VIRILITY DEFICIENCY AND THE SEX-RATIO TRAIT IN *DROSOPHILA PSEUDOOBSCURA*. II. MULTIPLE MATING AND OVERALL VIRILITY SELECTION

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ABSTRACT

Previous studies on fitness components of Drosophila have shown the overwhelming importance of virility selection. In this study, virility selection is further partitioned into two components—one with respect to virgin females and the other with respect to nonvirgin females. The relative importance of the two components to the overall virility selection depends on the remating tendency of females which is investigated here. A theoretical model is then proposed to estimate virility selection under the condition of frequent female remating. The model is tested experimentally. When this model is applied to the Sex-Ratio system of *D. pseudoobscura*, Sex-Ratio males are found to suffer substantial virility reduction. The significance of this finding to the Sex-Ratio problem is discussed.

IN the accompanying paper (WU 1983a), evidence is presented to show how underestimates of virtility selection would result if only matings of virgin females were considered. It was also shown that *Drosophila pseudoobscura* males carrying the Sex-Ratio *X* chromosome (denoted SR; SR males transmit 99% *X*-bearing sperm) suffer substantial virility reduction, compared with Standard (ST) males, when mated with nonvirgin females. This reduction is more pronounced at 14° than at 22°. The effect of temperature on the virility of SR males seems to be in agreement with the geographical distribution, temporal variation and altitudinal distribution of the SR chromosome in natural populations (DOBZHANSKY 1944; EPLING, MITCHELL and MATTONI 1957; BALDWIN 1979; BRYANT, BECKENBACH and COBBS 1982). The finding that the intensity of virility selection varies with the mating status of females makes virility estimation very complicated, and it has been well documented that *D. pseudoobscura* mate quite frequently under both laboratory and natural conditions (DOBZHANSKY and PAVLOVSKY 1967; COBBS 1977; LEVINE *et al.* 1981).

In this paper, a model that takes into account multiple inseminations is developed for virility estimation. In such cases, virility selection actually depends on the relative importance of its two components (the virgin and non-

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virgin component) which, in turn, depends on how often the females remate. By that, we are actually asking two questions. (1) What is the distribution of "waiting time" (in the mathematical sense) between two consecutive matings? (2) Does the waiting time change as the mating status changes? (Mating status is defined as the number of matings in which a female engages.)

Information on the first question is available in the literature. In this paper, I provide an answer to the second question. Based on the studies of female remating, a simple mathematical model is developed to estimate virility selection in Drosophila in general. This model incorporates many virility elements, including multiple inseminations and sperm displacement. A procedure for testing this virility model in laboratory populations is also established. This model is then applied to the Sex-Ratio system in *D. pseudoobscura*.

MATERIALS AND METHODS

Strains: The standardized stocks for determination of esterase-5 (*Est-5*) alleles were sent to our laboratory by S. BRYANT. The remating experiment described later used females from a newly extracted line fixed for the *1.00* allele. Males used in that experiment carrying other known *Est-5* alleles were derived by mating virgin females from the standardized *Est-5* stocks to males from the noninbred strains. This practice minimized the effect of inbreeding in the standardized stocks. The electrophoretic procedures used in determining *Est-5* alleles are identical with those of BECKENBACH (1983). The origin and maintenance of the SR and ST strains used here were described in the paper by WU (1983a).

The remating experiment: The procedure for this experiment was to give a female of a known genotype many chances in a sequence to mate with males of different genotypes. By examining the genotypes of the offspring throughout the mother's lifetime reproduction, we could determine how often mating recurs.

The marker gene in this experiment was the sex-linked esterase-5 locus with six identifiable alleles: *0.85*, *0.97*, *1.00*, *1.03*, *1.09* and *1.12*. All females used were homozygous for the *1.00* allele. The first matings of all 20 females were with males carrying the same *1.00* allele, and the matings were observed. Successful copulation in this species lasts about 4–5 min.

A typical sequence of mating trials of these females is the following. After the first mating with an $Est\text{-}5^{1.00}/Y$ male was observed, the female was confined with two males with the *1.12* allele in a vial with fresh food. Two days later, the female was transferred to a new vial containing two $Est\text{-}5^{0.97}/Y$ males. The same procedure was followed with males carrying the *1.03*, *0.85* and *1.09* alleles in that order. In the end, the female was allowed to lay eggs by herself in another new vial. Since *Est-5* is sex linked, the F_1 females emerging from the six vials contained information on paternity in that specific period when eggs were laid. It is, therefore, possible to detect the gametic contribution of most of the males before their sperm are displaced by the next male. The arrangement of the mating-trial sequence was always in such a way that the genotype of the copulating male was sufficiently different from that of the previous male for easy identification. For example, the sequence just described is *1.12-0.97-1.03-0.85-1.09*. The maternal allele, *1.00*, served as a very good reference point.

Three to six female offspring from each of the six vials were assayed for their *Est-5* alleles. A male was considered to have failed to mate if his allele was not detected in the progeny collected from subsequent egg samples.

Testing virility of SR males: Six laboratory populations were contrived. Five of them started with 30 virgin ST/ST females, ten ST males and ten SR males. One population consisted of 45 virgin females, 15 ST males and 15 SR males. On day 0, all flies were 3 days old. The experiment was done at 14°, and few eggs were laid in the first 3 days. Eggs were collected from the populations during the following time periods: days 4–6, days 8–9, days 11–12 and day 14. Fresh food medium was supplied between each egg sampling. F_1 progeny from these egg samples were counted and sexed.

Egg samples collected on day 14 were different from others: Females and males in each population were separated on day 14, and each female collected was put into a vial and allowed to lay eggs for 2–3 days. Sex composition from each family was recorded. The sum of the data from all of the families constitutes the sex composition of the samples of day 14. Due to the difficulty in retrieving the flies from the cages, only 115 of the original 195 females contributed to the collection of day 14. Data from all six cages were pooled in Table 2.

A control population with 30 ST/ST females and 20 ST males was also set up under the same conditions to estimate relative viability of larvae of both sexes.

RESULTS AND ANALYSIS

The tendency of females to remate

Fourteen of the 15 females that were offered males of six different genotypes consecutively in a 10-day period mated four or five times. The other one mated at least three times. Four other females that were offered males of five different genotypes all mated three or four times. The heterogeneity among females seems negligible. Table 1 gives the results of remating tendency of females according to their mating status. The last row suggests that the probability of a female remating in a 2-day period does not vary with her mating status. (None of the pairwise comparisons is significant.) The high receptivity of multiply mated females to males is an interesting observation. The answer to the second question posed in the beginning of this paper, therefore, is: No, a female does not become less receptive solely because of her mating status.

The "waiting time" between matings has been shown to follow an exponential distribution (BECKENBACH 1981). This observation together with data presented here suggests a simple approximation to the remating events: a Poisson process. Therefore, it is assumed that, at time t, the distribution of mating status of females follows a Poisson distribution with parameter λt, where λ is the remating rate. In the following section, λ is estimated from data of the experimental population.

TABLE 1

Proportions of females remated in a 2-day period

Genotype of males	Mating status of females				
	1	2	3	4	Total
0.85	1/1	7/7	6/8	2/3	16/19
0.97	4/8	8/10	0/1		12/19
1.03	0/1	1/9	0/7	0/2	1/19
1.09	5/5	1/1	6/6	3/3	15/15
1.12	8/12	2/2	3/5		13/19
Σ^a	18/26	18/20	15/20	5/6	
	=0.692	=0.90	=0.75	=0.833	
	(0.091)	(0.067)	(0.097)	(0.152)	

Mating status of a female is the number of times she has mated.

[a] Trials with 1.03 males are excluded. The numbers in parentheses are standard deviations.

A model on virility estimation

Definitions: Let the relative fertility of two genotypes of males, A and B, be 1 and c, respectively ($c \leq 1$). To be specific, A can be referred to as ST and B as SR.

Intensity of sperm displacement p or q is defined as the proportion of sperm in the female storage organ that is displaced if the last male she mated with is of genotype A or B, respectively. Let $p \geq q$. Therefore, $1 - p$ or $1 - q$ is the proportion of sperm remaining in the female tract from previous matings. The same "dilution" assumption is made if the female has engaged in more than two matings. Therefore, $(1 - p)^2$ is the proportion of sperm from earlier matings remaining undisplaced when the female has mated serially to two A males.

Let y be the frequency of genotype B among males.

Let $u_1(y)$ and $u_2(y)$ be the probabilities of a virgin female and a nonvirgin female, respectively, mating with a B male. Let v_1 and v_2 be the mating success of a B male relative to that of an A male with respect to virgin females and nonvirgin females, respectively. Then,

$$u_1(y) = \frac{v_1 y}{\{(1 - y) + v_1 y\}} \quad \text{and} \quad u_2(y) = \frac{v_2 y}{\{(1 - y) + v_2 y\}}. \tag{1}$$

In the remainder of this section, time t is defined as the age of an individual female with $t = 0$ being the time of her first mating rather than the time of eclosion.

The model: The random variable F_i is defined as the proportion of fertilized eggs of a randomly chosen female laid between her ith and $i + 1$th mating that were fathered by B males.

The distributions and variances of F_i's are required for the test of the model and are given in APPENDIX. The expectation of F_{i+1} conditional on F_i is

$$\begin{aligned} E(F_{i+1} \mid F_i) &= u_2(y)\{q + (1 - q)F_i\} + \{1 - u_2(y)\}\{(1 - p)F_i\} \\ &= u_2(y)q + \{1 - b(y)\}F_i \end{aligned}$$

where $b(y) = p - u_2(y)(p - q)$, $i = 1, 2, 3, \ldots\ldots$ Therefore,

$$E(F_{i+1}) = \frac{q u_2(y)}{b(y)} + \{1 - b(y)\}^i \frac{\{E(F_1)b(y) - u_2(y)q\}}{b(y)} \tag{2}$$

where $E(F_1) = \dfrac{u_1(y)c}{u_1(y)c + \{1 - u_1(y)\}}$.

To actually test the virility difference between two genotypes (*e.g.*, SR and ST), the F's have to be defined as a function of time.

Let $F(t)$ be the proportion of eggs fertilized by sperm of B males when females are aged $(t, t + dt)$. Let $I(t)$ be the number of matings a female has engaged in prior to time t. It was suggested previously that $I(t)$ constitutes a Poisson process, *i.e.*,

$$\text{Prob}\{I(t) = i\} = \exp(-\lambda t)(\lambda t)^{i-1}/(i - 1)! = g_{\lambda t}(i) \quad \text{and} \quad E\{I(t)\} = \lambda t + 1$$

where $1/\lambda$ is the mean waiting time between two consecutive matings.

The probability distribution of $F(t)$ is

$$\text{Prob}\{F(t) = z\} = \sum_i \text{Prob}\{F(t) = z, I(t) = i\} = \sum_i g_{\lambda t}(i)\text{Prob}\{F_i = z\},$$

therefore,

$$\begin{aligned} E\{F(t)\} &= \sum_z z\ \text{Prob}\{F(t) = z\} \\ &= \sum_i E(F_i)g_{\lambda t}(i) \qquad (3) \\ &= \frac{u_2(y)q}{b(y)}\{1 - \exp(-b(y)\lambda t)\} + \exp(-b(y)\lambda t)E(F_1). \end{aligned}$$

We are interested only in the contribution of a B male to the gametic pool relative to that of an A male. The relative virility is thus defined as

$$s(t) = \frac{E(F(t))/y}{\{1 - E(F(t))\}/(1 - y)}, \qquad (4)$$

and,

$$\lim_{t\to\infty} s(t) = (q/p)v_2. \qquad (5)$$

Equation 5 shows that, after repeated matings, the frequency of paternal genes in the zygotic pool reaches an equilibrium determined by the relative displacement ability of males and by mate choice by nonvirgin females. Note that c and v_1 disappear in (5). However, at a female's virgin mating, the relative virility is governed only by c and v_1:

$$s(0) = \frac{E(F_1)/y}{\{1 - E(F_1)\}/(1 - y)} = cv_1. \qquad (6)$$

The virility function, $s(t)$, monotonically decreases from cv_1 to $(q/p)v_2$. This virility function at $t = 0$ and at $t = \infty$ is a simple function of a small set of well-defined parameters. The actual virility of males, however, depends on how rapidly $s(t)$ approaches $(q/p)v_2$ from cv_1. Male virility is conventionally equated to $s(0)$ $(=cv_1)$ without reference to q/p or v_2. In contrast, this new virility function predicts that relative gametic contributions of males either increase or decrease as *females* grow older.

Frequency-dependent virility selection: The virility function (equation 4) is a complicated expression. We will deal with a special case in which the virility of males differs only in their mating success with respect to nonvirgin females. Let $c = v_1 = 1$ and $p = q$, we then obtain from (2), (3) and (4)

$$s(t) = \frac{v_2 + (1 - y)(1 - v_2)\exp(-p\lambda t)}{1 - y(1 - v_2)\exp(-p\lambda t)}. \qquad (7)$$

It follows that $\partial s/\partial y < 0$.

Equation (7) suggests that, as the frequency of B males increases, their virility decreases. Figure 1 gives an example of the effect of the genotypic frequencies on the relative virility.

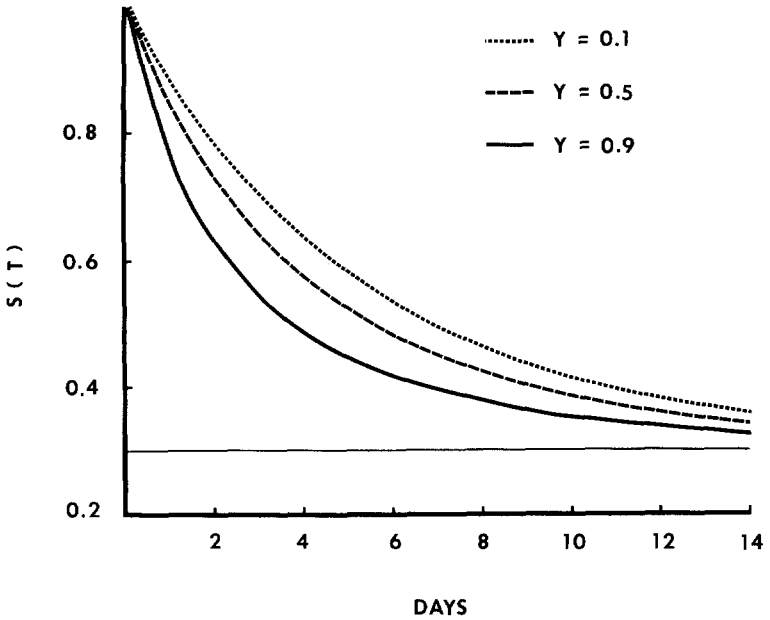

FIGURE 1.—The frequency-dependent virility function, $s(t)$. This function is the relative virility of males of different genotypes. Note that t is the age of females and Y is the frequency of the less virile males.

If the mate preference of nonvirgin females is in the opposite direction to that of virgin females (*i.e.*, $v_1 > 1 > v_2$), the effect of frequency dependence would be stronger than is reported here.

Estimation of overall virility: The virility function, $s(t)$, introduced previously is not readily applicable to the discrete generation model of population genetics. To approximate the virility of SR males, S, in the sense of a discrete generation model, two age-dependent traits of females have to be considered—the age-specific fecundity and the mortality of fecund females.

Let $z(t)$ be the proportion of eggs a female lays at age $(t, t + dt)$. Generally, $z(t)$ increases in the first several days and stays at a plateau for 1–2 weeks and then decreases gradually. Let $m(T)$ be the probability density that a female surviving to and dying on day T after its first mating.

For any female that dies at age T, the gametic contribution by B males to her lifetime zygotic output is

$$H(T) = \int_0^T E(F(t))z(t)dt$$

whereas her total output is

$$Z(T) = \int_0^T z(t)dt.$$

The relative male virility, S, with respect to a cohort of females in a population

with a stable age structure, is, therefore,

$$S = \frac{(\bar{H}/\bar{Z})/y}{(1 - \bar{H}/\bar{Z})/(1 - y)} \tag{8}$$

where $\bar{H} = \int_0^\infty H(T)m(T)dT$ and $\bar{Z} = \int_0^\infty Z(T)m(T)dT$.

Equation (8) is used to compute the relative virility of SR males. The age-specific fecundity, $z(t)$, at 22° obtained in this study is very similar to that reported by BECKENBACH (1978). At 14°, egg laying is delayed by about 2 days. The mortality of fecund females is usually quite small within 2 weeks in the laboratory populations. In nature, however, DOBZHANSKY and WRIGHT (1943) estimated the daily mortality of *D. pseudoobscura* adults to be about 8%. I will use their estimate of $m(T)$ in virility estimation. Both the upper limit of v_2 (=0.73) and the estimated one ($v_2 = 0.52$) are used to give an indication of the range of variation in estimates.

At 14°, with all virility elements incorporated, SR males are expected to be only about 30–37% virile compared with ST males. ($S = 0.30$ if $v_2 = 0.52$; $S = 0.37$ if $v_2 = 0.73$.) This low level of virtility is more than sufficient to offset the advantage of the meiotically driven SR chromosome.

At 22°, the estimated relative virility is as follows:

Frequency of SR males	$v_2 = 0.52$	$v_2 = 0.73$
0.1	0.52	0.65
0.5	0.50	0.64
0.9	0.48	0.63

Again, virility reduction in SR males is quite substantial. The implication of the low virility of SR males will be discussed.

A method of testing the virility model

An important prediction of this model is that, if *B* males are less virile than *A* males only with respect to nonvirgin females, the gametic contribution from *B* males decreases as a function of the mating status and, hence, the age of females. Let $A(t)$ represent the proportion of eggs fertilized by *B* males in the tth day collection of egg samples from a cohort of m females, and assume N eggs are collected in each sample. The mean and variance of $A(t)$ can be found in the APPENDIX. If we also know, in addition to $A(t)$, the proportion of eggs fertilized by *B* males in the egg pool of each *individual* female in the last collection of egg samples, then it is possible to estimate the remating rate λ in such an experimental population. The method is a numerical Maximum Likelihood Estimator (MLE) and is given in the APPENDIX.

Here are some numerical examples of $E(A(t)) \pm \sqrt{\mathrm{Var}(A(t))}$ with $y = 0.5$ and $\lambda = 0.25$ on days 2, 8 and 14, respectively: 0.471 ± 0.075, 0.377 ± 0.064 and 0.347 ± 0.059 if $m = 50$ and $N = 500$. The values become 0.471 ± 0.036, 0.377 ± 0.030 and 0.347 ± 0.029, respectively, if $m = 200$ and $N = 3000$. In these two examples, $p = 0.9$, $q = 0.6$ and $v_2 = 0.75$ such that virility of *B* males relative to *A* males gradually approaches 0.5 [$=0.6 \times 0.75/0.9$, see

(5)]. Because $\mathrm{Var}(A(t)) = E(F(t))/N + \mathrm{Var}(F(t))/m$, the standard deviation of $A(t)$ is usually large relative to the changes in $E(A(t))$. In this example, a collection of 500 individuals in each sample is not sufficient to detect a decrease of 50% in relative virility. [Although $A(t)$ is autocorrelated, incorporation of covariance terms will not change the conclusion; see WU (1982, p. 166).] To test a smaller virility difference, a very large sample is needed.

Testing the virility of SR males: Direct scoring of gametic contribution by SR males as a function of females' ages is very time consuming. Instead, I used the proportion of daughters in the zygotic pool at time t, $f(t)$, to represent the changes in $F(t)$. Formally, $f(t) = \frac{1}{2}(1 - F(t)) + (1 - D)F(t)$, where D is the proportion of sons among the progeny of SR males and is normally between 0.01 and 0.05. The mean and variance of the *observed* proportion of daughters in a sample of eggs collected from m females, $A'(t)$, are presented in the APPENDIX. The viability difference between male and female larvae is also incorporated in $A'(t)$. The limitation of this test will be discussed. The observed proportions of daughters at 14° are presented in Table 2.

The data of day 14 represent the sum of sex compositions of 115 families, and the distribution of this sex composition is shown in Figure 2. I then used the MLE method (APPENDIX) to estimate λ from these data. This method computes the likelihood, $L(\lambda)$, of obtaining the distribution given λ. Figure 3 gives the ratios of $L(\lambda)/L(\lambda_0)$, where $L(\lambda_0)$ is the maximum likelihood obtained. It suggests that the MLE of $1/\lambda$ (*i.e.*, the average waiting time between matings) is somewhere between 4 and 4.5 days. I will assume $1/\lambda$ to be 4 in the subsequent tests. Note that the likelihood of $\lambda = 0$ ($L(0)$) is infinitesimal relative to $L(\frac{1}{4})$. This means that it is fairly unlikely to obtain data with a profile like that of Figure 2 when females do not engage in multiple matings (*i.e.*, $\lambda = 0$).

The predicted and observed decrease in the virility of SR males at 14° is presented in Figure 4. The expected values are based on data of sperm displacement and sexual selection from the acompanying paper (WU 1983a) as well as λ from Figure 3. Observed values are from Table 2.

The horizontal line is what one would expect if females did not remate. Again, it can be seen that an overestimate of the virility of SR males would result if only virgin females are considered. The expected relative virility based

TABLE 2

A test of the male virility model at 14°

	*n*th day collection of eggs				
	0	5	8	11	14
No. of females		2554	2184	2302	5908
No. of males		1025	954	1075	3101
% Female	78.4	71.4	69.6	68.2	65.6

Sex composition in F_1 samples collected at 14°. Expected proportion of daughters on day 0 is based on equal fertility of SR and ST males and on the estimated relative viability of the two sexes ($\delta/\varphi = 0.81$). This estimate comes from the control population with 1296 females and 1045 males collected.

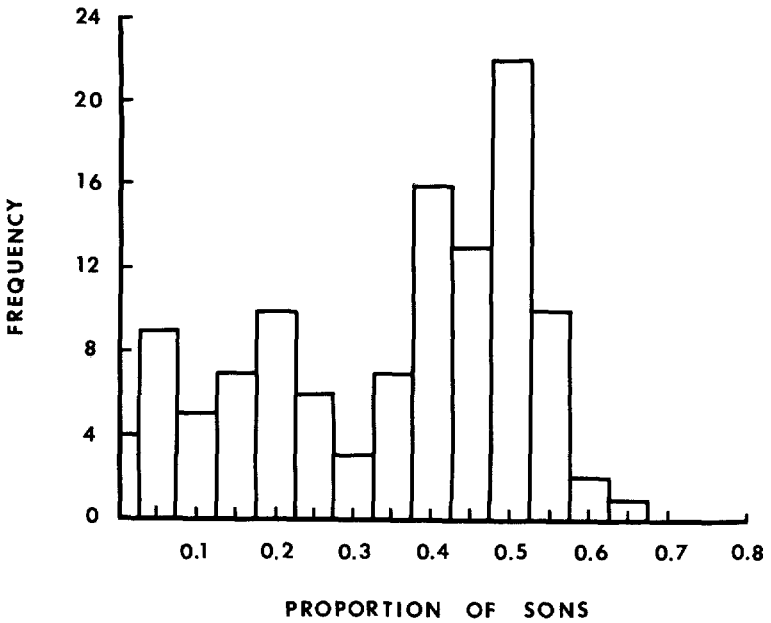

FIGURE 2.—The distribution of sex composition from 115 families.

on the estimated v_2 (=0.52) does not fit the observed pattern as closely as the expectation based on the upper limit of v_2 (=0.73) does.

Finally, it is desirable to forego the data of $A'(t = 0)$ (which were collected from the control experiment) and test whether the remaining four data points manifest a trend of decrease in the proportion of daughters. With the variances of $A'(t)$ calculated from equation A12, regression yields a z value of -2.10 ($P < 0.02$) against the null hypothesis of zero slope.

DISCUSSION

The virility model: The findings of this paper and the accompanying one are the following. (1) The intensity of virility selection increases as females become nonvirgin, and (2) females remate quite frequently, and the tendency to remate does not weaken even after they have mated many times. The consequence is that, in a population, relative virility of males of different genotypes (*e.g.*, SR *vs.* ST) decreases or increases as a function of the age of females.

The results of the remating tendency of Drosophila females have some interesting ramifications. Our understanding of the remating tendency of females in nature comes mainly from the studies of the mother-offspring genotypic relationship (MILKMAN and ZEITLER 1974; COBBS 1977; LEVINE *et al.* 1981). Because the frequency distribution of alleles for most loci is highly skewed, the probability of a female mating males of the same genotype is quite high. In addition, sperm stored from the first mating usually would not be recovered after the females remate more than twice. Consequently, the information about multiple paternity contained in the brood of a quintuply inseminated female would be very similar to that of a triply, or even doubly, mated female. To

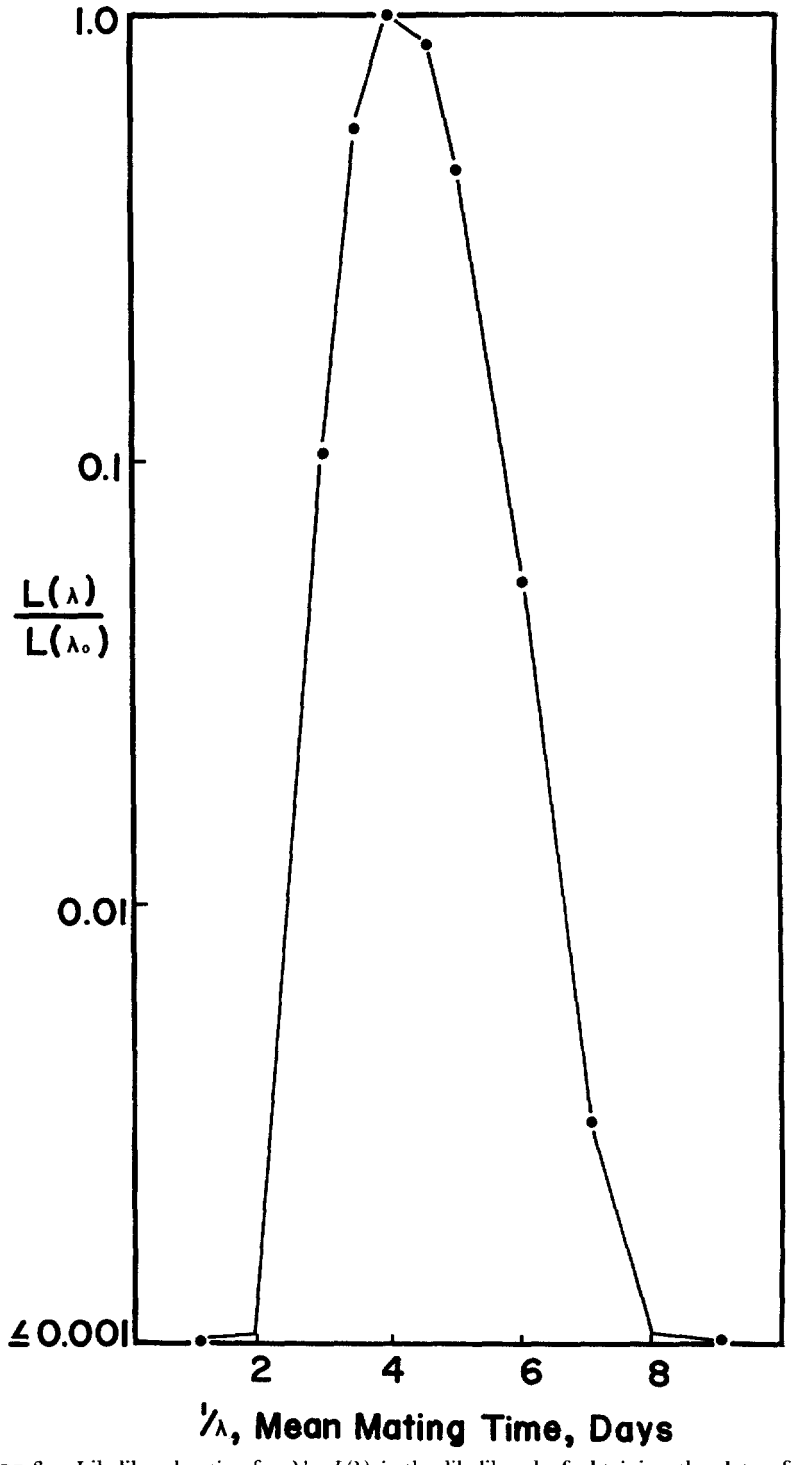

FIGURE 3.—Likelihood ratios for λ's. $L(\lambda)$ is the likelihood of obtaining the data of Figure 2 given the remating rate, λ.

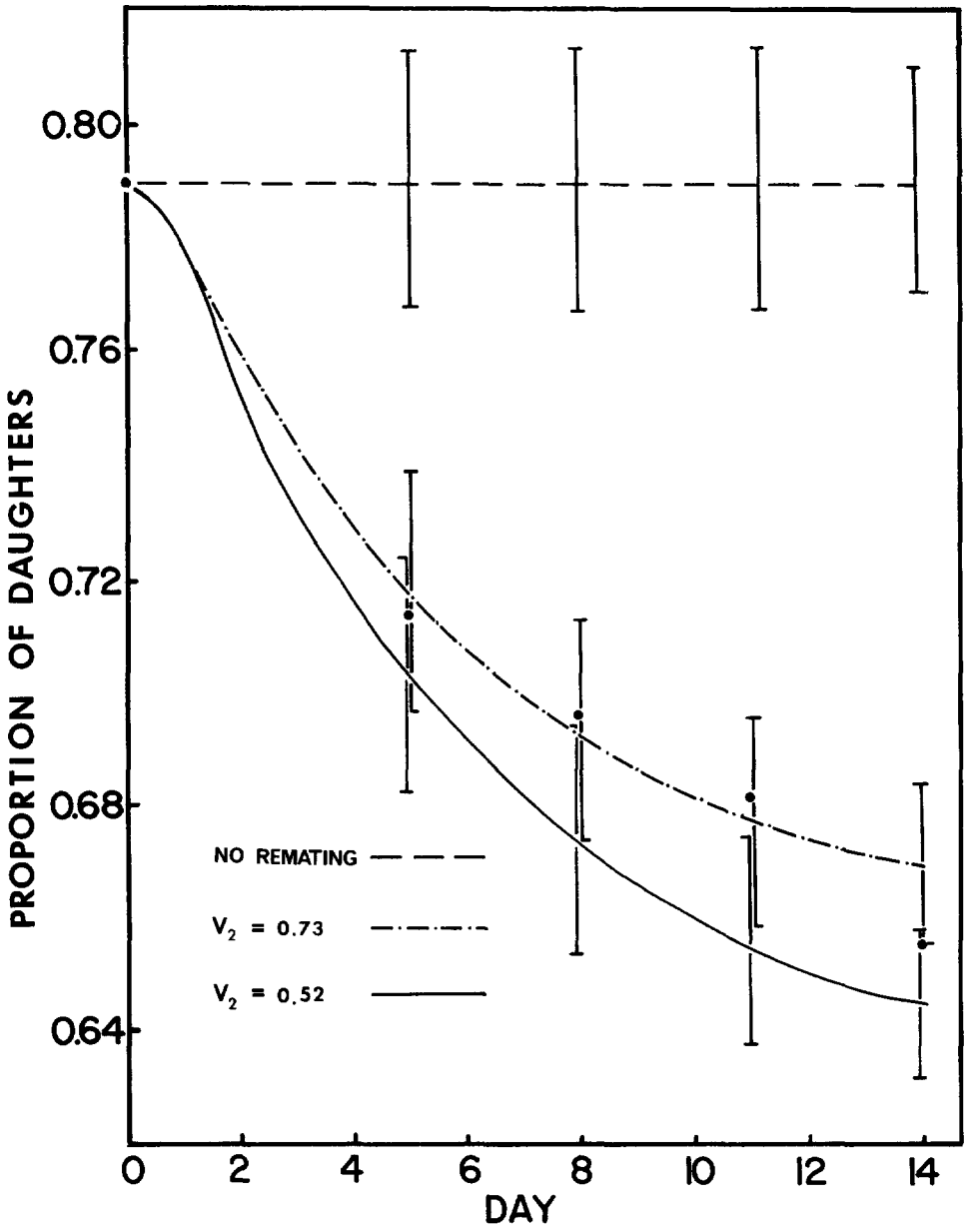

FIGURE 4.—Observed *vs.* expected proportions of daughters. The dots represent observed values in Table 2. The lines are expected values based on A11 of APPENDIX. Vertical bars are standard errors calculated with A12. Paremeter values used, if not specified, are adopted from WU (1983a).

study the phenomenon of multiple mating in detail, it is necessary to track the broods of each individual female through time. This procedure is possible in laboratory studies as were carried out in this investigation. In nature, a reliable technique in aging the Drosophila females is probably necessary.

The simulation of female matings by a Poisson process is only an approxi-

mation. It has been shown that Drosophila species exhibit strong diurnal variation in courting activity (HARDELAND 1972). Therefore, the probability of remating increases with time in a stepwise fashion, instead of a smooth exponential way. There are also reports of a refractory period after mating during which a female would not remate at all. This period is 2 hr in *D. melanogaster* (BUNDGAARD and CHRISTIANSEN 1972) and 12 hr in *D. pseudoobscura* (BECKENBACH 1981). Nevertheless, this refractory period is generally shorter than the periodicity of the diurnal cycle; hence, those newly mated females would have resumed the normal level of receptivity before the next activity peak.

The Poisson process also requires the renewal assumption of waiting time which is justified in this study with only 20 females from one recently isolated stock. The generality of this assumption requires more testing with flies from other stocks.

Figure 1 shows that virility selection depends on the frequencies of males of different genotypes, although none of the parameters governing virility is frequency dependent. It suggests that frequency-dependent virility selection can arise without "females carrying out the difficult task of discriminating between rare and common males" (O'DONALD 1977; BRYANT, KENCE and KIMBALL 1980).

The SR polymorphism: By applying the virility model to the SR polymorphism in *D. pseudoobscura*, it was found that SR males were indeed less virile than ST males, in contrast to the results of BECKENBACH (1978) and CURTSINGER and FELDMAN (1980). Virility selection by itself is not likely to be solely responsible for the SR polymorphism as EDWARD'S (1961) and CURTSINGER and FELDMAN'S (1980) theoretical studies showed. This study, however, throws some light on two puzzling questions concerning the SR trait of *D. pseudoobscura*. First, the search for modifiers in *D. pseudoobscura* has been fruitless (POLICANSKY and DEMPSEY 1978; BECKENBACH, CURTSINGER and POLICANSKY 1982). The absence of suppressor modifiers of sex-ratio expression suggests, in theory, an overall low fitness of SR males relative to ST males (WU 1983b). Previous estimates (WALLACE 1948; CURTSINGER and FELDMAN 1980) of this relative fitness are much too high to prevent the spread of autosomal modifiers in natural populations. The discrepancy between the theory and experimental results can be explained if previous estimates of viabilities are correct but virility selection was underestimated, leading to an overestimate of overall fitness of SR males. This experimental study on virility selection lends some support to such an explanation.

Second, the frequency of SR is in general inversely related with temperature in nature. This negative correlation, reflected in the geographical, temporal and altitudinal distribution of SR (DOBZHANSKY 1944; EPLING, MITCHELL and MATTONI 1957; BALDWIN 1979; BRYANT, BECKENBACH and COBBS 1982), suggests an increasing intensity of natural selection against SR carriers as temperature decreases. In this paper, it is estimated that SR males are 35–50% less virile than ST males at 22° but are 65% less virile at 14°. The virility difference at 14° is mainly due to a drastic reduction in the sperm-displacing ability of SR males despite their normal fertility.

Testing the virility model with SR vs. ST males: The test has many limitations and is suggestive, rather than conclusive, of the virility model. Data in Table 2 were analyzed in two ways to support the present model, and each has its limitation. First, because only a few eggs were laid in the first 2 days at 14°, the proportion of daughters, $A'(t)$, at $t = 0$ (Figure 4) was obtained by using the viability estimates from a control experiment (0.81 for relative viability of ST males *vs.* ST/ST females) and assuming SR and ST males are equally virile with respect to virgin females. Homozygous ST and heterozygous females were assumed to be equally viable. These procedures, although each with empirical basis, may become a major source of error when carried out simultaneously. In the second analysis, only data actually collected from the experimental cage were used. Regression analysis shows a significant trend of decrease in $A'(t)$ ($P < 0.02$). However, if the actual variances associated with $A'(t)$ are much larger than the expected variances (which do take into account many possible sources of variation), the trend may not be significant.

Differential mortality of males in the parental population may also cause some errors, although very minor. Mortality of adults in the population cage at 14° had been monitored and was found to be quite low. In addition, even if half of the cage populations lose 20% of the SR males, we would still expect $A'(t)$ to be only about 1% lower than when there is no differential adult mortality (from A11).

Although the test is not a sensitive one, it does support the idea that virility selection with respect to virgin females underrepresents the overall virility selection. This qualitative conclusion is the main point of this study. Calculations of average remating time (Figure 3) also suggest an important role female remating plays in virility selection.

Finally, I shall comment on the procedure of using the proportion of daughters [$A'(t)$] as an indicator of relative SR virility, in contrast with the direct method of scoring genotypes [*i.e.*, $A(t)$], such as examinations of polytene chromosomes or allozyme markers. As was emphasized, the stochastic component is very large in this system. The numerical example given in RESULTS shows a 12.5% decrease in $A(t)$ in 12 days associated with a standard deviation of 6–8%, if relative SR virility decrease from 1 to 0.5 and $N = 500$. Since, with $A'(t)$, a larger sample is feasible, *e.g.*, $N = 3000$, we find in the same situation that $A'(t)$ shows a 6% decline with a standard deviation of 2–2.25%.

Certainly, viability estimates, some variation in sex-ratio and occasional loss of adult males in the experiment all compound the task of testing this virility model, these difficulties, nevertheless, will not vanish with the adoption of the technically more cumbersome method of direct scoring. The causes of unexpected variations in sex-ratio (due to laboratory procedures, for example) may also result in similar unexpected variations in the frequencies of genotypes. The best solution to filter out these noises is to collect very large samples. The $A'(t)$ statistic is indeed a better one than $A(t)$ in this regard.

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APPENDIX

All of the notations in this section are identical with those in previous sections. The expected value of $F(t)$ is given in (3). By using the same procedure, we obtain

$$E[F(t)^2] = \alpha + [E(F_1^2) - \alpha - \beta]e^{-\lambda t\gamma} + \beta e^{-\lambda tb} \qquad \text{A.1}$$

where $b = b(y) = p - u_2(y)(p - q)$

$$\gamma = 1 - [u_2(1 - q)^2 + (1 - u_2)(1 - p)^2]$$

$$= p(2 - p) - u_2(p - q)(2 - p - q)$$

$$\alpha = \frac{u_2q^2[p + u_2(2 - p - q)]}{b\gamma}$$

and

$$\beta = \frac{2u_2q(1 - q)[E(F_1)b - u_2q]}{b(\gamma - b)}.$$

It follows that $\mathrm{Var}[F(t)] = E[F^2(t)] - [EF(t)]^2$.

Mean and variance of A(t)

In a cohort of m females and a mixture of A and B males, we want to know the proportion of eggs laid by all of the females at time t that are fertilized by B males.

Let n_j be the number of fertilized eggs collected from the jth female in the interval $(t - \Delta t, t + \Delta t)$ and x_j be the number of progeny of B males in that hatch. The total number of progeny is $N = \sum_{j=1}^{m} n_j$. The following quantity

$$A(t) = \sum_{j=1}^{m} x_j/N \qquad \text{A.2}$$

is what we observe in an experiment.

X_j's are independent identical variables and are binomially distributed given n and $F(t)$. Also, n and $F(t)$ are uncorrelated.

$$\begin{aligned} E(X) &= \sum_z \sum_\eta E(X \mid F(t) = z, n = \eta)\mathrm{Prob}(F(t) = z, n = \eta) \\ &= \sum_z \sum_\eta z\eta\ \mathrm{Prob}(F(t) = z)\mathrm{Prob}(n = \eta) \\ &= E(F(t))E(n) \end{aligned} \qquad \text{A.3}$$

$$\begin{aligned} EX^2 &= \sum_z \sum_\eta E(X^2 \mid F(t) = z, n = \eta)\mathrm{Prob}(F(t) = z)\mathrm{Prob}(n = \eta) \\ &= \sum_z \sum_\eta \{(z\eta)^2 + \eta z(1 - z)\}\mathrm{Prob}(F(t) = z)\mathrm{Prob}(n = \eta) \\ &= E(n^2)E(F^2(t)) + E(n)E(F(t)) - E(n)E(F^2(t)) \end{aligned} \qquad \text{A.4}$$

$$\begin{aligned} \mathrm{Var}\ X &= EX^2 - (EX)^2 \\ &= \mathrm{Var}(n)E(F^2(t)) + (E(n))^2\mathrm{Var}(F(t)) + E(n)(EF(t) - EF^2(t)) \end{aligned}$$

If there is no differential larval viability among genotypes, we can assume $E(n) = N/m$. (This is certainly not always the case, and viability differences will later be incorporated when the virility of SR males is tested.) It is further assumed that $\mathrm{Var}(n) = E(n)$. This would be true if, for example, the zygotic contribution of an individual female in the samples follows the Poisson distribution. We therefore obtain

$$E[A(t)] = \frac{mE(X)}{N} = E[F(t)] \qquad \text{A.5}$$

$$\mathrm{Var}[A(t)] = \frac{E[F(t)]}{N} + \frac{\mathrm{Var}[F(t)]}{m}. \qquad \text{A.6}$$

Estimation of remating rate, λ

This method computes the numerical values of the likelihood, $L(\lambda)$, of obtaining an observed set of data (like those of Figure 2) for each λ. First, we determine the distribution of $F(t)$ numerically.

The distribution of F_i's are easily obtainable. For example, let B - A - B be the mating sequence of a triply mated female.

$$\mathrm{Prob}\{B\text{ - }A\text{ - }B\} = u_1(1 - u_2)u_2 = \mathrm{Prob}\{F_3 = q + (1 - q)(1 - p)\}$$

and

$$\mathrm{Prob}\{A\text{ - }B\text{ - }A\} = (1 - u_1)u_2(1 - u_2) = \mathrm{Prob}\{F_3 = (1 - p)(q + (1 - q)F_1\} = \mathrm{Prob}\{F_3 = (1 - p)q\}$$

and so on.

$I(t)$, as defined, is the mating status of a female at time t. Therefore,

$$\mathrm{Prob}\{F(t) = z\} = \sum_i \mathrm{Prob}\{F_i = z\}\mathrm{Prob}\{I(t) = i\} = \sum_i \mathrm{Prob}\{F_i = z\}g_{\lambda t}(i) \qquad \text{A.7}$$

where g is the Poisson probability with parameter λt. For convenience, z is designated to be either 0.5 or the midvalues of the intervals $(0.5 + l0.05,\ 0.5 + 0.05(l + 1))$, $l = 0, 1, \ldots, 9$.

The computation of L(λ): Given the data of m female Drosophila, the jth one producing n_j offspring and among them x_j are fathered by B males, it is possible to calculate the likelihood of obtaining the given data. The likelihood is a function of remating rate λ.

$$L(\lambda) = \prod_{j=1}^{m} \mathrm{Prob}(x_j \mid n_j, \lambda).$$

For a given n_j and λ at time t (=14 in Figure 2),

$$\mathrm{Prob}(X = x_j) = \sum_z \mathrm{Prob}(X = x_j, F(t) = z) = \sum_z \mathrm{Prob}(F(t) = z)\mathrm{Prob}(X = x_j \mid F(t) = z)$$

$$= \sum_z \mathrm{Prob}(F(t) = z)B(x_j; n_j, z).$$

The first term is provided in A.7, and the second term is the binomial probability. $L(\lambda)$ can, therefore, be calculated.

Testing the virtility of SR males with A'(t)

Let $f(t)$ be the proportion of female progeny emerged from the eggs laid when the mother is aged between $(t - \Delta t,\ t + \Delta t)$ and let D be the proportion of sons among the progeny of SR males. Since $f(t) = \frac{1}{2}(1 - F(t)) + (1 - D)F(t)$, the mean and variance of $f(t)$ can be derived from the mean and variance of $F(t)$.

Mean and variance of A'(t): Let w be the larval viability of males relative to that of females (usually $w \leq 1$). Let n'_j and x'_j be the actual number of progeny and the actual number of daughters, respectively, of the jth female that emerged and were counted, and let n_j be the *corrected* number of progeny surviving to emergence if the relative larval viability of males is 1. Therefore, $n'_j = (n_j - x'_j)w + x'_j$.

We now define $A'(t) = \sum_{j=1}^{m} X_j'/N'$, where $N' = \sum_j n_j'$, and we want to know its mean and variance. We drop the subscript j in the derivation. In analogy to A.3 and A.4 (and let $\mathrm{Var}(n) = E(n)$),

$$EX' = E(f(t))E(n) \tag{A.8}$$

and

$$\mathrm{Var}\, X' = EX' + [E(n)]^2 \mathrm{Var}\, f(t). \tag{A.9}$$

What remains to be worked out is $E(n)$. We have

$$E(n') = \sum_z \sum_\eta E(n' \mid f(t) = z,\ n = \eta)\mathrm{Prob}(f(t) = z)\mathrm{Prob}(n = \eta)$$

and

$$\mathrm{Prob}\{n' = \eta' \mid f(t) = z,\ n = \eta = \mathrm{Prob}\{x' = (\eta' - \eta w)/(1 - w) \mid f(t) = z,\ n = \eta\}$$

$$= \begin{cases} B(x';\ z,\ \eta) & \text{if } x' \text{ is nonnegative integer} \\ 0 & \text{if otherwise} \end{cases}$$

where B is the binomial random variable. Also,

$$E(n' \mid f(t) = z,\ n = \eta) = \sum_{\eta'} \eta' B(x';\ z,\ \eta) = \sum_{x'} ((\eta - x')\, w + x')B(x';\ z,\ \eta) = (1 - w)z\eta + \eta w,$$

therefore, $E(n') = E(n)((1 - w)Ef(t) + w)$, and

$$E(n) = N'/m \cdot (1/\hat{w}) \tag{A.10}$$

where $\hat{w} = (1 - w)E(f(t)) + w$.

Putting A.8 and A.10 together, we obtain

$$E(A'(t)) = mE(X)/N' = E(f(t))/\hat{w}. \tag{A.11}$$

Similarly, combining A.9 and A.10, we obtain

$$\begin{aligned} \mathrm{Var}(A'(t)) &= 1/(N'\hat{w}) \cdot E(f^2) + 1/(m\hat{w}^2)\mathrm{Var}(f(t)) + 1/(N'\hat{w}) \cdot (E(f(t) - E(f^2(t)) \\ &= 1/(m\hat{w}^2) \cdot \mathrm{Var}(f(t)) + 1/(N'\hat{w})E(f(t)) \end{aligned} \tag{A.12}$$