IDENTIFICATION OF TWO LOCI CONTROLLING CROSSING OVER IN MALES OF *DROSOPHILA ANANASSAE*¹

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KIKKAWA (1938) and MORIWAKI (1940) summarized their independent discoveries of the spontaneous occurrence of crossing over in males of *Drosophila ananassae*, and both investigators concluded that the highly variable recombination frequencies observed in males were under genetic control. World War II terminated these studies and its havoc included most of the laboratory stocks of this species. Because the mechanism of crossing over and other components of chromosome behavior are still only vaguely defined, and because the resolution of these processes may be facilitated by genetic analyses, it has seemed desirable to reinvestigate this extraordinary phenomenon. This report will include a partial analysis of the autosomal cytogenetic correlations in *D. ananassae*, descriptions of recombination between linked markers in males, and the identification of two loci which contribute to the observed variation in recombination frequencies.

MATERIALS AND METHODS

Cultural procedures and cytological techniques used for *D. ananassae* were essentially the same as those developed for *D. melanogaster*. Typical experimental matings involved a single tested parent with two or three testers for seven days in a 25 × 95 mm shell vial containing about 5 ml medium. The medium contained cornmeal, molasses, dried brewer's yeast, agar, and propionic acid and was sprayed with live propionic acid resistant yeast. Cultures were maintained at 25 ± 2°C, and progenies were scored three or four times from the beginning of emergence through the eighteenth day of culture.

The project was initiated with an assortment of mutant and wild-type stocks supplied through the courtesy of Professors D. MORIWAKI, B. H. JUDD, J. S. F. BARKER, and D. FUTCH. This material has provided the basis for a general reconstruction of the genetics of *D. ananassae*, much of which is beyond the scope of this report. The markers of immediate interest included, in chromosome 2, *cd* (cardinal eye color), *j* (jaunty wing posture), *ca* (claret eye color), *b* (black body color), *se* (sepia eye color), *ba* (balloon wing structure), and *ey* (eyes reduced or missing). Chromosome 3 markers were *bri* (bright eye color), *pe* (peach eye color), *stw* (straw body color), *px* (plexus wing venation), *ru* (rough eye texture), and *cy* (curly wing posture). The mutants *px* and *cy* were not reliable markers in that their homozygotes sometimes appeared phenotypically wild; all other mutants listed were easily scored and recovered from testcrosses of heterozygotes in frequencies approximating 50%.

The foundation stocks were highly polymorphic for the autosomal inversion sequences described by KAUFMANN (1936) and KIKKAWA (1938) and recognized to be widely distributed in natural populations. FUTCH (1966) has designated the sequences found in the stock derived from a population on Majuro Island as standard (+) and the inversions as *In(2L)A* which involves

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$\frac{2}{3}$ of the longest element in the complement, *In(3L)A* with breakpoints at the distal end and about $\frac{1}{3}$ the distance from the chromocenter in *3L*, and *In(3R)A* which is small and proximally situated in *3R*. The existence of these inversions frequently confounded the construction of multiple marker stocks, but they were instrumental in establishing cytogenetic relations.

Three stocks were used extensively as genetic and cytological testers. A wild-type stock (labeled "Texas," but of obscure derivation) segregating for all three inversions was inbred to produce a structurally homozygous +; *In(3L+3R)A* stock called Texas-3. Through a series of crosses, all chromosome 3 mutants initially available were combined to produce a multiple marker stock designated 3 ple-*px* (*bri pe stw px ru*). Later, the *cy* mutant was discovered in a *v* (vermillion, X-linked) stock and transferred to 3ple-*px*, replacing *px* to produce a 3ple-*cy* (*bri pe stw ru cy*) stock. Both 3ple-*px* and 3ple-*cy* were diagnosed as homozygous for the *In(2L)A*; ++ sequences. These and all other marker stocks referred to herein were homozygous for the standard sequences in *XL*, *XR*, and *2R*.

RESULTS AND ANALYSIS

KIKKAWA (1938) and MORIWAKI (1938) derived the basic chromosomal and genetic relationships in *D. ananassae*, but the dissimilarity of their array of autosomal markers and those presently available required that these associations be re-established. The requisite information, summarized in Table 1, was accumulated from testcrosses of multiply marked heterozygous females produced from crosses between stocks whose karyotypes have been previously diagnosed; recombination frequencies involving the unreliable markers *px* and *cy* were obtained by progeny testing the offspring of heterozygous females. The elimination

TABLE 1
Cytogenetic correlations in Drosophila ananassae

Karyotype	Recombination frequencies in females												
	Chromosome 2						Chromosome 3						
	<i>cd</i>	<i>j</i>	<i>ca</i>	<i>b</i>	<i>se</i>	<i>ba</i>	<i>ey</i>	<i>bri</i>	<i>pe</i>	<i>stw</i>	<i>px</i>	<i>ru</i>	<i>cy</i>
$\frac{+}{+}; \frac{+}{+}$		.18	.16	.41		.18							
$\frac{In(2L)A}{In(2L)A}; \frac{+}{+}$		.27		.41	.06			.07	.38	.36	.02	.05	
$\frac{In(2L)A}{+}; \frac{+}{+}$		0		.47	.07			.09	.41		.38		
$\frac{In(2L)A}{In(2L)A}; \frac{In(3L)A}{+}$								0	.02		.41		
$\frac{In(2L)A}{In(2L)A}; \frac{In(3R)A}{+}$								.07	.40	.22	.02	.04	
$\frac{+}{+}; \frac{In(3L+R)A}{+}$	.15	.19											
$\frac{In(2L)A}{+}; \frac{In(3L+R)A}{+}$	0	0						.01	.03		.31		

of recombination in the *cd-j-ca-b* segment by heterozygosity for *In(2L)A* showed the loci represented by these mutants to lie in *2L*, and since this lengthy inversion hardly altered *b-se-ba* recombination, the *se*, *ba* and *ey* mutants probably reside in *2R*. Similarly, gross suppression of recombination in the *bri-pe-stw* intervals by heterozygosity for *In(3L)A* showed *bri* and *pe* to be *3L* markers. The small proximal *In(3R)A*, when heterozygous, reduced recombination only in the *stw-px* region indicating *px*, *ru* and *cy* to be in *3R*. Although the recombination frequencies entered in Table 1 were based on numerically adequate samples, the possibilities of uncontrolled genetic variables as well as rather long unmarked intervals preclude their adoption as standard maps for this species; they will, however, provide a basis for comparison with recombination in males of similar karyotypes and genotypes.

In the course of multiple marker stock construction, sporadic crossing over in males was indicated, usually by the presence of double recessive homozygotes in F_2 progenies from F_1 's bearing linked markers in repulsion. It was not until the discovery of the *cy* mutant that high and repeatable frequencies of recombination in male heterozygotes were found. The most notable aspect of the data from tests of a genotypic and karyotypic variety of males, as presented in Table 2, was the difference in recombination frequency depending upon the 3ple tester stock used to obtain the F_1 males. With one exception (*v* test), the total recombination between chromosome 3 markers ranged between 0 and .005 with 3ple-*px* as the tester parent but from .012 up to .306 with 3ple-*cy* as the tester. It seems remarkable that the highest recombination observed in males was for the structurally heterozygous Texas-3/3ple-*cy* tests; here the total frequency approached that of .35 observed in comparable females (Table 1). The consistency of this result is shown by the two entries for Texas-3/3ple-*cy* in Table 2 reflecting independent tests which also show, as expected, that the smaller the number of progeny scored per male, the greater the range of recombination between individual males. No differences in the behavior of F_1 males from reciprocal crosses have been found for either tester.

The difference between the 3ple-*px* and 3ple-*cy* testers extends to recombination in chromosome 2 as revealed by the data in Table 3 from F_1 males testcrossed to appropriate 2ple marker females. The maximum recombination frequency observed for 3ple-*px* males was .002 but 3ple-*cy* male values ranged up to .085 in the *j b se* test. Apparently, the behavior of *j ca b se*/3ple-*cy* males is exceptional; although heterozygosity for *In(2L)A*, which completely suppressed recombination in the *j-ca-b* intervals in females, partially accounts for this, the near absence of recombination in the *b-se* region adjacent to the inversion is not consistent with presumably comparable regions in chromosome 3. The extensive variability in recombination frequency between males within tests recorded in this table, and also for the parallel tests of these stocks presented in Table 2, probably does not reflect gonial propagation of mitotic exchanges. Examination of the distribution of recombinants within the progeny of single males revealed no conspicuous clustering with respect to either regions involved or complementary classes (see also KALE 1969). On the contrary, meiotic origin of the recombi-

TABLE 2
Male recombination frequencies in chromosome 3 of *Drosophila ananassae*

Tested stock	F ₁ male karyotype*		Tester stock	Number of		Recombination frequencies						Individual male range
	2L	3L3R		Tested F ₁ males	Progeny scored	bri	pe	stuv	ru	Sum		
<i>j ca</i>	<i>In</i>	+	3ple-pr	20	1263	.002	.002	.002	.002	.005	0-.04	
	+	+	3ple-cy	12	884	.009	.051	.065	.065	.124	.07-.21	
<i>ba</i>	<i>In</i>	+	3ple-pr	23	2008	0	0	.004	.004	.004	0-.02	
	+	+	3ple-cy	11	848	0	.165	.104	.104	.269	.20-.41	
<i>v</i>	<i>In</i>	+	3ple-pr	15	1697	.002	.131	.108	.108	.242	.14-.39	
	<i>In</i>	+	3ple-cy	10	1351	0	.068	.036	.036	.104	.07-.18	
Texas-3	<i>In</i>	<i>In</i>	3ple-pr	18	946	0	0	.004	.004	.004	0-.04	
	+	+	3ple-cy	16	3354	0	.110	.196	.196	.306	.25-.37	
	+	+	3ple-cy	35	3887	0	.106	.196	.196	.302	.06-.51	
	<i>In</i>	<i>In</i>	3ple-pr	8	854	0	0	0	0	0	0	
<i>b se ey</i>	<i>In</i>	+	3ple-cy	8	579	0	.010	.002	.002	.012	0-.06	
	<i>In</i>	+	3ple-cy	9	1177	0	0	0	0	0	0	
<i>j b se</i>	<i>In</i>	?	3ple-pr	8	981	0	.016	.020	.020	.036	.01-.08	
	<i>In</i>	+	3ple-cy	17	797	0	0	0	0	0	0	
<i>cd b se ba</i>	<i>In</i>	+	3ple-pr	23	1657	.001	.063	.038	.038	.102	.02-.19	
	<i>In</i>	+	3ple-cy	10	1232	0	0	0	0	0	0	
<i>cd b ba</i>	<i>In</i>	?	3ple-pr	8	1063	0	.034	.017	.017	.050	0-.16	
	<i>In</i>	+	3ple-cy	18	1453	0	0	.004	.004	.004	0-.06	
<i>j ca b se</i>	+	+	3ple-pr	24	4351	0	.087	.051	.051	.138	.05-.26	

* In some tested stocks, sequence heterozygosity prevented complete specification of karyotypes.

TABLE 3
Male recombination frequencies in chromosome 2 of *Drosophila ananassae*

Tested stock	F ₁ male karyotype*		Tester stock	Number of		Recombination frequencies								Individual male range
	2L	3L3R		Tested F ₁ males	Progeny scored	cd	j	ca	b	se	ba	cy	Sum	
<i>b se ey</i>	<i>In</i> + +	+	3ple-pr	6	764					0	0		0	0
	<i>In</i> + +	+	3ple-cy	9	1073					.017	.013		.030	0 -.07
<i>j b se</i>	<i>In</i> + ?	?	3ple-pr	8	1249			0		.001			.001	0 -.01
	<i>In</i> + +	+	3ple-cy	9	1230			.058		.027			.085	.02-.22
<i>cd b se ba</i>	<i>In</i> ? +	+												
	<i>In</i> + +	+	3ple-cy	22	692		.014			.009	.006		.029	0 -.10
<i>cd b ba</i>	<i>In</i> ?	?	3ple-pr	10	1289		0				.002		.002	0 -.01
	<i>In</i> + +	+	3ple-cy	9	630		.033				.006		.040	0 -.10
<i>j ca b se</i>	<i>In</i> + ?	?	3ple-pr	13	663		0	0		.001			.001	0 -.01
	<i>In</i> + +	+	3ple-cy	27	4144		0	.001		.002			.002	0 -.01

nants is supported by preliminary cytological observations of anaphase bridge and fragment configurations in both the first and second meiotic divisions in testes from Texas-3/3ple-*cy* males but not in testes from Texas-3/3ple-*px* males.

A comparison of the data in Table 1 with those of Tables 2 and 3 suggests, for marked regions unencumbered by inversion heterozygosity, that there was only a rough proportionality between the regional distribution of exchanges in females and males. The *pe-stw* and *stw-ru* intervals in females had the same amount of recombination and this may be true in the aggregate for males, although inequalities existed in individual tests. Each of these regions is at least 5 times larger than the *bri-pe* region in females, but the male data indicate a much greater ratio. Turning to chromosome 2, the *b-se* interval is the largest indicated for adjacent markers in the female, yet the recombination in males for this region was clearly exceeded by the *j-b* interval in the *j-b-se/3ple-cy* test (cf. the near absence of *b-se* recombination in *In(2L)A/+* males noted above). A prominent example of disproportionality occurred in the *pe-stw* and *stw-ru* intervals in *In(3L+3R)A/+* where their recombination frequencies were .03 + .31 in females as compared to .11 + .20 in males. Another way of examining the regional recombination behavior in males is provided by the occurrence of double crossover strands involving the *pe-stw-ru* intervals (DCO's involving other regions occurred, but in frequencies too low to be of quantitative significance). The coincidence fractions of 2/2.9, 11/14.6, 19/24.0, 1/3.3, and 16/19.3 for the *j ca/3ple-cy*, *ba/3ple-cy*, *v/3ple-px*, *v/3ple-cy* and *j ca b se/3ple-cy* tests, respectively, indicate positive chiasma interference. On the other hand, the two Texas-3/3ple-*cy* male tests yielded coincidence fractions of 96/72.5 and 81/81.0 (28/22.9 for comparable females) reflecting, if not heterogeneity between individual males, independence or even negative interference between exchanges on the two sides of the small heterozygous *In(3R)A*.

If the enhancement of recombination in male heterozygotes associated with the 3ple-*cy* tester stock were attributable to a single gene difference between that stock and the 3ple-*px* tester, the allele in the 3ple-*cy* stock would be dominant (symbolized *E* for Enhancer). The association of *E* with the transfer of *cy* from the *v* stock to 3ple-*px* stock suggests the origin of *E* from the *v* stock. Thus a cross between *v* (*E*) and 3ple-*px* (+) should produce *E/+* *F*₁ males exhibiting high recombination, as confirmed by the data for this test shown in Table 2. A test for segregation of *E/+* was performed by backcrossing *F*₁ Texas-3/3ple-*cy* to Texas-3 (+/+), according to the performance of Texas-3/3ple-*px* males), then testcrossing *F*₂ sons to 3ple-*cy* females to determine their recombination frequencies. There was no segregation among 37 sons bearing NCO *bri pe stw ru cy* strands from the *F*₁ parent (individual male recombination values ranged from .08 to .48), but among 15 sons bearing various diagnostic CO strands, 7 (all *bri pe stw*) produced maximum recombination of .01. The interpretation of these results, that *E/+* segregated in the distorted (45 *E* : 7 +) ratio due to linkage with the markers selected to diagnose for recombination, was then tested by the following procedure. Structurally homozygous females of the constitution *stw + ru cy E/+ px + + +* were mated to *bri* males and their sons, carrying either NCO or

CO strands, were double-crossed, first to 3ple-*px* and then to 3ple-*cy* females. These crosses produced diagnoses for marker constitution and *E*, the latter through measurement of recombination between *bri* and one or more of the other markers (where *px* was the only other marker, the performance of F_3 *px*/3ple-*px* males gave the diagnosis for *E*). The results of this experiment, summarized in Table 4, show that among 162 completely analyzed strands, *E*/+ segregation was precisely 1 : 1 and that *E* is located 1.2 map units to the right of the *cy* marker. This location, and that of 1.7 map units derived from an essentially redundant mapping experiment, supersede an erroneous report (HINTON 1970) giving, as a consequence of direct scoring of the unreliable *cy* marker, the locus of *E* as 2.3 map units to the left of *cy* in the sequence *stw cy ru*. Although the coincidence fractions shown in Table 4 are greater than 1.0 for the sequence specified, they are not statistically significant and any other assumed sequence produces even larger coincidence values.

The heterogeneity in recombination performances for individual males within tests, as noted above for Tables 2 and 3, is amplified by more extensive tests of the *b se* and *j se* stocks presented in Table 5. Thirty-one *b se*/3ple-*px* F_1 males assorted into two groups, 25 with 0 or .01 and six with .04 to .22 recombination between 3ple markers. Randomly selected F_2 sons of the low F_1 males produced maximum recombination of .02 whereas F_2 sons of high F_1 males could be classified as six low and one high. These observations suggested the *b se* stock to be segregating at the *E* locus. A rather more perplexing situation was found in the behavior of F_1 *b se*/3ple-*cy* males among which 59 produced maximum recombination of .02 and only two behaved as expected if the F_1 genotypes were *E*/+ or *E*/*E*. This

TABLE 4

Location of E, based upon recombination in stw + ru cy E/+ px + + + females

Type	Strands recovered Composition	Number	Analysis
NCO	<i>stw ru cy E</i>	52	
	<i>px</i>	48	
SCO 1	<i>stw px</i>	24	Region 1 = .327
	<i>ru cy E</i>	22	
SCO 2	<i>stw</i>	2	Region 2 = .019
	<i>px ru cy E</i>	1	
SCO 3	<i>stw ru</i>	2	Region 3 = .068
	<i>px cy E</i>	3	
SCO 4	<i>stw ru cy</i>	1	Region 4 = .012
	<i>px E</i>	0	
DCO 1,3	<i>stw px cy E</i>	3	coincidence = 6/3.6
	<i>ru</i>	3	
DCO 1,4	<i>stw px E</i>	0	coincidence = 1/0.6
	<i>ru cy</i>	1	

TABLE 5

Heterogeneity of male recombination responses to 3ple-px and 3ple-cy testers

Tested stock	F ₁ male karyotype			Tester stock	Number of		Recombination frequencies					Individual	
	2L	3L3R			Tested males	Progeny scored	<i>bri</i>	<i>pe</i>	<i>stw</i>	<i>ru</i>	Sum	male	range
<i>b se</i>	<i>In</i>	+	+	3ple- <i>px</i>	25	1496	0	.001	.003	.005	0	-.01	
	<i>In</i>	+	+		6	404	0	.035	.067	.102	.04	-.22	
				F ₂ from Low F ₁	17	2008	0	.001	.002	.003	0	-.02	
				F ₂ from High F ₁	6	292	0	0	.007	.007	0	-.02	
					1	108	0	.04	.17	.20			
				3ple- <i>cy</i>	59	4701	0	.002	.003	.006	0	-.02	
<i>j se</i>					2	152	0	.05	.04	.09	.08	-.10	
	<i>In</i>	?	?	3ple- <i>px</i>	20	2045	0	0	.001	.001	0	-.01	
	<i>In</i>	+	+	3ple- <i>cy</i>	7	1024	0	.004	.002	.006	0	-.02	
					1	110	0	.01	.11	.12			
								<i>j</i>	<i>se</i>				
				3ple- <i>px</i>	11	1405		0		0			
				3ple- <i>cy</i>	9	1572		0		0			

anomaly was also observed in tests of the *j se* stock; although there was no indication of the presence of *E* in this case, only one of 17 F₁ *j se*/3ple-*cy* males recombined in the second or third chromosome as predicted by their *E*/+ genotypes. A possible explanation, that the 3ple-*cy* stock was segregating at the *E* locus rather than being homozygous, was contraindicated by the high recombination manifested by every male in combinations of 3ple-*cy* with such stocks as *j ca*, *ba*, Texas-3, and *j ca b se*; an alternative explanation, involving suppression of *E*, is derived from the following results.

In an experiment designed for another purpose, F₁ females from the cross *b se* × 3ple-*cy* were crossed to Texas-3 males. Their F₂ sons, structurally heterozygous for *In*(2L)*A* and *In*(3L + 3R)*A*, were then testcrossed to 3ple-*cy* females. Fifty-two of these males carried from their mothers either NCO *bri pe stw ru cy* or various CO strands that permitted scoring for recombination in the *pe-stw-ru* regions of chromosome 3; the results were highly variable from male to male as shown by the distribution presented in Figure 1 which also includes for comparison the distributions of individual recombination frequencies of the Texas-3/3ple-*cy* and Texas-3/3ple-*px* males from Table 2. Testcrossing F₃ and F₄ wild-type noncrossover (*In*(3L + 3R)*A*/3ple-*cy*) males selected from the progenies of low (0-.01) F₂ and F₃ males showed this variability to persist in the next two generations, but F₄ sons from high (.18-.30) F₃ males produced a distribution curtailed at the lower end. These F₂, F₃, and F₄ distributions are clearly not unimodal, and a major high-low separation paralleling the nonoverlapping distributions of Texas-3/3ple-*cy* and Texas-3/3ple-*px* is indicated. Excluding the F₄ sons of high F₃ males, there were 51 males having recombination frequencies of .07 or above and 46 with .05 or below which points to the hypothesis of an autosomal locus segregating independently of chromosome 3 and with one allele

FIGURE 1.—Total recombination frequencies for the marked intervals *bri-pe-stw-ru* in individual males, all heterozygous for *In(3L+3R)* A. The distribution in column A is for Texas-3/3ple-*cy* males, in B for Texas-3/3ple-*px* males, in C for the F₂ sons from *b se*/3ple-*cy* × Texas-3, in D and E for the F₃ and F₄ sons of low recombination fathers, and in F for the F₄ sons of high recombination fathers.

FIGURE 2.—Total recombination frequencies for the marked intervals *bri-pe-stw-ru* in individual males, all structurally homozygous in chromosome 3. The distribution in column A is for *b se/3ple-cy* males, in B for *ba/3ple-cy* males. Columns C, D, E, and F give, respectively, the distributions for the ++, *b se*, *b +*, and *+ se* strand-bearing F_2 sons of *b se/3ple-cy* females.

acting as a dominant suppressor of the expression of *E*. This allele (symbolized *S*), if present in high frequency in the *b se* and *j se* stocks, would account for their anomalous behavior with the *3ple-cy* tester as well as part of the heterogeneity noted for other tests.

Because of semidominant expression of *b* observed among most of the progenies of the low recombination males in the experiment just described, it seemed probable that the *S* locus should be located in chromosome 2. Mapping of *S* was

attempted by crossing *In(2L)A, b se (S)/+*, *ba (+)* females to 3ple-*cy* males to produce F_2 males which were then doublecrossed, first to 3ple-*cy* females to determine recombination frequencies, then to *b se* females to diagnose for chromosome 2 markers (*ba* was ignored because of its proximity to *se*). Unfortunately, the smeared distribution of recombination frequencies precluded classification of all the 92 tested males as having either *S* or *+*; this is illustrated in Figure 2 in addition to the related distributions for *ba/3ple-cy* males from Table 2 and *b se/3ple-cy* males from Table 5. One can imagine a discontinuity in the distributions in the vicinity of .02 recombination to produce segregation ratios for *S/+* of 50:42 or 48:44. It is more conservative to rely on the weighted mean recombination frequencies of .107 for *++*, .017 for *b se*, .010 for *b +* and .148 for *+ se* for the conclusion that *S* is closely linked to *b*; but since *b* marks the region covered by *In(2L)A*, it is possible to conclude only that *S* probably resides in 2*L*. Obviously, improved resolution of this question is needed.

The variation in recombination frequencies between individual males in the experiment just described can hardly be explained by the segregation of a single pair of alleles, and in various tests where major discontinuities could be attributed to differences at either the *E* and/or *S* loci, there remained significant heterogeneity within a group (e.g., the distributions illustrated in Figure 1). The probability of modifiers of crossing over in males other than the *E* and *S* loci is further indicated by the distinctly higher recombination of *ba/3ple-cy* than of *j ca/3ple-cy* males and of *v/3ple-px* than of *v/3ple-cy* males (Table 2). Genotypic factors, rather than structural heterozygosity, might be responsible for the finding of maximum recombination frequencies in Texas-3/3ple-*cy* males.

That variability of male recombination frequency is not restricted to laboratory marker stocks of uncertain but probably nonrandom origins comes from a survey of stocks derived from seven natural populations on islands of the Pacific Ocean and one in Yucatan, Mexico. According to FUTCH (1966), these stocks either were homozygous for the standard sequence or, if polymorphic for an inversion, bore the standard arrangement most frequently. Each stock was sampled by test-crossing one F_1 male from each of several matings between wild stock males and 3ple-*px* or 3ple-*cy* females. The results of these assays, presented in Table 6, revealed the same kind of variability in recombination observed previously with these testers. Individual males with high recombination in the 3ple-*px* series suggested the existence of *E* in the Niue and possibly the dark Tutuila, Majuro, and Hawaii stocks, whereas all eight of the *+/3ple-cy* tests provided evidence for segregation of *S* in these stocks. If F_1 *+/3ple-px* males carried both *S* and *E*, the presence of *E* would not have been detected, but due to independent segregation, *E* would be manifested in one-half of the F_2 males. Eighteen F_2 males from each of the Truk, Rarotonga, and Palmyra 3ple-*px* tests yielded maximum individual recombination frequencies of .01, thereby failing to demonstrate the presence of *E* in any of these three stocks. Because of the restricted number of *Y*-autosome complements assayed for each stock, the variation exposed does not quantitatively reflect the situation in the stocks, much less the natural population.

TABLE 6

Assay of genomes from wild-type stocks for effects on recombination in males

Wild-type stock	Tester	Recombination frequencies, bri-ru Individual F ₁ males										All males	Progeny scored
Tutuila, dark	3ple- <i>px</i>	0,	.02,	.02,	.03,	.03,	.03,	.03,	.06			.031	622
	3ple- <i>cy</i>	0,	0,	0,	.03,	.03,	.05,	.11,	.11,	.11		.044	542
Niue	3ple- <i>px</i>	0,	0,	0,	.01,	.02,	.04,	.08,	.15,	.21		.049	818
	3ple- <i>cy</i>	.02,	.03,	.03,	.03,	.08,	.11,	.15,	.16,	.43		.131	496
Majuro	3ple- <i>px</i>	0,	0,	0,	0,	0,	0,	0,	.03,	.05		.007	776
	3ple- <i>cy</i>	0,	.02,	.02,	.07,	.09,	.10,	.16,	.33			.119	445
Truk	3ple- <i>px</i>	0,	0,	0,	0,	0,	0,	0,	0,	0		0	755
	3ple- <i>cy</i>	.01,	.02,	.04,	.05,	.08,	.08,	.21,	.23			.082	561
Rarotonga	3ple- <i>px</i>	0,	0,	0,	0,	0,	0,	.01,	.02			.005	624
	3ple- <i>cy</i>	0,	.02,	.04,	.04,	.07,	.07,	.08,	.09			.052	543
Hawaii	3ple- <i>px</i>	0,	0,	0,	0,	.01,	.02,	.05,	.05			.017	574
	3ple- <i>cy</i>	0,	0,	0,	0,	.03,	.04,	.08,	.12			.042	589
Palmyra	3ple- <i>px</i>	0,	0,	0,	0,	0,	0,	0,	0,	0		0	736
	3ple- <i>cy</i>	0,	0,	.01,	.02,	.02,	.02,	.06,	.22			.032	568
Yucatan	3ple- <i>px</i>	0,	0,	0,	0,	0,	0,	0,	0,	.01		.001	955
	3ple- <i>cy</i>	0,	0,	.03,	.05,	.08,	.14,	.16,	.16,	.24		.108	782

DISCUSSION

KIKKAWA (1938) observed recombination between *px* and either *Bb* (Barbed bristles) or *Pm* (Plum eye color, associated with a reciprocal translocation between chromosomes 3 and 4) in frequencies ranging from 0 up to .20 in F₁ males. He deduced the existence in the *px* stock of one or more dominant enhancers (*En*) of male crossing over and, from inadequate linkage data, the location of *En* in chromosome 3. One test for recombination between the *cd* and *e* (ebony body color, probably allelic to *b* of this study) markers in chromosome 2 was negative. In MORIWAKI's (1940) case, recombination was observed in males heterozygous for a variety of chromosome 2 markers with a mean frequency of .004 per 10 "map units" in the female, and he concluded that a dominant enhancer (*En-II*) residing in *2R* was responsible for male crossing over as well as increased recombination in females. More recently, MORIWAKI and TOBARI (1967, 1968) have rediscovered recombination for chromosome 2 markers in males with frequencies as high as .36 for individual males. Additional observations of high spontaneous recombination in male *D. ananassae* have been made by MUKHERJEE (1961) for a pair of markers in chromosome 3 and by KALE (1968) for a trio of chromosome 2 markers; both authors used wild-type stocks derived from Indian populations and reported that recombination frequencies increased in response to selection. In view of these precedents, it is not unexpected that the present study has yielded positive evidence for the occurrence of crossing over in males and for its genetic control.

The relationship between *E* and the *En* or *En-II* factors of KIKKAWA and MORIWAKI is problematical. The presumptive location of *En* in chromosome 3 is consistent with identity to *E*, and a *px* stock from Tokyo, presumably descended from that used by KIKKAWA, has been diagnosed as having *E* (along with *S*; sons of F_1 *px*/3ple-*px* males having 0 recombination segregated as two with .07 and .08 recombination frequencies to five with 0–.02). The failure to demonstrate an effect of *En* on crossing over in chromosome 2 is not critical since the males tested by KIKKAWA may have been heterozygous for either *S* or *In*(2*L*)*A*. In the case of *En-II*, MORIWAKI's assignment of 2*R* linkage would appear to deny identity with *E*; however, this association was derived from data showing heterogeneity of recombination frequencies among females which was not satisfactorily correlated with crossing over in males and which may have been caused by any of several possible and uncontrolled modifiers of crossing over in females. Thus, there is no decisive evidence either for or against the existence of more than one dominant enhancer of male crossing over. As for *S*, its demonstrated presence in a variety of marker and wild-type stocks makes it a likely candidate to explain some of the heterogeneity in male crossing over encountered in previous studies. *E* and *S* together satisfactorily account for the qualitative control of male crossing over, but still undefined modifiers must be invoked to explain the quantitative variability.

The mechanisms through which the *E* and *S* loci operate and interact are quite obscure at this juncture. Although the presence of pachytene bivalents in primary spermatocytes of Texas-3 males (HINTON 1968) is apparently exceptional in the genus *Drosophila*, this observation parallels the genetic data in only suggesting that crossing over occurs in a classical meiotic milieu rather than indicating a specific mode of action for either *E* or *S*. The wide distribution of *E* and *S* in natural populations and especially their effectiveness in male inversion heterozygotes stimulate intriguing questions about the role of these factors in the population dynamics of *D. ananassae*. Speculation on problems such as these may be prudently reserved until information from more detailed cytogenetic analyses is available.

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SUMMARY

The cytogenetics of chromosomes 2 and 3 in *Drosophila ananassae* was resurrected using female recombination frequencies for a new array of markers and their suppression by inversions. The spontaneous occurrence of crossing over in males was rediscovered and shown to depend on a dominant enhancer (*E*) which was mapped in the right arm of chromosome 3 and on the recessive allele of a dominant suppressor (*S*) which was tentatively located in the left arm of chromosome 2. The presence of *E*, *S*, or both, was diagnosed in a majority of the marker stocks assayed as well as in stocks derived from natural populations. Evidence suggestive of additional modifiers of crossing over in males was noted. In males

of the requisite genotype, meiotic crossing over occurred in both large autosomes and in the presence of heterozygous inversions in chromosome 3; in the latter case, the total recombination frequency of .31 was found to approach that of .34 observed in comparable females but the distribution of recombinants within marked regions differed between the sexes.

LITERATURE CITED

- FUTCH, D. G., 1966 A study of speciation in South Pacific populations of *Drosophila ananassae*. Univ. Texas Publ. No. 6615: 79-120.
- HINTON, C. W., 1968 Meiosis in *Drosophila ananassae* males. *Drosophila Inform. Serv.* **43**: 154. —, 1970 Genetic control of crossing over in males of *Drosophila ananassae*. (Abstr.) *Genetics* **64**: s29-s30.
- KALE, P. G., 1968 Spontaneous crossing over in the males of *Drosophila ananassae*: Two-way selection for recombination values. *Japan. J. Genet.* **43**: 27-31. —, 1969 The meiotic origin of spontaneous crossovers in *Drosophila ananassae* males. *Genetics* **62**: 123-133.
- KAUFMANN, B. P., 1936 A terminal inversion of *Drosophila ananassae*. *Proc. Natl. Acad. Sci. U.S.* **22**: 591-594.
- KIKKAWA, H., 1938 Studies on the genetics and cytology of *Drosophila ananassae*. *Genetica* **20**: 458-516.
- MORIWAKI, D., 1938 The genetics of some mutant characters in *Drosophila ananassae*. *Japan. J. Genet.* **14**: 1-22. —, 1940 Enhanced crossing over in the second chromosome of *Drosophila ananassae*. *Japan. J. Genet.* **16**: 37-48.
- MORIWAKI, D. and Y. N. TOBARI, 1967 Male crossing over in *Drosophila ananassae*. *Drosophila Inform. Serv.* **42**: 81. —, 1968 Unusual segregation of the four phenotypes in the dihybrid F_2 generation caused by male crossing over in *Drosophila ananassae*. *Drosophila Inform. Serv.* **43**: 134-135.
- MUKHERJEE, A. S., 1961 Effect of selection on crossing over in the males of *Drosophila ananassae*. *Am. Naturalist* **95**: 57-59.