ESTIMATION OF FITNESS COMPONENTS IN *DROSOPHILA MELANOGASTER*. I. HETEROZYGOTE VIABILITY INDICES

ALAN J. KATZ AND RICARDO A. CARDELLINO[1]

Department of Biological Sciences, Illinois State University, Normal, Illinois 61761

AND

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108

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ABSTRACT

We examine the assumption of "dominance" with regard to viability of the *Cy* and *Pm* marker chromosomes in *D. melanogaster*. This assumption is often invoked for the extraction of wild-type second chromosomes from natural populations and for the calculation of relative viability indices. Significant genotypic variances for viability are found among both $Cy/+_j$ and $Pm/+_i$ heterozygotes in California and Japanese populations. The magnitude of the $Pm/+_i$ genotypic variance is substantially less than that of the $Cy/+_j$ heterozygotes (less than one half). Significant reciprocal effects are also found to influence $Cy/+_j$, $Pm/+_i$ and $+_i/+_j$ viabilities. We conclude that viability indices of heterozygotes based on the Curly method are biased. We suggest that viability indices in the future be expressed relative to the viability of the *Cy/Pm* genotype (Curly-Plum method) or possibly that of the $Pm/+_i$ genotype (Plum method).

DURING the past 10–15 years much of the experimental research on natural populations of Drosophila has been of two sorts: (1) electrophoretic studies of single locus enzyme polymorphisms (LEWONTIN and HUBBY 1966 and others) and (2) studies of "whole" chromosome phenomena such as segregation distortion (HARTL and HIRAIZUMI 1976), mutator factors (KIDWELL, KIDWELL and NEI 1973) and viability effects (MUKAI 1964). This paper is concerned with the latter type of research and examines a critical assumption frequently encountered in studies of this kind.

A common procedure in studying whole chromosome phenomena is to extract wild-type chromosomes from a natural population. For example, to examine second chromosomes, one generally crosses newly collected individuals to a balanced lethal inversion stock such as *Cy/Pm* (Curly-Plum). *Cy/+* (or *Pm/+*) males from the F_1 are then backcrossed to *Cy/Pm* females. After repeated backcrossing, one has effectively extracted a randomly chosen wild-type second chromosome and placed it in a homogeneous genetic background of *Cy/Pm* origin.

[1] Present address: EMBRAPA, Beef Cattle Research Centre, Campo Grande, Mato Grosso, Brazil.

The technique of repeated backcrossing to *Cy/Pm* females has been employed by many researchers to accumulate spontaneous mutations affecting such fitness components as viability and developmental time on the wild-type second chromosome (CARDELLINO and MUKAI 1975; KIDWELL, KIDWELL and NEI 1973; MUKAI 1964, 1969; MUKAI *et al.* 1972; MUKAI, CHIGUSA and YOSHIKAWA 1964, 1965; MUKAI and YAMAZAKI 1968; YAMAGUCHI, CARDELLINO and MUKAI 1976; YOSHIKAWA and MUKAI 1970). Present in all these types of investigations is the basic assumption that nearly all mutations that occur on the extracted second chromosome will be protected from the scrutiny of natural selection through the supposed "dominance" of the *Cy* (and *Pm*) chromosomes *regardless of how deleterious the mutations might be in heterozygous combination with any other wild-type chromosome.*

Similarly, the experimental technique commonly employed to calculate indices of viability and developmental time for heterozygous chromosome combinations, known as the Curly method (*cf.*, WALLACE 1956; MUKAI 1964), assumes dominance of the *Cy* chromosome. In this method of measuring relative viability, crosses of the form $Cy/+_i \times Cy/+_j$ are performed. Mean viability of the $+_i/+_j$ progeny is calculated as the ratio of wild-type progeny to the sum of $Cy/+_i$ and $Cy/+_j$ flies. Since the standard genotypes appearing in the denominator are of $Cy/+$ constitution, the assumption of equal viability of these individuals becomes a critical one.

The Curly method is derived from the considerably more laborious Curly-Plum (or Curly-Lobe) method of WALLACE (1956). With this method the crosses are of the form $Cy/+_i \times Pm/+_j$. Mean viability indices of the $+_i/+_j$ progeny are calculated as the ratio of wild-type progeny to *Cy/Pm* individuals. No assumptions of dominance of *Cy* or *Pm* chromosomes are necessary. After WALLACE and DOBZHANSKY (1962) had shown the regression coefficient of $Cy/+_i$ viability on $+_i/+_i$ viability to be zero, it became generally accepted that, on the average, *Cy* and *Pm* chromosomes suppress the effects of deleterious genes on the homologous chromosomes. Subsequently, the simpler Curly method has become widely used, although not yet to the point of total exclusion of the Curly-Plum method (*e.g.*, WATANABE and YAMAZAKI 1976).

This paper reports the findings of an analysis of viability for different $Cy/+_j$, $Pm/+_i$ and $+_i/+_j$ heterozygotes, with the prime objective of examining the validity of the assumption of dominance for *Cy* and *Pm* chromosomes.

MATERIALS AND METHODS

Theory

(A) *Curly Method*: The Curly method of viability estimation involves crosses of the type $Cy/+_i \times Cy/+_j$. The genotypes, numbers and viabilities of the progeny are designated as follows:

Genotype:	Cy/Cy	$Cy/+_j$	$Cy/+_i$	$+_i/+_j$
Expected no. zygotes:	$\frac{1}{4} N_z$	$\frac{1}{4} N_z$	$\frac{1}{4} N_z$	$\frac{1}{4} N_z$
Viability:	0 (lethal)	V_{cj}	V_{ci}	V_{ij}
Observed no. adults:	0	N_{cj}	N_{ci}	N_{ij}
Expected no. adults	0	$\frac{1}{3} N_z V_{cj}$	$\frac{1}{3} N_z V_{ci}$	$\frac{1}{3} N_z V_{ij}$

Only two phenotypic classes can be distinguished among the viable progeny, of course, (1) *Cy* and (2) wild type. Assuming that chromosomes act in a multiplicative manner with regard to viability (*e.g.*, MUKAI *et al.* 1974), one may write the above viabilities in terms of an exponential model:

$$V_{cj} = \exp(-S_c - S_j - d_{cj})$$
$$V_{ci} = \exp(-S_c - S_i - d_{ci}), \text{ and}$$
$$V_{ij} = \exp(-S_i - S_j - d_{ij})$$

where the S's represent the "additive" effects of the second chromosomes and the d's denote the interaction or "dominance" effects for pairs of chromosomes. The expected number of adults for each viable genotype is a function of the number of fertilized eggs (N_z) and is dependent upon the fertility of the parents; therefore, one cannot make a direct comparison between the viability of $+_i/+_j$ and that of $+_k/+_l$ (from another cross). Instead one usually calculates the viability index (v_{ij}) of the $+_i/+_j$ genotype relative to those of the $Cy/+_i$ and $Cy/+_j$ classes as follows:

$$v_{ij} = \ln\left\{\frac{\text{no. wild-type progeny}}{\text{no. } Cy \text{ progeny}}\right\} = \ln\left\{\frac{N_{ij}}{N_{ci} + N_{cj}}\right\}.$$

It is easily shown that the above index v_{ij} estimates the following quantity:

$$E(v_{ij}) = -S_i - S_j - d_{ij} - \ln\{\exp(-S_c - S_i - d_{ci}) + \exp(-S_c - S_j - d_{cj})\}.$$

Once obtained, the viability indices are commonly analyzed by analysis of variance to estimate the genotypic variance for viability. For the above viability indices to be valid estimators, one should expect that the difference $(v_{ij} - v_{kl})$ actually measure $(S_k + S_l + d_{kl} - S_i - S_j - d_{ij})$. However, one finds that

$$\begin{aligned} E(v_{ij} - v_{kl}) &= (S_k + S_l + d_{kl} - S_i - S_j - d_{ij}) + \ln\{\exp(-S_c - S_k - d_{ck}) + \exp(-S_c - S_l - d_{cl})\} \\ &\quad - \ln\{\exp(-S_c - S_i - d_{ci}) + \exp(-S_c - S_j - d_{cj})\} \\ &= (S_k + S_l + d_{kl} - S_i - S_j - d_{ij}) + \ln\left\{\frac{\exp(-S_k - d_{ck}) + \exp(-S_l - d_{cl})}{\exp(-S_i - d_{ci}) + \exp(-S_j - d_{cj})}\right\}. \end{aligned}$$

Therefore the viability indices obtained under the Curly method are unbiased estimators only if one assumes that the viabilities of $Cy/+$ heterozygotes are constant, irrespective of the genetic constitutions of the wild-type chromosomes; *i.e.*, one must assume that $(S_k + d_{ck}) = (S_l + d_{cl}) = (S_i + d_{ci}) = (S_j + d_{cj})$. Biologically, this would require that the *Cy* chromosome contain a completely dominant allele at each second chromosome locus influencing viability.

(B) *Curly-Plum Method*: With the Curly-Plum method of viability estimation, one initiates crosses of the type $Cy/+_i \times Pm/+_j$. The genotypes, numbers and viabilities of the progeny are as follows:

Genotype:	Cy/Pm	$Cy/+_j$	$Pm/+_i$	$+_i/+_j$
Expected no. zygotes:	$\frac{1}{4} N_z$	$\frac{1}{4} N_z$	$\frac{1}{4} N_z$	$\frac{1}{4} N_z$
Viability:	V_{cp}	V_{cj}	V_{pi}	V_{ij}
Observed no. adults:	N_{cp}	N_{cj}	N_{pi}	N_{ij}
Expected no. adults:	$\frac{1}{4} N_z V_{cp}$	$\frac{1}{4} N_z V_{cj}$	$\frac{1}{4} N_z V_{pi}$	$\frac{1}{4} N_z V_{ij}$

where (again employing an exponential viability model),

$$V_{cp} = \exp(-S_c - S_p - d_{cp})$$
$$V_{cj} = \exp(-S_c - S_j - d_{cj})$$
$$V_{pi} = \exp(-S_p - S_i - d_{pi}), \text{ and}$$
$$V_{ij} = \exp(-S_i - S_j - d_{ij}).$$

The viability index of the $+_i/+_j$ genotype is defined as v_{ij} where

$$v_{ij} = \ln\left\{\frac{\text{no. wild-type progeny}}{\text{no. } Cy/Pm \text{ progeny}}\right\} = \ln\left(\frac{N_{ij}}{N_{cp}}\right)$$
$$\text{and } E(v_{ij}) = (S_c + S_p + d_{cp} - S_i - S_j - d_{ij}).$$

It follows that $(v_{ij} - v_{kl})$ measures $(S_k + S_l + d_{kl} - S_i - S_j - d_{ij})$. Thus the above viability indices are unbiased and do not require the additional assumption that v_{cj} $(j = 1, 2, \ldots, n)$ equals a constant.

Using the Curly-Plum method, one may also calculate the viability indices of the $Cy/+_j$ (or $Pm/+_i$) genotypes by v_{cj} such that

(i) $v_{cj} = \ln\left(\frac{N_{cj}}{N_{cp}}\right)$

(ii) $E(v_{cj}) = (S_p + d_{cp} - S_j - d_{cj})$, and

(iii) $E(v_{cj} - v_{ck}) = (S_k + d_{ck} - S_j - d_{cj})$.

One can thereby test the validity of the assumption implicit in the Curly method of viability estimation, namely that $(S_j + d_{cj}) = (S_k + d_{ck}) =$ a constant.

Stocks: 32 wild-type second chromosomes were extracted from a natural population of *D. melanogaster* in California by the *Cy/Pm* technique described above. Similarly, 26 second chromosomes were extracted from a Japanese population. Eight generations of backcrossing *Cy/+* males to *Cy/Pm* females transpired during the extraction process for the California population, and nine generations of backcrossing occurred for the Japanese population. All stocks in this study were reared at 25° and 40% relative humidity.

The *Cy/Pm* stock used in this study is from North Carolina State University and is formally designated C-160. Details of its synthesis can be found in MUKAI (1964). Briefly, C-160 is an isogenic stock except for the two marker second chromosomes. Both the *Cy* and *Pm* chromosomes have multiple inversions and act as effective crossover suppressors (LINDSLEY and GRELL 1968).

Experimental crosses: After the extraction procedure was completed, each line was again crossed to *Cy/Pm* and both *Cy/+* and *Pm/+* males and virgin females were collected. Reciprocal crosses were then performed among lines *within populations* in a circulant design as follows:

$$\begin{array}{ccccc} & (\varphi\varphi) & (\sigma\sigma) & (\varphi\varphi) & (\sigma\sigma) & \\ \ldots & Cy/+_{i-1} & \times\ Pm/+_i & Cy/+_i & \times\ Pm/+_{i+1} & \ldots \\ & & \text{and} & & \text{and} & \\ & Pm/+_i & \times\ Cy/+_{i-1} & Pm/+_{i+1} & \times\ Cy/+_i & \end{array}$$

Five pairs of flies were employed in each cross, and each reciprocal cross was replicated twice. After 5 days, the parents within each vial were placed in a fresh transfer vial. After 5 days in the transfer vials, the parents were discarded. Counts of progeny were made on the 11th, 13th, 15th, 17th and 19th day after the original matings (or transfers) were made. The total numbers of flies counted in each phenotypic class were summed over both the original and the transfer mating vials and regarded as one observation.

Estimation of viability: Among the progeny of each cross, as outlined above, we expect the following four genotypic classes:

$$Cy/+_i \times Pm/+_j$$
$$\downarrow$$
$$Cy/Pm : Cy/+_j : Pm/+_i : +_i/+_j$$

We calculated the viability indices of each of the three classes involving a wild-type chromosome as follows:

$$v_{cj} = \ln\left\{\frac{\text{no. } Cy/+_j \text{ progeny}}{\text{no. } Cy/Pm \text{ progeny} + 1}\right\}$$

$$v_{pi} = \ln\left\{\frac{\text{no. } Pm/+_i \text{ progeny}}{\text{no. } Cy/Pm \text{ progeny} + 1}\right\}$$

$$v_{ij} = \ln\left\{\frac{\text{no. } +_i/+_j \text{ progeny}}{\text{no. } Cy/Pm \text{ progeny} + 1}\right\}$$

where the 1 in the denominator is HALDANE's (1956) correction factor.

Statistical analyses: Analyses of the $Cy/+_j$ viabilities were first performed on an intrapopulational basis. The following linear model was assumed to describe each observation:

$$v_{jkl} = \mu + g_j + r_{jk} + e_{jkl}$$

where μ = overall mean viability $= S_p + d_{cp}$

g_j = effect of the jth (j=1,2, . . . ,n) genotype $= -(S_j + d_{cj})$

r_{jk} = effect of the kth (k=1,2) reciprocal ($r_{j1} = -r_{j2}$), and

e_{jkl} = experimental error of the lth (l=1,2) replication.

The effects g, r and e are assumed to be random variables normally distributed with means equal to zero and variances equal to σ^2_g, σ^2_r, and σ^2_e respectively. The genotypic variance is comprised of the variance of the S_j's and that of the d_{cj}'s; *i.e.*, $\sigma^2_g = \sigma^2_S + \sigma^2_{d_c}$.

The expected mean squares of the analyses of variance are as follows:

MS	E(MS)
Genotype	$\sigma^2_e + 4\sigma^2_g$
Reciprocal	$\sigma^2_e + 2\sigma^2_r$
Error	σ^2_e

Experimental error variances were compared between populations and, if homogeneous, the data were then pooled and analyzed on an interpopulational basis. Analyses of the $Pm/+_i$ viabilities were performed in a manner analogous to that described for $Cy/+_j$.

Generally the $+_i/+_j$ viabilities obtained from circulant mating designs can be analyzed by methods appropriate for a partial diallel cross (KEMPTHORNE and CURNOW 1961; CURNOW 1963). Such methods enable one to estimate both σ^2_S and σ^2_d. Unfortunately the specific circulant design employed in this study results in singular least-square equations and cannot be analyzed as a partial diallel cross. Consequently, the $+_i/+_j$ viabilities were analyzed in a manner analogous to that described above for $Cy/+_j$ (and $Pm/+_i$). In regard to the linear model we now have,

(i) $\mu = S_c + S_p + d_{cp}$;
(ii) $g_j = -(S_i + S_j + d_{ij})$ where $i = j-1$; and
(iii) $\sigma^2_g = 2\sigma^2_S + \sigma^2_d$.

Covariances were calculated between viability of the $+_i/+_j$ heterozygotes (v_+) and the average viability of the corresponding $Cy/+_i$ and $Cy/+_j$ genotypes (v_c). The expected covariance may be calculated as follows:

$$\begin{aligned}\text{Cov}\{v_c, v_+\} &= \text{Cov}\{\tfrac{1}{2}(v_{ci}+v_{cj}),\ v_{ij}\} \\ &= \text{Cov}\{S_p + d_{cp} - \tfrac{1}{2}(S_i + d_{ci} + S_j + d_{cj}),\ S_c + S_p + d_{cp} - S_i - S_j - d_{ij}\}.\end{aligned}$$

Assuming that S_c, S_p and d_{cp} are constants, one finds that

$$\begin{aligned}\text{Cov}\{v_c, v_+\} &= \tfrac{1}{2}\text{Cov}\{S_i, S_i\} + \tfrac{1}{2}\text{Cov}\{S_j, S_j\} \\ &= \sigma^2_S.\end{aligned}$$

Regression coefficients $b_{v_c \cdot v_+}$ were also estimated within populations and later pooled. The $Pm/+_i$ viability indices were analyzed in a similar manner.

RESULTS

A summary of the analyses of variance of $Cy/+_j$, $Pm/+_i$ and $+_i/+_j$ viabilities, performed on an intrapopulation basis, is presented in Table 1. Reciprocal effects are significant in both populations. Hence we have not pooled the reciprocal and error sums of squares to obtain larger degrees of freedom as is commonly done. Nevertheless, the genotypic effects for all viabilities are significant in both populations.

Since tests of homogeneity of error variances of the two populations result in nonsignificant differences ($F_{64,52}$ of 1.12, 1.51 and 1.27 for $Cy/+_j$, $Pm/+_i$ and $+_i/+_j$ viabilities, respectively), the data from both populations are pooled. A summary of the combined data is presented in Table 2. Again the genotypic and reciprocal effects are highly significant for each of the three viabilities. Estimates of the genotypic variance components are presented in Table 3 for the California, Japanese and pooled data.

Estimates of regression coefficients of the average viabilities of Cy and Pm heterozygotes on the viabilities of their corresponding wild-type heterozygotes

TABLE 1

Intrapopulation analyses of variance of Cy/+$_j$, Pm/+$_i$ *and* +$_i$/+$_j$ *viabilities*

Population	Source of variation	DF	*Cy*/+$_j$ MS	*Cy*/+$_j$ F	*Pm*/+$_i$ MS	*Pm*/+$_i$ F	+$_i$/+$_j$ MS	+$_i$/+$_j$ F
California	Genotype	31	0.0941	3.79***	0.0680	1.75*	0.1731	5.08***
	Reciprocal	32	0.0910	3.67***	0.0910	2.35**	0.0667	1.96*
	Error	64	0.0248		0.0388		0.0341	
Japanese	Genotype	25	0.0957	4.31***	0.0553	2.15**	0.1173	4.38***
	Reciprocal	26	0.0564	2.54***	0.0375	1.46	0.0604	2.25**
	Error	52	0.0222		0.0257		0.0268	

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

TABLE 2

Interpopulation (pooled) analyses of variance of Cy/+$_j$, Pm/+$_i$ *and* +$_i$/+$_j$ *viabilities*

Source of variation	DF	*Cy*/+$_j$ MS	*Cy*/+$_j$ F	*Pm*/+$_i$ MS	*Pm*/+$_i$ F	+$_i$/+$_j$ MS	+$_i$/+$_j$ F
Population	1	0.0024	0.03	0.1963	3.07	0.1094	0.74
Genotype	56	0.0948	4.02***	0.0623	1.89**	0.1482	4.81***
Reciprocal	58	0.0755	3.20***	0.0670	2.04***	0.0639	2.07***
Error	116	0.0236		0.0329		0.0308	

** $P \leq 0.01$.
*** $P \leq 0.001$.

TABLE 3

Estimated genotypic variance component of Cy/+$_j$, Pm/+$_i$ *and* +$_i$/+$_j$ *viabilities*

Population	Genotypic variance component ($\hat{\sigma}_g^2$) *Cy*/+$_j$	*Pm*/+$_i$	+$_i$/+$_j$
California	0.0173 ± 0.0061	0.0073 ± 0.0047	0.0348 ± 0.0111
Japanese	0.0184 ± 0.0068	0.0074 ± 0.0041	0.0226 ± 0.0084
Pooled	0.0178 ± 0.0045	0.0074 ± 0.0031	0.0294 ± 0.0071

TABLE 4

Linear regressions of average viabilities of Cy (*and* Pm) *heterozygotes on their corresponding wild-type heterozygotes (see text for explanation)*

Population	Heterozygous combinations with *Cy* b†	Cov	r^2	Heterozygous combinations with *Pm* b†	Cov	r^2
California	0.49 ± 0.07	0.021	0.63	0.23 ± 0.07	0.010	0.26
Japanese	0.45 ± 0.11	0.014	0.39	0.40 ± 0.08	0.012	0.51
Pooled	0.48 ± 0.06	——	——	0.30 ± 0.05	——	——

† All regression coefficients are significant at $P \leq 0.005$.

are shown in Table 4. Regressions are positive and significantly different from zero within each population. Since no heterogeneity was found between populations, pooled regression coefficients were calculated and are presented.

DISCUSSION

The results presented above (Tables 1, 2 and 3) show that there exists significant genotypic variance for viability among $Cy/+_j$ individuals. Its magnitude is appreciable, being 61% of the genotypic variance observed among wild-type $+_i/+_j$ heterozygotes. The results agree quite well for both populations studied (California and Japan), which were chosen to represent two widely different sources of second chromosomes.

The evidence upon which researchers have inferred the dominance of the *Cy* chromosome is Figure 1 of WALLACE and DOBZHANSKY (1962) in which the viability of $Cy/+_i$ heterozygotes is regressed on the viability of $+_i/+_i$ homozygotes. A nonsignificant regression was found, and it was inferred that there was no genotypic variance in viability among $Cy/+_i$ heterozygotes. In the present study, a significant positive regression is found of the average viability of the two *Cy* heterozygotes ($Cy/+_i$ and $Cy/+_j$) on their corresponding $+_i/+_j$ wild-type heterozygote. The results are strikingly similar in both populations (Table 4). As pointed out by WALLACE and DOBZHANSKY (1962), the statistics presented in Table 4 may be biased upwards due to an intrinsic correlation between the wild-type heterozygote viabilities and those of *Cy* heterozygotes that come from the *same* replicate. However, we are confident that this possible bias cannot fully account for those positive regressions found since the covariances are expected to be positive provided there is *any* "additive" genetic variance (σ_S^2). Indeed the regression of $Cy/+_i$ heterozygotes on $+_i/+_j$ individuals that originate in different crosses, and therefore *different* vials, was estimated as $b = 0.32 \pm 0.10$ ($P \leq 0.005$).

CARDELLINO and MUKAI (1975) reported that recently analyzed data (MUKAI and COCKERHAM, unpublished) of newly arisen mutations affecting viability revealed that the genotypic variances among $Cy/+_j$ and $Pm/+_i$ heterozygotes were not significantly different from zero, while that of the $+_i/+_j$ individuals was large. Unlike the present experiment, however, all of their chromosome lines were derived from one original wild-type second chromosome, the difference among the lines resulting from mutations accumulated during approximately 60 generations. Their results, however, could have been obtained even if *Cy* and *Pm* were not dominant, since chromosomes carrying mutations that were not completely suppressed by their homologous *Cy* (or *Pm*) might tend to be eliminated from the populations during the process of mutation accumulation. MUKAI *et al.* (1974) reported that recent experiments (MUKAI, unpublished) showed "normal" variance among individuals heterozygous with *Cy*. CARDELLINO and MUKAI (1975) noted that the assumption of dominance of *Cy* may not always be true for chromosomes extracted from a natural population. Our results certainly confirm these latter findings.

The results obtained in this study involving the *Pm* chromosome follow, in general, the same pattern as those obtained for *Cy* (Tables 1 and 2) but are not as clear-cut in some instances. There is evidence that the genotypic variance among $Pm/+_i$ individuals is significantly different from zero (Table 3), but the standard errors are uncomfortably large. Taken at face value, the estimated genotypic variance among $Pm/+_i$ heterozygotes is 25% of that for $+_i/+_j$ individuals and 42% of that for $Cy/+_j$ heterozygotes. The regression of the average of $Pm/+_i$ and $Pm/+_j$ viabilities on that of $+_i/+_j$ is positive (Table 4), but smaller than that found in the case of *Cy*.

A second source of significant variation found among viabilities in this study are the reciprocal effects (Tables 1 and 2). Similar findings in regard to $+_i/+_j$ viability have been reported previously (MUKAI *et al.* 1974). Although we cannot determine the specific cause(s) of this variation, we can offer at least three possible explanations. Firstly, the reciprocal effects may result from a distortion of the expected gametic proportions, presumably among the male parents (SANDLER and NOVITSKI 1957). Assuming that the mean frequency of the wild-type chromosome among the paternal gametes was less than one half (HIRAIZUMI 1971), one would expect a decrease in the mean frequency of $+_i/+_j$ genotypes relative to that of $Cy/+_j$ or $Pm/+_i$. Mean viability indices of the three genotypic classes are presented in Table 5 for both populations examined. The mean viability of $+_i/+_j$ genotypes is substantially less than that of $Cy/+_j$ within each population, in spite of the fact that the *Cy* chromosome would tend to lower viability. The opposite is true when comparing $+_i/+_j$ to $Pm/+_i$. However, the lowered viability associated with *Pm* may have counteracted any influence of segregation distortion.

A second interpretation of the reciprocal effects involves the fact that the extracted chromosome lines also differ among themselves with respect to the *Y* chromosome (during the extraction process one typically backcrosses a $Cy/+$ male progeny to one or more *Cy/Pm* females). Hence, any differential effects among the *Y* chromosomes on the viabilities of males could result in significant reciprocal effects. Lastly, the observed differences between reciprocal crosses might be attributable to maternal effects (*not* to sex-linked and/or cytoplasmic factors since the extracted chromosome lines should be uniform for the *X* chromosome and cytoplasm—both of *Cy/Pm* origin).

The Curly-Plum method of viability estimation does not require uniformity of viability among $Cy/+_j$ heterozygotes as does the Curly method. However, the Curly-Plum technique does require that *Cy/Pm* progeny be genetically uniform.

TABLE 5

Mean viability indices within California and Japanese populations

Population	$Cy/+_j$	$Pm/+_i$	$+_i/+_j$
California	0.124 ± 0.027	0.005 ± 0.023	0.030 ± 0.037
Japanese	0.131 ± 0.030	0.063 ± 0.023	0.074 ± 0.034

How valid is this assumption in practice? Given that the *Cy/Pm* stock is isogenic at the start of the extraction process, after several generations of backcrossing one may not be able to ignore the accumulation of spontaneous mutations throughout the entire genome. Furthermore, one generally ignores the possibility of recombination occurring on the other linkage groups during repeated backcrossings of *Cy/+* males, although male recombination factors are known to exist in natural populations of Drosophila (HIRAIZUMI 1971). Thus the assumption of genetic uniformity of *Cy/Pm* progeny among crosses may, in some instances, be rather tenuous. Nevertheless, we believe the Curly-Plum procedure is to be preferred over the Curly method since the *Cy/Pm* progeny are undoubtedly more uniform over crosses than are $Cy/+_j$ heterozygotes.

We conclude that viability indices of heterozygotes based on the Curly method, which assumes that the genotypic variance among $Cy/+_j$ individuals is zero, are biased. We have not yet assessed the consequences of using this experimental method in the estimation of genetic parameters. MUKAI *et al.* (1974) and CARDELLINO and MUKAI (1975) have suggested that even if the assumption of dominance of *Cy* does not hold, the estimate of dominance genetic variance may not be affected.

We suggest that the Curly-Plum method of viability estimation be used in future studies since no assumption of dominance is involved. If this method proves to be too laborious, then viabilities expressed relative to $Pm/+_i$ genotypes (Plum method) are recommended. Although viability indices are generally not calculated with $Pm/+_i$ genotypes used as a standard, our results indicate that there may be less genotypic variance for viability (*i.e.*, greater uniformity) among $Pm/+_i$ heterozygotes than among $Cy/+_i$ individuals.

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Corresponding editor: J. F. KIDWELL