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Homozygous-viable pericentric inversions for genetic control of *Lucilia Cuprina*

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Summary. The isolation of homozygous-viable pericentric inversions for inclusion in field-female killing (FK) systems in *Lucilia cuprina* is described. From 7,236 irradiated chromosomes screened, 16 pericentric inversions were isolated. Four of these were viable as homozygotes. One of these, In(3LR)14, possesses the properties required for inclusion in FK systems (tight linkage of one inversion break-point to the white-eye gene and substantial genetic exchange within the inversion in heterozygous females).

Key words: Genetic control – Genetic sexing – Pericentric inversions – *Lucilia cuprina*

Introduction

Genetic sexing systems that eliminate field females form the basis for proposed large-scale population control of the Australian sheep blowfly, *Lucilia cuprina* (Foster 1989). These and other genetic sexing systems are usually based on sex limitation of the expression of certain autosomal mutations by combining them with Y-autosome translocations (Whitten 1969, 1979; Curtis et al. 1976; Whitten et al. 1977; Foster et al. 1978, 1985, 1988; Kaiser et al. 1978; Baker et al. 1979, 1980; Rossler 1979; Suguna et al. 1981; Robinson and Van Heemert 1982; Saul 1984).

In most mosquitoes, which frequently have high levels of male recombination, crossover-suppressing inversions are routinely included in genetic sexing strains to maintain the nexus between sex and the critical mutation (Curtis et al. 1976; Kaiser et al. 1978; Baker et al. 1979, 1980). In species with low levels of male recombination, it was originally believed that such measures would not be necessary to preserve the phenotypic separation of the sexes (Whitten 1969; Robinson and Van Heemert 1982). However, experience has led to rejection of this assumption.

The genetic sexing systems tested so far in *L. cuprina* and the Mediterranean fruit fly, *Ceratitis capitata,* have proved unstable in mass-rearing colonies. Breakdown of the linkage between sex and the deleterious autosomal mutations is generally caused by some form of recombinational event in males, followed by selection of certain recombinant genotypes (Foster et al. 1980a, 1985, 1991; Rossler 1982a, b; Hooper et al. 1987; Busch-Petersen 1989). To overcome this problem, the approach adopted in *L. cuprina* has been to incorporate homozygous-viable pericentric inversions on the mutation-bearing chromosomes, to eliminate recombinants between sex and the critical mutation (Foster 1991a). In addition to reducing recombination, inclusion of such inversions increases the genetic death caused by field-female killing systems (Foster 1991a, b).

The present report describes the synthesis and properties of several pericentric inversions on the third chromosome of *L. cuprina*. One-fourth of the inversions isolated were homozygous viable.

Materials and methods

Mutations and strains

The names and symbols of the *L. cuprina* mutations mentioned in the present report are as follows: featherless aristae (ar), crooked bristles (ck), molten eyes (me), rusty body (ru), Scalloped wings (Scl), white eyes (w), white-mustard eyes (w^m), wavy wings (wy), and yellowish eyes (yw) (all on linkage group 3). Descriptions of these mutations are contained in Maddern et al. (1986) and cytological map positions of most of them are summarized in Fig. 1.

Genetic map positions of all except ck, me, and Scl are contained in Foster et al. (1981). Genetically, ck maps 4.3 map

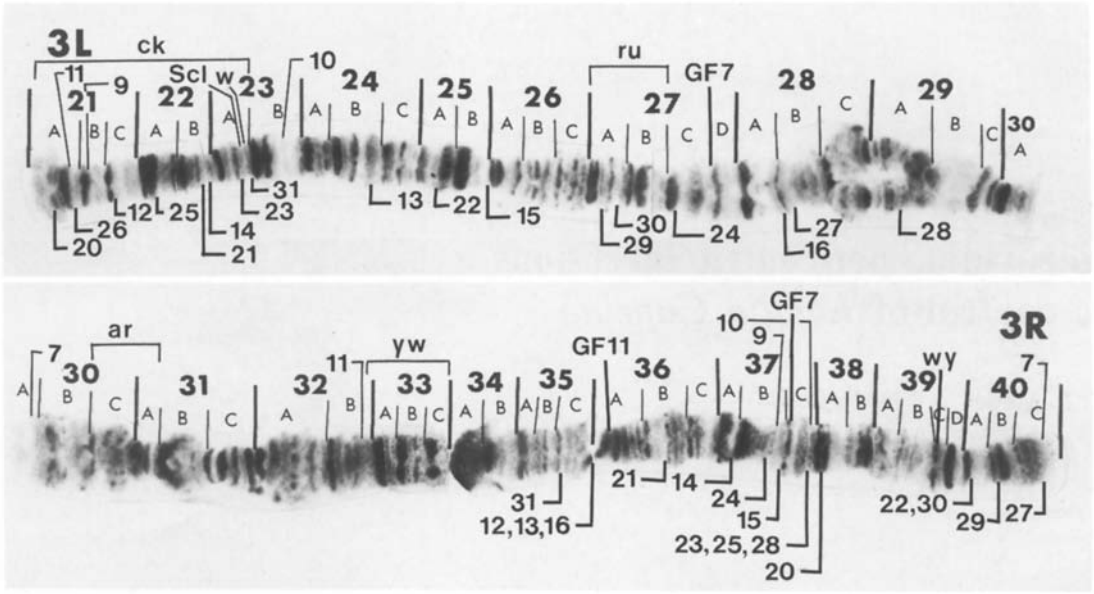

Fig. 1. Inversions and genetic markers plotted on the standard chromosome-3 polytene map (Foster et al. 1980 b). The centromere is located in the constriction at the boundary of 33C–34A. Genetic markers, translocation breaks, and balancer-chromosome inversions are shown above the chromosome. Newly generated pericentric inversions are shown below the chromosome

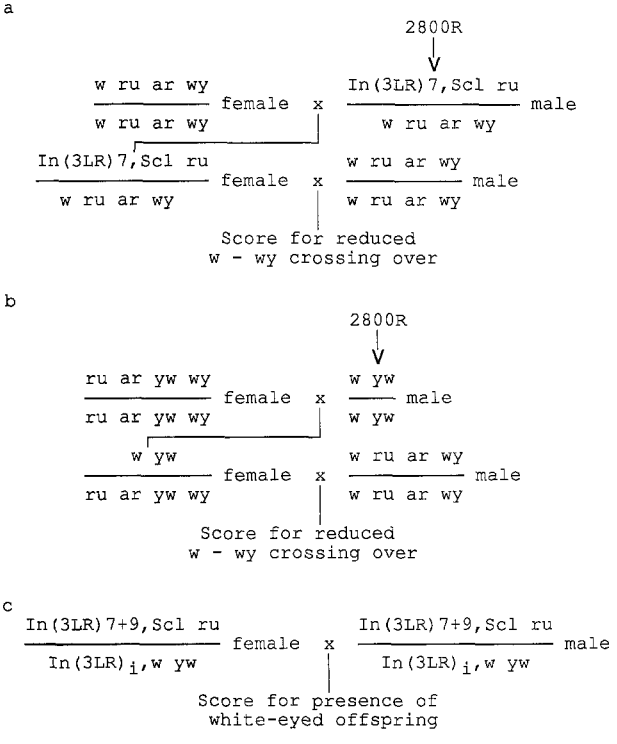

Fig. 2a–c. Crossing schemes used in the present study: **a** method to construct the balancer chromosomes In(3LR)7+9, In(3LR)7+10, and In(3LR)7+11 (see 'Results'); **b** screen for induction of pericentric inversions; **c** test for homozygous viability of pericentric inversions (subscript "i" indicates inversion number) using the balancer chromosome In(3LR)7+9

units to the left of (distal to) w, and me maps 10.2 units to the right of ru and approximately 20 units to the left of ar (unpublished results). Scl is associated with a single-band cytological deficiency in polytene region 23A (Fig. 1) and maps 0.2 map units to the left of w; it is homozygous lethal at the egg stage, and heterozygous adult flies possess thickened wing veins and serrated wing edges (unpublished results). It is similar and probably homologous to the *Drosophila melanogaster* Notch mutations (Lindsley and Grell 1968; Maddern et al. 1986).

The inversion In(3LR)7 (Foster and Whitten 1991) is a homozygous-lethal pericentric inversion spanning the right half of chromosome 3 (Fig. 1). It was isolated in 1974 (induced by irradiation with 2,800 rad gamma rays from a ^{60}Co source) by selection for reduced crossing-over between the mutations w, yw, and wy (G. G. Foster and M. J. Whitten, unpublished results). Other inversions mentioned in this paper are described in 'Results'.

Irradiation

All irradiation treatments involved adult males aged at least 5 days at 27 °C post eclosion. At this stage only mature sperm are treated. The average dose was 2,800 rad gamma rays from a ^{137}Cs source (dose rate 1,725 rad/min).

Construction of balancer chromosome

In order to simplify the screen for homozygous viability of new inversions, a crossover-suppressing balancer chromosome was constructed (Fig. 2a). Mature In(3LR)7, Scl ru males were irradiated and mated to w ru ar wy females. Virgin Scl females were collected from the offspring of this cross and mass-mated to w ru ar wy males. Broods of progeny from individual females were reared and scored for significantly reduced crossing-over between w and wy.

Isolation of pericentric inversions

Pericentric inversions were isolated using the crossing scheme outlined in Fig. 2b. Males homozygous for a lethal-free chromosome carrying the mutations w and yw were irradiated and mass-mated to virgin ru ar yw wy females. Virgin F_1 daughters were mass-mated to w ru ar wy males, broods of progeny were reared from individual females, and progeny were scored for reduced crossing-over between the segregating markers.

The following quick scoring procedure was adopted to save time. The first ten white-eyed flies encountered were examined for the wy phenotype and the culture was discarded if three or more were mutant. The remaining cultures were scored further to determine whether or not the culture should be discarded (generally more than 30% crossovers). Using this procedure, 95% of cultures could be screened at a rate of less than 1 min per culture.

The trichogen-cell polytene chromosomes (Bedo 1982) of putative crossover suppressors were examined cytologically for the presence of inversions or other rearrangements during the generation following the screen. Confirmed inversions were then screened for homozygous viability using the balancer chromosome In(3LR)7+9 (Fig. 2c). In this cross, if the chromosome containing the newly induced inversion [In(3LR), w yw] carried a lethal mutation, all offspring would have wild-type eye color (brownish red). If the newly induced inversion did not contain a lethal mutation, approximately one-third of offspring should be white-eyed.

Scoring recombination in inversion heterozygotes

The following crosses were used to obtain data regarding the effect of the inversions on crossover recovery on chromosome 3 (where the subscript "i" refers to inversion number):

$In(3LR)_i$, w yw/ru ar wy × w ru ar wy

$In(3LR)_i$, w yw/ru wy × w ar wy

$In(3LR)_i$, w yw/ck w^m ru × ck w^m ru yw

$In(3LR)_i$, w yw/ck Scl ru × ck ru yw

$In(3LR)_i$, w yw/ck Scl × ck yw.

In all crosses, females were mass-mated to marker males. Eggs were obtained from individual females and progeny from each female were reared separately. Larvae were reared on a mixture of sheep liver and commercial pet food. All stages were incubated at 27° ± 2 °C.

In crosses where both w and yw were used as genetic markers, special procedures were adopted for scoring crossover frequency, because w is epistatic to all other eye color mutations. Use was made of interactions beween these mutations and the w^m allele of w, in order to detect recombination between w and yw. The eye colors of flies homozygous or heteroallelic for w/w, w/w^m, and w^m/w^m are white, orange, and dark ruby, respectively (Maddern et al. 1986). The mutant combinations w^m yw/w yw and w yw/w yw are both white-eyed. The combinations w^m +/w^m yw and w +/w^m yw are ruby and orange-eyed, respectively. Since white-eyed flies did not give unambiguous genotype determination, only results from nonwhite (i.e., yw^+) offspring are used from these crosses.

In most crosses where recombination in the w – yw region was measured, Scl was used as the marker, since the Scl – w distance is small (see above).

Measurement of egg hatch and fecundity

Egg masses from individual females (on average 208 eggs) were wetted and teased apart, using a soft paintbrush, onto moistened blotting paper in a petri dish, and incubated for 24 h at 27 °C, after which hatched and unhatched eggs were counted. Larvae were then transferred to rearing medium and allowed to complete development. The resulting adult flies were counted to determine fecundity.

Results

Balancer construction

From 644 irradiated In(3LR)7, Scl ru chromosomes scored, 12 gave reduced crossing-over and were saved for retesting. Nine either failed to reproduce or did not breed true as crossover suppressors. The remaining three chromosomes each contained a new inversion which overlapped In(3LR)7: In(3LR)9, In(3LR)10, and In(3L)11 (Fig. 1).

The In(3LR)7+11 chromosome was lost before further testing. Crossover data from the original single inversion and the remaining balancer chromosomes In(3LR)7+9 and In(3LR)7+10 are summarized in Table 1. Although some recombination was detected in each double-inversion chromosome, all were considered suitable for use in the screen for homozygous viability of pericentric inversions (Fig. 2c).

Table 1. Crossover recovery from crosses with chromosome-3 balancer and marker chromosomes

Inversion	Markers	No. scored	Single crossovers[a]				Double crossovers						Triples
			1	2	3	4	1,2	1,3	2,3	1,4	2,4	3,4	
In(3LR)7	Scl ar yw wy	470	163	1	6	–	0	3	11	–	–	–	13
	w ru ar wy	417	63	96	8	–	4	4	16	–	–	–	9
In(3LR)7+9	ck Scl w ar wy	587	0	0	5	1	0	2	1	0	0	0	0
	Scl w me ar wy	157	0	0	0	6	0	0	2	0	2	3	0
In(3LR)7+10	ck Scl w ar wy	613	13	2	4	7	0	0	0	0	0	0	0
	Scl w me ar wy	101	0	1	0	0	0	0	0	0	0	0	0

[a] Crossover regions are numbered from left to right: e.g., for w ru ar wy, regions 1, 2, and 3 are w – ru, ru – ar, and ar – wy, respectively

Table 2. Summary of properties of isolated crossover suppressors

Crossover suppressor	Inversion type[a]	Initial crossover freq.[b]	Screen number scored	Inversion length[c]	Viability[d]
In(3LR)20	AD	0.04	48	88	I
In(3LR)26	AD	0.08	117	86	I
In(3LR)12	AD	0.07	57	74	I
In(3LR)25	AD	0.09	35	82	I
In(3LR)21	AD	0.00	96	72	I
In(3LR)14	AD	0.04	72	75	V
In(3LR)23	AD	0.02	56	77	I
In(3LR)31[e]	BD	0.15	54	65	V
In(3LR)13	BD	0.05	56	55	I
In(3LR)22	BE	0.13	84	75	I
In(3LR)15	BD	0.11	36	64	I
T[In(3LR);5]29	BE/CE	0.22	82	62	–
In(3LR)30	BE/CE	0.26	76	65	V
In(3LR)24	CD	0.21	102	53	V
In(3LR)16	CD	0.22	91	40	I
In(3LR)27	CE	0.15	117	60	I
In(3LR)28	CD	0.34	50	45	I
T(3;6)GF11	–	0.18	22	–	–
T(3;5;6)GF7	–	0.29	21	–	–

[a] For inversion types, see Fig. 3; [b] crossover frequency between w and wy; [c] length is given as percent of standard polytene map (Foster et al. 1980); [d] I = inviable, V = viable as homozygote; [e] originally carried a T(X;3) translocation within the inversion

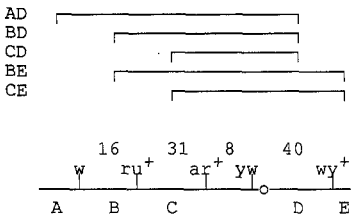

Fig. 3. Inversion type, classified in relation to genetic marker position. Break types A, E – distal to w, wy, respectively; break types B, C, D – between w – ru, ru – ar, yw – wy, respectively. Numerals indicate approximate crossover frequencies between adjacent genetic markers, in the absence of chromosome rearrangements

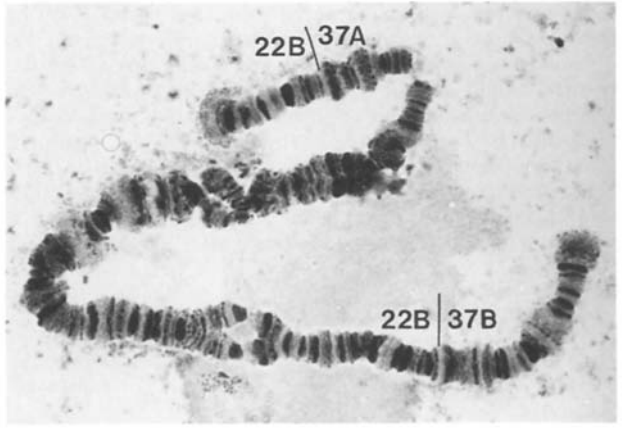

Fig. 4. Polytene chromosome preparation of In(3LR)14 homozygote with inversion break-points marked

Pericentric inversions

From 7,236 irradiated w yw chromosome lines scored for crossover suppression, 29 with reduced recombination were saved and examined cytologically. Of these, 19 carried one or more rearrangements. These rearrangements and the w – wy crossover distance observed in the initial screen are listed in Table 2.

Of the original 29 lines with reduced recombination, 16 had less than 20% crossing-over between w and wy. Of these, 11 carried a single pericentric inversion, 1 carried a pericentric inversion plus an X;3 translocation (Table 2, footnote e), 1 was a reciprocal translocation (T(3;6)GF11), and 3 carried no rearrangement. The remaining 13 original lines had more than 20% crossing-over between w and wy. Four of these had a single pericentric inversion, 1 (T(In(3LR);5)29) carried a pericentric inversion and a 3;5 translocation, 1 carried the complex reciprocal translocation T(3;5;6;)GF7, and 7 carried no rearrangement.

Cytological break-points of all rearrangements recovered are indicated in Fig. 1. Inversions are listed in Table 2 according to the cytological position of the left break-point, from left to right on the standard map (Fig. 1). Inversion type (Table 2) summarizes break-point positions with respect to the major genetic markers used in the present study (Fig. 3).

In the complex rearrangement T(In(3LR);5)29, the inversion and translocation shared one of the breaks. This complex was not tested for homozygous viability. The other complex, T(X;3); In(3LR)31, contained an X;3 translocation with the chromosome-3 break approximately halfway between the inversion breaks. The translocation and inversion In(3LR)31 subsequently separated spontaneously, presumably by a double-crossover event, and a stock was made of the single inversion.

Of the 16 pericentric inversions recovered, 4 were homozygous viable: In(3LR)14, In(3LR)24, In(3LR)30, and In(3LR)31 (Table 2). Figure 4 shows a chromosome pair homozygous for In(3LR)14.

Crossover recovery from pericentric inversions

Crossover data for chromosome-3 genetic markers in inversion heterozygotes are presented in Table 3, with sample data for non-inversion crosses involving w ru ar wy and w ar wy. In general, the frequency of double crossover greatly exceeded that of single crossovers. Crossover recovery was lower in the presence of inversions and was influenced strongly by break-point posi-

Table 3. Crossover recovery from pericentric inversions and marker chromosomes

Genetic markers & inversion	Inversion type	No. scored	Single crossovers*			Double crossovers			Triples 1, 2, 3
			1	2	3	1,2	1,3	2,3	
w – ru – ar – wy									
Noninversion[a]	–	819	70	144	173	13	40	74	5
In(3LR)20	AD	1,209	0	15	61	27	178[b]	211	0
In(3LR)26	AD	1,040	4	30	42	35	127[b]	254	2
In(3LR)25	AD	838	0	2	3	27	71	51	0
In(3LR)21	AD	560	2	0	4	21	82	20	0
In(3LR)23	AD	824	0	0	27	15	46	26	0
In(3LR)31[c]	BD	102	1	2	5	9	13	8	0
In(3LR)22	BE	1,034	94	0	0	0	10[b]	207	7
In(3LR)24	CD	933	221	1	4	0	2	18	0
In(3LR)28	CD	415	100	40	1	1	0	11	4
T(In(3LR);5)29	BE/CE	446	55	82	67	7	20	36	7
w – ar – wy									
Noninversion	–	900	225	214	–	134	–	–	–
In(3LR)20	AD	862	24	58	–	214[b]	–	–	–
In(3LR)14	AD	1,014	1	35	–	228	–	–	–
In(3LR)31	BD	1,258	27	229	–	103	–	–	–
In(3LR)30	BE/CE	981	137	19	–	141	–	–	–
In(3LR)24	CD	834	160	18	–	58	–	–	–
ck – Scl/w – ru – yw									
In(3LR)12[d]	AD	539	2	0	3	0	5	44	0
In(3LR)14[e]	AD	1,328	0	30	58	0	1	94	0
In(3LR)13[d]	BD	993	83	0	3	0	0	13	0
In(3LR)13[e]	BD	1,129	57	10	3	0	1	26	0
In(3LR)15[e]	BD	717	95	33	50	0	3	4	0
In(3LR)16[e]	CD	615	93	165	6	0	0	0	0
ck – Scl – yw									
In(3LR)14	AD	392	0	7	–	0	–	–	–
In(3LR)14	AD	677	1	21	–	0	–	–	–
In(3LR)31	BD	684	6	4	–	0	–	–	–
In(3LR)30	BE/CE	454	58	135	–	11	–	–	–
In(3LR)24	CD	901	177	210	–	12	–	–	–

* See footnote a, Table 1; [a] data from Foster et al. (1981); [b] unequal recovery of reciprocal phenotypic crossover products; [c] crossing-over measured with T(X;3) translocation contained in inversion; [d] markers used – ck w^m ru yw; [e] markers used – ck Scl ru yw

tion. For example, in type AD inversions, in which the Scl/w – ru and ru – ar regions are within the inversion, frequencies of phenotypic single crossovers in these regions are very low. Single crossovers increase in these regions in inversions with more proximal break-points (types B and C, respectively). Similarly, single crossovers in the ar – wy region increase in inversions with type D breaks, with increasing distance of the break from the wy locus (Fig. 1, Table 3).

Crossover recovery in regions outside the inversions was suppressed in the immediate region of the inversion break-point. Crossover recovery in the ck – Scl region was less than 0.1% in crosses with In(3LR)14 and In(3LR)31 (Table 3), compared to the standard crossover frequency of 4.3% (unpublished results). However, where the break-point is further removed, crossover recovery outside the inversions was considerably higher than in noninversion crosses. For example, the frequency of ck – Scl crossovers ranged from 13.7 to 21.0% in crosses with In(3LR)15, In(3LR)16, In(3LR)24, and In(3LR)30.

The crosses with In(3LR)13 show considerable differences in crossover recovery distal to the inversion between the two marker chromosomes, ck Scl ru yw and ck w^m ru yw (Table 3). In the cross with Scl, the ck – Scl crossover frequency was 5.0% and that between Scl and ru 0.9%. In the cross with w^m, the ck – w^m crossover frequency was approximately 3% greater (8.4%), while the w^m – ru crossover frequency decreased to zero. In standard crosses, the Scl – w crossover frequency is 0.2%, with Scl distal to w (unpublished results). Since the Scl and w loci are only a few bands apart on the polytene

chromosomes (Bedo and Howells 1987; and see Fig. 1), it appears that the suppression of crossing-over in regions near inversion break-points may end rather abruptly.

Unequal recovery of reciprocal phenotypic classes

With three of the inversions, certain reciprocal phenotypic classes were recovered in unequal frequencies (Table 4).

Table 4. Unequal recovery of reciprocal crossover products from certain inversions: (a) In, w + +/+ ar wy × w ar wy; (b) In, w + + +/+ ru ar wy × w ru ar wy

Cross-over	Genotype	In(3LR)20	In(3LR)22	In(3LR)26
(a)				
NCO	w + +	334***		
	+ ar wy	232		
1	w ar wy	21***		
	+ + +	3		
2	w + wy	18**		
	+ ar +	40		
1,2	w ar +	168***		
	+ + wy	46		
(b)				
NCO	w + + +	376	364	272
	+ ru ar wy	341	352	274
1	w ru ar wy	0	41	3
	+ + + +	0	53	1
2	w + ar wy	11	0	23**
	+ ru + +	4	0	7
3	w + + wy	26	0	19
	+ ru ar +	35	0	23
1,2	w ru + +	16	0	21
	+ + ar wy	11	0	14
1,3	w ru ar +	124***	3	82***
	+ + + wy	54	7	45
2,3	w + ar +	177***	83**	226***
	+ ru + wy	34	124	28
1,2,3	w ru + wy	0	5	2
	+ + ar +	0	2	0

, * Reciprocal classes significantly different from 1:1 expectation using G-test for goodness of fit, $P<0.01$, $P<0.001$, respectively

In the In(3LR)20 × w ar wy cross (Table 4a), the frequency of reciprocal phenotypes differed significantly from the 1:1 expectation in all four cases. In the In(3LR)20 × w ru ar wy cross (Table 4b), the frequency of reciprocal products differed significantly from expectation in two of the phenotypic double-recombinant classes. Similar differences were observed with In(3LR)22 and In(3LR)26. In the crosses with In(3LR)20 and In(3LR)26, the chromosome carrying the w mutation was usually more prevalent where significant departures from equality occurred, but with In(3LR)22 the other chromosome was favored.

Fertility/fecundity of inversion heterozygotes

Data on fertility (egg hatch) and fecundity (survival to adult stage) of females heterozygous for selected inversions are contained in Table 5. Hatch levels were high, with that for In(3LR)14 not significantly different from the control. Hatch values for In(3LR)12 and In(3LR)16 were significantly different from one another and from In(3LR)14 and the control (Table 5). However, the number of adult offspring reared from inversion heterozygotes ranged from 52 to 60% of those in the control.

Discussion

The preponderance of inversions among the crossover suppressors isolated in the present study (Table 2) contrasts with the results obtained in some other studies. Of the 102 suppressors isolated by Roberts (1970) in *Drosophila melanogaster*, 70 were translocations. In *Ceratitis capitata* all five crossover suppressors recovered by Busch-Petersen (1990) were translocations. These differences could reflect the positions of the genetic markers used with respect to chromosomal sites susceptible to breakage, or they could reflect other procedural variations. Differential susceptibility of chromosome regions to breakage has been well documented in *D. melanogaster* (e.g., Lefevre 1974) and in mosquitos (Rabbani et al. 1977; Robinson et al. 1986). There is some evidence for this in the present study. Three rearrangements were recovered with break-points at the 35C/36A constriction of chromosome 3, and three more with breaks at the 37C/38A boundary (Fig. 1).

Table 5. Egg hatch and survival to adult data from inversion heterozygotes

Female genotype	No. of egg masses	Total eggs	Egg hatch		Adult emergence	
			number	proportion	number	survival
w yw/+ +	12	2,657	2,592	0.976	2,309	0.869
In(3LR)12, w yw/+ +	12	1,994	1,731	0.868	1,041	0.522
In(3LR)14, w yw/+ +	7	1,711	1,677	0.980	794	0.464
In(3LR)16, w yw/+ +	12	2,603	1,891	0.726	1,180	0.453

From Lefevre's (1974) estimate that 50% of rearrangement break-points produced by 2,000–3,000 rad X-rays in *D. melanogaster* are viable, Boussy (1988) inferred that 25% of two-break translocations should be homozygous viable. The present finding, that one fourth of the inversions isolated (with 2,800 rad gamma rays) are viable as homozygotes (Table 2), fits this prediction. Suguna et al. (1981) found 2 homozygous-viable pericentric inversions out of 14 isolated in *Anopheles albimanus*. Boussy (1988) found that 32% ($N=113$) of two-break translocations produced by 5,000 rad X-rays in *D. melanogaster* were viable as homozygotes.

With the exception of the inversions that gave unequal recovery of reciprocal classes, all of the phenotypic single crossovers occurring within the limits of the inversions arise from two exchange events within the inversion: one in the marked region concerned, and one in an unmarked region (e.g., in crosses with w ru ar wy, distal to the w locus). Double crossovers were recovered much more frequently than singles and, except as noted above, represent the products of double-exchange events.

The high frequency of exchange occurring within the larger inversions (Table 3) suggests that the frequency of inviable aneuploid offspring arising from exchange within the inversions should approach 50% (Roberts 1967; Robinson 1975; Rabbani et al. 1977; Suguna and Seawright 1980). The fecundity data (Table 5) support this hypothesis, with adult recovery from inversion heterozygotes approximately half that from noninversion females.

The egg hatch data (Table 5) suggest that a considerable proportion of the aneuploid zygotes generated by crossing-over in these inversions survives the egg stage but fail to reach adulthood. This is expected from inversions with breaks near the chromosome ends, since the terminal duplications and deficiencies from such inversions should be small (Rabbani et al. 1977; Suguna and Seawright 1980). It can be predicted that where such aneuploids hatch, the hatch frequency should be positively correlated with the size of the inversion, since the larger inversions would have smaller (and therefore more viable) distal segments. The data are consistent with this hypothesis, with the longest inversion, In(3LR)14, giving the highest hatch, followed by In(3LR)12 and In(3LR)16 (Tables 2 and 5).

Of the inversions that gave unequal reciprocal progeny classes, all have one break-point very close to one end of the chromosome (3L in the case of In(3LR)20 and In(3LR)26, and 3R in the case of In(3LR)22) (Fig. 1). This suggests that the unequal reciprocal phenotypic classes may arise from the production of viable aneuploid products by exchange events.

Single exchange within In(3LR)20 gives rise to aneuploid chromosomes (Fig. 5) carrying either two copies of polytene map region 38A–40C and deficient for the distal part of region 21A, or the reciprocal duplication/deficiency pair (Fig. 1). The size of the duplicated (wy^+) region generated by In(3LR)20 is well within the range of known viable terminal hyperploids (Lindsley et al. 1972; Konovalov et al. 1983). The terminal deficiency in this chromosome is less than 1% of the total polytene complement, i.e., within the range of viable deficiencies in *D. melanogaster* (Lindsley et al. 1972). The combination of small deficiency and medium-sized duplication is probably not sufficient to prevent survival to adulthood of heterozygotes. The reciprocal product, however, carries a large deficiency that is almost certainly incapable of survival to the adult stage (Lindsley et al. 1972; Konovalov et al. 1983).

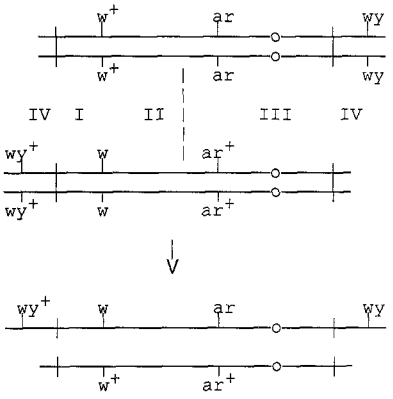

Fig. 5. Generation of aneuploid products by crossing-over within In(3LR)20 heterozygotes

Thus, the reciprocal products recovered more frequently (Table 4) comprise both euploid and viable aneuploid offspring. These may arise from single or double (three-strand and four-strand) exchanges. In the viable aneuploid product, one of the duplicated 38A–40C regions carries the mutant wy allele and the other carries the wild-type wy^+ allele. Hence, these aneuploid chromosomes will give rise to a wy^+ phenotype. Thus, exchange in region I yields + ar +, exchange in region II gives w ru +, and exchange in region III gives w + + aneuploids (Fig. 5). The excess of w ar wy flies (Table 3a) probably results from a three-strand double exchange, with one exchange in region II within the inversion and one outside the inversion (region IV) (Fig. 5). Both duplicated regions in the aneuploid chromosome generated by this type of event carry a mutant wy allele. Because of the relatively small sizes of regions I and IV and possibly interference (since regions I and IV are adjacent in the inversion, and III and IV are adjacent in the standard chromosome), double exchanges involving regions I, IV and III, IV (yielding + ar wy and w + wy aneuploid offspring, respectively) are probably rare.

The reciprocal classes recovered less frequently (Table 4) thus probably consist entirely of euploid genotypes. In the In(3LR)20 × w ar wy cross (Table 4a), the

wild-type (+ + +) offspring arise from double (two-strand or three-strand) exchanges in regions I and II (Fig. 5); w + wy offspring may arise from single exchange in region IV or double exchange in regions I and III; + + wy offspring arise from double exchange in regions II and III; and + ar wy offspring are non-crossovers.

Other inversions with near-terminal break-points, such as In(3LR)7, In(3LR)12, and In(3LR)30, did not give rise to unequal reciprocal recombinant classes. With In(3LR)7, the distal segment at the left end of the inversion is half the length of chromosome 3. With the other two inversions, the distal segments at each end of the inversions were longer than in the inversions that showed distorted recovery (Fig. 1). Presumably the resulting combinations of small deficiencies and large duplications were sufficient to cause mortality before adult eclosion.

Of the homozygous-viable pericentric inversions, In(3LR)14 is the most suitable for inclusion in a field-female killing (FK) strain (Foster 1991 b). The left break-point of this inversion is very close to the w locus (Fig. 1) and is unlikely to recombine with this locus. This is essential for optimum performance of inversions in FK systems, as recombination between the inversion and w would lead to lower genetic death in the field (Foster 1991 b).

In(3LR)14 is also the longest of the viable inversions (Fig. 1, Table 2), and gives substantial levels of recombination between w and yw (Table 3), which comprise an important source of genetic death in this population control system (Foster 1991 b).

The crossover data involving the ck – Scl – ru – yw regions (Table 3) can be used to estimate the frequencies of exchange in the various regions within the inversion, and thus enable estimation of the genetic death possible from a FK strain containing this inversion (Foster 1991 b). Since the recovered double crossovers represent half of the double-exchange tetrads, then if x, y and z are, respectively, the frequencies of exchange between w – ru, ru – yw, and yw and the right break-point of the inversion, the double-exchange frequencies are given by:

$$x \cdot y = 2(D_{xy})$$

$$y \cdot z = 2(D_{yz})$$

$$x \cdot z = 2(D_{xz}),$$

where (D_{xy}), (D_{yz}) and (D_{xz}) are the observed frequencies of double crossovers (from Table 3, classes 2,3; 3+1,3; and 2). The frequencies of double exchange thus estimated are 0.142, 0.089, and 0.045, respectively. Solving the set of simultaneous equations gives $x=0.268$, $y=0.529$, and $z=0.168$.

The frequency of single-exchange tetrads for each marked region, x_s, y_s, and z_s, are calculated as:

$$x_s = x - x \cdot y - x \cdot z$$

$$y_s = y - x \cdot y - y \cdot z$$

$$z_s = z - x \cdot z - y \cdot z,$$

giving values of 0.081, 0.440, and 0.034, respectively. The estimated triple-exchange frequency, obtained from the product of x, y, and z, is 0.024.

The frequency of exchange between the left inversion break-point and yw is estimated from the above data at 0.679, and that between yw and the right break-point at 0.192. The total frequency of exchange within the inversion is thus estimated as the sum of the singles, doubles, and triples, or 0.713. This gives a predicted inversion semisterility (fecundity) level of approximately 36%. This is somewhat lower than the average value of 47% (range of 39 to 52%) suggested by the survival data (Table 5). However, this may simply reflect sampling error.

The results of preliminary trials indicate that flies carrying In(3LR)14 are competitive. These will be the subject of a separate report. Strains carrying In(3LR)14 and various sex-linked translocations are currently being assembled for large-scale testing.

Although In(3LR)14 is the best viable inversion currently available for FK systems, the low frequency of exchange within the inversion to the right of yw restricts both inversion semisterility and the frequency of homozygosis of yw, each of which are important elements in the total genetic death available from such systems (Foster 1991 b). Thus, the search for homozygous-viable pericentric inversions is continuing, with the goal of isolating one whose right break-point is distal to that of In(3LR)14, which should in turn give a higher frequency of exchange within the inversion.

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