Two Tests of *Y* Chromosomal Variation in Male Fertility of *Drosophila melanogaster*

Andrew G. Clark

Department of Biology and Genetics Program, Pennsylvania State University, University Park, Pennsylvania 16802

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ABSTRACT

Deficiency mapping with *Y* autosome translocations has shown that the *Y* chromosome of *Drosophila melanogaster* carries genes that are essential to male fertility. While the qualitative behavior of these lesions provides important insight into the physiological importance of the *Y* chromosome, quantitative variation in effects on male fertility among extant *Y* chromosomes in natural populations may have a significant effect on the evolution of the *Y* chromosome. Here a series of 36 *Y* chromosome replacement lines were tested in two ways designed to detect subtle variation in effects on male fertility and total male fitness. The first test involved crossing males from the 36 lines to an excess of females in an attempt to measure differences in male mating success (virility) and male fecundity. The second test challenged males bearing each of the 36 *Y* chromosomes to competition in populations with males bearing a standard, phenotypically marked ($B^S Y$) chromosome. These tests indicated that the *Y* chromosome lines did not differ significantly in either male fertility or total fitness, but that interactions with autosomes approached significance. A deterministic population genetic model was developed allowing *Y* autosome interaction in fertility, and it is shown that, consistent with the experimental observations, this model cannot protect *Y*-linked polymorphism.

DESPITE the fact that the *Y* chromosome constitutes about 14% of the genome of male *Drosophila melanogaster*, there is a remarkable lack of phenotypic variation associated with the *Y* chromosome. In reviewing the literature, WILLIAMSON (1976) concluded that there were no definitive examples of polygenic variation associated with naturally occurring *Y* chromosomes. In a massive quantitative genetic study, TORO and CHARLESWORTH (1982) failed to find any additive genetic variation for sex ratio. Finally, in the search for morphological mutants in Drosophila, the *Y* chromosome has presented us with a paucity of morphological markers.

Although these observations suggest that the *Y* chromosome is lacking in gene functions, laboratory genetic manipulations have identified several critical functions of the *Y* chromosome. The sterility of *XO* males suggested that the *Y* chromosome was required for male fertility, and deficiency mapping has identified six distinct factors necessary for normal spermiogenesis (BROSSEAU 1960; GATTI and PIMPINELLI 1983). *Y*-linked rRNA genes can alleviate the bobbed phenotype associated with a deficit of *X*-linked rDNA, and there is considerable variation in the number of copies of *Y*-linked rDNA repeats (LYCKEGAARD and CLARK 1989). That we might expect to find phenotypic variation associated with this copy number variation is supported by observations of variation in the *X*-linked rDNA array, where spacer length variation is associated with variation in developmental rate (CLUSTER *et al.* 1987), and selection on bristle length resulted in rDNA changes (FRANKHAM, BRISCOE and NURTHEN 1980). The *Y* chromosome also has sequences that are necessary for chromosome pairing, but recent evidence suggests that the rDNA array may serve in this role as well (MCKEE and LINDSLEY 1987; MCKEE and KARPEN 1990). In *Drosophila affinis*, the *Y* chromosome appears to be involved in interactions with the *X* chromosome in producing a "sex ratio" phenomenon (VOELKER 1972).

Further studies of the *Y* chromosome have suggested its role in interactions with the expression of genes on other chromosomes. The suppression of position effect variegation has long been known to be associated with the *Y* chromosome (SPOFFORD 1976), and recent evidence suggests that the degree of suppression does not depend on the amount of *Y* chromosome present (DIMITRI and PISANO 1989). A number of *X*-linked recessive mutations have been recovered that are lethal in *XX* females and *XO* males, but are viable in *XY* males, presumably due to suppression of position effect variegation (LINDSLEY, EDINGTON and VON HALLE 1960). Finally, *X*-linked bobbed mutations are often unstable, and reversion is associated with increases in germline copy number of rDNA. This magnification of the rDNA array has been shown to require the presence of a portion of the long arm of the *Y* chromosome (KOMMA and ENDOW 1986, 1987).

From a population genetic perspective, one would

expect *a priori* that the patriclinous transmission of the *Y* chromosome would result in a lower opportunity for selectively maintained polymorphism. Simple models with constant fitnesses fail to maintain polymorphisms in panmictic populations (CLARK 1987b), and even allowing recombination with the *X* chromosome, the conditions for polymorphism are stringent (CLARK 1988). Selective polymorphism could be maintained by some form of frequency dependence, or by appropriate population subdivision and migration, but again, the stringency of conditions for polymorphism suggests that variation among *Y* chromosomes is even more likely to be neutral than autosomal variation.

An intriguing case of variation associated with the *Y* chromosome is found in *D. affinis*, which has a naturally occurring polymorphism for *XO* and *XY* males, both of which are fertile. Rather than exhibiting an all-or-none effect on fertility, the *Y* chromosome was shown to provide a quantitative advantage in male fitness (VOELKER and KOJIMA 1971). Although purely theoretical arguments suggest that *Y*-linked variation is likely to have little phenotypic consequence, the presence of so many functions affecting male fertility demands a careful look at this component of fitness. The objective of the study reported here is to test for the existence of subtle quantitative variation in male fertility among an array of naturally occurring *Y* chromosomes of *D. melanogaster*.

METHODS

Drosophila stocks: The 72 *Y*-chromosome replacement lines were constructed following the procedure described in CLARK (1987a). There were a total of 36 distinct *Y* chromosomes among these lines, with each *Y* chromosome in two different lines having different genetic backgrounds. The marked *Y* chromosome used in assays of total male fitness was the B^SY from the stock $B^SY/C(1)DX$ *y f/* $In(1)sc^{4L}sc^{8R}$, *y* $sc^{4L}sc^{8R}$ *w Tu* obtained from the Bowling Green Stock Center. Males possessing this *Y* chromosome were readily identified by the Bar eye phenotype. The *Roth-15* females used in the net male fitness experiment were from the same line that was used in constructing the background replacement for the *Y*-replacement lines (CLARK 1987a). The stocks used in all of the competitive tests had the P cytotype.

Test of male fertility: Male fertility was directly assayed by placing individual 2–4-day-old virgin males of the 72 replacements lines reared at controlled density in vials with 10 virgin females. After 4 days the females were placed individually into 95-mm vials with fresh medium and allowed to lay eggs for 4 more days. On days 14–17 after females first laid eggs, the offspring were scored to determine the total number of offspring sired by individual males. Females of both genetic backgrounds used in constructing the *Y* replacement lines were used, and nine independent replications of the experiment were performed. The data were structured as (36 *Y* chromosomes) × (2 male genetic backgrounds) × (2 female genetic backgrounds) × (10 females per male) × (9 replicates), yielding a total of 12,960 vials. These data were analyzed by analysis of variance using the following model:

$$Y_{ijkl} = \mu + Y_i + M_j + F_k + YM_{ij} + YF_{ik} + YMF_{ijk} + R_l + \epsilon_{ijkl}$$

where Y_{ijkl} is the progeny count from the lth replicate of the cross with the ith *Y* chromosome in a male with background j and a female with background k. The model assumes additivity of the effects of the ith *Y* chromosome with the jth male background, the kth female background, the lth replicate, several interaction terms and a random error term, ϵ. All the effects are random effects. Model fitting and computation of significance tests were done with the procedure GLM in the SAS statistical package.

Test of net male fitness: Net male fitness, which includes components of viability, fertility, and meiotic drive, was assessed by coculturing males from each of the *Y* chromosome replacement lines with males bearing a *Y* chromosome with the Bar^{Stone} B^SY translocation. Changes in the relative frequencies of the Y^+ and B^SY may reflect differences in relative fitness. Only one genetic background of the *Y*-replacement lines was used (*Roth-15*), and one standard *Y* line (B^SY) was used, so differences among *Y*- replacement lines should reflect differences due to the *Y* chromosome. Effects of rearing environment were minimized by rearing the F_1 flies (Y_i^+ and B^SY) within the same bottle.

Virgin *Roth-15* females were crossed to B^SY males and to males from each of the *Y*-replacement lines. Equal numbers of females from the B^SY and Y_i^+ crosses were placed into bottles, setting up one bottle for each of the *Y*-replacement lines. After 4 days, the adults were transferred to fresh bottles. Male F_1 progeny of both phenotypes were collected from these bottles and crossed to virgin *Roth-15* females from the common background. Matings of the two types were kept separate, then equal numbers of inseminated females (bearing sperm of Y_i^+ and B^SY males) were placed into five replicate 95-mm vials. These too were transferred to fresh vials after 4 days. At this point, each of the 36 lines has five replicates, each with two duplicate vials. On days 14–17 after these females started laying eggs, the adult progeny were scored by eye phenotype. These counts were called generation 1 data, and gave a baseline frequency for the F_2 progeny of the original cross. Female progeny from each of these vials, which had been inseminated by males that had emerged, were transferred to five fresh vials and allowed to lay eggs for 4 days. When the progeny from these vials emerged, they were scored as generation 2. The generation 1 data consisted of counts from (36 lines) × (5 replicates) × (2 duplicates) = 360 vials. Flies from each of these vials were transferred to five more vials for a total of 360 + 1800 = 2160 vials.

A comparison of the frequencies of Y_i^+ and B^SY chromosomes at generations 1 and 2 reflect differences in net effects on fitness of the two *Y* chromosomes. Estimates of fitness were obtained from a maximum likelihood model described in RESULTS, and these estimates were analyzed by analysis of variance, fitting the following model:

$$Y_{ijkl} = \mu + Y_i + R_{ij} + D_{ijk} + \epsilon_{ijkl}$$

where Y_{ijkl} is the observation of the lth vial of the kth duplicate of the jth replicate of the ith *Y* chromosome. The replicate effect, R_{ij}, is nested within *Y* chromosomes, and the duplicate effect, D_{ijk}, is nested within replicates and *Y* chromosomes. All the effects are random, and ϵ is a random error term.

Deterministic model of *Y* autosomal effects on fertility: The possibility for interactions between the *Y* chromosome and the autosomal background suggested the following model. The simplest case with these two types of variation has two *Y* chromosomes and one autosomal locus with two

TABLE 1

Means, standard errors and sample sizes of fertility estimates

Line	Male fertility			Net male fitness	
	N	Log (mean fecundity)	Virility	*N*	Log (fitness)
R1	11,393	2.515 ± 0.214	1.174 ± 0.061	2,650	0.084 ± 0.127
R2	12,090	2.934 ± 0.163	1.276 ± 0.054	3,251	0.163 ± 0.145
R3	10,952	2.501 ± 0.211	1.154 ± 0.061	2,372	0.006 ± 0.117
R4	13,122	2.744 ± 0.221	1.195 ± 0.059	2,781	0.031 ± 0.139
R5	11.194	2.661 ± 0.174	1.226 ± 0.049	3,972	0.207 ± 0.060
R6	10,765	2.408 ± 0.255	1.131 ± 0.082	3,025	0.247 ± 0.168
R7	11,057	2.655 ± 0.157	1.201 ± 0.053	3,342	0.079 ± 0.048
R8	9,966	2.538 ± 0.182	1.196 ± 0.062	4,383	0.183 ± 0.095
R9	10,867	2.581 ± 0.188	1.193 ± 0.056	2,549	0.052 ± 0.067
R10	11,597	2.633 ± 0.196	1.191 ± 0.062	2,875	0.172 ± 0.109
R11	10,462	2.511 ± 0.165	1.190 ± 0.056	3,082	0.157 ± 0.140
R12	11,106	2.630 ± 0.182	1.204 ± 0.056	4,168	0.239 ± 0.084
R13	8,412	2.125 ± 0.192	1.034 ± 0.062	2,544	0.048 ± 0.137
R14	10,675	2.672 ± 0.165	1.242 ± 0.059	3,984	0.107 ± 0.095
R15	12,035	2.871 ± 0.166	1.297 ± 0.055	3,003	0.159 ± 0.064
R16	9,652	2.487 ± 0.180	1.199 ± 0.056	3,444	0.240 ± 0.091
R17	8,992	2.397 ± 0.195	1.152 ± 0.068	3,680	0.166 ± 0.120
R18	11,553	2.804 ± 0.167	1.258 ± 0.052	3,480	0.205 ± 0.049
R19	13,539	3.217 ± 0.096	1.357 ± 0.033	3,571	0.181 ± 0.084
R20	10,856	2.619 ± 0.203	1.189 ± 0.064	3,513	0.038 ± 0.116
R21	10,617	2.473 ± 0.209	1.111 ± 0.073	2,316	0.068 ± 0.136
R22	10,632	2.580 ± 0.184	1.174 ± 0.061	2,752	0.074 ± 0.115
R23	12,069	2.741 ± 0.204	1.230 ± 0.062	3,491	0.011 ± 0.083
R24	9,378	2.589 ± 0.183	1.175 ± 0.075	2,862	0.014 ± 0.086
R25	10,971	2.640 ± 0.178	1.249 ± 0.063	3,277	0.217 ± 0.066
AH41	13,153	2.813 ± 0.205	1.244 ± 0.062	3,318	0.045 ± 0.146
AH198	11,918	2.735 ± 0.193	1.206 ± 0.060	3,870	0.013 ± 0.092
Eg-1	6,986	2.076 ± 0.193	1.102 ± 0.067	1,133	0.350 ± 0.191
Fan6	9,796	2.598 ± 0.184	1.214 ± 0.060	2,628	0.128 ± 0.220
GB13	10,582	2.553 ± 0.188	1.202 ± 0.055	3,455	0.002 ± 0.084
GB41	9,362	2.462 ± 0.187	1.190 ± 0.055	3,439	0.173 ± 0.070
Hikone	8,930	2.295 ± 0.222	1.143 ± 0.067	1,865	0.115 ± 0.121
Samark	11,875	2.736 ± 0.194	1.204 ± 0.062	2,360	0.234 ± 0.135
St-4	10,851	2.627 ± 0.185	1.236 ± 0.059	3,236	0.234 ± 0.151
Wd-4	9,629	2.395 ± 0.200	1.146 ± 0.064	3,717	0.138 ± 0.128
Wd-7	10,056	2.277 ± 0.198	1.080 ± 0.066	4,057	0.039 ± 0.083

Mean fecundity is reported as the log of the mean number of offspring per female, and virility is the arcsine transformed fraction of inseminated females.

alleles. This results in six male genotypes, whose fertility may be written:

	Y	*y*
AA	f_{11}	f_{12}
Aa	f_{21}	f_{22}
aa	f_{31}	f_{32}

In the absence of selection, when all fertilities are 1, the recursions for the frequencies of the four gametic types are identical to those for a model with cytoplasmic variation and one autosomal locus (CLARK 1984). With selection, however, the models differ. A recursion system having one equation for each of the six genotypes can be written directly from a mating table. This recursion system produces the same frequency dynamics as the following system of four equations, which specifies the frequencies of the four gametic types:

$$\bar{F}s_1' = s_1[(s_1 + s_2)f_{11} + \tfrac{1}{2}(s_3 + s_4)f_{21}] + \tfrac{1}{2}s_3(s_1 + s_2)f_{21}$$

$$\bar{F}s_2' = s_2[(s_1 + s_2)f_{12} + \tfrac{1}{2}(s_3 + s_4)f_{22}] + \tfrac{1}{2}s_4(s_1 + s_2)f_{22}$$

$$\bar{F}s_3' = s_3[\tfrac{1}{2}(s_1 + s_2)f_{21} + (s_3 + s_4)f_{31}] + \tfrac{1}{2}s_1(s_3 + s_4)f_{21}$$

$$\bar{F}s_4' = s_4[\tfrac{1}{2}(s_1 + s_2)f_{22} + (s_3 + s_4)f_{32}] + \tfrac{1}{2}s_2(s_3 + s_4)f_{22}$$

where s_1, s_2, s_3 and s_4 are the frequencies of the gametes *AY*, *Ay*, *aY* and *ay*, respectively, and $\bar{F}$ is the sum of the right hand sides. There are six trivial equilibria to this model, including the four fixation states and the two "edge" equilibria having an autosomal polymorphism but fixed for one or the other *Y* chromosome. Analysis of local stability and numerical simulations were done to assess the ability of such a system to maintain *Y* chromosome polymorphism.

RESULTS

Male fertility: Two components of male fertility were assayed by the test that presented males with an excess of females. The proportion of females that

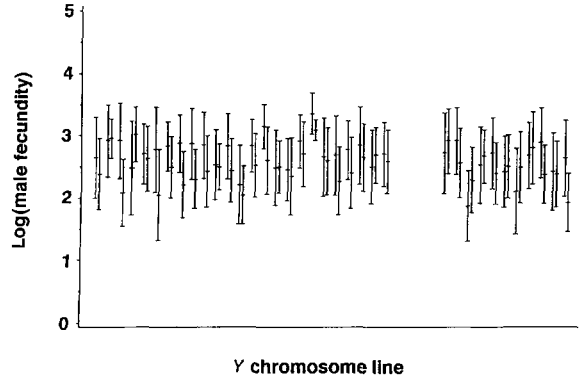

FIGURE 1.—Means ± 1 SE of the log transformed mean fecundities of males from the 36 *Y* replacement lines. Each pair of bars represents the *Harwich* (left) and *Roth-15* (right) male genetic background for the given *Y* chromosome. The first 25 bars are the 25 Rothrock *Y* chromosomes, and the 11 rightmost bars are the lines of diverse geographic origin. The lines are in the same order as in Table 1.

were inseminated indicated the virility of the males, and this statistic was arcsine transformed to make the means independent of the variances. The mean number of offspring per female sired by the male was a measure of the fecundity of the mating, and since the female genotype was common within a background, differences among matings reflect variation in male fecundity. Log transformation of mean fecundity data was found to best stabilize variances. The means of these two components are given for each line in Table 1, and a plot of the mean fecundity appears in Figure 1. The experiment was performed with each sex having two different genetic backgrounds, and the influence of each genetic background on fecundity could be accurately assessed. To test the significance of *Y* chromosome and background effects on fecundity and virility, the linear model described in MATERIALS AND METHODS was fitted to the data. The results of this analysis of variance appear in Table 2. Analyses were done separately for each component, and separately for the 11 lines of diverse geographic origin, the 25 lines whose *Y* chromosome originated from the Rothrock State Forest in Pennsylvania, and the complete set of 36 lines. The two components yielded the same result: all of the background effects were highly significant, and none of the *Y* chromosome effects or interactions was significant. The only exception to this pattern was the lack of significance of the female background effect in mean fecundity among the diverse lines.

The failure to find any quantitative effect of *Y* chromosome variation on male fertility must be interpreted in light of the sensitivity of the experiment to detect such variation. This was a large experiment, with over 10,000 flies scored for each line, and a total sample size of 387,090. The observation that with only one exception, every opportunity to detect an effect of the genetic background on both fertility components revealed highly significant effects indicates that the design could detect genetic differences in both fertility components. The pooled standard errors of transformed virility and fecundity were 0.058 and 0.176, which represent 4.8% and 6.1% of the respective normalized means. When the analysis of variance was repeated on untransformed mean fecundity and virility data, as well as total fecundity (number of progeny sired by each male), the *Y* chromosome effects remained insignificant.

TABLE 2

Analysis of variance of male fecundity data

	Diverse			Rothrock			Pooled		
Source	SS	F	P	SS	F	P	SS	F	P
Mean fecundity									
Y	18.773	1.92	0.158	36.508	1.30	0.265	58.912	1.55	0.099
M	34.522	28.62	<0.001	66.258	61.01	<0.001	100.622	89.62	<0.001
Y × M	19.380	1.99	0.147	43.988	1.56	0.141	63.526	1.67	0.066
F	0.297	0.25	0.620	14.325	13.19	<0.001	11.939	10.63	0.001
Y × F	12.333	1.26	0.359	19.381	0.69	0.817	34.397	0.91	0.613
M × F	29.108	24.13	<0.001	63.856	58.80	<0.001	92.959	82.80	<0.001
Y × M × F	9.760	0.81	0.619	28.182	1.08	0.358	37.949	0.97	0.526
Virility									
Y	1.039	0.95	0.528	3.451	0.91	0.590	4.612	0.94	0.566
M	3.231	25.87	<0.001	3.466	28.76	<0.001	6.478	53.16	<0.001
Y × M	1.399	1.29	0.349	3.749	0.99	0.511	5.369	1.10	0.390
F	0.911	7.30	0.007	3.131	25.98	<0.001	4.010	32.90	<0.001
Y × F	1.248	1.15	0.416	2.486	0.66	0.846	3.767	0.77	0.776
M × F	1.059	8.49	0.004	2.175	18.05	<0.001	3.233	26.54	<0.001
Y × M × F	1.088	0.87	0.560	3.792	1.31	0.146	4.882	1.14	0.260

Analyses of variance were done for the 11 lines of diverse origin, the 25 Rothrock lines, and the pooled set of 36 lines separately. Sums of squares, the *F* statistic and tail probability of *Y* chromosome effects, male background effects, female background effects and interactions are reported.

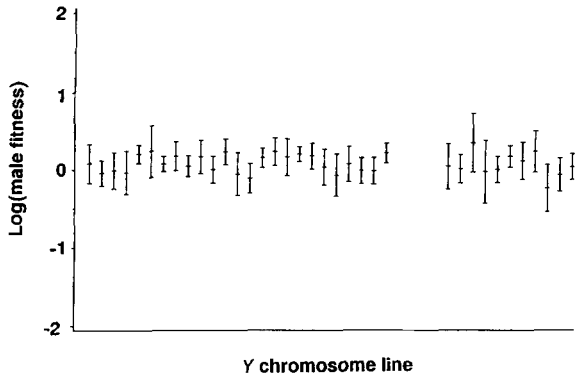

FIGURE 2.—Means ± 1 SE of the log-transformed fitness of males from the 36 *Y* replacement lines estimated by competition with B^SY. Only the *Roth-15* genetic background was examined. The first 25 bars are the 25 Rothrock *Y* chromosomes, and the 11 rightmost bars are the lines of diverse geographic origin, as in Figure 1.

Net male fitness: The test of male fitness using the B^SY yielded counts of progeny at two times, spanning one complete generation. If the counts from the generation 1 data are n_1 of the Y^+ and n_2 of the B^SY, and the respective counts at generation 2 are n_3 and n_4, then we can define the frequencies of Y^+ at the two generations as

$$p_1 = n_1/(n_1 + n_2)$$

$$p_2 = \frac{p_1 w}{p_1 w + (1 - p_1)}$$

where w is the relative fitness of the Y^+ chromosome. The likelihood of the sample is

$$L = p_2^{n_3}(1 - p_2)^{n_4}.$$

After substituting, the derivative of the logarithm of the likelihood is

$$\frac{d \log(L)}{dw} = \frac{-wn_1n_4 - n_2n_3}{wn_2 + w^2n_1}$$

which yields a maximum likelihood estimator for w of

$$\hat{w} = n_2n_3/n_1n_4.$$

Interestingly, the *Y* chromosome exhibits haploid transmission, and a means of estimating relative fitness in bacterial cultures is to calculate the slope of the regression of $\log(p/q)$ against time, where p and q are the frequencies of two competing strains (KUBITSCHEK 1974). The equivalence of this measure of fitness and $\hat{w}$ can be seen by taking the logarithm of $\hat{w}$, which can be expressed as $\log(p'/q') - \log(p/q)$, where p is the frequency of Y^+ and q is the frequency of B^SY. This is the slope of the line of $\log(p/q)$ plotted against generations.

For each combination of *Y* chromosome, replicate and duplicate estimates of $\log(\hat{w})$ were calculated. The means and standard errors of these measures appear in Table 1, and Figure 2 presents a graph of the estimates. The nested analysis of variance was performed as described above, and results are presented in Table 3. The results can again be succinctly summarized: there was no evidence for a *Y* chromosome effect. The significant duplicate effects, which represent one level of sampling error, indicate that the allele frequencies of the two duplicates could be detected as different when data from five descendant vials of each duplicate were scored. In this study 113,445 flies were scored, and the pooled standard error was 0.084, or 7.8% of the normalized mean fitness.

***Y* autosome fertility model:** The development of a model allowing *Y* autosome interactions was motivated by the observation that the *Y* × male background effect was weakly significant ($P = 0.066$) in the mean fecundity of the pooled data (Table 2). The analysis of the local stability of the equilibrium with fixation of the *AY* gamete ($\hat{s}_1 = 1$) proceeds by calculating the Jacobian matrix of partial derivatives evaluated at this point and solving the characteristic equation of this matrix. The characteristic equation is:

$$(f_{12}/f_{11} - \lambda)(f_{21}/f_{11} - \lambda)(f_{22}/2f_{11} - \lambda) = 0$$

having the roots $0, f_{22}/2f_{11}, f_{21}/f_{11}$ and f_{12}/f_{11}. If all of these roots are less than one, then this fixation is stable. Similarly, the conditions for the stability of the other three fixations are:

Equilibrium	Stability conditions
$\hat{s}_2 = 0$	$f_{11}/f_{12} < 1, f_{21}/2f_{12} < 1$, and $f_{22}/f_{12} < 1$
$\hat{s}_3 = 0$	$f_{21}/f_{31} < 1, f_{22}/2f_{31} < 1$, and $f_{32}/f_{31} < 1$
$\hat{s}_4 = 0$	$f_{21}/2f_{32} < 1, f_{22}/f_{32} < 1$, and $f_{31}/f_{32} < 1$

In addition to the four fixations, there are two edge equilibria that maintain a polymorphism at the autosomal locus. The $s_1 - s_3$ edge has an equilibrium at

$$\hat{s}_1 = \frac{f_{31} - f_{21}}{f_{11} - 2f_{21} + f_{31}}$$

and $\hat{s}_3 = 1 - \hat{s}_1$. This equilibrium exists if there is classical overdominance or underdominance among the genotypes *AAY*, *AaY* and *aaY*, but stability requires more than simple overdominance. The conditions for the stability of this edge equilibrium have been solved explicitly, but they are algebraically cumbersome. Instability of the four fixations guarantees the stability of one or both of the edges. All orderings of the six fertilities also guarantee stability of one or more of the six equilibria described above. These results prove that this model is not capable of protecting a polymorphism. Numerical testing of 100,000 randomly chosen sets of fertility parameters verified that there are seven possible configurations of equilibria, and in all 100,000 cases, at least one edge or corner was stable (Figure 3 and Table 4). Numerical iteration of

TABLE 3

Analysis of variance of net male fitness

Source	Diverse			Rothrock			Pooled		
	SS	F	P	SS	F	P	SS	F	P
Y	4.112	0.53	0.858	10.208	1.12	0.335	15.087	0.87	0.683
Replicate	31.761	0.73	0.843	35.979	0.81	0.844	67.739	0.76	0.946
Duplicate	46.641	3.75	<0.001	43.868	1.90	<0.001	90.509	2.55	<0.001

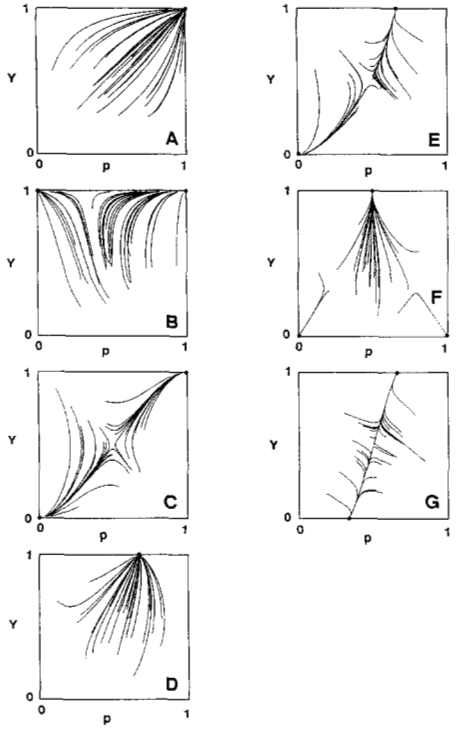

FIGURE 3.—Phase planes showing all seven possible qualitative behaviors of the *Y* autosome model. The *X* axis represents the frequency of the *A* autosomal allele, and the *Y* axis is the frequency of the *Y* allele. For each panel, one set of fitnesses is given and 30 random starting points were iterated until the population attained an equilibrium. No case with a stable interior equilibrium was found. A. One corner stable ($f_{11} = 1, f_{21} = 0.9, f_{31} = 0.8, f_{12} = 0.9, f_{22} = 0.8, f_{32} = 0.7$). B. Two corners with same *Y* stable ($f_{11} = 1, f_{21} = 0.9, f_{31} = 0.95, f_{12} = 0.8, f_{22} = 0.9, f_{32} = 0.9$). C. Two opposite corners stable ($f_{11} = 1, f_{21} = 0.9, f_{31} = 0.8, f_{12} = 0.8, f_{22} = 0.9, f_{32} = 1$). D. One edge stable ($f_{11} = 0.9, f_{21} = 1, f_{31} = 0.8, f_{12} = 0.9, f_{22} = 0.8, f_{32} = 0.9$). E. One edge and one opposite corner stable ($f_{11} = 0.9, f_{21} = 1. f_{31} = 0.8, f_{12} = 0.9, f_{22} = 0.9, f_{32} = 1$). F. One edge and two opposite corners stable ($f_{11} = 1, f_{21} = 1.1, f_{31} = 1, f_{12} = 1.05, f_{22} = 1, f_{32} = 1.05$). G. Two opposite edges stable ($f_{11} = 0.9, f_{21} = 1, f_{31} = 0.8, f_{12} = 0.8, f_{22} = 1, f_{32} = 0.9$).

the recursion system was followed for another 100,000 random fertility matrices, and each case was iterated until the frequency of the *Y*-linked allele was either greater than 0.9999 or less than 0.0001. This happened in all cases, suggesting that not only is it impossible to protect a polymorphism, but also that there are no stable interior equilibria.

DISCUSSION

The experiments reported here failed to detect significant variation among 36 *Y* chromosomes in their effects on male fertility or on net male fitness. This conclusion gains credence when it is recognized that the tests were large enough to have the statistical power to detect differences in fitness of just a few percent. Natural selection may have a significant impact on allele frequencies if the selection coefficients are larger than the reciprocal of the effective population size. The experiments do not achieve this level of precision, which means that despite the negative result, there may still be genetic variation among naturally occurring *Y* chromosomes that is influenced by selection. The rate of fixation of *Y*-linked variants with a given magnitude of selective effect can be much greater than that of autosomes (CHARLESWORTH, COYNE and BARTON 1987), leaving a smaller opportunity to detect transient *Y*-linked polymorphism with differences in fitness. The experiments reported here, which involved scoring 500,535 flies, were easily large enough to detect significant variation among *X* chromosomes (CURTSINGER 1984) or among second chromosomes (SEAGER and AYALA 1982; CLARK 1986). Regardless of the true magnitude of variation in male fitness, the *Y* chromosome clearly exhibits far less variation than other chromosomes.

TABLE 4

Results of numerical simulations of the *Y* autosome fertility model

Equilibrium	Frequency
A. One corner	20,979
B. Two corners, same *Y*	20,717
C. Two diagonally opposed corners	21,539
D. One edge autosomal polymorphism	15,780
E. One edge and opposite fixation	15,748
F. One edge and both opposite fixations	3,992
G. Both autosomal edges	1,245
Total	100,000

These results are consistent with simple population genetic theory which finds it difficult to maintain stable *Y*-linked polymorphism (CLARK 1987b). The failure to detect variation among geographically diverse populations may be more surprising. In a sufficiently subdivided population, one would expect the rapid fixation of favorable mutations to result in strong subdivision of *Y*-linked variation. Data of WILLIAMS *et al.* (1987) do suggest that the *Y*-linked rDNA array exhibits greater subdivision than the *X*-linked

array. The magnitude of subdivision of *Y* chromosomes that differ in fitness will depend on the population sizes, on the rates of adaptive mutation, and gene flow, but there is need for formal theory on this problem. With an average F_{ST} of autosomal and *X*-linked genes of 0.116 ± 0.130 and 0.153 ± 0.159, respectively (SINGH and RHOMBERG 1987), we might expect molecular *Y*-linked variation to exhibit subdivision, but no prediction can be made about subdivision of selectively different variants.

Our test of male fertility could have revealed differences due to several different components of fitness, including mating speed, remating latency, amount of sperm transferred, and possibly sperm competition. Although the genes on the *Y* chromosome have functions relating to sperm maturation, variation in those genes might result in sperm having, for example, different properties of sperm competition (PROUT and BUNDGAARD 1977). Models that incorporate more biological complexity are often better able to maintain polymorphism. For example, stable *Y*-linked polymorphism can be maintained under some restrictive conditions of mating success, sperm displacement, refractory period and viability (T. PROUT, personal communication). The variance in the net effects of components of male fertility may be greater than or less than the variance in any single component, because natural selection may maintain a low level of net fitness with variants having antagonistic tradeoffs among components. This motivates the study of the individual components, particularly those that might be related to sperm maturation including sperm competition.

Significant variation was detected among the same set of *Y* chromosomes in their segregation properties (CLARK 1987b), and the reason these differences were not detected in the assay of net fitness probably lies in the increased statistical power of that study. Although the magnitude of variation that was detected amounted to only a 3% deviation in segregation values, this level was clearly larger than $1/N$ for Drosophila populations. The maintenance of such variation is likely to be transient, since the introduction of a mutant *Y* chromosome with a 3% advantage should result in a change in frequency from 0.01 to 0.99 in 311 generations. One mechanism for maintaining stable *Y*-linked polymorphisms requires recombination with the *X* (CLARK 1988), and although *X-Y* exchanges are rare in Drosophila, they may have important consequences in the maintenance of *Y*-linked variation.

One biological complication that cannot stabilize a *Y*-linked polymorphism is interaction with the autosomes, exemplified by the model presented above. Interactions between autosomes and the *Y* chromosome in effects on male fertility have been documented in *Drosophila mojavensis* × *Drosophila arizonensis* hybrids (VIGNEAULT and ZOUROS 1986), and the specificity of the interaction has been demonstrated by mapping an autosomal factor responsible for the hybrid sterility (PANTAZIDIS and ZOUROS 1988). Population genetic theory of *Y*-autosome interaction in hybrid sterility fails to recover conditions for *Y* polymorphism, and this property, along with the rapid fixation of alternate *Y* chromosome types, has suggested the possible importance of *Y*-autosome interactions in speciation (ZOUROS 1986). Whether *Y*-autosome interactions provide a general explanation of Haldane's rule for hybrid male sterility has been questioned by COYNE (1985), who found that among hybrids of species in the melanogaster group, *X-Y* mismatches always produced immotile sperm, while *Y*-autosome mismatches often had motile sperm. With respect to the maintenance of intraspecific *Y* chromosomal polymorphism, *Y*-autosome interactions may have an influence on the duration of transient polymorphism, but the formal theory remains to be done.

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