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James N. Thompson, Jr.
Ronny C. Woodruff, *Bowling Green State University*

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Increased mutation in crosses between geographically separated strains of *Drosophila melanogaster*

(genetic variation/hybrid dysgenesis/insertional sequences)

JAMES N. THOMPSON, JR.*, AND R. C. WOODRUFF†

*Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019; and †Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403

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ABSTRACT **Mutator activity associated with the common male recombination (MR) chromosomes in *Drosophila melanogaster* appears to be suppressed in natural populations. Crosses between geographically separated populations, however, lead to the release of mutator activity as measured by a significant increase in visible mutations. Such an increase in mutation in hybrid individuals may be a powerful factor in inducing or releasing variation in nature, and in more extreme instances may contribute to the separation of microdifferentiated populations.**

Although genetic variation provides the basis for adaptation to changing environments, an incomplete picture of adaptability is given by measuring levels of variation alone. It is incomplete because it assumes that the rate at which new variants are produced in natural populations is low and essentially constant—assumptions that have been brought into question by studies of mutator factors in *Drosophila melanogaster* (1–4).

Although it is clear that various environmental and genetic factors can influence mutation rates (5), genetic factors have been difficult to study because of the technical problems involved in their identification and in the measurement of their activity in natural populations. Recently, however, the study of mutator activity has been given a significant boost. Hiraizumi (6) found that, contrary to common belief, low levels of recombination can occur in the hybrid male progeny of some wild-caught *Drosophila melanogaster* strains crossed to laboratory marker stocks. Male recombination (MR) was subsequently found to be correlated with mutator activity, chromosome breakage, hybrid sterility, distortion of segregation, and other genetic events (2, 7). Thus, MR can be used as a simple assay for mutator strains, providing a unique opportunity to survey mutator activity in natural populations. One of the most surprising discoveries to come from these surveys is the finding that the factors responsible for MR activity are extremely common in nature, being found world-wide in up to 100% of the wild isofemale strains tested (2).

In considering their potential impact upon the genetic structure of natural populations, a second important discovery was that the activity of mutators appears to be genetically suppressed within any particular population (4, 8). Upon crossing a wild strain and a laboratory stock, however, suppression breaks down, resulting in the release of mutator activity and an explosive increase in genetic variation. This breakdown in suppression of mutator activity has been called hybrid release (4).

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The initial studies of MR strains have mainly been concerned with understanding possible mechanisms of mutator activity and have been limited to crosses between a laboratory stock and a wild strain. But in order to extrapolate from the laboratory to natural populations, one must also demonstrate that crosses between different natural populations can increase mutator activity. Unfortunately, what defines the boundaries of a natural population in this cosmopolitan species is notoriously difficult to establish, and we shall simply define different populations on the basis of collections from points widely separated geographically or temporally. In this paper, therefore, we shall describe experiments that show a significant increase in mutation rate in hybridized natural population samples of *Drosophila melanogaster* and we shall discuss briefly some of the influences that hybrid release of mutator activity might have upon the genetic structure and evolution of populations.

MATERIALS AND METHODS

The objective of this study was to measure the spontaneous mutation rate of sex-linked visible mutations in sampled natural populations and in hybrids produced by crossing individuals from these populations. The following wild-caught isofemale lines were used: *M-4* (collected by P. A. Parsons, Melbourne, Australia, 1978); *N-8* and *N-34* (Noble, Oklahoma, July 1977); *N-1316* (Noble, Oklahoma, June 1978); *OK1* [the primary MR line of our earlier studies (see refs. 2, 9)]; *S-4* (Stratford, Oklahoma, July 1977); and *T-25* (Tishomingo, Oklahoma, July 1977). All of these strains show high levels of MR. The laboratory stocks *Canton-S* and *Oregon-R*, which do not show MR activity, were used as controls.

The mutation rate of sex-linked visible loci was measured by a simple breeding program (Fig. 1) similar to that used by Woodruff *et al.* (4). Either males from a wild strain or hybrid males from a cross between two wild strains were mated to females carrying the attached-X chromosome denoted *C(1)DX, y f*. The males produced by such a cross carry the X chromosome contributed by their father. These males can be scored visually for changes in bristle shape, eye and body color, wing or vein expression, and other morphological changes. All presumptive mutations are again mated to attached-X females and retested for expression or stability. Allelism is tested by crossing to phenotypically similar laboratory stocks or by mapping the new mutants. Thus, in a single generation one can measure the frequency of new sex-linked visible mutations and contrast the mutation rates estimated in a series of population samples and in their hybrids.

Abbreviation: MR, male recombination.

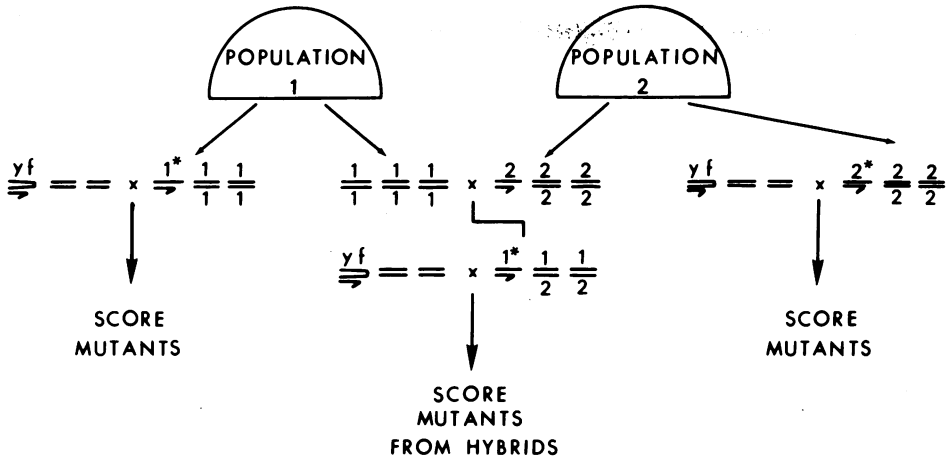

FIG. 1. Breeding program to measure mutation frequencies in two populations and in the hybrid males produced by crossing parents from those populations. Female genotypes are on the left and male genotypes are on the right for each cross. *C(1)DX, y f* females have an attached-X and a free Y chromosome. *, X chromosome on which mutants are detected.

RESULTS

Our primary hypothesis is that mutator activity can be induced or released from suppression by hybridization between individuals drawn from different natural populations. The present experiments extend the study done by Woodruff *et al.* (4), in which mutation frequencies were compared before and after hybridization between individuals from a mutator or control strain and a laboratory assay stock. In this earlier study, males from a tested base population were mated to *C(1)DX, y f* females. The mutant individuals appearing in the first generation represented mutations occurring during sperm development under the genetic and environmental conditions of the base population sample. The F_1 male progeny differ from the parental male, however, in that they are now heterozygous for all autosomes as a result of the hybridization between the base population and the attached-X laboratory stock. Thus, a second cross to *C(1)DX, y f* allows one to measure mutation frequencies in outcrossed or "hybridized" males. Approximately an order of magnitude increase in mutation frequency was found for visible loci after outcrossing. In a similar test of X-linked lethals, about a 3-fold increase was found.

The present experiment is conceptually similar to the earlier study, except that it makes the critical step of inducing hybridization by crossing males and females from geographically separated populations and comparing mutation frequencies in the hybrids with mutation frequencies measured in the original population samples. Because hybridization occurs through matings between individuals drawn from two different populations, the assay cross to *C(1)DX, y f* is limited to a single generation (Fig. 1), so that no direct influence of laboratory genotypes is involved. The series of crosses can be divided into two groups: an initial group in which large samples of chromosomes are assayed in order to establish the role of hybridization in increasing mutation and a second group of smaller data sets in which reciprocal crosses are compared.

The increase in mutation frequencies in hybrid males can be surprisingly high (Table 1). The Stratford and Tishomingo samples were collected at about the same time from roadside fruit stands nearly 45 miles apart. The two Noble samples were collected 1 year apart in the same location (a fairly isolated, forested area near a fisheries research station). The habitat does not maintain a permanent population here, and these temporally separated samples are almost certainly from genetically disconnected populations. All lines showed high MR activity when the mutation assays were done (November 1978 to April 1979). Although occasional mutants were detected in the assays of the four Oklahoma base populations, mutation frequencies were almost an order of magnitude higher in the hybrid males. It is important to recognize, however, that not all interpopulation crosses result in increased mutation, as shown by the cross of *M-4* and *N-34* (Table 2). Because the basis of mutator induction is not yet understood, however, its variation among crosses should not surprise us.

A strong reciprocal cross effect has been reported for chromosome breakage, MR, sterility, and other events correlated with mutator activity in MR strains (2, 7, 8). A reciprocal difference is also observed in the magnitude of mutation frequencies in crosses between populations (Table 2). Of the MR strains in these three sets of crosses, *OK1* is by far the most potent mutator line. Not surprisingly, the frequency of mutation is higher when *OK1* is used as the male parent, which is consistent with the relationship described for other correlated MR events in crosses with laboratory lines (2). The most significant point, however, is that mutator activity is induced in crosses of both directions; the difference is only in magnitude. The possible exception is the cross between *OK1* females and *M-4* males. Mutation frequencies are not significantly higher in the hybrids than in the *M-4* base population from Melbourne, in which mutation is high even before hybridization. An explanation for this might, however, be found in some peculiarity of the population sampled or in its culture or transportation. It is also worth emphasizing that any particular population sample may include individuals derived from hybridization between separate microgeographic races or between migrants and the base population. Until population structure is better understood, there will always be this ambiguity.

Table 1. Frequencies of visible mutations produced in males from four sampled natural populations and in F_1 males from crosses between pairs of populations

Population		Population 1			Population 2			Hybrid (1 × 2)		
1	2	Mutants	Total	%	Mutants	Total	%	Mutants	Total	%
S-4	*T-25*	0	8,905	0.0	2	10,024	0.02	22	11,931	0.18
N-8	*N-1316*	9	11,611	0.08	1	8,033	0.01	58	10,054	0.58

Each of the comparisons of a base population to hybrid mutation rates is significant at least at the 0.01 level by the Kastenbaum and Bowman test (10).

Table 2. Mutation frequencies in reciprocal crosses between wild lines and in the base populations and control lines

Cross		Mutations in hybrid males		
Female parent	Male parent	Mutants	Total	%
OK1	*OK1*	0	13,355	0
M-4	*M-4*	13	6,319	0.21
N-34	*N-34*	3	5,259	0.06
Canton-S	*Canton-S*	0	8,590	0
Oregon-R	*Oregon-R*	0	3,170	0
N-34	*M-4*	4	4,386	0.09
OK1	*N-34*	18	6,314	0.28
N-34	*OK1*	38	2,762	1.38
OK1	*M-4*	8	3,886	0.20
M-4	*OK1*	14	2,635	0.53
OK1	*Canton-S*	5	4,342	0.12
Canton-S	*OK1*	61	2,982	2.04
Oregon-R	*Canton-S*	0	3,654	0
Canton-S	*Oregon-R*	1	3,339	0.03

The array of mutations produced in this series of crosses resembles that reported by others (4, 11). The most common mutations were *singed* alleles and "reduced bristle" mutations, some of which appear to be allelic to *bobbed*. A large number of dominant, deformed eye (*Lobe*-like) mutants were found in some crosses, and *Notch* wing was also frequent. Others included *yellow*, *white*, *miniature*, *lozenge*, *carnation*-like, and several types with deformed or blistered wings. Not all apparent mutations bred true in backcrosses to *C(1)DX, y f* females; those that did not were not included in the final counts, though they may represent somatic mutation. Indeed, several examples of somatic *singed*-like mutant mosaics were identified in our screens.

As reported by others (11, 12), some of the mutants produced by MR outcrosses were unstable, whereas others were not. Not all mutants were tested for stability, but among the samples that were, *singed* alleles were particularly unstable, often mutating to a phenotypically less extreme allele. Limited estimates of backmutation rate show that it is approximately the same as the forward mutation rate (e.g., 1:100 to 1:1000). Occasionally, mutations appeared in small clusters, including a cluster of seven *miniature*, a series of clusters of various sizes in different crosses totalling 72 individuals with deformed eyes, and several clusters of three or four *singed*. Single occurrences of visible mutants also appeared in each of these crosses. Because the question we are asking focuses directly upon the *impact* (i.e., relative number) of MR hybrid-induced mutations, however, clustering does not produce a problem, though it is clearly relevant to discussion of mechanisms. Evidence for premeiotic versus meiotic action of MR factors is often ambiguous (2), and MR lines may even vary in the timing of MR-induced events.

DISCUSSION

Given the large number of individuals typical of most species, even a low mutation rate insures that a large number of new variants will arise each generation. These new variants are insignificant, however, when compared to the existing reserves of genetic variation found in most species. Thus, simple assumptions about recombination and innate reserves of variation lead to the prediction that mutation *per se* would not make an important contribution to changes in rates of evolution. But simple assumptions are not necessarily the best reflection of natural situations. If mutation rates vary in time or in space, changes in mutation rate could have a significant influence upon local population structure. Thus, one important contribution of these MR mutator studies is the attention they attract to possible geographic variation in mutation rates in natural populations.

The results reported here confirm that mutation rate can vary significantly as a function of hybridization between individuals from geographically separated populations. But any attempt to generalize from these studies rests upon the demonstration that MR mutator factors are widespread. Happily, this requirement is well-satisfied by extensive surveys of *Drosophila melanogaster* world-wide (2), and similar phenomena have been reported from at least five other species of *Drosophila* including *D. simulans* and from various other organisms (2).

Given the extensive distribution of mutator factors, the hypothesis of hybrid release (4) has a number of interesting implications and suggests many experiments. The suppression of mutator activity within each tested population is consistent with the expectation that mutation rates are, at least in part, under genetic influence and that rates have been minimized by natural selection. The suppression of mutation rates, like other quantitative traits, can be accomplished by a large variety of different modifier combinations. Indeed, numerous mechanisms could be involved, including cytoplasmic influences, as suggested by the reciprocal cross difference. Thus, the mode of suppression would often be expected to differ somewhat from population to population, depending upon the degree of separation between the gene pools. Hybridization between two populations that differ in the suppressor alleles they carry could, consequently, lead to a disruption of coadapted suppressor gene complexes. The result would be an explosive increase in genetic variation. Such periods of mutational drought and explosion have been documented for *Drosophila* (13, ‡).

Hybrid release of mutator activity would be effective only where the species is composed of reasonably distinct microgeographic populations. Effective population size is, in part, a function of the movement of individuals and of their mating activity. Limited dispersal or mating before dispersal tends to decrease effective population size and can result in microdifferentiated populations. *Drosophila melanogaster* appears to show both limited dispersal and early mating (14) and microdifferentiation of *Drosophila* populations has been reported (15).

Dispersed food resources, particularly during the early part of the growth season for the population, could also result in temporary subdivision of the population. In *Drosophila melanogaster*, Wallace (14) has shown that dispersed food resources can attract migrants from long distances, and migrants appear to have a mating advantage. Thus, one might predict that the mutation rates in new colonies or on a fresh food resource early in the season might be higher than those in later, more established populations, due to the mixing of coadapted gene pools of migrants. In a comparable situation but on a different scale, hybrid zones of introgression between two species might be another situation in which increased mutation rates could be sought (cf. ref. 16). Indeed, mutator activity provides an alternative explanation for the often observed increase in phenotypic variation associated with some hybrid zones. The chromosome breakage that is correlated with mutator activity in at least some MR lines (8) might also come into play here by contributing to isolation between microgeographic races.

‡ Berg, R. (1965) in "Mutation in Population," *Symposium on the Mutation Process (Prague)*.

It is difficult to discuss mutator activity without some consideration of possible mechanisms by which it could occur. The results described here show that heterozygosity at the mutating locus is not a necessary prerequisite, because the breeding program focuses upon X-linked loci in hemizygous males. The mutations, however, are commonly unstable like those reported by others (11, 12). One of the most compelling hypotheses at the moment is that MR mutator factors are similar to the insertional sequences (IS elements) described in procaryotes (17). Several families of dispersed, translocatable, repeated gene sequences are known to be polymorphic in strains of *Drosophila melanogaster*—e.g., copia, 412, 297, and other middle repeated gene families (18, 19). The support for the comparison of MR with such sequences is mainly circumstantial, but includes the fact that insertional sequences can induce mutations by insertion within structural genes or between a structural and a control gene; insertional mutations are often unstable, presumably due to the excision of the element; small deletions, chromosome breakage, and rearrangements are produced by both; both show some site specificity, although insertional sequences at least can recognize many sites at low frequencies; and MR factors are translocatable among chromosomes (20) and may be transmissible by injection or other physical means (21).

An interesting alternative hypothesis is that hybridization between MR lines may break down a coadapted complex involved in the regulation of normal nuclear division and stability. Tests of both hypotheses and further answers to the questions raised by this system can hardly help but clarify the complex relationships that interconnect mutation rate, variation, and evolution in natural populations.

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