the *X* chromosome.

4. *Transmission ratio distortion*: HIRAIZUMI (1971) observed that in T-007/*cn bw* males, the wild type T-007 chromosomes were transmitted less frequently than the *cn bw* chromosomes. KIDWELL and KIDWELL (1976) and J. JENKINS and M. G. KIDWELL (unpublished) have also observed transmission ratio distortion in several crosses of the A type between Cranston and Harwich and two multiply marked stocks. It should be emphasized that the transmission ratio distortion in these cases results in *reduced* transmission of wild-type, paternally-derived chromosomes. It is not clear whether this effect is in any way related to the so-called *segregation distorter* system (HARTL and HIRAIZUMI 1976). In that case, distortion is dependent on the mutations *Sd* and *Rsp* at two closely linked second chromosome loci. *Rsp/Rsp*$^+$ heterozygous males produce an excess of *Rsp* bearing sperm when these individuals are also *Sd/Sd*$^+$ or *Sd/Sd*. Homozygous *Rsp/Rsp* males exhibit reduced fertility.

In dysgenic males, the distortion effect appears not to be restricted to association with a specific chromosome, as in the case of the SD system. An example of this is provided by a comparison of ratios between A and B type crosses for both chromosomes II and III. F_1 males from reciprocal crosses between Harwich and *cn,e* were backcrossed to *cn,e* females, with the results shown in Table 2. The frequency of the wild type phenotype is independently and significantly reduced for both chromosomes II and III in the A cross as compared to the B cross.

5. *Female recombination*: HIRAIZUMI *et al.* (1973) initially reported no observable changes in female recombination value in crosses associated with male recombination. Later SLATKO and HIRAIZUMI (1975) reported that female recombination changes, including lowered interference values, are associated with male recombination induction. KIDWELL (1977) observed female recombination in intervals spanning almost the entire length of the three major chromosomes. Reciprocal crosses were made between Cranston and multiply marked stocks appropriate for each chromosome. In both major autosomes recombination values in proximal intervals were considerably higher in F_1 A cross females than in F_1 B cross females. There was a slight opposite tendency in the most distal autosomal intervals, and in all *X* chromosome intervals where F_1 B recombi-

TABLE 2

The proportions of progeny classes from crosses of F_1 cn e/*Harwich males and* cn e/ cn e *females*

P_0 mating	*cn e*	*cn* +	+ *e*	++	Total progeny
A. *cn e/ cn e* ♀ × Harwich ♂	0.354	0.224	0.270	0.152	1997
B. Harwich ♀ × *cn e/ cn e* ♂	0.253	0.245	0.255	0.247	4286

Heterogeneity test between A and B crosses—
cn : + $\chi_1^2 = 34.1$, $P < 0.001$
e : + $\chi_1^2 = 74.2$, $P < 0.001$
Test of independence of *cn*: + and *e*: + segregation—
A cross $\chi_1^2 = 1.57$, $0.20 < P < 0.30$
B cross $\chi_1^2 = 0.001$, $0.95 < P < 0.98$

nation values tended to be higher than those of F_1 A. The data given in Table 5 (to be described in a later section) also demonstrate that reciprocal differences in female recombination values in an autosomal centromeric interval, associated with hybrid dysgenesis, are the result of A cross increases above, rather than B cross decreases below, the parental intra-strain values.

6. *Chromosomal aberrations*: Gross chromosomal changes such as paracentric inversions, pericentric inversions, translocations and transpositions have been observed at high frequencies in various mutable chromosome lines (VOELKER 1974; YAMAGUCHI and MUKAI 1974; CARDELLINO and MUKAI 1975). Whilst reciprocal differences in aberration induction have not been reported, it is doubtful that they have been tested due to the nature of the accumulation procedure involved. It is considered significant that the accumulation techniques require successive backcrosses of the A type which in parallel experiments of our own have produced other dysgenic traits (*e.g.*, KIDWELL and KIDWELL 1976).

7. *Nondisjunction*: An experiment was carried out to detect nondisjunction of *X* chromosomes in F_1 females from reciprocal crosses between Harwich and Canton-S. (Canton-S females × Harwich males is designed cross A and Harwich females × Canton-S males is cross B). F_1 tester females were individually mated with two males from an H-41 stock (MUKAI and BURDICK 1959) and allowed to deposit eggs for nine days. Their progeny were scored for exceptional *B* w^a males and +/+ females. Table 3 indicates that a high frequency of exceptional progeny was observed from F_1 A mothers but not from F_1 B mothers. All exceptional males were tested for fertility and proved to be sterile, indicating their *XO* constitution as a result of primary nondisjunction. Test matings of the exceptional females were not possible because they had already mated before scoring. High sterility was observed in the F_1 A females in which the frequency of nondisjunction was high, providing another example of associated dysgenic traits.

It should be emphasized that more than one dysgenic trait can be expressed in the same group of F_1 A hybrids. In fact, it is suspected that the expression of multiple traits is the general rule and their joint detection may be limited only by the impossibility of using a single balancer/marker stock for all traits. Two

TABLE 3

Frequency of B w^a *exceptional male progeny and* B^+w^+/B^+w^+ *female progeny in crosses between H-41 males and Canton-S/Harwich* F_1 *females*

		Direction of F_1 cross		
		A	B	
% F_1 female sterility	63.8	(80)*	5.0	(40)*
% exceptional male progeny	2.19	(366)†	0	(951)†
% exceptional female progeny	0.54	(368)†	0	(1167)†

* Total number tested.
† Total number examined.

or more associated dysgenic tratis have been observed in many experiments, *e.g.*, male recombination and transmission ratio distortion (HIRAIZUMI 1971), male recombination and sterility (KIDWELL and KIDWELL 1975a; SVED 1976b), mutation and sterility (KIDWELL, KIDWELL and IVES 1977) and male recombination, female recombination, distortion of transmission frequencies and sterility (M. G. KIDWELL, unpublished results). In addition, KIDWELL and KIDWELL (1976), after selection for male recombination, demonstrated correlated responses in both sterility and transmission ratio distortion. These associations might be explained by a single underlying genetic event such as chromosome breakage.

Hybrid manifestation

From the foregoing sections it is clear that dysgenic traits have been observed many times in F_1 hybrid progeny of crosses between males from various wild-derived strains and females from a number of special laboratory stocks synthesized to detect such traits. It may be convenient to refer to particular traits as being a property of the wild-type strain involved, *e.g.*, "male recombination strains." However, we feel that this may be misleading since hybrid dysgenesis is a property of the apparent interaction between two strains. We have direct evidence for four traits that dysgenesis does not occur within strains at the high frequencies characteristic of some hybrids:

1. The results of female sterility tests for many inter- and intra-strain cross combinations are described in detail in a later section of this paper. We refer specifically to the results shown in Figure 1 where intrastrain cross sterility frequencies appear on the diagonals, F_1 A hybrids above the diagonals and F_1 B hybrids below the diagonals. Despite the possibility that factors other than dysgenesis may contribute to sterility in intra-strain combinations (*e.g*, inbreeding depression), none of the parental intra-strain combinations exhibited the high frequencies of sterility observed in interacting A type hybrids (*e.g.*, progeny of Canton-S female × Harwich male).

2. Normally, male recombination cannot be detected in intra-strain crosses because of the absence of suitable markers. The following procedure was under-

		1					2			3			4		
	♀ \ ♂	AE	CA	NE	OR(B)	PR	CE	OT	PA	CR	HA	OX	WE	WG	WI
1	AE	0	0	0			1		10		27	5		2	3
	CA		4		0				0	8	41	39	5		0
	NE			0		2	3			10	48	15		12	
	OR(B)	0			5			2			0	3	2		
	PR					2			0	12	20	23	0		
2	CE				3		7			30	8	23		20	15
	OT	0	0			0		5		13	20	3	7	5	2
	PA	0		0					0	7	76	42	13	20	
3	CR	1	1	0	0	1	7	0	0	1	0	0	1	0	0
	HA	1	0	1	2	2		0	2	5	2	0	0		
	OX	7	0		0	0	2	0	0	2	3	6	3		
4	WE		0			2	7	0	2	0	0		0	0	2
	WG	0		0			2	0	0	0				8	
	WI	2					0	0	0	5	2	0	1		5

FIGURE 1.—Percent female sterility in 14 strains and in the progeny of some reciprocal crosses among them. All flies were raised at 25° in the Brown laboratory and at least 60 females per cross were tested for sterility.

taken to introduce a suitable chromosome, bearing the dominant markers Glued and Stubble, into the genetic backgrounds of two wild-type strains known to show nonreciprocal F_1 sterility. *Gl-Sb*/++ males were successively backcrossed to wild-type females from Harwich and from Canton-S strains. From each of the two backcross lines, at generations 5 and 12, forty males were tested for recombination between *Gl* and *Sb*. At the same time, using reciprocal crosses, male recombination was also tested in *Gl-Sb* (Harwich)/Canton-S hybrids (cross A) and in *Gl-Sb* (Canton-S)/Harwich hybrids (Cross B). (The genetic background of the *Gl-Sb* male parent is shown in parenthesis). The results are presented in Table 4. Only the *Gl-Sb* (Harwich)/Canton-S hybrid males from cross A produced substantial frequencies of recombination in both generations tested. The *Gl-Sb* (Harwich) males (from an essentially intra-strain cross) had only low levels of male recombination. Note that the male recombination found in this experiment demonstrates that recombination in chromosome III can be caused

TABLE 4

Mean percentages of male recombination between Gl *and* Sb *in Harwich (HA) and Canton-S (CA) backcross lines and in hybrids between them*

Parental cross	Generation number 5	12
HA♀ × *Gl Sb* (HA) ♂	0.13 (3735)*	0.03 (3728)
CA♀ × *Gl Sb* (CA) ♂	0 (2737)	0 (3779)
CA♀ × *Gl Sb* (HA) ♂	0.90 (3780)	0.72 (3329)
HA♀ × *Gl Sb* (CA) ♂	0.12 (2443)	0.03 (3690)

* Number of progeny counted.

by a paternal contribution which does not include the Harwich chromosome III.

3. As indicated eariler, large centromeric increases in female recombination values are associated with hybrid dysgenesis and provide another indication of occurrence of this phenomenon. From the same set of matings described in section 2 above, female recombination values between *Gl* and *Sb* were also measured after each of the first five, the ninth and the twelfth generations of backcrossing to the Harwich and Canton-S strains and in their hybrids. Detailed results of this experiment are given by KIDWELL (1977), but a summary is provided in Table 5. This indicates that the overall pattern is consistent with the male recombination results. A notable feature was that female sterility increased dramatically and productivity decreased in Canton-S female × Harwich male hybrids compared with the reciprocal cross and the Harwich and Canton-S backcrosses which remained at similar low levels with increasing generation number. In addition to demonstrating that backcrossing has no significant effect on recombination frequency, these data provide clear evidence that hybrid reciprocal differences in female recombination are the result of increases in the type A cross rather than decrease in the B cross.

4. KIDWELL, KIDWELL and IVES (1977) found no evidence for increased frequencies of spontaneous *X*-linked recessive lethals in nonhybrids from either the Harwich or South Amherst strains. This was in contrast to F_1 A hybrids from crosses with *Basc* females in which the lethal frequency was increased 5–10 times above the expected level.

TABLE 5

Mean female recombination (%) between Gl *and* Sb *in Harwich (HA) and Canton-S (CA) backcross lines and in hybrids between them*

Parental cross	Generation number 5	9	12
HA♀ × *Gl Sb* (HA) ♂	12.1 (1314)*	9.6 (1433)	9.3 (900)
CA♀ × *Gl Sb* (CA) ♂	12.2 (1344)	12.7 (2087)	13.2 (1030)
CA♀ × *Gl Sb* (HA) ♂	20.0 (925)	27.4 (776)	23.8 (227)
HA♀ × *Gl Sb* (CA) ♂	11.7 (1834)	12.2 (1798)	10.1 (898)

* Number of progeny counted.

Strong independent evidence that dysgenesis is restricted to hybrids is provided by WOODRUFF, HENDERSON and THOMPSON (personal communication). They observed widespread chromosome breakage at male meiosis in F_1 inversion heterozygotes derived from male parents of a wild-type Oklahoma line (OK1) and female parents from a stock having a second chromosome paracentric inversion. Fragmentation did not occur in the OK1 base stock homozygotes nor in the reciprocal (OK1 female parent) heterozygotes. However, more information on dysgenic traits in crosses among natural populations is needed.

NATURE OF THE DYSGENIC INTERACTION

In an earlier section, a classification of strains into P and M types was introduced.On the basis of data from both male recombination and sterility tests, strains can be essentially divided into two classes. We have also found that the existence, but not necessarily the strength, of interaction between any pair can usually be predicted according to the types of the parents involved. The results of a large number of crosses between United States strains and also between United States and Australian strains are given below to justify this approach. Sterility tests have been used as an indicator of hybrid dysgenesis since they have the advantage of not requiring the use of specialized balancer and marker stocks. However, it should be noted that the observation of nonreciprocal sterility is sufficient but not necessary for a cross to be classified as dysgenic. Many dysgenic crosses exhibit some but not all dysgenic traits.

Crosses among United States strains

In an experiment conducted in the Brown laboratory, the progeny of reciprocal combinations and intra-strain crosses among a number of wild-type strains from different sources were tested for sterility. Males and females from parental strains were mated *en masse*. F_1 females were first allowed to mate with their brothers for at least 24 hours and then individually mated in shell vials with two males from a known fertile strain. Figure 1 indicates percent female sterility measured at 25°. At least 60 female progeny per cross were tested. Intra-strain combinations are seen on the diagonal with reciprocal crosses appearing in symmetrical positions above and below the diagonal. Strains were *a priori* grouped according to their history and ability to induce another dysgenic trait, third chromosome male recombination, in interacting combinations with appropriate marker stocks (KIDWELL and KIDWELL 1975a; KIDWELL and IVES, in preparation):

Group 1. Long-established "classic" wild-type stocks which previously had been associated with only trivial frequencies of male recombination ($< 0.05\%$).

Group 2. Strains collected from the wild in the mid 1960's and which also produced only trivial frequencies of male recombination.

Group 3. Strains collected from the wild in the mid 1960's but associated with relatively high levels of male recombination. ($> 1.00\%$).

Group 4. Strains collected from the wild in 1974, associated with low to moderate levels of male recombination ($0.05 - 1.00\%$).

In Figure 1, it is seen that above the diagonal, group sterility means corre-

spond fairly closely to the original male recombination groupings, sterility and male recombination tending to be positively associated, however there were exceptions in some individual strain combinations. Also, like male recombination, sterility frequencies in reciprocal crosses (below the diagonal) were consistently at a low level. Intra-strain sterility did not in any case exceed the ten percent level.

In general, the results shown in Figure 1 justify the classification of strains into the two types, P and M, proposed earlier. On the basis of either male recombination or sterility tests or both, groups 1 and 2 can be classified as M strains and groups 3 and 4 as P strains. Oregon R-C (B) is one possible exception to the general rule; this strain did not produce appreciable levels of sterility when crossed with any of a number of other strains from different groups, nor did it produce male recombination when crossed with several marker stocks. Further tests will be required to determine whether this strain represents a third, neutral, type.

Crosses between United States and Australian strains

We were interested in learning whether the same types of interactions observed in intercrosses of strains from within the United States, and of strains collected within Australia were also found in crosses between strains from the two countries. Therefore, the strains Harwich and Canton-S, previously shown in intercrosses to be two of the most reactive strains with respect to female sterility, were mailed from Brown to Sydney in December, 1975. These with the Hunter Valley and *cn, e* strains, which together were also known to produce high nonreciprocal hybrid sterility, were subsequently crossed in all possible combinations. The results are given in Figure 2, and fall into a pattern very similar to that seen in Figure 1.

Perhaps the most interesting aspect of the results of Figure 2 concerns the

♀ \ ♂	CA (B)	cn e (S)	HA (B)	HV (S)
CA (B)	2	0	92	0
cn e (S)	0	5	100	98
HA (B)	2	11	10	7
HV (S)	2	0	3	3

FIGURE 2.—Percent female sterility in all F_1 combinations of four strains, two each from the Brown (B) and Sydney (S) laboratories. All flies were raised at 25° in the Sydney laboratory and 60 females per cross were tested for sterility.

crosses between the United States and Australian wild type strains, which showed little or no sterility. This and the similar findings from Figure 1 indicate that hybrid dysgenesis is not related in a simple way to genetic divergence between the two parent strains. It seems unlikely that the Harwich and Hunter Valley strains have exchanged genes for some considerable time, although it is of course impossible to be certain about this. If dysgenesis in crosses between the laboratory and wild-type strains is attributed to random genetic divergence, this implies that there ought to be a corresponding divergence beween the Austrailian and United States strains. The failure to detect dysgenesis in these crosses seems to imply that some special change which usually occurs in laboratory strains, perhaps related to inbreeding, is necessary for hybrid dysgenesis to occur.

Two further aspects relevant to the nature of the dysgenic interaction should be mentioned here. First, the interaction is not always confined to crosses involving a wild-type strain. For example the *cn, e* laboratory stock leads to hybrid dysgenesis when used as female parent in crosses to a number of laboratory stocks. In particular the cross *cn, e* (female) × Oregon R-C (S) (male) gives 100% sterile female offspring, and no sterility in the reciprocal cross. The second aspect is that the restriction to two classes (P and M) may be an oversimplification. The same Oregon R-C (S) laboratory stock, unlike the Oregon R-C (B) stock, when used as female parent in crosses to Harwich gives 70% sterile daughters, and therefore cannot be unambiguously assigned to either the P or M class. This result suggests that there might be some sort of hierarchical classification of strains, the *cn, e* and Harwich strains being at the two extremes, and that the P and M classification delineates the major subdivision of this scale.

ENVIRONMENTAL INTERACTIONS

Preliminary experiments indicate that environmental interactions may have a large effect on the manifestation of dysgenic traits. These interactions appear to be highly complex and a complete description will require considerable experimentation. A first effort to determine the effects of two factors, developmental temperature and age, on two dysgenic traits in a limited number of strain crosses is described below.

Temperature

A demonstration of the effect of temperature on sterility is provided by reciprocal crosses between Canton-S and Harwich strains. F_1 females were concurrently raised at 18°, 25° and 29° and the F_2 offspring from the F_1 25° group were also raised at 25°. The F_2 offspring of the 18° and 29° group were themselves assigned to 18°, 25° and 29° temperatures for raising. The temperature shift occurred at the time the F_1 females were assigned to individual test vials. Percent female sterility under these varying temperature regimes is given in Table 6. Temperature was found to be effective in influencing "all-or-none" sterility only during the developmental period of hybrid individuals. Once these individuals reached maturity, temperature (within the range 18–29°) was no longer a factor in their fertility, as here defined. Cross A F_1 females varied from

TABLE 6

Percent F_1 female sterility in reciprocal P_0 crosses A and B between Canton-S and Harwich at three developmental temperatures

F_1 raising temperature (deg. C.)	F_2 raising temperature (deg. C)					
	A			B		
	18	25	29	18	25	29
18	3.3 (30)*	0 (59)	0 (30)	0 (30)	0 (60)	0 (30)
25		41.4 (350)			1.2 (170)	
29	100.0 (30)	100.0 (50)	100.0 (30)	6.7 (30)	0 (60)	0 (30)

* Figures in parentheses denote numbers of F_1 females tested.

complete sterility when raised at 29° to complete fertility when raised at 18° with a somewhat intermediate value when raised at 25°. Female sterility in the B cross was zero or trivial at all temperatures.

Seven other crosses were tested in reciprocal combinations for F_1 female sterility at 25° and 29° (Table 7). At least 60 F_1 females were tested for each parental combination. There was a general tendency for those crosses with substantial sterility at 25° to show an increase at 29°. In contrast, those crosses having low sterility at 25° showed, at the most, only small increases at 29°.

TABLE 7

Percent F_1 female sterility in eight reciprocal strain crosses raised at temperatures of 25° and 29°

P_0 cross	Cross A		Cross B	
	25	29	25	29
CA x HA	41	100	0	0
CA x OX	39	53	0	0
CA x PA	0	0	0	2
CE x WG	20	13	2	0
OT x CR	13	70	0	2
PA x OX	42	85	0	5
PA x WE	13	48	2	10
HA x OX	0	0	3	-

TABLE 8

Minimum percent male recombination events at three temperatures in reciprocal crosses between rucuca *and Harwich*

Temperature (deg. C.)	Cross	
	A	B
18	0.35 (7774)*	0.52 (7100)
25	2.19 (4664)	0.25 (3623)
29	0.43 (4593)	0.13 (4457)

* Number of progeny tested.

The effect of temperature on male recombination was investigated in another series of experiments. Reciprocal crosses between Harwich and *rucuca* were raised at 18°, 25° and 29°. F_1 males from both A and B crosses were individually backcrossed to three *rucuca* females in shell vials and their progeny raised at 25°. Male recombination frequencies for combined intervals of the third chromosome are presented in Table 8. It is seen that reciprocal differences are essentially eliminated at the two extreme temperatures. F_1 A values were at a maximum at the intermediate 25° temperature. F_1 B values increased with decreasing temperature. Unfortunately, humidity was not controlled and dessication did occur in some of these tests. Therefore caution in the interpretation of these results is advisable until further tests are made.

These results indicate that in strain crosses with a predisposition for hybrid dysgenesis, environmental variables have a critical effect on its manifestation. This interaction has not yet been clearly described and many questions remain, such as the shape of the temperature-response curves for different traits and the developmental stage when environmental factors are critical. However, from a practical standpoint, high temperature increases in sterility have provided a useful method of assay for hybrid dysgenesis in some strongly interacting strain combinations.

Age of male

We have frequently observed that in crosses with a high percent of hybrid sterility, those hybrids which are fertile produce low numbers of progeny. In order to investigate this further, sterility frequencies were measured as a function of age of male in reciprocal crosses between *Gl-Sb/LVM* and Harwich strains. F_1 males were brooded with successive harems of five females on alternate days. The cumulative percentage of sterile males is plotted against age in days in Figure 3. It is seen that the sterility differential between reciprocal crosses A and B is not only present during the first age period, but actually increases dur-

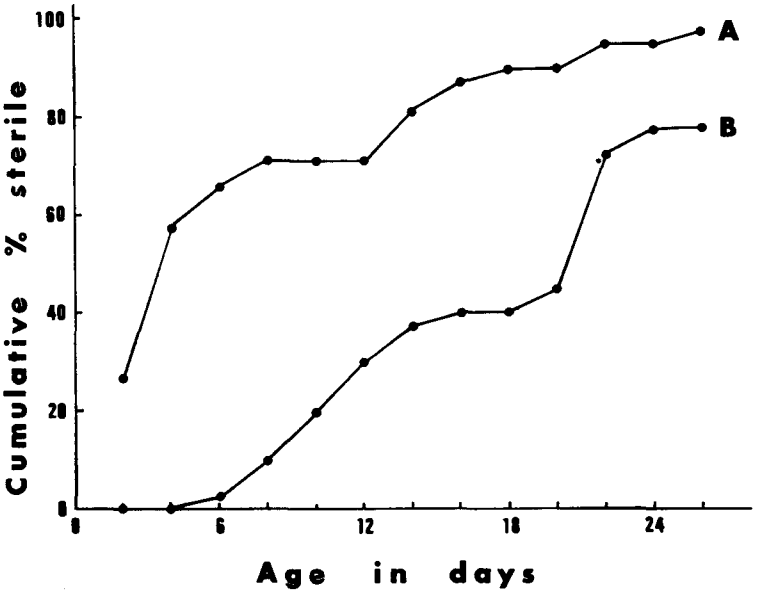

FIGURE 3.—Cumulative percentage of sterile F_1 males from reciprocal crosses A and B between Harwich and *Gl Sb*/LVM.

ing the first six days of life. The productivity differential between reciprocal crosses during this period therefore may be a reflection of the premature sterility of so-called "fertile" males from the A cross. This result explains the commonly observed phenomenon that a significant proportion of dysgenic individuals produce just a few progeny. Thus the all-or-none classification of life-time sterility grossly underestimates overall sterility, because individuals which are early fertile but prematurely sterile are not distinguished from normal fertile individuals.

In a second experiment, we tested male recombination in successive broods of progeny from a group of dysgenic hybrid males in order to find out if age of male was a factor which also influenced the frequency of this trait. The experiment was initiated by crossing *en masse* virgin *rucuca* females with Harwich males. Each of thirty F_1 heterozygous male progeny, within 24 hours of eclosion, were mated with five homozygous *rucuca* females. These males were successively brooded with a new harem of five *rucuca* females every five days until they were 30 days of age. Male recombination was measured for each brood and in all *rucuca* intervals. The data are given in Table 9 and the results are depicted graphically in Figure 4. There was a clear trend for mean recombination to decrease with increasing age of male. This trend was maintained when mean recombination was calculated only within the group of 13 males which remained fertile to the last brood (Table 9). Figure 4 incidently demonstrates the advantage of correcting for clustering of recombinants; the graph of mean frequency of male recombination for all tested males is seen to fluctuate somewhat erratically mainly due to a single large cluster in the fourth brood (20 days of age);

TABLE 9

*Mean frequencies of male recombination in successive five day broods of Harwich/*rucuca *F_1A hybrids*

	All fertile males				13 males, fertile to 30 days		
Male age	No. fertile males	No. progeny	Minimum % events	Total % recombination	No. progeny	Minimum % events	Total % recombination
1-5	28	1442	2.15	3.88	775	1.94	4.65
6-10	27	1364	1.39	1.61	723	1.80	2.07
11-15	27	948	0.84	1.48	524	0.76	1.53
16-20	23	1065	1.03	3.66	666	1.05	1.20
21-25	17	874	0.80	0.94	672	0.89	1.04
26-30	13	556	0.36	0.36	556	0.36	0.36

the graph for the minimum frequency of recombination events (KIDWELL and KIDWELL 1975b) is considerably smoother. This latter measure corrects for the "noise" caused by large clusters. The influence of male age on recombination demonstrated here is not confirmed by WOODRUFF and THOMPSON (1977). It is possible that the effect is specific to this particular cross.

DISCUSSION

A striking similarity exists between the strain interaction phenomena described

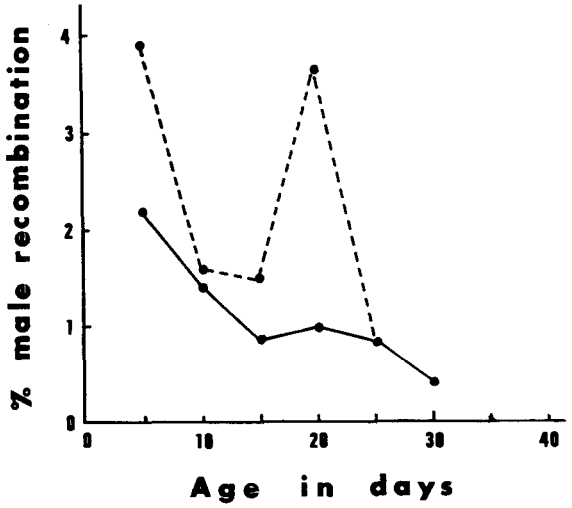

FIGURE 4.—Mean frequency of recombination in the third chromosome in F_1A *rucuca/* Harwich males mated with successive broods of *rucuca* females. The dashed line depicts percent male recombination. The solid line depicts minimum percent male recombination events. The two measures give identical frequencies for the last brood.

here and those recently reported by PICARD (1976). He tested many combinations of strains obtained from European laboratories and others collected from the wild. He classified strains as either inducer (I), reactive (R) or neutral (N). Of 93 strains, collected from the wild, all were classified as I strains (PICARD *et al.* 1976). Laboratory strains were divided among all three categories. It is not yet clear whether the P and M classification proposed in the present paper can be equated to the I and R classification used by PICARD. In the French tests, various degrees of sterility were observed in F_1 female hybrids when females from reactive strains were crossed with males from inducer strains. The similarity of this system to our own seemingly extends to the chromosomal association of the inducer factor in successive generations of backcrossing heterozygous inducer/reactive males with females from a reactive strain. The absence of other dysgenic traits associated with female sterility in the French tests, *e.g.*, segregation distortion and female recombination (PICARD *et al.* 1977) and male sterility (BUCHETON 1973), may not reflect a difference in genetic determination between their system and hybrid dysgenesis. It may merely reflect the dependence of dysgenic trait manifestation on temperature. Likewise, the increase in fertility with high-temperature shocks and aging, which is seemingly the reverse tendency to that in our own system, may be due to differences in procedure. Further experiments are clearly required before it can be established whether the two systems are fundamentally the same.

For many years it has been well known that male recombination can occur spontaneously in *Drosophila ananassae* and at an even higher frequency than has more recently been discovered in *D. melanogaster*. Several other dysgenic traits such as dominant mutation and chromosomal aberration have recently been observed at high frequencies in association with male recombination in *D. ananasse* (HINTON 1974, 1975), suggesting a strong similarity to the hybrid dysgenesis syndrome here described in *D. melanogaster*. The similarity extends to the characteristics of nonreciprocality and hybrid manifestation. Male recombination has also been reported in *D. simulans* (WOODRUFF and BORTOLOZZI 1976), *D. subobscura* (PHILLIPS 1944), *D. virilis* (KIKKAWA 1935) and *D. willistoni* (FRANCA, DA CUNHA and GARRIDO 1968). STURTEVANT (1939) observed an enhanced mutation rate in the offspring of backcrosses from hybrids between *D. pseudoobscura* and *D. persimilis*. Conventional studies of nonreciprocal hybrid male sterility, *e.g.*, DOBZHANSKY (1974) in *D. pseudoobscura*, could also conceivably be related to hybrid dysgenesis.

In the present study there are some interesting exceptions to the restriction of M strains to long-established laboratory stocks. Three strains collected in the 1960's (group 2 of Figure 1) are now clearly classifiable as M strains. This is in contrast to three other strains collected at approximately the same time (group 3 of Figure 1) which include the strongest interacting strains of the P type. Whether these present-day differences reflect the concurrent existence of the two types in natural populations in the 1960's or whether they represent evolution in response to a laboratory environment is a question of some significance. The first interpretation suggests that the potential for reproductive isolation may exist

among geographically diverse natural populations. The second interpretation suggests divergence between strains related to differences between laboratory and natural environments. It may be of significance that the group 2 (M) strains were collected in areas to the north and west of the group 3 (P) strains, but with so few strains tested we can do little more than speculate. Of course our results with crosses between United States and Australian strains show that there is no simple relationship between geographical isolation and strain interaction. Further tests of present day strains from different geographical areas are required, followed by monitoring over a period of years in a laboratory environment.

One observation which may have relevance to the role of hybrid dysgenesis in speciation is that the two environmental conditions which we have shown to be least dysgenic (*i.e.*, 18° temperature and aged males) are the two environmental conditions which are effectively used in the synthesis of hybrids between the two sibling species *D. melanogaster* and *D. simulans* (D. WEISBROT, personal communication).

Due to the drastic reduction in population fitness expected to accompany high frequencies of dysgenic traits, it has been difficult to understand how mutator mechanisms are maintained over long periods within natural and laboratory populations (KIDWELL 1975). CARDELLINO and MUKAI (1975) observed that although chromosome aberrations and recessive lethal mutations were induced at a very high rate in their accumulation experiments, the frequencies of these traits in natural populations were not high (MUKAI and YAMAGUCHI 1974). These difficulties can be at least partially resolved if it is no longer assumed that dysgenic traits occur at equally high frequencies in hybrids and nonhybrids. One of the main aims of the present paper has been to present evidence that dysgenic traits do not in fact occur in nonhybrids at the high rates found in F_1 cross A hybrids. However, a fitness advantage to carriers of dysgenic potential in natural populations must be present in the long term if this phenomenon has any generality. Perhaps the required advantage could be partially related to the development of reproductive isolating barriers to maintain genetic integrity within incipient species.

There are many questions of considerable interest related to the etiology of hybrid dysgenesis. A number of observations indicate the complex nature of the underlying mechanism and its phenotypic manifestations. Chromosomal associations can be traced when heterozygous males are used as male parents in successive generations (*e.g.*, KIDWELL and KIDWELL 1976) but such close associations appear to break down when heterozygous female parents are employed. Cytoplasmic transmission through several generations has been demonstrated in some cases (*e.g*, SVED 1976a) but not beyond the F_1 in other cases (*e.g.*, KIDWELL, KIDWELL and IVES 1977). Considering the results contained in the present paper, it seems that within the category of interacting F_1 A hybrids the strength of interaction between any pair of strains is to some extent at least a specific property of each combination itself, as is the spectrum of intensity of interactions affecting different traits in cases where it is possible to test several traits concurrently. Reproductive isolation may provide a partial explanation for these observations,

but there are many outstanding questions relating to specificity of interaction and the effect of laboratory maintenance on interaction potential.

Currently, the most commonly considered hypothesis to explain hybrid dysgenesis and related phenomena is in terms of transmissable factors. This hypothesis has several variants including controlling elements (KIDWELL, KIDWELL and NEI 1973) and episomes or viruses (*e.g.*, VOELKER 1974; ROBERTS 1976; see also MINAMORI 1969). The most direct evidence for the existence of some type of transmissable factor is provided by the results of SOCHACKA and WOODRUFF (1976). They induced recombination in Canton-S/*dp b cn bw* males by injection with whole-fly and ovarian extracts from a line which previously had yielded male recombination when outcrossed to multiply marked strains. In addition WOODRUFF, HENDERSON and THOMPSON (personal communication) claimed that their observations of widespread chromosome fragmentation were similar to previous reports of those induced by certain chemicals and virus infections. An alternative hypothesis to that of transmissable factors has been proposed by SVED (1976b) in terms of spatial organization of chromosomes.

Hybrid dysgenesis has potential practical implications for experimentation involving strain crosses. We have found that laboratory balancer and marker strains are often of the M type. Thus when these stocks are used as female parent, which is the general rule in many experiments, they may be expected to produce dysgenesis if the male parent is from a strain of the P type. Considerable care should therefore be taken in the design of experiments employing such crosses. Specifically, it is suggested that reciprocal crosses be made to detect the possible existence of strain interactions.

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