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Induced and natural break sites in the chromosomes of Hawaiian *Drosophila*

(radiation/mutagenesis/chromosome rearrangements/genetic variability)

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ABSTRACT **γ-Irradiation of a laboratory strain of the Hawaiian species *Drosophila heteroneura* yielded 310 breaks in the five major acrocentric polytene chromosomes. Their map positions conform to the Poisson distribution, unlike most of the 436 natural breaks mapped in 105 closely related species endemic to Hawaii. Genome element *E* is longer and has more induced breaks than the others. Both in Hawaiian and related species groups, this element shows increased polymorphism and fixation of naturally occurring inversions. The X chromosome (element *A*) also accumulates many natural breaks; the majority of the resulting aberrations become fixed rather than remain as polymorphisms. Although size may play a small role in initial break distribution, the major effects relative to the establishment of a rearrangement in natural populations are ascribed to the interaction of selection and drift. Nonconformance of the natural breaks to the Poisson distribution appears to be due to the tendency for breaks to accumulate both in the proximal euchromatic portion of each arm and in heterochromatic regions that are not replicated in the polytene chromosomes.**

Most sexually reproducing organisms carry extensive genetic variability in their populations. Among the species of *Drosophila*, chromosome rearrangements are commonly found and are useful in establishing phylogenetic continuities (1). The chromosomes of the Hawaiian *Drosophila* have been extensively studied, and the polytene karyotypes have been arranged into lineages reflecting a series of inversions within chromosome arms (2). Since breakage–fusion events account for the origin of inversions, the nature and distribution of break sites may, among other things, relate to special structural and genetic properties of the chromosomes. The occurrence of break sites has been correlated to the proximity of other breaks (3), to the length of chromosomes (4, 5), to the differential radiosensitivity of heterochromatin and euchromatin (5–8), to mutator factors (9–11), and to mobile genetic elements (12, 13). In studies analyzing the distribution of break sites, various patterns emerge depending upon the perspective of the study. These include distal clusters of breaks in natural polymorphisms (3), random distributions of radiation-induced breaks (7), nonrandom clustering of natural and radiation-induced breaks in centromeric and intercalary heterochromatin (6, 8, 14–17), and associations with sites of mobile genetic-element insertion (12, 13, 18).

In the present paper, we compare the pattern of breaks in natural populations accounting for fixed and polymorphic inversions in 105 Hawaiian *Drosophila* species to the pattern of breaks induced by γ-irradiation of male germ cells of one of these species, *Drosophila heteroneura*. If we divide the polytene chromosomes into sectors in an arbitrary but unbiased manner, there are essentially two possible properties of a given division that could explain its exhibition of either an apparent excess or deficiency of observed breakage events. These can be referred to as content and positional properties. Content differences might result from variable amounts of DNA per chromosomal division, the presence of genes capable of mutating to dominant lethality, or unusual DNA sequence organization. Any of these could produce apparent differences in target size. Positional properties would include the location of the chromosomal region relative to heterochromatic blocks or to the centromeric or telomeric ends of chromosome arms. In general, while content properties alone might dictate the rate at which transmissible breaks occur, both content and positional properties could play a role in influencing the probability of establishment of new rearrangements.

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MATERIALS AND METHODS

Adult fertile males of *D. heteroneura* stock T94B18 from the Volcano Experiment Station, Olaa, Island of Hawaii, were irradiated on day 30 after eclosion. A ^{60}Co source was used at a dose rate of 118.3 rads/sec (1 rad = 0.01 Gy) to impart total doses of 1000, 3000, 6000, and 9000 rads to different samples of 15 males each. Once irradiated, the individual males were placed with two nonirradiated females and maintained in serial cultures. Chromosome aberrations induced in the male germ cells were scored by analyzing chromosomes from salivary gland cell dissected from F_1 larvae, fixed in ethanol/acetic acid, 3:1 (vol/vol), and stained in 2% (wt/vol) acetolactoorcein. The location of all breakpoints on each of the five chromosomes was identified on newly prepared photographic chromosomal maps of *D. heteroneura*.

The X chromosome and the four major autosomes of the Hawaiian picture wing group of *Drosophila* species were divided into arbitrary divisions of approximately equal size, based on their appearance in polytene chromosome preparations, in a process as analogous as possible to the methods of Bridges (19). This resulted in a total of 68, 86, 71, 92, and 86 divisions for chromosomes 1 (or X) through 5, respectively. Divisions were numbered from the telomeric to the centromeric ends of these telocentric chromosomes. The numbers of breaks recovered for each division, either those induced by γ-irradiation or naturally occurring in various species, were recorded.

For some statistical tests, it was necessary to have each chromosome partitioned into an equal number of intervals. For these situations, we took the X chromosome with its 68 divisions as the standard and reduced each of the four autosomes to the same number by combining a suitable number of randomly chosen pairs of neighboring divisions. For example, since chromosome 2 has 18 excess divisions, it was necessary to condense 1 in every 4⅞ (=86/18) divisions. This was accomplished by choosing 1 of the 86 divisions as an arbitrary starting point and then condensing either the third and fourth, or fourth and fifth divisions as

one moves along the chromosome. In this manner, third and fourth divisions were condensed 4 times and fourth and fifth divisions 14 times, with the order of the 18 condensations chosen randomly.

The number of natural breaks in 103 species was compiled from Carson (2) and the references therein. Also included are unpublished breakpoints from both *Drosophila mimica* and one undescribed new species (the *Pritchardia* fly from the island of Hawaii). However, there are approximately 34 breaks in the X chromosome and 18 breaks in chromosome 4 of *D. mimica* that could not be mapped in *D. heteroneura* and have not been included in Table 1. The distribution of both natural (436) and induced (310) breaks within each of the 340 divisions is recorded in Table 2.

RESULTS

Divisional Differences in Frequency of Breakage Events. We first performed a simple test of goodness of fit of the observed pattern of breaks in Table 2 to a Poisson distribution, both at the level of individual chromosomes and for all five arms combined. The results are summarized in Table 1. It should be noted that the number of breaks induced in the X chromosome would be expected to be approximately double the figure shown. This is due to the fact that about half of the F_1 larvae examined for breaks were males, in which no irradiated X chromosome appears.

For induced breaks there is an excellent fit to the Poisson distribution, providing no support for the existence of either "hot" or "cold" mutational spots. That is, there is no evidence for gross content differences along the chromosomes. Conversely, breakpoints observed in natural populations of species tend to be more clustered, interspersed with regions showing few or no breaks. While this is not easily perceived in Table 2, it is quantitatively measured by the significant χ^2 statistics in Table 1 for all chromosomes except number 5.

However, some forms of clustering would not be detectable by this simple approach. For example, although the number of divisions with five or more observed breaks might approach the number expected under the Poisson distribution, these divisions might lie near each other in the same general chromosomal positions, producing a higher level of clustering. This would represent a positional rather than content property of the chromosome. To identify such higher-order clusters, it is necessary to use a statistical test that is sensitive to spatial correlation in break number for neighboring divisions. These divisions might be adjacent to one another within an individual chromosome or in the same relative position across chromosomes after they are standardized for division number.

Positional Properties of Breakpoint Distributions. To make the examination of the breakpoint distribution more tractable, the 68 divisions were reduced to 17 intervals by combining each 4 successive divisions in each of the five chromosomes (Table 3). Within each interval the numbers of breaks were then converted to ranks and adjusted for ties. The resulting 5 × 17 matrix of ranks served as the basic data set for the nonparametric H test of Kruskal and Wallis in ref. 20. Table 3 lists only rank totals adjusted for ties for both columns and rows. It is clear from the table that there are no significant column effects for either natural or induced breaks; that is to say, there appears to be no support for the notion that "hot spots" for breakage are occurring in similarly positioned divisions across these telocentric chromosomes.

However, there is a significant row effect for both data sets. This can be at least partly attributed to an excess of breaks in both data sets (measured by high row rank totals) contributed by chromosome 4. Since we have no good basis for comparing the actual length of the five chromosomes at the DNA level, it is not possible to determine whether this excess represents a simple overall length effect or an individual chromosome difference in susceptibility to chromosome breakage. There is, in fact, some evidence for a greater size for chromosome 4. In our analysis, it was assigned the greatest number of divisions (92) before standardization. Based on allozyme loci comparisons, the five rod-shaped chromosomes seen in the Hawaiian species appear to correspond to the five basic elements of the *Drosophila* genome: *A*, *C*, *B*, *E*, and *D*, respectively (see Table 1). These correspond to *X*, *2R*, *2L*, *3R*, and *3L*, respectively, of *Drosophila melanogaster* (21). This means that the homologue to chromosome 4 is arm *3R*, the longest arm in *D. melanogaster* (19).

A second contribution to the row effect comes apparently from chromosome 1 (the X), which is underrepresented in induced breaks but overrepresented in the naturally occurring rearrangements. Because a bias against recovery of X rearrangements arises as a consequence of the screening protocol for the induced but not the natural breakpoints, it is difficult to accurately interpret this difference. To add to the complexity, for both chromosomes 1 and 4, there are a number of naturally occurring breaks that were not mapped and consequently were excluded from this analysis, as noted

Table 1. Numbers of induced and natural chromosome breaks resulting in rearrangements

Chromosome	Genome element	Break(s) per chromosome division												Total breaks	χ^2 (df)	P
		0	1	2	3	4	5	6	7	8	9	10	11			
Induced break(s)																
1	*A*	39	21	7	1									38	0.0512 (1)	>0.80
2	*C*	45	31	9	0	0	1							54	0.314 (1)	>0.50
3	*B*	29	24	13	3	1	1							68	0.297 (2)	>0.80
4	*E*	39	29	17	7									84	1.07 (2)	>0.50
5	*D*	39	31	13	3									66	0.0417 (1)	>0.80
Total		191	136	59	14	1	2							310	0.785 (2)	>0.60
Natural break(s)																
1	*A*	24	14	13	8	5	2	1	0	1				108	13.3 (3)	<0.005
2	*C*	59	16	5	2	2	0	2						52	8.49 (1)	<0.005
3	*B*	40	13	7	6	3	0	0	0	0	0	1	1	78	23.8 (2)	<0.001
4	*E*	35	20	11	13	4	6	1	1	1	0	0	0	148	29.6 (3)	<0.001
5	*D*	49	29	5	2	0	1	0						50	0.417 (1)	>0.50
Total		207	92	41	31	14	9	4	1	2	0	1	1	436	129 (3)	<<0.001

df, Degrees of freedom.

Table 2. Distribution of breaks along the length of the polytene chromosomes

Chromosome	Break(s) in each division,* no. 1	11	21	31	41	51	61	68
	Induced break(s)							
1	0 1 1 1 0 2 1 1 1 1 0 1 2 0 2 0 0 0 0 0 0 0 0 1 0 1 0 2 0 0 1 0 0 2 1 1 1 0 0 1 1 0 0 0 0 0 2 0 0 0 1 1 1 0 1 0 0 0 3 0 0 1 0 0 0 0 0 2
2	2 0 1 1 1 1 1 0 2 0 1 1 0 2 0 1 0 0 1 0 1 0 1 1 0 0 0 0 3 1 1 0 0 1 2 2 0 0 0 2 0 1 0 6 0 0 2 0 1 0 0 1 1 0 2 1 0 0 2 0 1 0 2 1 0 0 1 2
3	1 3 0 1 5 0 0 0 1 4 0 0 1 1 1 0 0 1 0 3 0 2 4 0 1 2 0 0 2 2 1 2 1 1 0 1 1 3 0 0 0 1 4 0 0 0 0 0 0 0 2 0 1 1 1 1 1 2 0 2 0 0 1 1 1 0 1 2 1
4	2 3 0 1 1 2 4 1 2 1 3 1 0 0 0 2 1 1 1 0 3 0 0 2 1 3 0 3 2 0 2 4 3 1 0 0 3 3 1 0 2 1 0 0 1 1 0 1 2 0 0 2 0 2 1 0 2 0 0 0 2 3 1 2 0 1 1 2 0
5	2 0 0 0 3 0 1 0 1 0 2 2 0 0 1 1 1 0 4 1 0 0 1 2 3 2 0 0 1 1 2 0 0 2 1 1 2 1 1 0 1 1 2 3 0 1 1 0 0 0 0 0 2 1 1 2 1 1 2 1 1 1 0 0 4 1 0 0
	Natural break(s)							
1	2 2 0 0 4 1 3 0 1 4 0 1 0 0 2 0 1 2 1 0 0 2 0 0 1 2 2 1 3 0 2 1 4 3 1 0 2 2 3 1 3 0 0 0 5 0 0 1 3 2 1 1 3 0 4 1 5 2 6 0 3 0 0 2 4 0 0 8
2	1 0 0 0 0 0 0 0 0 0 1 0 0 1 0 1 0 0 0 0 0 0 0 0 3 2 0 2 1 1 0 0 4 0 0 0 0 1 0 1 1 1 1 6 0 0 0 0 0 1 0 1 0 0 2 1 0 1 4 0 2 4 0 0 2 0 0 6
3	1 0 1 0 0 0 0 2 3 2 0 1 0 2 0 0 1 2 0 1 0 0 0 0 0 0 0 0 0 0 2 1 1 3 0 1 1 3 3 0 0 1 4 0 0 0 0 4 11 1 0 2 1 3 0 0 1 0 1 0 3 0 0 0 0 2 0 0 4 10
4	0 0 0 1 0 1 2 0 1 0 1 3 3 1 1 5 0 5 3 0 4 3 2 4 2 3 1 0 11 1 1 7 1 5 3 0 1 1 0 2 6 3 1 0 0 4 2 5 5 0 5 0 0 2 6 4 5 2 0 1 1 1 5 0 0 3 1 8
5	1 0 0 1 0 0 1 1 1 1 0 3 0 0 0 0 0 0 0 0 0 0 2 3 1 1 1 1 0 2 0 0 1 3 0 0 0 1 1 1 0 1 1 1 0 0 1 0 1 1 1 1 0 1 2 0 1 0 0 0 2 1 1 0 1 0 1 0 1 5

*The number of divisions is adjusted to the length of the X chromosome (see text).

earlier. However, this exclusion simply tends to make the statistical tests for frequency differences between induced and naturally occurring breaks more conservative and would not alter any of our qualitative conclusions concerning these chromosomes.

Even though there is no significant intercolumn variation (as seen earlier), there may still be higher patterns of breakpoint association (e.g., columns with high breakpoint totals may lie near each other). Thus, there could be a general tendency for breaks to be concentrated in columns either in the proximal or distal ends of chromosomes. This can be tested by both the mean successive difference test and the median test (20), both of which check for clumping of like-valued columns along the chromosome intervals. The

Table 3. Statistical tests of break-site distribution

Chromosome	Break(s) in each interval, no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Rank totals
	Induced break(s)																	
1	3	4	3	4	0	1	3	1	4	2	1	2	2	2	3	1	2	402
2	4	3	4	3	1	3	0	5	5	2	7	2	2	4	2	4	3	628.5
3	5	5	5	3	4	6	3	7	3	4	5	0	3	4	4	3	4	846
4	6	8	7	2	3	5	7	8	4	7	3	3	4	3	4	6	4	980.5
5	2	4	5	2	6	3	5	4	4	4	7	2	0	6	5	2	5	798
Rank totals adjusted for ties	243	287	298.5	141	169	214.5	215	291	254	217.5	268	86	120.5	227.5	219.5	183.5	219.5	3655
Median test	A	A	A	B	B	B	B	A	A	B	A	B	B	A	M	B	M	
Succ. diff. test		44	11.5	−157.5	28	45.5	0.5	76	−37	−36.5	40.5	−182	34.5	107.5	−8	−36	36	
	Natural break(s)																	
1	4	8	6	2	4	2	6	6	8	8	3	6	7	8	13	5	12	917.5
2	1	0	1	2	0	0	7	2	4	2	9	0	2	3	5	6	8	496
3	2	2	6	2	4	0	0	4	5	6	5	15	6	1	4	2	14	649
4	1	3	5	10	8	13	6	20	9	4	10	11	10	12	8	7	12	1084
5	2	2	5	0	0	6	3	3	3	3	3	2	3	3	3	2	7	508.5
Rank totals adjusted for ties	101	143.5	216.5	138	159	165.5	210	233	261	216	259	240.5	244.5	221.5	270	204.5	371.5	3655
Median test	B	B	M	B	B	B	B	A	A	B	A	A	A	A	A	B	A	
Succ. diff. test		42.5	72	−78.5	21	6.5	44.5	23	28	−45	43	−13.5	4	−23	48.5	−65.5	167	

Statistical analyses

Median Test			*H* test		
Induced	$u_p = 0.016$	$P = 0.50$	Induced		
Natural	$u_p = 1.294$	$P = 0.098$	Rows	$\chi^2_4 = 19.77$	$P < 0.001$
Succ. mean diff.			Columns	$\chi^2_{16} = 19.36$	$P > 0.25$
Natural	$u_p = -2.428$	$P = 0.008$	Natural		
Induced	$u_p = -1.01$	$P = 0.158$	Rows	$\chi^2_4 = 26.15$	$P > 0.001$
			Columns	$\chi^2_{16} = 20.76$	$P > 0.15$

Letter values for the median test reflect whether a column total was above (A), below (B), or equal to the median (M) value for all columns. Entries for the mean successive difference test (Succ. diff. test) represent the value obtained when the column total immediately to the left is subtracted from the column total above the appropriate entry. For both these tests, the test statistic produced is a unit normal deviate (u_p). Significance levels for these and the χ^2 statistics produced from the *H* test of Kruskal and Wallis appear at the bottom of the table. See text for further discussion.

results of these tests are also summarized at the bottom of Table 3. The median test is not significant for either data set, although there is some apparent tendency for lengthy runs above (A) or below (B) the median (M) for the data set of natural breakpoints. This is revealed more strikingly in the mean successive difference test, which is significant only for natural breakpoints. This apparently reflects a tendency for natural breaks to concentrate in the proximal halves of chromosomes (see the column totals of Table 3).

DISCUSSION

The naturally occurring chromosome breaks reviewed in this paper amount to a historical record of past mutational activity. The resulting gene rearrangements have accompanied the natural processes of adaptation and speciation in this group of species. Most breaks appear to have arisen two at a time and to result in paracentric inversions. Some inversions are clearly recent; for example, some are found in only one species that is geographically confined to the geologically most recent island. Other inversions are fixed in many species; geological, geographical, and phylogenetic information on these species suggests an age for some of the chromosomal mutants of about 5 million years before the present, although some may be even older (2).

The record of past mutational activity has been studied by comparing natural breakage patterns with those obtained by induced breaks. Statistical analysis shows that the induced breaks are distributed along each chromosome according to the laws of the Poisson distribution. In contrast, the natural breaks, with the exception of chromosome 5, are not so distributed. Some chromosomal divisions accumulate an excess of breakpoints (Table 1), and there is a tendency for them to be concentrated in the proximal halves of the chromosomes. Chromosome 4 accumulates excess breaks in both data sets.

The fact that the frequency and distribution of natural breaks is different from freshly-induced ones is not surprising. When a mutant occurs naturally, it becomes subject to the laws of selection in populations; these laws will determine whether the structural change will be eliminated, fixed, or balanced in the heterozygous condition. Only breaks in the latter two categories will be observed in the descendent natural populations.

A partial interpretation of differential break distribution is provided by chromosome 4 (element *E*). Its slightly greater length, suggested by our study, has also been observed in phylads of other related species groups of the subgenus *Drosophila* (22), where it is also principally an acrocentric chromosome.

The extra length of element *E* cannot account, however, for the large excess number of natural breaks in this element; its tendency to display intraspecific chromosomal polymorphism is strong both in the Hawaiian species and in the *Drosophila melanica* and *Drosophila repleta* groups (Table 4). There is also good agreement between the chromosomal arm location of heterozygous inversions and fixed inversions in both the *D. repleta* group (22) and the Hawaiian group (2).

Table 4. Distribution of polymorphic inversions among comparable chromosome arms in several groups of *Drosophila* species (22)

Species group	Chromosome element					Total inversions	No. of species
	A	*B*	*C*	*D*	*E*		
repleta	7	15	5	5	86	118	62
melanica	12	4	1	7	56	80	6
hawaiian	13	18	9	11	35	86	103

By differentially favoring polymorphism in certain chromosomes, natural selection appears to play a role in establishing such patterns in nature.

The data in Tables 1 and 4 show that the X chromosome (element *A*) has a higher proportion of fixed inversions than the other chromosomes. We suggest that interaction between the hemizygous state of the X chromosome in males and the periodic population size reductions in these island- and volcano-hopping species facilitates the fixation of inversions in this group.

In contrast to element *E*, both elements *C* and *D* are relatively invariable in gene order across many species of the subgenus *Drosophila* (Table 4); this tendency may partially explain why our chromosome 5 (*D*) shows a Poisson distribution of natural breaks.

A review of the distribution of natural break sites on the chromosome maps gives certain qualitative indications that are somewhat concealed by the system of establishing divisions for statistical purposes. Thus, breaks arise (or are preserved) at certain apparent highly localized hot spots that undoubtedly contribute to the failure of these distributions to fit the Poisson distribution. Some of these are found at the proximal ends of each chromosome (see division 68 in Table 2). As in other species of *Drosophila*, it appears that these breaks have occurred somewhere within the unreplicated heterochromatin at the base of each polytene chromosome arm; thus, they probably do not represent strictly localized events occurring at a hot spot.

A similar interpretation may be applied to the 15 breaks in divisions 47–48 of chromosome 3 (Table 1). These breaks occur on each side of a single band that has been identified as containing the genes for the 18/28S ribosomal RNA (23). These RNA-encoding genes appear to have reached this interstitial position by one or more inversions that have moved them from a site within the basal heterochromatin. One other possible hot spot draws attention; this is division 29 on chromosome 4, showing 11 breaks. There is no evidence for interstitial heterochromatin in this region. Thus, this is the only area that may be differentially sensitive to breakage. Induced breakage shows nothing unusual in any of these regions.

Finally, natural breaks tend to be concentrated in the proximal halves of the chromosomes. Such a distribution reflects a bias that is most likely to be attributable to an intrinsic positional rather than a content property. There is as yet no evidence that transposable elements play a role in the induction of chromosome breaks in the Hawaiian *Drosophila* species as they do in some other species (12, 13, 18).

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