EXPERIMENTAL POPULATION GENETICS OF MEIOTIC DRIVE SYSTEMS
II. ACCUMULATION OF GENETIC MODIFIERS OF SEGREGATION DISTORTER (*SD*) IN LABORATORY POPULATIONS[1]

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ABSTRACT

The accumulation of modifiers of the meiotic-drive locus Segregation Distorter (*SD*) in *Drosophila melanogaster* was monitored by measuring the changes in the mean and variance of drive strength (in terms of "make" value) that occur in laboratory populations when *SD* and *SD+* chromosomes are in direct competition. The particular *SD* lines used are *T(Y;2),SD* translocations showing pseudo-*Y* drive. Four sets of population cages were analyzed. Two sets were monitored for changes in *SD* fitness and drive strength (presumed to be positively correlated) and analyzed for the presence of autosomal dominant or *X*-linked modifiers after long periods of time. The remaining two sets were made up of cages either made isogenic or variable for background genetic material, and these were used to test whether the rate of accumulation of modifiers was dependent on initial genetic variability.—Contrary to previous studies in which most suppression of *SD* action could apparently be attributed to a few dominantly acting modifiers of large effect, the conclusion here is that laboratory populations that are initially free of such major dominant loci evolve to suppress *SD* action by accumulating polygenic, recessive modifiers, each of small effect, and that much of the required genetic variability can be generated *de novo* by mutation. Possible explanations for these seemingly incompatible results and the evolutionary implications for *SD* are considered.

SANDLER and NOVITSKI (1957) defined a meiotic-drive locus as one that, as a result of the mechanics of the meiotic divisions, leads to a nonrandom excess recovery of the driven allele in the gametes of heterozygous individuals. Considerable effort has been expended in the intervening years on generating a comprehensive picture of the theoretical population genetics of meiotic drive, including specifying the conditions for maintenance of polymorphism at drive loci (HIRAIZUMI, SANDLER and CROW 1960; LEWONTIN 1968; HARTL 1970a, b), the relationship of various fitness functions to the strength of drive (HARTL 1972), and the population dynamics of modifier loci, either linked or unlinked to the main drive locus (PROUT, BUNDGAARD and BRYANT 1973; HARTL 1975a, b; KARLIN and Mc-

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GREGOR 1974). For most of this theory, the meiotic drive element Segregation Distorter (*SD*) of *Drosophila melanogaster* has proved to be the most valuable experimental model available. This is primarily because the investigation of the biological mechanisms involved in the *SD* phenomenon has proceeded at a faster pace than that with other known cases of meiotic drive. (See PEACOCK and MIKLOS 1973, and especially HARTL and HIRAIZUMI 1976, for excellent reviews of the *SD* system.)

SD seems to consist of a complex of loci located on chromosome *2* of *D. melanogaster*, consisting of at least an *Sd* locus, currently mapped to the basal of euchromatin of *2L*, where the action of *SD* is presumed to originate; a Responder (*Rsp*) locus, which is the target for the *Sd* action and is located in the centromeric heterochromatin of *2R*; and a series of loci that act as polygenic modifiers of *Sd* strength. These are of varying effect and are located throughout the genome (SANDLER and CARPENTER 1972; HARTL 1974, 1975b; KATAOKA 1967; TRIPPA and LOVERRE 1975; GANETZKY 1977). The current interpretation of the *SD* phenomenon is that the action of *Sd* is directed to cause dysfunction of any sperm carrying a sensitive Rsp^+ allele (TOKUYASU, PEACOCK and HARDY 1972). Consequently, the *SD* chromosome is thought to carry the *Sd* allele and an insensitive Responder (*Sd Rsp*), while an SD^+ chromsome carries a sensitive Responder (Sd^+ Rsp^+). *SD* operates in SD/SD^+ males to give an excess of *SD*-bearing sperm, the extent of the SD^+ sperm dysfunction generated in a particular line being measured by the *K*-value, defined in Table 1.

TABLE 1

Definition of pertinent parameters and variables

f = fertility of a *T(Y;2)SD* male relative to a standard male (usually *cn bw* in these studies).

v = viability of a *T(Y;2)SD* male relative to a standard male.

c = frequency of alternate segregation ($Y + SD$ from $X +$ *cn bw*) in a *T(Y;2)SD* male. This quantity is defined to be 0.5 in nontranslocated lines.

z = probability of *SD*-induced dysfunction of a SD^+-bearing sperm in a SD/SD^+ male.

k = the observed proportion of *SD*-bearing sperm among all functional sperm of a SD/SD^+ male.

K = the mean k value of a stock or line.

M = the mean "make" value of a SD/SD^+ male measured in probits. A reduction in this quantity is presumed to reflect accumulation of *SD* suppressors.

The quantities z, k and M are related by the equation

$$\frac{(2k-1)}{k} = z = \int_{-\infty}^{M-5} N(0,1)\,dx$$

$W = \dfrac{2fcv}{2-z}$ = total fitness of a *T(Y;2)SD* male relative to a standard male.

$\ln\left(\dfrac{q_{g+1}}{p_{g+1}}\right) = \ln\left(\dfrac{q_g}{p_g}\right) = \ln(W)$ = one successive difference in a cage,

where

$$\ln\left(\frac{q_g}{p_g}\right) = \ln\left(\frac{\#\ T(Y;2)SD \text{ males at generation } g}{\#\ cn\ bw \text{ males at generation } g}\right)$$

Segregation Distorter was first isolated from a natural population near Madison, Wisconsin (SANDLER, HIRAIZUMI and SANDLER 1959). In the 20 years since that time, elements of the *SD* system have been found in practically every population of *D. melanogaster* surveyed (HARTL 1975b; HARTL and HIRAIZUMI 1976). Populations harboring *SD* are always polymorphic, suggesting that the strength of drive in males and the schedule of fitnesses for the *SD/SD*, *SD/SD*$^+$, and *SD*$^+$/*SD*$^+$ genotypes are in such balance as to satisfy the general requirements for polymorphism given by HARTL (1970b). The polymorphism is maintained primarily because the reduced fitness of *SD/SD* homozygotes (which are often lethal, or, in the case of males, sterile owing to the action of *SD*) offsets the drive advantage enjoyed by *SD* when heterozygous in males (HIRAIZUMI, SANDLER and CROW 1960; HARTL 1970b, 1975a, 1977). Consequently, polymorphism for *SD* generates a sizable genetic load. Investigations of this load in experimental populations yield estimates in the range of 4 to 7% (HIRAIZUMI 1962; HARTL 1970a). Clearly, if genetic variability for drive suppressors is available, a population challenged by *SD* might be expected to fix these suppressors until K is reduced to such a level that the haploid drive advantage of *SD* is no longer great enough to overcome its diploid fitness disadvantage. As a consequence, the complex and the load it generates are eliminated from the population. In the case of *SD*, these could be of two main types: (1) *Rsp* alleles segregating to allow *SD*$^+$ chromosomes to become insensitive to *Sd* action, or (2) true suppressors segregating at loci distinct from Responder. The population dynamics of such suppressors and their allelic counterparts, drive enhancers, have been investigated for the case of autosomal drive in a series of papers (HARTL 1975a; PROUT, BUNDGAARD and BRYANT 1973; THOMPSON and FELDMAN 1974, 1975), which have demonstrated the following general conclusions: (1) Suppressors unlinked to the drive locus will become fixed. (2) Enhancers sufficiently tightly linked to the drive locus, while not fixed, will tend to be found coupled to the driven allele (linkage disequilibrium exists). (3) A genetic factor increasing the linkage (*e.g.*, a locus modifying recombination, or an inversion) between a drive locus and an enhancer locus will tend to increase in frequency.

Although the above conclusions were derived explicitly for cases of autosomal drive, they apply to sex-chromosome drive also. In fact, conclusions (1) and (2) have long been employed as arguments for the maintenance of a 1:1 sex ratio, starting with FISHER (1930) and continuing more recently with NUR (1974) and THOMSON and FELDMAN (1975). The classic argument is that autosomal suppressors of deviant sex ratios (sex-chromosome drive) selectively increase in frequency to return the sex ratio to 1:1 (conclusion 1), while enhancers of distortion located on the sex chromosomes themselves are selected to be in coupling with the driven chromosome (conclusion 2).

A distillation of all these arguments is that, for both autosomal and sex-chromosomal drive, unlinked suppressors should increase in frequency to reduce the segregation load, while linked enhancers should evolve to increase that load. The fact that natural selection can act in this direction of increasing load should make us wary of assuming that diploid population fitness always increases (*cf.*,

PROUT, BUNDGAARD and BRYANT 1973). If prezygotic selection is strong, as it is in many cases of drive, it may overcome a sizable zygotic selection in the opposite direction. Since load is measured from the diploid point of view, it is thus not surprising that we may find load increasing in such situations.

Populations segregating for *SD* fulfil many of these theoretical predictions. For example, polygenic enhancers of *Sd* are known to occur in the centromeric region and throughout the right arm of the *SD* chromosome (MIKLOS and SMITH-WHITE 1971), and are often found coupled to the *Sd* locus by means of a series of inversions (see GANETZKY 1977 and HARTL and HIRAIZUMI 1976, for details); whereas, suppressors of *SD* action accumulate either on the unlinked *X* (KATAOKA 1967) or third chromosomes (TRIPPA and LOVERRE 1975), or on the second chromosome, but in repulsion to the *Sd* locus as *Rsp* alleles occurring in *SD*+ chromosomes. Studies surveying either natural (HARTL 1970c; HARTL and HARTUNG 1975) or laboratory populations (HIRAIZUMI, SANDLER and CROW 1960; WATANABE 1967; HARTL 1977) segregating for *SD* indicate that these *Rsp* alleles are the most important source of drive suppression. Presumably the insensitive *SD*+ chromosomes (*Sd*+ *Rsp*) arise primarily from rare recombinant events in *Sd Rsp/Sd*+ *Rsp*+ females (HARTL 1975b; 1977). Since most *SD* suppression is due either to these *Rsp* alleles or to strong *X*-linked (KATAOKA 1967) or third-chromosome (TRIPPA and LOVERRE 1975) suppressors, the conclusion of these investigations has generally been that suppressors are usually dominant, of strong effect, and are confined to a few major loci.

The investigation reported here involved a series of experiments designed to monitor the population dynamics of the accumulation of drive modifiers, both suppressors and enhancers, in laboratory populations segregating for *SD*. The *SD* strain used was a special *T(Y;2),SD* stock, which exhibits "pseudo-*Y* drive" (LYTTLE 1977) through the effective coupling of the *Y* chromosome to the *SD* chromosome *via* chromosomal translocation. *T(Y;2),SD* males consequently produce only male offspring, a fact that offers several advantages for the study of modifier accumulation: (1) The large genetic load incurred as a result of extreme deviations from the 1:1 sex ratio introduces intense selective pressure for suppressors of drive (see, for example, LYTTLE 1977). (2) The fact that the *Y-SD* complex occurs only in males ensures complete linkage for the whole *SD* chromosome, thus increasing the opportunity for the selection of enhancers of drive on that chromosome. (3) Since the converse of (2) is that *Sd*+ *Rsp* chromosomes cannot arise from *SD* by recombination, it is possible in this system to monitor only those modifiers that were either part of the initial variability in the *SD*+ population challenged by *SD*, or that arose *de novo* by way of mutation.

The conclusion presented below will be that populations do appear to accumulate drive modifiers according to the predictions of PROUT, BUNDGAARD and BRYANT (1973), discussed above, that considerable background variation for drive suppressors exists in *SD*+ populations that have not been previously challenged by *SD*, and that most of the reduction in *K* value that occurs in *T(Y;2)*, *SD* cage populations appears to be due to the increase in frequency of autosomal recessive, polygenic suppressors of small effect, rather than to the accumulation of dominant, major locus suppressors reported by past investigators.

MATERIALS AND METHODS

Standard stocks (for complete descriptions, see LINDSLEY and GRELL 1968): *cn bw*: a stock carrying two second chromosome mutants. The *cn bw* chromosome is an *SD+* in this study. *cn bw* (iso): a stock isogenic for the whole genome and maintained since 1963 by single sib matings. *cn bw*; *e*; *spa*^{pol}: a stock carrying mutant markers for all autosomes. *XX/Y·bw+*; *cn bw* = *C(1)FMA4*, *In(1)w*^{m4} + *AB/In(1)FM7*, *y*$^{-}$*/Y·bw+*; *cn bw*, *Su(SD)*; *cn bw*: a tester stock carrying KATAOKA's (1967) *X*-linked SD suppressor. *FM7* = multiply inverted first chromosome balancer. *CyO* = multiply inverted chromosome balancer.

SD *stocks* (unless otherwise noted, these lines are maintained by constant backcrossing of *SD/cn bw* males to *cn bw/cn bw* females): *SD/cn* = R(*cn*)-14,*cn*: a line derived from R(*SD-36*)-1. This is a medium distorter ($K \approx 0.914$) used as a tester SD stock. *T(Y;2),SD L*^{2}: a "pseudo-*Y* drive" *SD* line used in the pilot cage population, derived from irradiation of *SD-72 L*^{2}. The autosomal breakpoint is in section 58 of the standard salivary map of *2R*. Figures 1 and 2 describe the segregational mechanics and analysis of relative fitness, respectively, for such a pseudo-*Y* drive line (see also LYTTLE 1977 for further details), which will be discussed subsequently. This stock is a medium level distorter ($K = 0.940$). *T(Y;2),SD L*^{2}-A: an *SD* line derived from the pilot cage population of *T(Y;2),SD L*^{2}, which shows an enhanced *K value* = 0.986. *T(Y;2),SD L*^{2} (iso): an *SD* line derived from *T(Y;2),SD L*^{2} with the isogenic background of *cn bw* (iso) introduced. This line is a weak distorter ($K = 0.735$).

Estimation of k *values*: The *k* values of individual *SD* males were normally determined by matings with two to three *cn bw* females, unless otherwise noted. The matings were brooded after eight days, the parents being discarded from the broods after eight more days. Progeny from the matings were counted for no more than 19 days, thus avoiding contamination from a second generation. Since the progeny of *T(Y;2),SD L*^{2} ♂ × *cn bw* ♀ crosses are deficient for one lethal class resulting from fertilizations by unbalanced *X;SD* sperm (see Figure 1), the *Y;SD* class of progeny was doubled in number when calculating *k* value in order to compensate for the missing individuals, giving $k = 2(\#SD \text{ males})/[2(\#SD \text{ males}) + \#SD+]$. The justification for this is the fact that these two classes are equal in frequency in the progeny of *T(Y;2)SD L*^{2} ♂ by *XX/Y·bw+*; *cn bw* ♀ matings, where all four classes of sperm are represented in the progeny (Figure 1; *Y;SD* = 2404, *X;SD* = 2442). Such *k*-values were then used in calculating both the unweighted mean and median *K* values for a given *SD* line.

Competition cages: Figure 2 depicts the one-generation changes in frequency of *cn bw*, *T(Y;2),SD* and hyperploid males when competing for *cn bw* females in population cages. Setting the *cn bw* male fitness to one, we find the ratio of *T(Y;2),SD* to *cn bw* males after one generation to be:

$$\frac{q_{g+1}}{p_{g+1}} = \frac{2fcv}{2\text{-}z} \cdot \frac{q_g}{p_g} \tag{2}$$

(The various terms are defined in Table 1.) Taking logarithms and rearranging gives:

$$\ln\left(\frac{q_{g+1}}{p_{g+1}}\right) - \ln\left(\frac{q_g}{p_g}\right) = \ln\left(\frac{2fcv}{2\text{-}z}\right) = \ln(W) \tag{3}$$

If the parameters *f*, *c*, *v* and *z* are all constant, the difference equation (3) can be used to estimate the relative fitness (*W*) of the *T(Y;2),SD* male line involved. When competition cages are monitored through time, several such independent estimates can be obtained to give a more accurate average value for fitness (see LYTTLE 1977 for a more detailed discussion). However, since *z* is a direct measure of the strength of drive (Table 1), if a change in *SD* activity occurs, it will be reflected not only directly by changes in *K* value, but also indirectly by changes in these cage-derived fitness estimates.

Table (2) describes four experimental groups of competition cages that were used in this investigation. Groups A and B were monitored through time to measure changes in drive strength as reflected both by changes in *T(Y;2),SD* fitness, as well as by direct analysis of various chromosomal components for the presence of drive modifiers (see next section). Groups G and D were designed to measure the relationship between the changes in mean and variance of drive strength

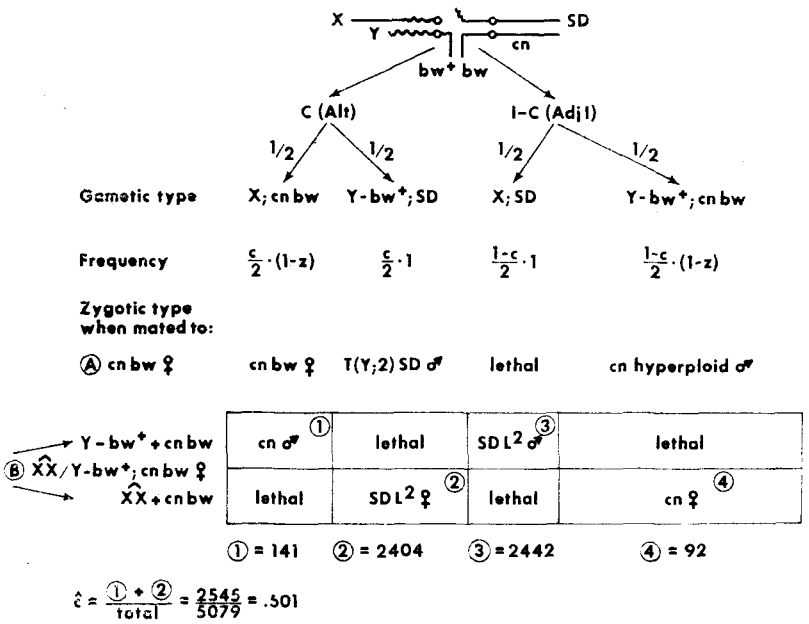

FIGURE 1.—The segregational mechanics in *T(Y;2),SD L²* males. The body of the figure gives the type and frequency of the expected progeny when such males are mated to (A) *cn bw*'s or (B) *X̂X/Y-bw+;cn bw*'s. The frequency of alternate segregation (*C*) is estimated using the numbers of the four progeny classes from cross (B), as depicted above. (See Table 1 for definitions of symbols.)

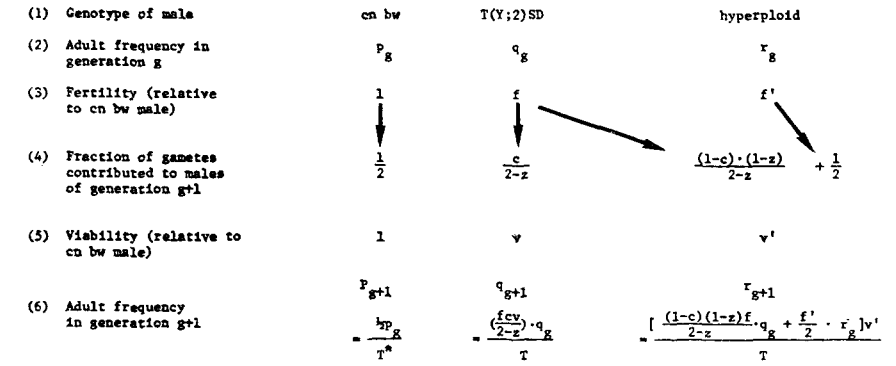

FIGURE 2.—One generation change in *T(Y;2),SD* male frequencies in competition cages. Note that the *T(Y;2),SD* males contribute to two males classes in the next generation, while the other male types contribute only to their own class. The values for the gamete fractions from the *T(Y;2),SD* males are determined as the frequency of that class among all gametes in Figure 1, divided by the total frequency of surviving gametes.

TABLE 2

Distribution of competition cages to the experimental groups

Experimental group	A	B	C	D
(1) *T(Y;2)SD* used	*T(Y;2),SD L²* (Fig. 6)	*T(Y;2),SD L²*-A (Fig. 7)	*T(Y;2),SD L²*-A (Fig. 7)	*T(Y;2),SD L²* (iso)
(2) Number of replicates	1	3	2	3
(3) $\ln\left(\frac{SD\,\delta}{SD+\,\delta}\right)$ monitored?	+	+	—	—
(4) a. Analyzed for changes in *K*?	+	+	+	+
b. Analyzed for presence of modifiers?	+*	+	—	—

Initial *k* value distributions can be obtained from the referenced figures .(+) indicates that the particular analysis was carried out on the experimental group in question; (—) that it was not.

* Not reported here.

and the presence or absence of initial background genetic variability in the cage population being tested. The isogenic cages, having less of this variability, might be expected in general to show a less rapid loss in *K* value, while variability in *K* should also be diminished. Group C acts as a control for Group D.

Each competition cage (LYTTLE 1977) was initiated with 200 males [*T(Y;2),SD* and *cn bw*] and 500 *cn bw* females, except for Group C, where cages were begun by adding small "inoculations" of 50 *T(Y;2),SD L²*-A males to *cn bw* cage populations that had been maintained for over a year in order to maximize genetic variability. The initial frequency of *T(Y;2),SD* males in the other cages was either 50% (for A and replicate B3), 30% (for D cages and replicate B2), or 10% (for replicate B1). The carrying capacity for each cage is about 1500 to 2000 flies. Change in *T(Y;2),SD* frequency in experiments A and B was obtained by total cage counts performed at approximately 25-day (two generation) intervals. In addition, aliquots of flies were collected from discard vials of the various cages at the times specified in Table 2 and used for *K* value or modifier component analyses. All cages were maintained in a walk-in incubator with diurnal light cycling, constant humidity, and a constant temperature (25 ± 0.5°).

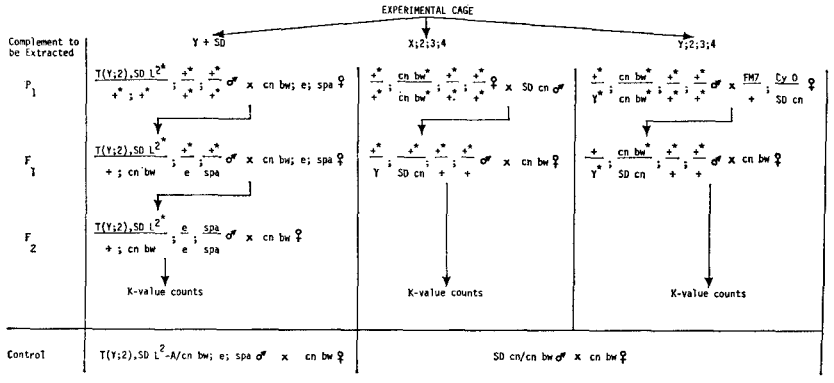

FIGURE 3.—Crossing scheme for extracting autosomal dominant, *X*-linked and *Y* + *SD* components of the *T(Y;2),SD* genome.

Isolation of modifier components: Figure 3 describes the crossing scheme modeled after one used by HARTL (1970c), designed to isolate drive modifiers in the $Y + SD$, X, and autosome components of the *D. melanogaster* genome. Note particularly that only dominant autosomal modifiers can be detected by this scheme, but this was not anticipated to cause problems since past studies (HARTL 1970c; HIRAIZUMI, SANDLER and CROW 1960; TRIPPA and LOVERRE 1975) had suggested these were the most important. In general, the k values were measured for each chromosome complement tested and then converted into "make" values (see next section) for further analysis. Loss or gain in "make" attributable to the *Y*;*SD* or *2*;*3*;*4* mean change in "make" from that measured for the *X*;*2*;*3*;*4* complement. This is legitimate because "make" values should be additive, even though K values are not.

Probit transformation of data: It has been argued in a series of papers (MIKLOS 1972a, b; MIKLOS and SMITH-WHITE 1971) that the variable of interest in studies of *SD* strength should not be the k value itself, but rather the fraction of $SD+$ sperm that are caused to dysfunction (defined as z in Table 1). Of course, z and k for a particular *SD* male are directly related (Figure 4b), but k is simply an observational transformation of the real biological event measured by z. In particular, k values are difficult to work with quantitatively, since they are not additive and the variance in k (V_k) is generally not independent of the mean K value of a line. To remedy this, MIKLOS (1972a, b) has suggested using the probit transformation of z (Table 1) as the proper metric for *SD* activity. The underlying biological variable involved in *SD* action is assumed to be a normally distributed "make" (or potency) which acts in the primary spermatocyte to determine whether a $SD+$ sperm will dysfunction or not; dysfunction being dependent on the surpassing of a certain threshold of "make" (see Figure 4a). The fraction of spermatocytes exceeding this threshold in a given male is related to k by the equation $z = (2k - 1)/k$. The mean "make" of an individual *SD* male can be equated to the extinction probability z by the probit transformation $z = \int_{\infty}^{M} N(0,1)dx$, where M is the difference between the mean "make" and the threshold value of make expressed in units of standard deviations, and $N(0,1)$ is the unit normal density function. To avoid negative values, $M = M + 5$ is used in calculations, and this is called the "probit" of z (FISHER and YATES 1963). A distribution of k's can therefore be transformed into a distribution of M's, and the variation in M is an accurate representation of the male-to-male genetic variability in an *SD* line. Figure 4b represents the relationship between k and mean "make" (in probits). We can also define the relationship between the variance in k and the variance of its transform, M, by using the asymptotic propagation of error equation:

$$V_k = V_M(dK/dM)^2. \quad (4)$$

Exact values for dK/dM are tabulated in MIKLOS and SMITH-WHITE (1971), but for our purposes

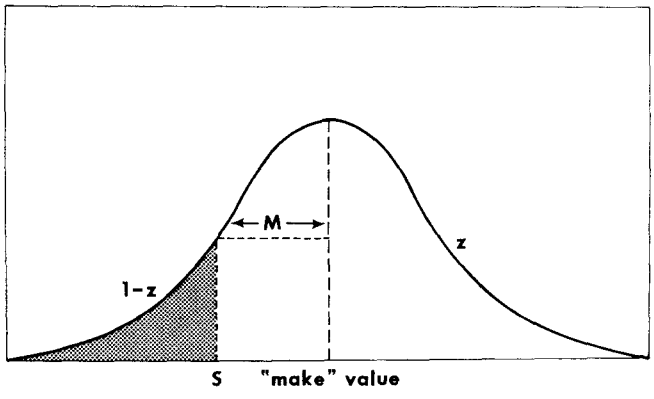

FIGURE 4a.—The relationship between "make" value and the probability of dysfunction (z) of $SD+$-bearing sperm.

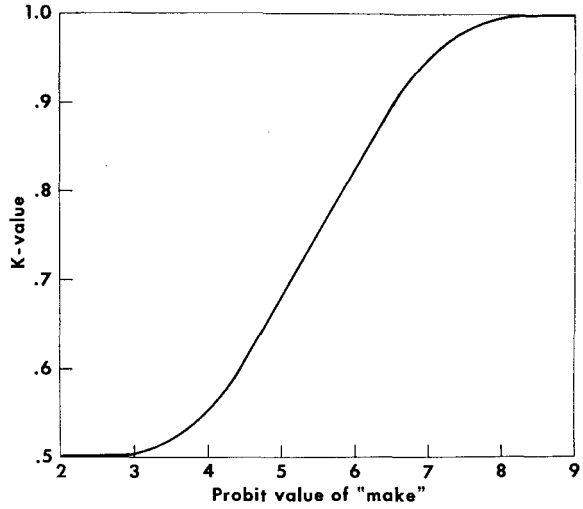

FIGURE 4b.—The relationship between the probit value of "make" (M or $\bar{M}$) and the fraction of SD progeny (k or K).

it is enough to note that dK/dM is maximal for intermediate values of K (around $K = 0.77$). Therefore, even when V_M is constant from line to line there will be an apparent increase in V_k as K decreases from 1.00 to 0.77, or increases from 0.50 to 0.77.

A quantitative modifier of drive is assumed to act by shifting the mean "make" value of a male by a fixed amount, thus changing z and ultimately, k. While such a modifier has a fixed effect on M, its effect on k will vary depending on the initial K value of the SD line being modified (Figure 4b). This illustrates why modifier analysis in terms of M values is considered more appropriate.

For the purposes of this investigation, individual k values were transformed to probits of "make," and M and V_M were calculated directly from the resulting distribution. In addition V_M was also calculated directly from V_k using equation (4) as a check on the method. When used, theoretical distributions of k values were generated from M and V_M in the following manner. The normal deviates of the class boundaries of k were generated in terms of M; that is, as $(M - \bar{M})/V_M$. Successive differences of such class boundaries were then calculated, and the relative area under the part of the normal curve included was taken as the corresponding height of the k histogram for the class interval in question. Theoretical histograms so generated can then be compared to the observed distribution of k values within a stock or line.

RESULTS

Experimental cage sets A and B: Table 3 and Figure 5 summarize the changes in K value and SD male frequency (see equations 2 and 3) for the pilot cage (A) and the replicated B cages, using the high-K $T(Y;2)$,SD L^2-A line derived from cage A. Only the three B cages are represented in Figure 5. The fact that the B cages gave similar rates of change for values of ln (q_g/p_g) per generation (see equation 3), taken with the results of LYTTLE (1977), which demonstrated that similar data from replicated competition cages could be legitimately pooled, were used as justification for combining the three values of ln (q_g/p_g) at each sample time. This method of representation serves to emphasize any changes in SD male

TABLE 3

Changes in K *and* M *values for experimental cages*

T(Y;2)SD stock or line	Day tested	Number of males tested	Reference figure for *k* distribution	*K*-value	*M*	V_M	Control *M*	$\Delta M(s_{\overline{\Delta M}})$
T(Y;2)SD L^2								
(control)	1	43	6	0.940	6.63	0.189	na	na
T(Y;2)SD L^2-A								
(control)	1	55	7	0.986	7.29	0.128	na	na
T(Y;2)SD L^2								
(iso) (control)	1	30		0.735	5.35	0.057	na	na
Experimental cages								
Pilot *T(Y;2)SD* L^2								
(Cage A)	255	24		0.812	5.83	0.614	6.63	—0.80 (0.17)
	1100	43		0.797	5.71	0.613	6.63	—0.92 (0.14)
T(Y;2)SD L^2-A cages								
B1	350	40		0.907	6.49	0.818	7.29	—0.80 (0.15)
B2	350	39		0.929	6.61	0.493	7.29	—0.68 (0.12)
B3	350	38		0.939	6.78	0.480	7.29	—0.51 (0.12)
B3	484	30		0.829	5.85	0.763	7.29	—1.44 (0.17)
T(Y;2)SD L^2-A								
short term cages								
C1	122	68		0.927	6.68	0.793	7.29	—0.61 (0.12)
C2	122	76	8	0.940	6.77	0.438	7.29	—0.52 (0.09)
T(Y;2)SD L^2 (iso) cages								
D1	157	66		0.687	4.97	0.640	5.35	—0.38 (0.11)
D2	157	38		0.704	5.10	0.544	5.35	—0.25 (0.13)
D3	157	47	9	0.635	4.65	0.462	5.35	—0.70 (0.11)

When pertinent, the distribution of *k* values for a particular sample is given by the referenced figure. The column headed "Day tested" refers to the age of the particular cage when the sample of tested males was removed.

frequency over time and makes values of the dependent variable in Figure 5 roughly three times as large as would be expected if the data from only one cage were used. The pilot cage (A) gave very similar results, but could not be legitimately pooled since it employed a different stock *T(Y;2),SD*, and the sampling was performed on different occasions. The asterisk in Figure 5 indicates the time at which the genome component analysis was performed on the B cages (see Table 4), and the arrows indicate times at which *k*-values were measured for *T(Y;2),SD* males sampled from these cages (Table 3).

It is clear from Figure 5 that, if the change in $\Sigma \ln (q_g/p_g)$ is used as a measure of $3 \cdot \ln(W)$, then *T(Y;2),SD* L^2-A males begin with a fitness advantage ($W > 1$), as measured by the upward slope of the graph through sample 11, but eventually come to have a fitness disadvantage ($W < 1$) relative to the *cn bw* males. Since $W = 2fcv/2\text{-}z$, it is logical to assume that most of the decrease in W is a direct result of a decrease in z, the probability of dysfunction of SD^+ sperm, brought about by the accumulation of suppressors of *SD* action. An internal

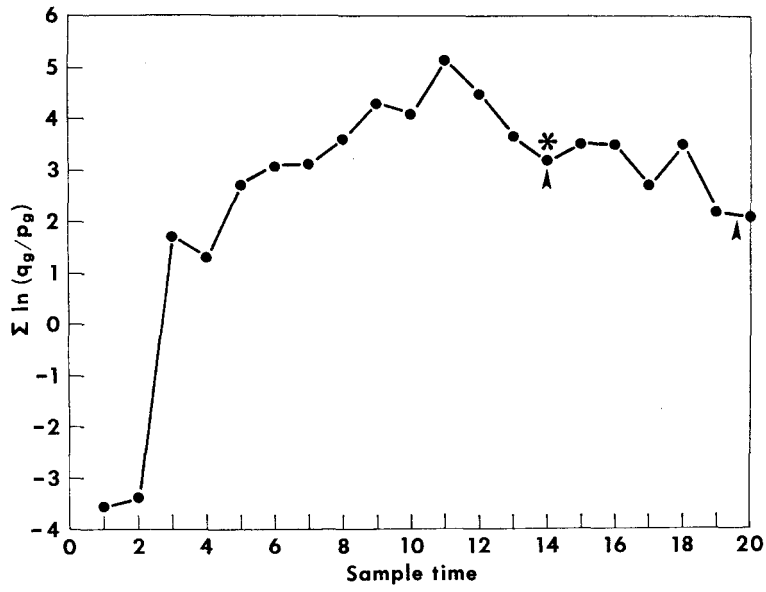

FIGURE 5.—Changes in $\Sigma \ln(q_g/p_g)$ with sample time for cages of experiment B. Values of the log transformed ratios of *SD* to *cn bw* males are pooled in order to emphasize the common trend exhibited by $\ln(q_g/p_g)$ measurements in all cages (see text for details).

check of this assumption can be derived from the k value measurements performed at sample time 14 (day 350). If the upward part of the slope in Figure 5 is used as a measure of initial *SD* fitness, then we obtain a fitness of $\hat{W} = 1.075$ from averaging the estimates derived from equation (3) over sample times 3 to 11. (The initial large jump in *SD* frequency between samples one and three was assumed to be due to the process of reaching population size and age equilibrium, and was consequently not included in the analysis.) Thus, *T(Y;2),SD* L^2-A males start with a small fitness advantage. From Table 1, and using $K = 0.986$ (Table 3) for these males, we calculate $z = 0.986$ and thus $2fcv = 1.060$. For W to be < 1 and *SD* frequency to decline (a downward turn for the curve of Figure 5), z must drop to < 0.940, or $K < 0.944$. The K-values for the B cages at day 350 (sample 14), after the downward turn has occurred, all satisfy this requirement of $K < 0.942$. Thus the drop in K value alone is sufficient to explain the downward shift in $\Sigma \ln(q_g/p_g)$ after sample 11.

Table 3 also gives the measured loss in *SD* activity in terms of "make" values for the A and B cages, which in all cases is significant. The pilot cage seems to have had a tendency to lose *SD* strength faster in the earlier stages and more slowly in later stages, compared to the B cages. (This retardation in "make" loss with time exhibited by the pilot cage will be discussed in a companion paper, in preparation.) Nevertheless, there has clearly been a considerable loss in *SD* potency through time in all these cages whether measured by K or $\overline{M}$ values.

Table 4 gives the results of genomic component analysis of loss in $\overline{M}$ value performed on flies collected at day 350 (sample 14) according to the protocol of

TABLE 4

Component analysis of modifier accumulation in B group cages at day 350

Stock or line		Number tested	*K*-value	$\overline{M}$	V_M	$\Delta\overline{M}(s_{\Delta\overline{M}})$
SD-cn (control)		36	0.914	6.38	0.406	na
T(Y;2)SD L^2-A/*cn bw; e; sp*^{pol} (control)		40	0.962	6.99	0.345	na
Experimental values						
T(Y;2)SD L^2 pilot *X;2;3;4* complement from day 1100 (cage A)		64	0.915	6.43	0.225	+0.05 (0.12) a
X;2;3;4 complements of *T(Y;2)SD* L^2-A cages—day 350	B1	51	0.885	6.21	0.280	—0.17 (0.13) b
	B3	51	0.895	6.30	0.305	—0.08 (0.13) c
2;3;4 complements of *T(Y;2)SD* L^2-A cages—day 350	B1	66	0.900	6.36	0.380	—0.02 (0.13) d
	B3	53	0.925	6.53	0.251	+0.15 (0.13) e
Y + SD complements of *T(Y;2)SD* L^2-A cages—day 350	B1	33	0.967	7.10	0.379	+0.11 (0.14) f
	B2	41	0.956	6.98	0.462	—0.01 (0.14) g
	B3	38	0.962	7.05	0.430	+0.06 (0.14) h

Contributions of various genomic components to total reduction in "make" at day 350

T(Y;2)SD L^2-*A*

cage B1—*X* contribution	$b - d$	$= -0.15$	Total of tested components	$= -0.07$
—*2;3;4* (dominant)	$1.6\ d$	$= -0.03$	(compared to total genome value	
—*Y + SD* contribution	f	$= +0.11$	of —0.80 in Table)	
cage B3—*X* contribution	$c - e$	$= -0.23$	Total of tested components	$= +0.07$
—*2;3;4* (dominant)	$1.6\ e$	$= +0.24$	(compared to total genome value	
—*Y + SD* contribution	h	$= +0.06$	of —0.51 in Table 3)	

Note that the total *2;3;4* (dominant contribution) is multiplied by 1.6 to take into account the presence of two third and two fourth chromosomes in the whole fly genome.

Figure 3. The lower part of the table gives the best component estimate for total genome change in $\overline{M}$ for cages B1 and B3. B2 was not fully analyzed for the various genomic components, and is omitted here. It is apparent that none of the three components (*X*-linked, *SD*-linked, or autosomal dominant) contribute enough to the suppression of *SD* action to explain the large losses in "make" value exhibited by the whole genomes assayed in Table 3. None of the estimated changes in $\overline{M}$ for Table 4 are statistically significant, but all but one of the six final values presented in the lower part of the table are at least in the predicted direction. That is, we expect unlinked suppressors to accumulate, and three of the four measured *X* or *2;3;4* (dominant) contributions are negative. Conversely, both the *Y + SD* contributions are positive, fulfilling the prediction that linked enhancers of drive will accumulate (PROUT, BUNDGAARD and BRYANT 1973; HARTL 1975a, b).

Moreover, examination of the distribution of k values obtained for each of the three components analyzed shows that there is no bimodality or other indication that would suggest segregation of any major modifier of *SD* is occurring. In all cases it would seem that any suppression or enhancement must be polygenic

in origin. We can therefore summarize the results of this section by noting that strong suppression of *SD* is clearly occurring, but the source of the suppression is apparently not the few dominantly acting loci that have been observed in other reported studies of populations segregating for *SD*.

Experimental cage sets C and D: Table 3 also summarizes the changes in K and $\overline{M}$ observed for the cages with initially isogenic (D) and variable (C) genetic backgrounds, as well as V_M values for all experimental populations. Examination of the V_M column of the table yields some interesting comparisons. First, as expected, the *T(Y;2),SD* L^2 isogenic control line has a drastically reduced V_M compared to the *T(Y;2),SD* L^2-A control line used in the C cages, indicating the low male-to-male genetic variability of the isogenic stock. Surprisingly, however, the variability in the isogenic cages increased rapidly, so that after only a short period of time (155 days, or about 12 generations), it was comparable to that observed in the nonisogenic cages. At the same time, average "make" had decreased by a surprising amount in the isogenic cages, although perhaps not by as much as in the nonisogenic controls. There is also apparently more variability in total "make" loss among the former than the latter. This may reflect the fact that different isogenic cages have qualitatively different types of genetic variability for *SD* suppression.

Overall, the four sets of cages tended to show an increase in V_M with time and a generally negative correlation of V_M and ΔM, suggesting that new genetic variation for suppression of *SD* activity was constantly arising and being selected in these populations. It is worth noting that the pilot cage showed an initial increase in V_M, but then no subsequent change over two years. Explanations for this anomalous behavior will be considered elsewhere (paper in preparation); it is sufficient to remark here that the stagnation in V_M parallels the similarly small amount of change in ΔM between the two sample times.

It seems, then, that isogenic populations are able to generate suppressor variability and select for suppressor substitution almost as rapidly as populations with extensive background genetic variability. This is contrary to our expectation of slow selective response for the isogenic cages, based on the assumption that suppression was due mostly to the action of a few loci with large effect, and that isogenic populations would thus require a long time to accumulate mutational variability at these loci. Clearly that is not the case here. The results of the C and D cages tend to support the hypothesis derived from analysis of the A and B cages in the previous section: most suppression of *SD* activity evolving in cage populations is the result of polygenic modifiers of small effect.

The tabled changes in M and V_M can also be represented by k value distributions so as to more easily visualize the dynamic changes in *SD* action occurring. Figures 6 to 9 (constructed according to the methods described earlier) give such distributions for *T(Y;2),SD* L^2 (control), *T(Y;2),SD* L^2-A (control), C1 and D3, respectively. The figures illustrate several points. First, they demonstrate the power of the probit transformation method for constructing theoretical k value distributions from knowing only M and V_M values. However, there tends to be an element of circularity in this process, since the M and V_M values used

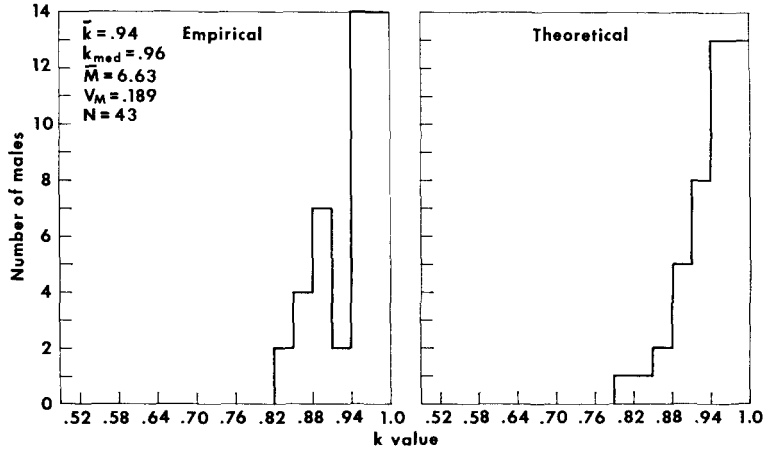

FIGURE 6.—Distribution of k values obtained from $T(Y;2),SD\ L^2$ (control) males.

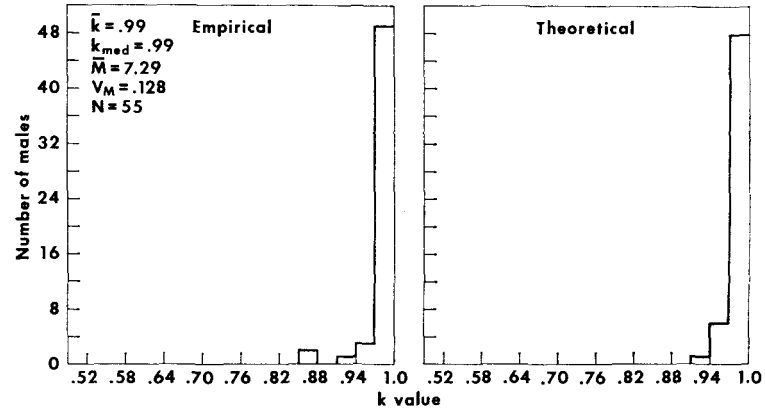

FIGURE 7.—Distribution of k values obtained from $T(Y;2),SD^2$-A (control) males. This line was derived from cage A, and is presumably the result of the accumulation of *SD* enhancers on its *SD* chromosome. (Compare to Figure 6)

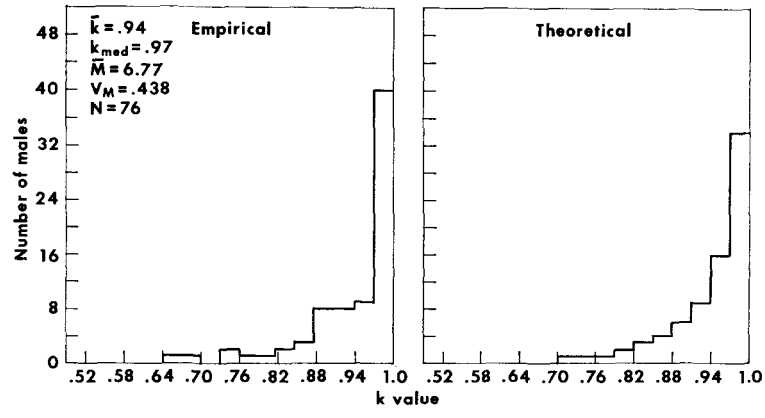

FIGURE 8.—Distribution of k values obtained from cage C2 (day 122). Compare to Figure 7 for control distribution.

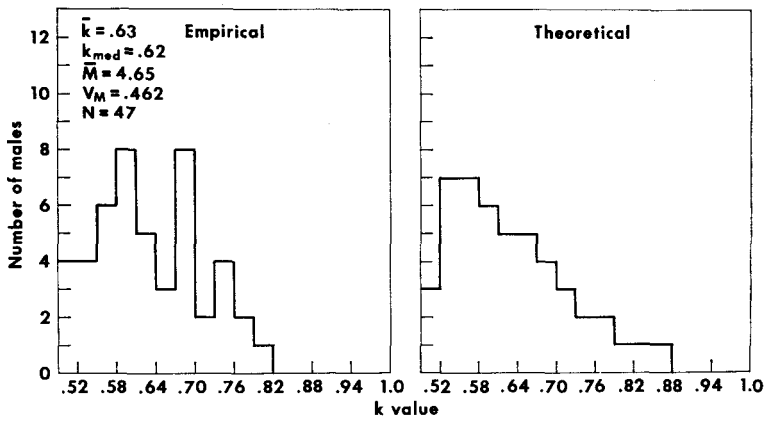

FIGURE 9.—Distribution of k values obtained from cage D3 (day 157). See Table 3 for the pertinent values for *T(Y;2),SD L²* (iso) (control) for comparison.

to construct a theoretical histogram must be estimated from the same empirical distribution we wish to test against the theoretical histogram. It should be remembered that when we compare such histograms, we are really only testing the validity of the assumption that M values are normally distributed.

The shift in k values resulting from supressor accumulation is exemplified by comparing the figures for the C2 cage distribution and its respective stock *SD* control (Figures 7 and 8). Other cross comparisons also provide information on modifier accumulation. Figure 7 shows the distribution for the high-K *SD* line (*T(Y;2),SD L²*-A) derived from the pilot cage employing the standard *T(Y;2), SD L²* of Figure 6. These two lines differ genetically only in their *Y* and *SD* chromosomes, indicating that at least one chromosome line evolving in the pilot cage was able to fix linked enhancers of *SD* action, as predicted by theory. Figures 6 and 8 exhibit a case where *SD* lines with similar K values (0.94) can have very different V_M values (0.189 and 0.428, respectively). Conversely, Figures 8 and 9 show a case where two lines have similar V_M values (0.438 and 0.462), but quite different K values (0.94 and 0.63). This also emphasizes how misleading analysis of k values alone can be, since from the k histograms we might erroneously conclude that the D3 cage shows a great deal more genetic variability for *SD* action than does the C2 cage, when in fact their V_M's are quite similar. This error arises from the fact that dK/dM in equation (4) is dependent on K, as discussed above (see also MIKLOS 1972a, b; and MIKLOS and SMITH-WHITE 1971).

DISCUSSION

HARTL 1975b; 1977) summarized a series of studies of both laboratory and natural populations that have been assayed for either the presence of *SD* or suppressors of distortion, or both. He suggests that the generally high frequency of suppressors can be attributed primarily to the production of *Sd⁺ Rsp* chromosomes through recombination in *Sd⁺ Rsp⁺/Sd Rsp* females. Once such recombinants have arisen, even at low frequency, the dynamics of drive modification

discussed above ensure their rapid increase in frequency (PROUT, BUNDGAARD and BRYANT 1973; HARTL 1975a; 1977). The *X*-linked suppressor also is found in high frequency in some natural populations, but is not present (or has not been analyzed) in others (KATAOKA 1967; HARTL 1970c).

Most analyses have been concerned only with measuring the frequencies of such major suppressors; indeed, the present study was designed in anticipation of monitoring primarily just such single-locus dominant suppressors. The fact that none of these major suppressors was a source of the observed reduction in *SD* strength initially came as something of a surprise. However, both natural and laboratory populations that are conventionally surveyed for *SD* modifiers are different in fundamental ways from the cage populations examined here. First, the latter populations are known to be free of any pre-existing major suppressors of drive. Moreover, no new *Sd*$^+$ *Rsp* "suppressor" chromosomes can arise through recombination, owing to the fact that the *Y*;*SD* translocation effectively prevents the *SD* chromosome from appearing in females (an exception to this rule is discussed below). Previous studies of laboratory populations involving standard *SD* chromosomes showed rapid accumulation of dominant second chromosome suppressors (frequency 20% after about 300 days for HIRAIZUMI, SANDLER and CROW 1960, and 30% after 92 days for WATANABE 1967). HARTL's (1975b; 1977) analyses of similar suppressors in a laboratory population maintained since 1967 by HIRAIZUMI indicates that these suppressors are almost certainly recombinationally derived *Sd*$^+$ *Rsp* chromosomes. Such recombinants, though of rare occurrence, would enjoy a tremendous selective advantage and could easily increase in frequency to the observed levels after the short times recorded. Natural selection apparently took advantage of a reservoir of genetic variability in those populations which was unavailable in the experimental cages of the present study.

On the other hand, natural populations have available not only the recombinationally produced *Sd*$^+$ *Rsp* suppressors, but the strong *X*- and chromosome 3-linked suppressors described in the introduction. It is perhaps not surprising that these elements would be missing from laboratory populations such as the ones described here, where only one to three years (30–90 generations) have available for the *de novo* production by mutation and subsequent selection of such single-locus suppressors.

In short, populations exhibiting segregation of major dominant suppressors of *SD* either have the genetic variability for the suppressors present initially, or are presumed to have had enough evolutionary time to produce them *de novo*. Laboratory populations that are not so fortunate (such as the ones described here) must rely on whatever polygenic suppressor activity is already segregating or can be generated quickly by mutation.

Table 3 indicates that considerable amounts of background genetic variability for suppression may already exist in laboratory strains (as measured by the V_M values of the control cases). In addition, the rapidity with which the isogenic populations were able to catch up with their controls in terms of genetic variability and suppressor selection is an indication that mutation is quickly able to supply

the necessary variability. All this would seem to argue for the action of a large number of modifier loci, each of small effect. The results of Table 4, as discussed above, also argue that these modifiers must be primarily recessive. This in turn implies that they are located primarily on chromosomes *3* and *4*, since chomosome *2* recessives would never be selected (owing to the permanent heterozygosity for that chromosome in *SD* males), while *X*-linked modifiers always act hemizygously and would consequently have been detected in the genomic partition of Table 4. Studies are currently underway to test cage populations specifically for the presence of these postulated chromosome *3* suppressors.

Evidence for the weak action of individual suppressors also comes from *k*-value distributions, such as those of Figures 6 to 9, which show a smooth gradation towards the low-*k* end of the scale rather than the bi- or multi-modality expected were major suppressors segregating (see HARTL 1970c for examples of the differences). It might also be worthwhile to mention here that it would be very difficult to detect the sort of minor polygenic effects dealt with in this study if major suppressors were also segregating. Consequently, this makes it difficult to design procedures for analyzing such modifiers in natural populations.

The theoretical predictions of HARTL (1975a) and PROUT, BUNDGAARD and BRYANT (1973) discussed above were roughly supported, or at least not called into question, by the component analyses of Table 4. While not statistically significant, there was a marked tendency for chromosome complements that were unlinked (or in repulsion) to *SD* to fix suppressors, while the *Y* and *SD* chromosomes (completely linked by the translocation) seemed to accumulate drive enhancers. Such enhancers clearly can arise, as evidenced by the *T(Y;2)*, *SD* L^2-A line, where the increased *k*-value over the original *T(Y;2),SD* L^2 stock can only come from changes in the *Y;SD* genetic component.

Our prediction about the rate of modifier accumulation is that it should decrease with time, either for the trivial reason that genetic variability for modifiers is exhausted or simply because the selection in favor of drive suppressors presumably weakens as *k*-value drops. The present data is not extensive or homogeneous enough to test this prediction, but separate investigations on this topic are currently being pursued.

A problem with *T(Y;2),SD* lines, which has been mentioned before now, is the "leakage" of *Y;SD* complexes into females caused by primary nondisjunction of the sex chromosomes in *SD* males. (This is the exception to the rule mentioned earlier.) In general, translocation males might be expected to show a high frequency of such nondisjunction, but in these studies the rate was <0.01%. Most cages showed few, if any, of the *XXY* females, which are the expected result. In only one case (the long-term pilot cage) was there a problem with recombinational breakdown from such females contaminating the results. However, this apparent disaster turned out to be serendipitous, since subsequent analysis of the process of breakdown led to some interesting observations on the mechanisms employed by natural selection to return populations to 1:1 sex ratios. The analysis of this special case will be reported in a companion paper now in preparation.

LYTTLE (1977) speculated on the possibility of employing the type of pseudo *Y* drive described here as a technique for eliminating or at least decreasing the fitness of target populations. The present investigations offer both good news and bad news bearing on that speculation. The bad news is that even when major dominant suppressors are eliminated from consideration, we can still expect populations to have or be able to generate enough polygenic suppressor variability to reduce the strength of drive. The good news is that, if the drive is intense enough to absorb such a relatively minor polygenic suppression effect, it may still be able to ensure fixation of the drive element. This follows as a consequence of the apparently long time required for the *de novo* generation of the dominant major suppressors that natural populations normally rely on to inactivate and eliminate deleterious drive systems.

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