rum), rat serum was found to be best from days 7 to 9. Although rat serum could be used prior to day 7 to replace fetal calf serum and human cord serum, the success rate of culturing mouse blastocysts to the limb bud stage decreased (*7*). The reason for this is unknown. Rat serum is an excellent culture medium for explanted early somite rat embryos (*8*) as well as mouse embryos (*9*). Continuous agitation of the culture medium and embryos may have greatly facilitated the exchange of nutrients, metabolites, and the gases between the embryo and the medium. On day 8 of culture, the embryos rapidly expanded in size and began to establish the blood circulation. A higher percentage of oxygen in the incubator at this stage appeared to be beneficial.

Although mouse embryos can be grown continuously in culture from the blastocyst stage to the limb bud stage, the success rate remains low. A higher success rate will require further improvements of the culture conditions.

L. T. CHEN

Department of Anatomy,
University of South Florida,
College of Medicine, Tampa 33612

Y. C. HSU

Laboratory of Mammalian
Development and Oncogenesis,
Johns Hopkins University,
School of Hygiene and Public Health,
Baltimore, Maryland 21205

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4. Female CF1 mice were superovulated by injecting 5 I.U. of gonadotropin from pregnant mare serum and, 48 hours later, 5 I.U. of human chorionic gonadotropin. Blastocysts, after 3.5 days of gestation, were flushed out of each uterine horn with 1 ml of culture medium (CMRL 1066) and pooled in a 35-mm plastic culture dish containing 2 ml of culture medium which was constantly aerated with 5 percent CO_2 and 95 percent air.
5. Whole mouse embryos were fixed in 0.1*M* cacodylate buffer (pH 7.2) containing 4 percent glutaraldehyde for 2 hours at room temperature and washed in the same buffer for 2 hours. Embryos were then dehydrated in ethanol and embedded in butoxylethanol glycol methacrylate (Polysciences, Warrington, Pa.). Sections 3 μm in thickness were stained with hematoxylin and eosin.
6. Fetal calf serum was obtained from Gibco; human cord serum was prepared from the umbilical cord blood [Y. C. Hsu, *Dev. Biol.* **68**, 453 (1979)], and rat serum was prepared from blood centrifuged immediately after extraction from the rat [C. E. Steele and D. A. New, *J. Embryol. Exp. Morphol.* **31**, 707 (1974)].
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Decay of Female Sexual Behavior Under Parthenogenesis

Abstract. *A laboratory strain of* Drosophila mercatorum *has existed for 20 years without males and therefore without natural selection operating to maintain the genetic basis of female mating behavior. The females of this strain have recently experienced a genetic impairment of mating capacity. This observation exemplifies the mode of evolution of vestigial characters and supports Muller's theory that random mutation will tend to destroy the genetic basis of a character from which selection has been removed.*

Thirty years ago, Muller (*1*) proposed that random mutations would destroy the genetic basis of a character unless natural selection functioned to maintain its integrity. According to the concept, if selection is removed, a character will decay down to the level at which some kind of positive natural selection becomes reimposed. This hypothesis provides a simple genetic explanation for such curious biological facts as the decline of wings to vestiges in flightless birds and the disappearance of eyes in cave animals. Evidence for such an apparently fundamental principle of genetic change in evolution is virtually lacking. Microevolutionary data that directly support Muller's prediction are presented here.

Unisexual (all female) laboratory strains of *Drosophila mercatorum* were established about 20 years ago by selection from normal wild bisexual ancestors (*2*). In the absence of male courtship (the selective agent in this case), a loss of mating propensity in females of an otherwise vigorous unisexual strain was observed. This loss is genetically based and is permanent.

Table 1. Mating speed of females from laboratory strains of *Drosophila mercatorum*. Control bisexual Salvador females (sets A, B, and C) and a control unisexual strain K-23 (sets D and E) are compared with strain S-1, a unisexual derivative of Salvador stock.

Data set	Strain	Year	Females tested (No.)	Mating speed*
		Bisexual		
A	Salvador	1973	167	90.4
B	Salvador	1980	87	84.0
C	Salvador	1981	149	81.9
		Unisexual		
D	K-23	1973	99	80.8
E	K-23	1981	150	74.6
F	S-1	1973	150	85.3
G	S-1	1980	90	20.0
H	S-1	1981	150	30.0
I	S-1-T	1981	160	18.1

*Percent mated in 30 minutes.

The unisexual laboratory strains that we used were established by isolating virgin females from bisexual laboratory stocks and by selecting for the enhancement of a naturally present, low-grade facultative parthenogenesis. Most strains produce occasional sterile males, but these usually number less than 0.5 percent. One strain in particular (S-1) has been maintained in the laboratory by routine culture since July 1961 when it was originally established (*2*, *3*).

All females in these strains are diploid; most oviposit well and produce daughters that are diploid like their mothers. Meiosis occurs normally and a haploid pronucleus is formed. In most strains, including the ones described here, diploidization occurs predominantly by duplication of this single haploid nucleus. This produces a diploid female that is isogenic, that is, homozygous at all loci (*4*–*6*). An alternative mode of diploidization, fusion between the pronucleus and a possibly nonidentical polar nucleus, is ordinarily infrequent. Soon after founding, therefore, each laboratory strain comes to be composed of one or more clones within each of which the females are genetically identical. This effect was demonstrated in experiments in which genetic variants artificially introduced into a stock were observed to survive as a series of coexisting parallel clones for a number of parthenogenetic generations (*7*). Some clones survived longer than others.

Sexual behavior in this species is influenced by an extensive polygenic basis. This has been demonstrated by artificial selection experiments (*8*) and by crosses between bisexual strains (*9*). Among a number of the unisexual strains produced in the laboratory, the mating propensity of the females is not different from that displayed by the ancestral bi-

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sexual strains. Others, however, show lowered propensities, which are permanent genetic properties of these unisexual strains (*10*).

Bisexual stock "S" (collected in El Salvador in 1954 by W. B. Heed) has served as the exclusive source of four separate unisexual strains established from it by selection at different times in 1961 and 1962. Behavioral tests on females from these strains and the control bisexual S stock were carried out in 1973 (*10*). Further tests, with an identical design, were done in 1980–81 and are reported here.

Freshly emerging flies were collected under light ether anesthesia. These were then aged in groups of ten of the same sex, females for 7 days and males (exclusively from S stock) for 14 days. Each experimental test unit was handled as follows. At zero time, 10 females and 20 males were allowed to fly into a clean glass test chamber, and the flies were watched for 30 minutes. As each copulation occurred, the pair was removed by aspiration and the time was recorded. Only one kind of female and one kind of male (the latter were always from S stock) were used at any one time in each test chamber. Control groups consisting of 10 S females and 20 S males were run simultaneously with the experimental groups. An additional control consisted of females from a unisexual strain from Kamuela, Hawaii (K-23). When tested in 1973, females of this strain showed behavior not significantly different from females of the bisexual Salvador control. The K-23 strain was used in 1981 tests as a control on the possible effects of the parthenogenetic mode of reproduction.

Table 2. Kruskal-Wallis tests for heterogeneity of samples. Tests are for heterogeneity among the data sets (from Table 1) listed. Parentheses indicate pooled data; NS, test with $P > .10$.

Data sets compared	Comparisons: Including unmated females		Comparisons: Excluding unmated females	
	χ^2	d.f.	χ^2	d.f.
ABC	88.03***	2		
DE	4.36*	1		
CE	1.36 NS	1		
CEHI	9.12*	3		
(CE) (HI)	183.20***	1		
GHI	8.50*	2		
(GH) I	0.51 NS	1		
HI	7.65*	1	1.73 NS	1
CH	80.07***	1	2.64 NS	1
CI	130.03***	1	5.70*	1
BG	83.91***	1	18.82***	1

*Significant at $P = .05$ (approximately, see text).
***Significant at $P = .0001$ level.

As reported in (*10*), the 1973 tests showed that females from four unisexual strains (S-1, S-6, S-7, and S-11) had mating propensities lower than the control Salvador stock from which they had been derived. Whether these differences had become established at the time each strain was originally founded, or whether they arose at a later time, is unknown. Conditions in S-1 are of special interest. The first behavioral tests were done in 1973, 12 years after the establishment of the strain. At that time it was slightly but significantly lower in mating propensity than the bisexual Salvador control. Since that time, however, females of this line have undergone a new drastic change which serves as the focal point of this report.

This major change is indicated by the behavioral data in Table 1, which shows the raw mating speeds (percent mated in 30 minutes) through time for the bisexual and unisexual controls and S-1. Included are tests of S-1-T, derived from a subculture of S-1 which was sent to the laboratory of A. R. Templeton (then at the University of Texas) in 1975. At Texas, in order to reduce the stock to a single clone, the strain was passed through a single, randomly chosen female for each of eight successive generations and then massed. It was returned to our laboratory and tested by us in 1981.

The low mating level of S-1 (and S-1-T) in 1980 and 1981 as compared with 1973 is apparent by comparing data set F with G, H, and I in Table 1. More detailed presentation and analyses of the performance of the females over the 30-minute test period will be found in Table 2 and Fig. 1.

As the data are clearly non-normally distributed, we used the Kruskal-Wallis test (a nonparametric analog of simple one-way ANOVA) which allows for the simultaneous comparison of multiple data sets with adjustment for ties (*11*). For each test, times until mating (with unmated females arbitrarily assigned a time score of 31 minutes) are pooled for k samples, then ranked in descending order of time until mating. The Kruskal-Wallis test then computes a statistic which has a χ^2_{k-1} distribution under H_0: no mating differences among samples. Improbably large values for the test sta-

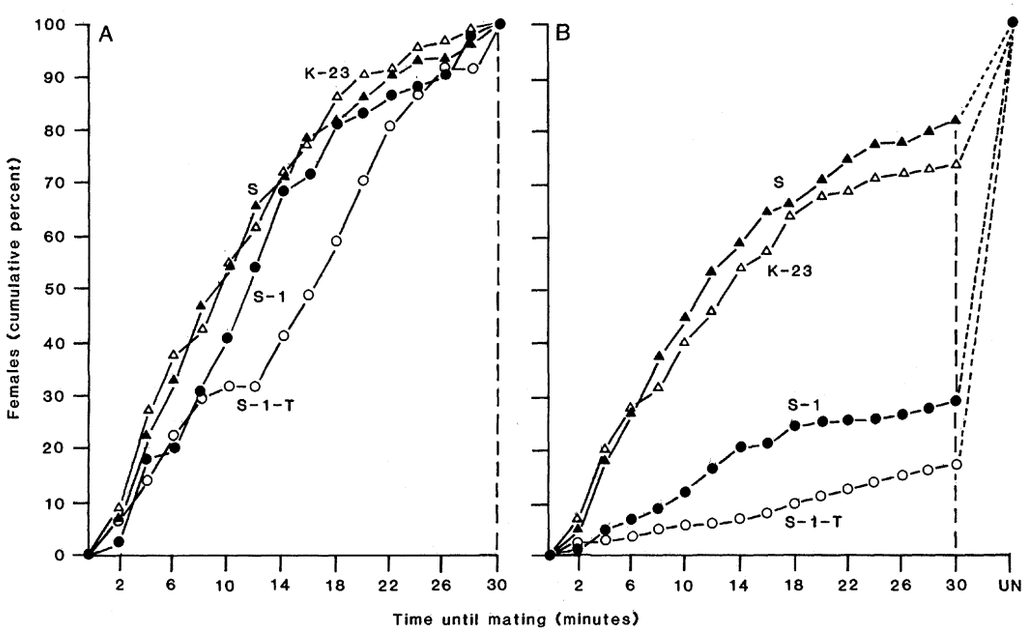

Fig. 1. Mating propensity of four laboratory strains of *Drosophila mercatorum*, 1981 data. (A) Cumulative percent of females mating in 30 minutes; females not mating in 30 minutes have been excluded. (B) Same, but including the class of females unmated after 30 minutes (*UN*). *S*, Salvador bisexual control (▲; C in Table 1); K-23, unisexual control (△; E in Table 1); S-1, unisexual strain (●; H in Table 1); S-1-T, Texas strain of S-1 (○; I in Table 1).

tistic are thus indicative of heterogeneity among the data sets. The multiple comparisons were performed with the use of a standard computer program (MRANK of SAS 79.5; *12*). Since unmated females might either represent "slow" females, that is, simply not inclined to mate in the 30-minute observation period, or qualitatively different "nonmaters", several samples were analyzed with the "unmated" class of females both included and deleted. The results of the Kruskal-Wallis tests for heterogeneity of sample means are presented in Table 2. The significance levels of the various tests were determined with full cognizance that multiple a posteriori comparisons have true significance levels (p values) somewhat higher than those determined from the standard tabled distributions of the χ^2 statistic for single a priori tests.

The 1981 test distributions of Fig. 1 present an interesting problem in interpreting the heterogeneity tests, since the two slow-mating lines S-1 and S-1-T exhibit different patterns depending on whether unmated females are included (Fig. 1B) or excluded (Fig. 1A) from the analysis. In the former case, the two controls (Salvador and K-23) are very similar, and the two slow maters (S-1 and S-1-T) are mildly different; these pairs, however, differ widely from each other [CE, HI, and (CE) (HI), respectively, in Tables 1 and 2]. With unmated females excluded, on the other hand, S-1 becomes indistinguishable from the Salvador controls while S-1-T remains significantly different (see Fig. 1A and CH, CI, right side of Table 2). It is as if the slower overall mating speed of S-1 was attributable not to a rightward shift of the whole mating time distribution, but to the mixture of two impaternate clones with different mating profiles. One of these may mate in a way similar to the controls and includes a peak of nonmaters resulting from the threshold effect of measuring mating for only 30 minutes. The other clone would appear to show little or no tendency to mate at all. Thus, S-1-T, having gone through eight generations of isogenization, may represent a pure strain of slow maters while the relatively large differences between the 1980 and 1981 tests of S-1 might be attributed to stochastic shifts in the relative number of females tested from each of the "mating" and "nonmating" clones.

The general similarity of S-1-T to S-1 permits inference of the approximate time of the major alteration of the behavior of the original S-1 females as occurring some time after the 1973 tests but before 1975, when the subsample was sent to Templeton. Contamination of the S-1 strain by another low-propensity strain can be ruled out, since each of the latter present in the laboratory was carrying a marker gene in homozygous state. Decay of mating behavior as found in the S-1 strain is not unprecedented. Incidental to another study, Henslee (*13*) noted a significant drop in mating propensity in the course of his experiments with a derivative of the unisexual stock RS-3. At the time of his work, the isogenizing nature of the genetic system operating in *D. mercatorum* was not fully understood.

Although we favor a genetic interpretation of this behavioral change, an extrinsic cause, such as a microbial infection, cannot be ruled out. We contend that unisexually reproducing stocks artificially made from bisexual ones provide an exceptional opportunity to test the genetic decay of characters pertaining to sexual reproduction. Females of *Drosophila* are well known to exercise choice in mating (*14*, *15*). Permanent removal of males from the environment of females, therefore, appears to serve as a sensitive test for Muller's hypothesis, outlined earlier. A stock that is automatically isogenizing in each generation may experience a number of random mutations, some of which may be expected to be deleterious to the proper functioning of mating behavior. If they do not have dysgenic side effects, such mutants may become fixed purely by chance, since normalizing natural selection will not function to remove them. Theory relating to the fixation of neutral mutations is well developed (*16*); the present case appears to provide an example; a destructive mutation need not be deleterious and can become incorporated much as a neutral gene change would be. The "decline and fall of the female" in the S-1 strain of *D. mercatorum* serves as a useful microevolutionary model for the regressive evolution of characters to vestiges which have become functionless with regard to the Darwinian fitness of the individual.

HAMPTON L. CARSON
LINDA S. CHANG
TERRENCE W. LYTTLE
Department of Genetics,
University of Hawaii at Manoa,
Honolulu 96822

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Interoceanic Differences in the Reproduction of Coral-Reef Fishes

Abstract. *Eggs of demersal spawning coral-reef fishes of the tropical western Atlantic are smaller than those of related species in the western Pacific. Decreased egg volume may result in increased fecundity per unit body weight of Atlantic species, a factor that may underlie apparent differences in the stability of the respective coral-reef fish communities.*

Interoceanic differences in the ecology of marine animals have been suggested (*1*) but have not been widely documented. Investigators of coral-reef fishes often assume that differences between western Atlantic and western Pacific faunas are minimal (*2*, *3*). This assumption is based primarily on broad systematic overlap at the generic level. Comparison of life histories to test this assumption is difficult because of the often lengthy planktonic larval stages characteristic of these fishes—a stage of development about which little is known. One aspect of reproduction, however, is amenable to comparison: the size of eggs produced by fishes from each area. Egg size has been linked to fecundity, size of newly hatched larvae, and duration of the planktonic larval stages (*4*, *5*). An analysis of egg-size distributions for fishes in the two ocean regions showed that there are statistically significant differences for several important families, which might

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