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The Use of Bridging Systems to Increase Genetic Variability in Compound Chromosome Strains for Genetic Control of *Lucilia cuprina* (Wiedemann)

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Summary. Genetic variability in the non-compound portion of the genomes of compound-chromosome (CC) strains intended for genetic control can be increased by the use of bridging strains which can be crossed to both CC and normal strains. Two bridging systems are described for chromosome-5 CC strains of *Lucilia cuprina.* The first system relies on the established viability and fertility of males trisomic for chromosome 5R. Males carrying the (5L.YL)23 half-translocation, a C(5R), and a normal chromosome 5 were crossed successfully to a CC strain and a normal strain. The second system uses a pair of reciprocal whole-arm 4;5 translocations to generate gametes disomic for 5R and nullosomic for 5L, which in combination with C(5L)-bearing gametes form viable near-euploid offspring with only small duplications and deficiencies. These offspring (C(5L);(4L.5R)357;(4R.5R)194;(4L.4R)) were crossed successfully with both CC and T(4;5)357/+ individuals. The latter were in turn crossed successfully with normal strains. The T(Y;5)23 system allows replacement of the non-CC genome with wild material more rapidly than the T(4;5)357/T(4;5)194 system, but unlike the latter does not allow replacement of the Y chromosome in the CC strain. The double translocation system is currently being used in *L. cuprina.*

Key words: Genetic control – Compound autosomes – Bridging systems – Inbreeding – *Lucilia cuprina*

Introduction

The ability of compound chromosome (CC) strains to displace chromosomally normal strains under laboratory conditions has led to considerable interest in their use for genetic manipulation and control of pest populations, both in *Lucilia cuprina* (Wiedemann) and *Drosophila melanogaster* (Meigen) (Foster et al. 1972, 1976; Fitz-Earle et al. 1973, 1975; Cantelo and Childress 1974; McKenzie 1976; Whitten et al. 1977). However, despite several attempts with *D. melanogaster* (Cantelo and Childress 1974; Fitz-Earle et al. 1975; McKenzie 1976) and *L. cuprina* (unpublished results), successful replacement of an uncaged field population by a CC strain has not yet been achieved.

The inability to replace *Drosophila* field populations with CC strains has been attributed either to inappropriate genetic backgrounds (Cantelo and Childress 1975) or to ecological factors such as immigration of native flies (Fitz-Earle et al. 1975; McKenzie 1976). The CC strains of *L. cuprina* so far tried in the field have invariably proved to be less trappable (Smith et al. 1981) than other strains (Whitten et al. 1977; Smith and Konovalov unpublished), which suggests that they were less fit. Several hypotheses (genetic and non-genetic), have been advanced in explanation of the low fitness of the CC strains (Whitten and Foster 1975; Whitten et al. 1977; Whitten 1979).

Inbreeding is one probable cause of low fitness in the *L. cuprina* CC strains. Two independent lines of evidence lead to the conclusion that inbreeding can seriously reduce fitness in *L. cuprina.* Firstly, an attempt to isolate an isogenic strain by a scheme of progressive inbreeding involving brother-sister pair matings (in a chromosomally normal wild-type strain) had to be abandoned because fertility and fecundity declined rapidly despite selection in each generation of matings giving high (>81%) egg to adult survival (Foster and Whitten, unpublished). Secondly, it has been frequently observed (Foster and Whitten, unpublished; Maddern, unpublished) that marked improvements in fecundity occur following hybridization between CC strains in which fecundity had declined over several generations. Presumably, the occurrence of genetic bottlenecks either during or after CC strain construction had restricted genetic variability, resulting in increased levels of homozygosis for sub-optimal

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mutations. The observed improvement of fecundity following outcrossing was probably due to increased heterozygosity in the non-compound portion of the genome, since heterozygosity within the compound arms cannot be increased by such hybridization of strains.

There are two major potential sources of inbreeding in CC strains. The first, as noted above, presumably results from accidental restriction of genetic variability in the non-compound portion of the genome. The second type of inbreeding results from meiotic crossing over within the compound chromosomes. On the average, half of all single exchanges within a compound chromosome will lead to homozygosis for the region distal to the exchange, and half of all double exchanges will lead to homozygosis for the region between the two exchanges (Baldwin and Chovnick 1967). If a recessive lethal or sterile mutation is contained in a segment made homozygous, the resulting individual fails to survive or reproduce, and that segment of chromosome will tend to be eliminated from the population. Segments of chromosome which do not contain lethal or sterile mutations will tend to become homozygous, and the proportion of individuals homozygous for the genes on the compounded chromosomes should asymptotically approach unity.

The only methods so far used to introduce new genetic variability into CC strains involve irradiation of wild type flies and mating them to CC flies in order to capture newly induced compound arms (Foster et al. 1972, 1976; Fitz-Earle et al. 1975; McKenzie 1976; Maddern 1981). Offspring carrying such new arms also contain a complete set of non-compounded chromosomes from the wild strain. In theory it should be possible to maintain this level (50% wild genomes) by crossing together individuals carrying newly recovered compound arms. In practice, however, Maddern (personal communication) found in *L. cuprina* that the low frequency of recovery of new compounds made this approach impractical, and necessitated crossing such isolates to established laboratory strains, in some cases for several generations. Thus, although Maddern (1981) was able to recover large numbers of new compound chromosome-5 arms from recently colonized strains, the amount of new genetic material introduced into the non-compound portion of the genome was probably much lower than 50%. In addition, it is uncertain what effect the radiation used to induce new CC arms may have had on the fitness of the resulting strains.

The present paper describes a solution to the problem of inbreeding in the non-compound portion of the genome, and discusses possible approaches to eliminate or minimize the effects of inbreeding within the compound arms. Two systems have been developed in *L. cuprina* for increasing heterozygosity in the non-compound portion of the genome of CC strains, without the use of radiation. Both systems involve the use of "bridging" strains, which are able to successfully breed with CC strains and chromosomally normal strains.

Materials and Methods

Mutations and Strains

The symbols and names of mutations mentioned in the present paper are as follows: Sh (Short bristles) and gl (golden body) on chromosome 4R; to and to^2 (topaz eyes), Sut (Suture), bz (bronze body) and mv (M1-veinless) on chromosome 5L; and sby (stubby bristles) on chromosome 5R. All these mutations except Sut have been described and mapped elsewhere (Witten et al. 1977; Foster et al. 1980b, 1981). Sut (gap in the median transverse thoracic suture) is a dominant visible homozygous lethal mutation which maps to the left of to (Arnold, unpublished).

All translocations were isolated following γ-irradiation of mature sperm using a Co^{60} source. They were detected by pseudolinkage of the appropriate genetic markers (in the present case gl, mv, or maleness) (Foster and Whitten 1974; Foster et al. 1980b).

T(Y;5)23 (Foster et al. 1978) has been partially described by Bedo (1980). The autosomal break is proximal on chromosome 5L, in polytene chromosome region 73C (Fig. 1).

The break points of T(4;5)194 and T(4;5)357 and the approximate positions of the genetic mutations used are indicated in Fig. 1. T(4;5)357 was isolated in a strain carrying the mutation Sh, and carries this mutation on the (4R.5L) half-translocation. The mutation Sut was inserted into T(4;5)194 by meiotic crossing over, and is carried on the (4L.5L) half-translocation.

The CC arms C(5L)1,to^{2}bz mv and C(5R)1,+ were isolated after irradiation of oocytes (Foster et al. 1976). C(5L)3,to, C(5R)2,sby, C(5R)3,sby and C(5R)5,sby as well as the unmarked C(5L)+ and C(5R)+ arms contained in the mass-rearing (MR) lines, were isolated following irradiation of immature spermatocytes (Maddern 1981, Maddern, unpublished). Each MR line was constructed by combining into a single strain 8 new C(5L)+ arms and 8 new C(5R)+ arms, each of which in turn had been isolated following irradiation of a recently colonized (within 12 months) field strain.

Rearing Methods

Rearing conditions were as described by Foster et al. (1981), except that the larval rearing medium used in the present study contained dried meat-meal, minced fresh sheep or beef liver, cotton linters and water in the approximate proportions of 30 : 20 : 3 : 40 by weight.

Results

Sex-linked Translocations as Bridging Strains

Recent studies (Foster et al. 1980b; Konovalov et al., unpublished) have revealed that many individuals carrying Y-linked autosomal duplications, some as large as 11% of the polytene chromosome complement,

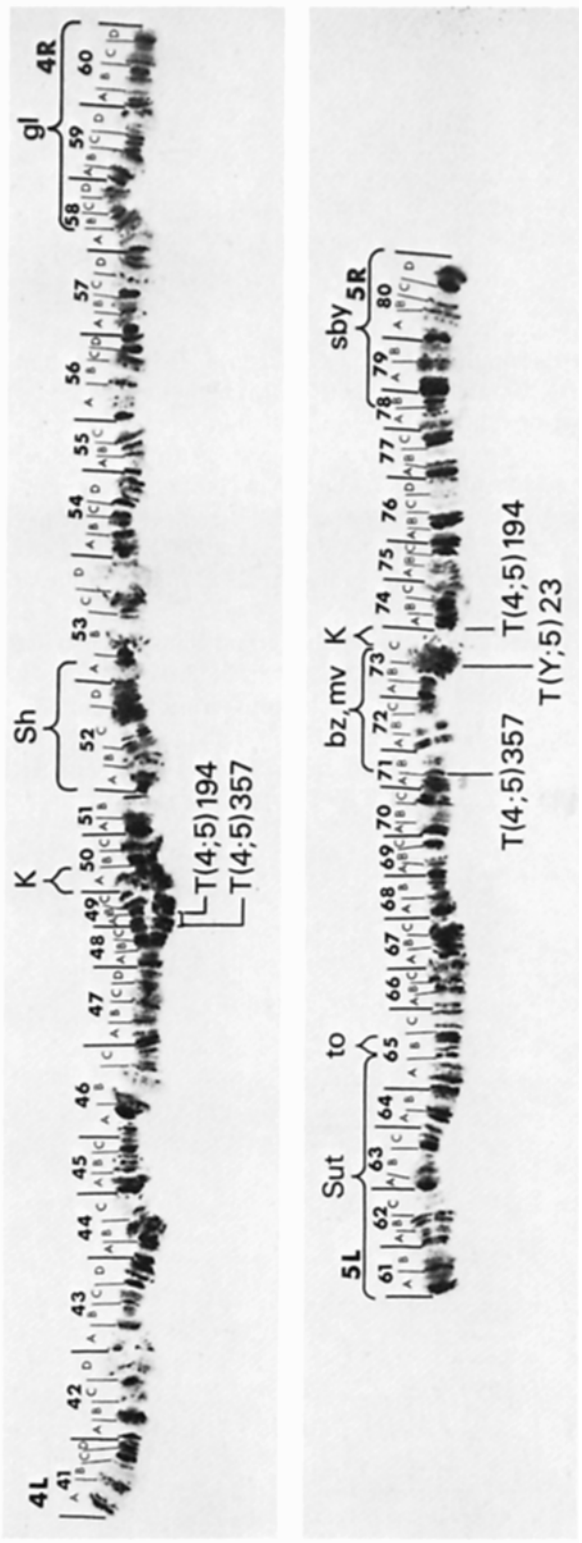

Fig. 1. Cytological positions of break points of translocations and genetic markers. K indicates centromere regions

are capable of surviving to adulthood and are fertile males. Specifically, two duplications for the entire right arm of chromosome 5 (7% of the genome) were viable and fertile (Konovalov et al., unpublished). The proximity of the autosomal break of T(Y;5)23 to the chromosome 5 centromere suggested that a fly carrying the (5L.Y^L)23 half-translocation (containing the Y-chromosome 5, and a C(5R) arm should be a viable male (Bedo and Foster, unpublished)), a normal chromosome 5, and a C(5R) arm should be viable male trisomic for 5R, capable of fertile matings with both chromosomally normal and CC females.

The results of a preliminary series of crosses confirmed the above predictions. Thirteen C(5L)3,to; C(5R)3,sby females crossed to a mixture of T(Y;5)23/to^2 and T(Y;5)23/to^+ males produced (in single-female cultures) a total of 21 wild type male and 5 yellow-eyed (to/to) female offspring. Three females died within a week, before oviposition was attempted, and two laid eggs, after being mated to males carrying a different sex-linked translocation, which failed to hatch. These females probably were trisomic for 5R (C(5L)3,to; C(5R)3,sby;(Y^S.5R)23,sby^+). The testes of three of the males were examined cytologically. One proved to be triploid (Maddern 1981), but the other two were diploid and contained a long autosomal segment attached to a heterochromatic element (presumably (5L.Y^L)23, Bedo 1980) and a small metacentric chromosome (presumably C(5R)3). Four of the remaining males, when crossed individually to CC and chromosomally normal females, produced offspring from both.

One of the above four males was crossed to both a chromosomally normal to sby/to sby female and a C(5L)1,to^2bz mv;C(5R)1,+ female. The to sby/to sby female produced 16 sby^+ female offspring with the eye colour characteristic of to/to^2 (Maddern 1981) and 7 to^+sby males. The CC female produced 4 to^2 bz mv sby female and 3 $to^+bz^+mv^+sby^+$ male offspring. These results confirm genetically the presence of C(5R)3,sby in the F_1 male. This series of crosses and the presumed karyotypes of the offspring are summarized in Fig. 2.

The above results led to a series of crosses designed to test the feasibility of using T(Y;5)23 as a genetic bridge between CC and non-CC strains. As was observed in the peliminary experiment, this type of cross was very infertile (normally a female is capable of laying a batch of 200–250 eggs), and among the offspring males outnumbered females. A series of 6 mass-matings, each involving 60–70 MR-line CC females and 15–20 T(Y;5)23 males, produced a total of 127 wild-type males, 10 wild-type females, plus 14 males and 1 female with a coppery-brown body colour phenotype with wings curled down at the tips, identical to the phenotype associated with most of the 5L duplications studied by Foster et al. (1980 b) and Konovalov et al.

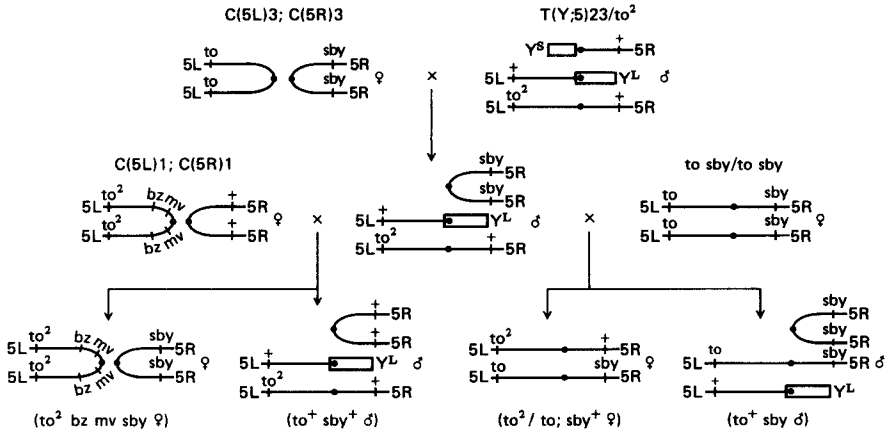

Fig. 2. Transmission of C(5R) arms through males bearing (5L.Y^L)23

(unpublished). Presumably these flies were trisomic for 5L. Their fertility was not examined, but Konovalov et al. (unpublished) found in non-compound strains that most male genotypes carrying 5L duplications which gave this phenotype were sterile. The meiotic segregation products and viable offspring expected from a CC×T(Y;5)23 cross are summarized in Fig. 3.

The low apparent fertility of these crosses is due mainly to a frequency of alternate segregation in T(Y;5)23 males of approximately 90% (unpublished data based on egg-to-adult survival). Thus there is a low incidence of sperm carrying the products of adjacent segregation, which are needed to form viable duplication-bearing zygotes (Fig. 3). The lower incidence of Dp(5R) (wild-type body) female offspring is due primarily to the low frequency of non-segregant gametes produced by CC females (Maddern and Foster, unpublished). The much higher recovery of copper-brown males (from a non-segregant×adjacent-2 zygote) compared to copper-brown females (segregant×adjacent-2) appears to contradict this interpretation. However, the copper females only carry one X chromosome (from the CC parent), and the low incidence of such females may reflect lower viability of this karyotype.

Crosses of chromosomally normal wild-type females to presumptive (5L.Y^L)23;C(5R);(5L.5R) males (Fig. 4, second generation) are much more fertile. Seven mass-matings, each involving 45–50 females and 15–25 males, produced a total of 2,297 wild-type females,

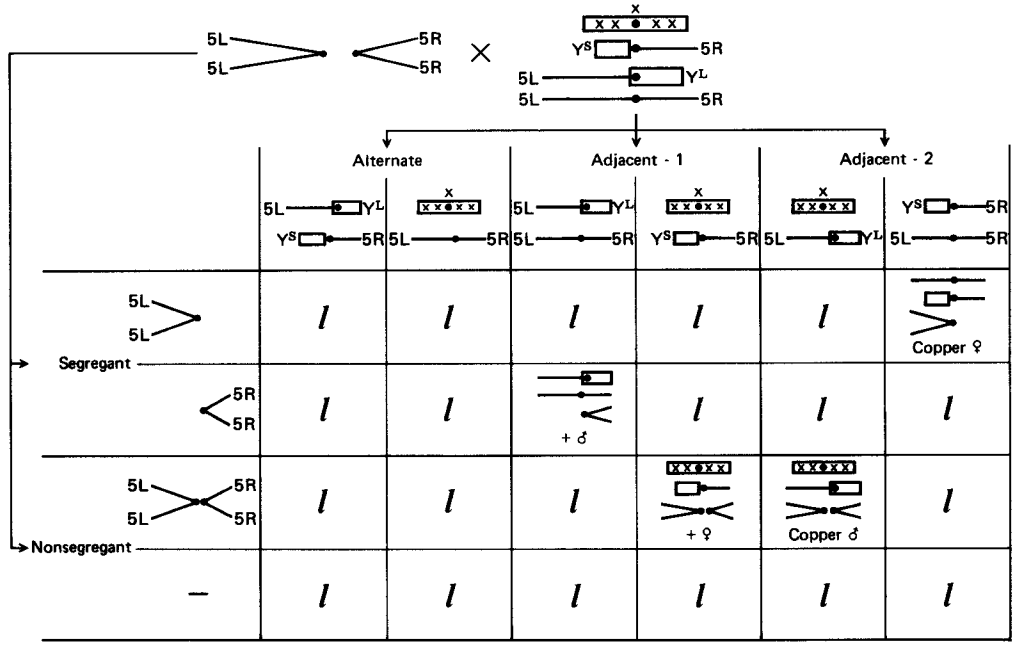

Fig. 3. Segregation products and viable offspring expected from a cross of CC females to T(Y;5)23 males

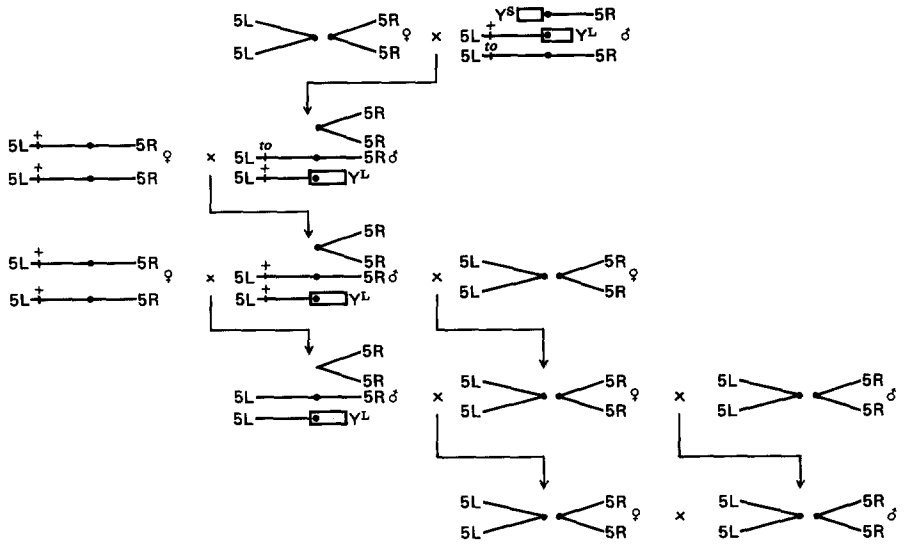

Fig. 4. The use of (5L.Y^L)23 in a bridging system

1,346 wild-type males and 120 copper-coloured males. The wild-type females are karyotypically normal and the wild-type males trisomic for 5R, both resulting from regular segregation of the (5L.Y^L) and C(5R) elements from the (5L.5R) chromosome. The copper-coloured males presumably result from segregation of (5L.Y^L) and (5L.5R) from C(5R), producing males trisomic for 5L. All other karyotypes produced by this cross are probably inviable.

Counts were not made of the offspring of subsequent crosses of CC females to wild-type duplication-bearing males (Fig. 4), but all such crosses were fertile and no difficulty was experienced in obtaining sufficient numbers of offspring for subsequent crosses. Both wild-type females and males, and copper-coloured males (but not females) were obtained from this type of cross. The wild-type (presumptive CC) females, when crossed to CC males, produced offspring in numbers similar to those expected of known CC♀ × CC♂ crosses (Maddern and Foster, unpublished).

The series of crosses outlined in Fig. 4 was followed for 14 different MR lines and 3 genetically marked CC lines, through to the fourth-generation offspring, and no difficulties with fertility were experienced in any but the initial crosses. From these results it was concluded that (5L.Y^L)23;C(5R);(5L.5R) males can be successfully used as a genetic bridge between chromosomally normal and CC strains. This series of crosses was terminated at generation 4.

Double Translocation Heterozygotes as Bridging Strains

The second type of bridging system which has been developed involves the use of a pair of whole-arm autosome:autosome translocations similar to T(4;5)A77 and T(4;5)A89, which were used to recover the first compound arm in *L. cuprina*, C(5L)1,to^{2}bz mv (Foster et al. 1976). These translocations are no longer available, but two others, T(4;5)194 and T(4;5)357, appeared to meet the requirements (Foster et al. 1976).

A single C(5L)3,to;C(5R)5,sby female crossed to a T(4;5)357, Sh/T(4;5)194 male produced 10 yellow-eyed

Table 1. Results of crosses between C(5L);C(5R) and C(5L);(4L.5R);(4R.5R) flies

Cross[a]	Number of offspring			
	to sby	to^{2}bz$^+$mv$^+$sby$^+$	to sby$^+$	to^{2}bz mv sby
A[b]	4	2	2	0
	3	5	3	5
	5	4	3	2
	5	8	1	4
	4	2	1	2
Totals	21	21	10	13
B[b]	13	17	0	1
	10	8	0	0
	14	20	0	0
	12	16	1	2
	8	10	0	0
	11	7	0	0
	15	5	0	0
	9	10	0	1
	1	0	0	0
	12	7	0	0
	7	6	0	0
	7	2	0	0
Totals	119	108	1	4

[a] Each line represents offspring from a single female

[b] Cross genotypes: A–C(5L)3,to;(4L.5R)357;(4R.5R)194 ♀ × C(5L)1,to^{2}bz mv:C(5R)2,sby ♂;
B–C(5L)1,to^{2}bz mv;C(5R)2,sby ♀ × C(5L)3,to;(4L.5R)357;(4R.5R)194 ♂

(to/to) progeny (7♀,3♂) with wild-type (Sh^+sby^+) bristles (i.e. with the phenotype expected of C(5L)3,to; (4L.5R)357;(4R.5R)194). Crosses of these flies to C(5L)1,to^2bz mv;C(5R)2,sby confirmed the presence of C(5L)3,to and (4L.5R)357 in the yellow-eyed F_1 flies (Table 1). The to sby and to^2bz mv sby offspring are C(5L)3;C(5R)2 and C(5L)1;C(5R)2, arising respectively from segregation or non-segregation of C(5L) from C(5R) in the CC parents (Fig. 5). The $to^2bz^+mv^+sby^+$ and to sby^+ flies are C(5L)1;(4L.5R);(4R.5R) (segregants) and C(5L)3;(4L.5R);(4R.5R) (non-segregants). The mv^+ phenotype of the C(5L)1;(4L.5R);(4R.5R) flies is expected in flies carrying (4L.5R)357 since this element includes 5L region 71A/B-73C, which is the site of the mv^+ locus (Foster et al. 1980b). The bz^+ phenotype of these flies indicates that the bz^+ allele is also contained in this region. No suggestion of the coppery-brown body colour phenotype associated with other 5L duplications (Foster et al. 1980b) was evident in any of the C(5L);(4L.5R);(4R.5R) genotypes examined.

The reciprocal crosses reported in Table 1 differ strikingly in the frequency of non-segregant offspring. This is consistent with other findings (Maddern and Foster, unpublished) that for most C(5L);C(5R) combinations non-segregation in females is generally much less frequent than in males. The high recovery of non-segregants in cross A (35%) indicates that 3 : 1 segregations (Fig. 5) occur frequently in C(5L)3;(4L.5R)357; (4R.5R)194;(4L.4R) females, as was observed in C(5L)1;(4L.5R)A89;(4R.5R)A77;(4L.4R) males (Foster et al. 1976).

The success of the above series of crosses prompted further investigation of the practicability of using segregation products from T(4;5)194/T(4;5)357 males as a genetic bridge between CC strains and chromosomally normal strains. An outline of the sequence of crosses tested is presented in Fig. 6.

In the initial cross of T(4;5)357,Sh/gl;mv ♀♀ × T(4;5)194,Sut/gl;mv ♂♂, Sh can be used fairly reliably as an indication of the presence of T(4;5)357 in the offspring, although some crossing over probably occurs

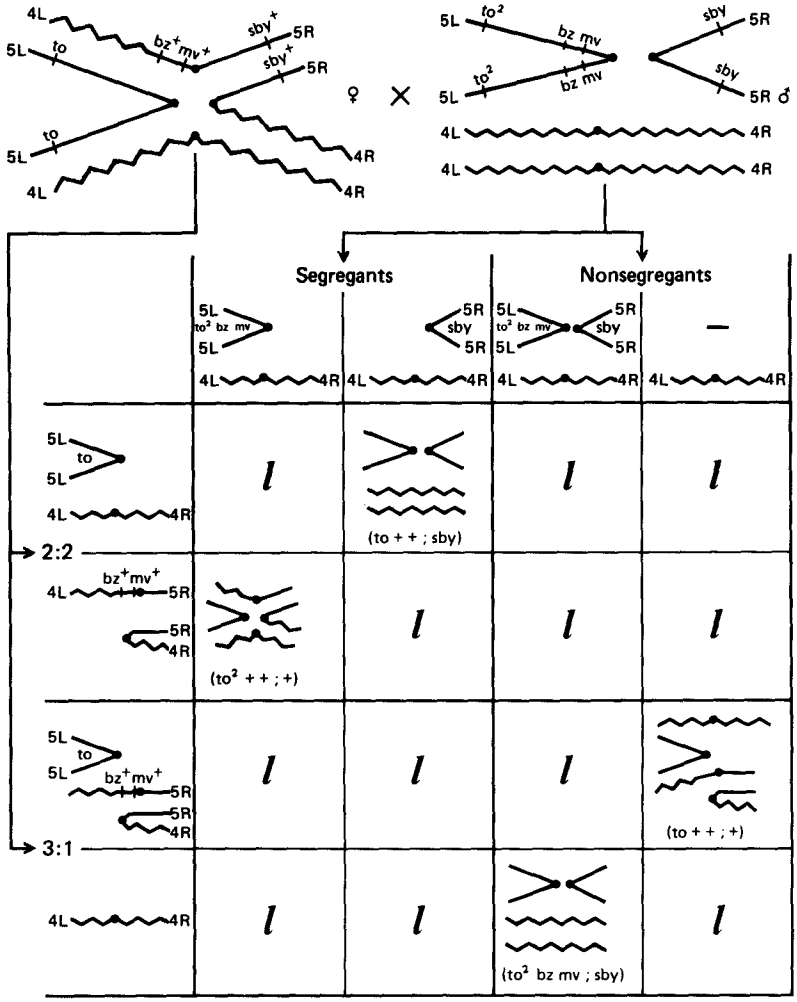

Fig. 5. Viable offspring expected from the cross: C(5L)3: (4L.5R)357; (4R.5R)194 ♀♀ × C(5L)1;C(5R)2 ♂♂

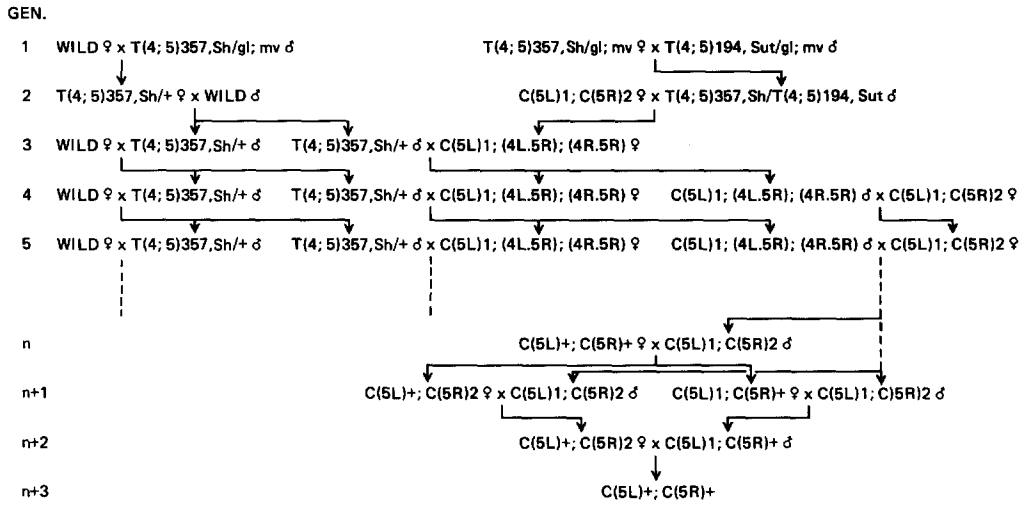

Fig. 6. The use of (4L.5R)357 and (4R.5R)194 in a bridging system

between the translocation break point and Sh (Fig. 1, Table 2). The Sut$^+$ offspring of this cross carry the normal chromosomes 4 and 5 from the male parent, with the mutations gl and mv respectively, as meiotic crossing over in males is rare or absent (Foster et al. 1980a). Thus crossing over in the region of the T(4;5)357 break point can be measured in the Sut$^+$ offspring, since the flanking markers mv and Sh are heterozygous in the female parents. The data (Table 2) suggest that a maximum of 5 of the 102 Sh Sut$^+$ offspring could have lacked T(4;5)357. Thus it appears reasonably safe to assume that nearly all Sh Sut progeny carry both translocations. Any such flies which lacked T(4;5)357 through crossing over would almost certainly fail to breed successfully with C(5L);C(5R) flies.

Table 2. Offspring of the cross T(4;5)357,Sh/gl; mv ♀× T(4;5)194, Sut/gl; mv ♂

Phenotype	Number of offspring[a]	Crossover class[b]
Sh+; Sut+	86	–
+ +; Sut+	103	–
Sh+; + +	55	NCO or DCO (1,2)
+gl; +mv	57	
+ +; +mv	36	SCO (3)
Sh gl; + +	42	
+gl; + +	2	SCO(1)+SCO(2)
Sh+; +mv	4	
+ +; + +	1	DCO(1,3)+DCO(2,3)
Sh gl; +mv	1	

[a] Pooled offspring of 10 single-female cultures
[b] NCO = noncrossover, SCO = single crossovers, DCO = double crossovers; numbers in parentheses refer to the following crossover regions: (1) mv–T(4;5)357 breakpoint; (2) T(4;5)357 breakpoint – Sh, (3) Sh-gl

In a mass mating of 85 C(5L)1,to^{2}bz mv;C(5R)2,sby females to 30 putative T(4;5)357,Sh/T(4;5)194,Sut males, 240 to^{2}bz$^+$mv$^+$sby$^+$Sh$^+$Sut$^+$ (presumably C(5L)1;(4L;5R);(4R;5R)) and 1 to^{2}bz$^+$mv$^+$sby$^+$Sh Sut$^+$ offspring were recovered. The latter was not examined further, but may have resulted from an exchange event in a male (Foster et al. 1980a). No sby offspring (presumptive C(5R);(4R.5L);(4L.5L) individuals) were recovered from either this or the preliminary cross. This genotype thus appears to be inviable, presumably due mainly to heterozygous deficiency for 71A/B-73C (Fig. 1).

The crossing of wild females to T(4;5)357,Sh/+ males (Fig. 6) involves no problems with regard to fertility. Although rare exchanges in males could lead to dissociation of Sh from T(4;5)357 (see above), in practice this has not proved to be a problem (after 24 generations of the crossing sequence outlined in Fig. 6) in matings of this type.

The second-generation cross between wild and T(4;5)357,Sh/+ flies (Fig. 6) involved mating translocation females to wild males, in order to ultimately replace the Y chromosomes in the CC strains with unirradiated Y chromosomes from the wild strain. Possible crossovers between the translocation and Sh in this cross were eliminated by crossing individual Sh males to both wild females and C(5L)1;(4L.5R);(4R.5R) females in the third generation, and collecting eggs separately from the two types of female. Of 35 such pairs of crosses in which both types of female produced offspring, 29 of the C(5L)1 females produced to^{2}bz$^+$ mv$^+$Sh$^+$ and Sh offspring, and 6 produced only Sh offspring. Sh male offspring of the wild ♀×Sh ♂ crosses

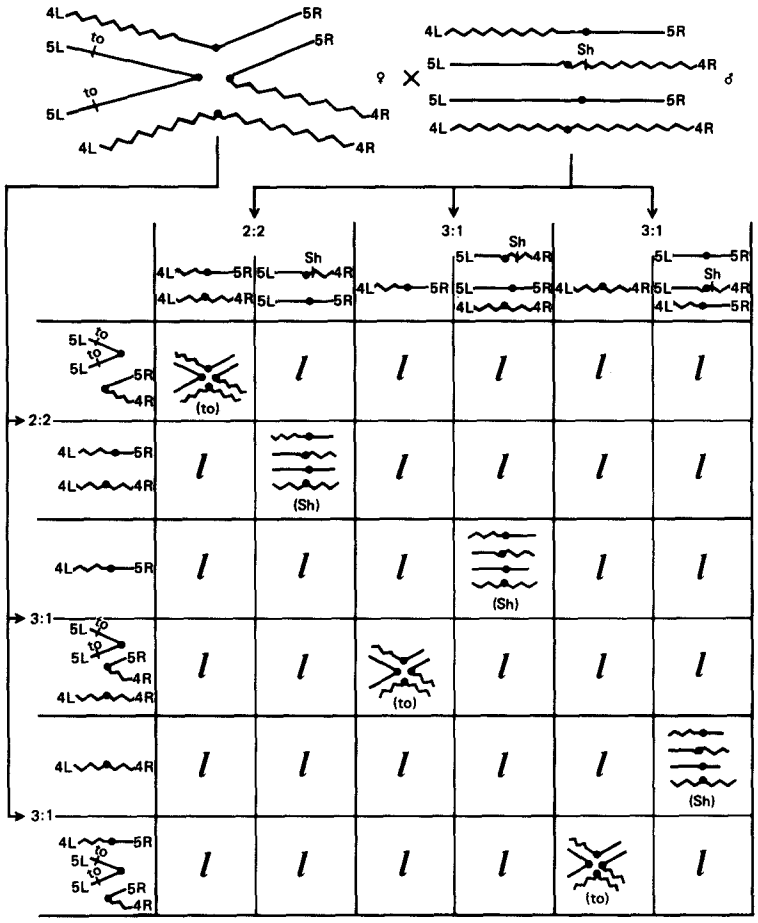

Fig. 7. Viable offspring from the cross: C(5L)3; (4L.5R)357; (4R.5R)194 ♀♀ × T(4; 5)357.Sh/+ ♂♂

were saved (and pooled for further crosses) only if the corresponding C(5L)1♀ × Sh♂ cross produced $to^2bz^+mv^+Sh^+$ offspring, indicating positively that the male parent carried T(4;5)357.

The C(5L);(4L.5R);(4R.5R)♀ × T(4;5)357,Sh/+♂ crosses are the least fertile of all the crosses in this series, presumably because the types of segregation events required to produce viable offspring (Fig. 7) occur relatively infrequently. Of the 3 classes of 2:2 segregation and 4 classes of 3:1 segregation theoretically possible in both parents, only those shown in Fig. 7 lead to viable offspring. In a typical set of results, from the 29 females mentioned above, 89 to^2 progeny (approximately half males) were obtained, giving only a net increase per generation of 1.5-fold so far as C(5L) females are concerned. Occasional infertility and preoviposition mortality frequently reduce this figure to below 1.0. Thus this step of the bridging system is the most difficult to maintain and constitutes the most likely site of a genetic bottleneck in the system. In practice this problem was overcome by obtaining two ovipositions each generation. As indicated in Table 1 (cross B) there is no problem with fertility in C(5L); C(5R) ♀ × C(5L);(4L.5R);(4R.5R) ♂ crosses.

At any stage from generation 5 onward, the genetic material from the wild strain (Y chromosomes plus non-CC autosomes) can be transferred from the genetically marked CC strain to a wild-type CC strain (Fig. 6). The method shown (generations n to n+3, Fig. 6) involves crossing females of the latter to males of the former (which carry the wild Y chromosomes), followed by backcrossing of the C(5L)1;C(5R)+ and C(5L)+;C(5R)2 offspring to the marked CC (wild background) strain, reconstituting the C(5L)+;C(5R)+ strain when the desired amount of wild genetic material has been transferred.

In theory wild genetic material could also be introduced directly from T(4;5)357/+ males into a wild-type CC strain by mating such males to C(5L)+; (4L.5R);(4R.5R) females. However, spontaneous detachment of the C(5L) arm in crosses of this type produces viable and highly fertile (when mated to T(4;5)357) offspring either carrying a reconstituted chromosome 5 or a new half-translocation which is compatible with (4R.5R)194 (Fig. 8). If the C(5L) element is genetically marked the breakdown products are detectable as wild-type Sh^+ offspring (unpublished data) and can be eliminated, but if the C(5L) is

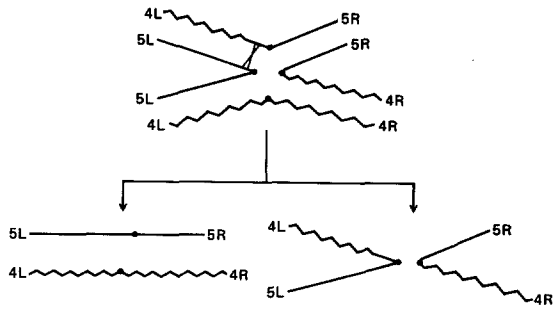

Fig. 8. Spontaneous detachment of C(5L) by crossing over in region 71A/B–73 C in C(5L);(4L.5R)357;(4R.5R)194 females

unmarked the breakdown products are phenotypically identical to C(5L)+;(4L.5R);(4R.5R) flies. Because of their high fertility the breakdown products increase rapidly in frequency relative to C(5L)+ (Foster et al. 1980a), resulting either in loss of the series of crosses (i.e. sterility of the subsequent cross of C(5L)+;C(5R)+ ♀♀×♂ breakdown products) or in considerable extra work to recover the desired karyotypes. Breakdown attributable to this mechanism was observed after 6 generations in 2 of 17 C(5L)+ lines carried through a crossing procedure analogous to that shown in Fig. 6, but using C(5L)+;C(5R)+ instead of C(5L)1;C(5R)2.

The scheme outlined in Fig. 6 is currently being followed using 3 different genetically marked C(5L) arms, and will be continued indefinitely, with crosses to appropriate C(5L)+;C(5R)+ strains being performed as wild-type CC strains for field trials are required.

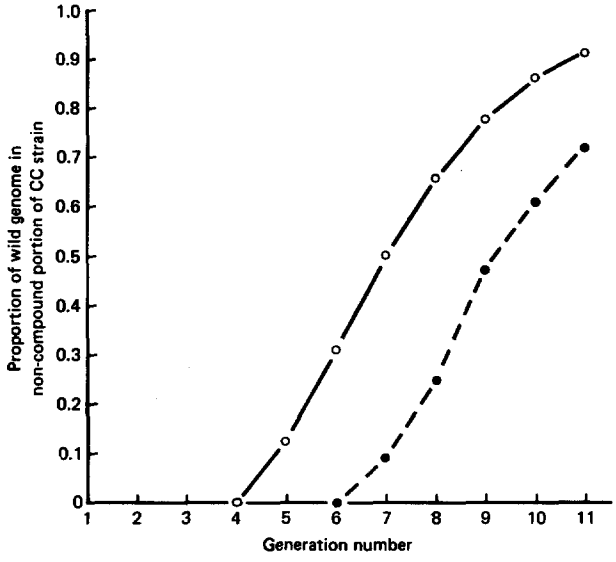

Fig. 9. Comparison of rate of replacement of non-compound portion of the genome in C(5L)+;C(5R)+ strains using the two bridging systems: O – O Chromosomes 2, 3, 4, 6, using the T(Y;5)23 bridging system; . – – – . chromosomes 2, 3, 6 using the T(4;5)357/T(4;5)194 bridging system, starting crosses with C(5L)+;C(5R)+ in generation 6 (Fig. 6). See text for rate of replacement of chromosome 4

Discussion

The theoretical increase of wild genetic material in the non-compound portion of C(5L)+;C(5R)+ strains derived using the bridging systems outlined in Figs. 4 and 6, is shown in Fig. 9. The T(Y;5) system is more rapid for the non-CC autosomal material than the T(4;5) system. However, as the number of generations increases the proportion of wild-derived genetic material will approach the theoretical maximum in both systems, and differences in level of heterozygosity remaining in strains derived by the two methods will tend to disappear. Moreover, unlike the T(Y;5) system, the T(4;5) system allows replacement of the Y chromosomes of the CC strain.

In the T(4;5) bridging system the rate of replacement of chromosome 4 is likely to be different from that of chromosomes 2, 3 and 6. One of the 3 : 1 segregation types depicted in Fig. 7 does not result in transmission of the chromosome 4 from the male parent to the C(5L);(4L.5R);(4R.5R) offspring. If this occurs rarely the replacement of chromosome 4 will be more rapid than that of chromosomes 2, 3 and 6. If it occurs frequently the replacement of chromosome 4 will be slower. The other 3 : 1 segregation type depicted in Fig. 7 has already been inferred to occur in C(5L);(4L.5R);(4R.5R) females (Table 1, Fig. 5). This, as well as 2 : 2 segregation, results in transmission of the paternal chromosome 4 to the C(5L)-bearing offspring.

The two bridging systems outlined in the present paper are by no means the only possible ones. For example, systems using a combination of a sex-linked translocation and an autosome-autosome translocation (such as one with a centromeric break on one chromosome and a telomeric break on the other) can be envisaged, and no doubt other methods can be devised. Holm et al. (1980) found in *D. melanogaster* that one of the products of recombination between a standard chromosome and one containing a pericentric inversion, acted as a genetic bridge between a C(2L);F(2R);F(2R) and a chromosomally normal strain.

The bridging system discovered by Holm et al. (1980) led to a stable equilibrium between the C(2L) and the normal strain in a mixed population, presumably because the fitness of the hybrid (inversion heterozygote) exceeded that of the C(2L) and normal strains (Robinson 1977). A stable equilibrium would not be expected to arise from the bridging systems described in the present paper, because (1) trisomic (5L.Y^L)23; C(5R);(5L.5R) males are less viable than euploids and are not likely to be competitive with either normal or CC males, and (2) low fertility of the intermediate-stage crosses in the T(4;5) system, particularly that of C(5L) females×T(4;5)357/+ males, makes such hybrid matings operationally much less fit than wild×wild or CC×CC matings. Thus in mixed populations of normal and CC flies contaminated with elements of either bridging system, either the CC or the normal strain should be rapidly eliminated, although elements of the intermediate strains may persist in the surviving population for several generations.

The spontaneous detachment of C(5L) (Fig. 8) may depend in the present case on meiotic crossing over within

region 71A/B-73 C, which is common to both C(5L) and (4L.5R)357. As noted earlier, both the bz and the mv loci lie within this region; the frequency of meiotic recombination between these loci in structurally normal females is 4.6% (Foster et al. 1981). However, there is no reason to believe that detachments could not occur in the absence of such a duplication, i.e. by heterologous exchange (Foster et al. 1980a).

The genetic analysis of detachment products may provide valuable information concerning aberrant recovery of certain compound arms (Foster and Maddern, unpublished). In addition, if the reverse of detachment can be achieved, i.e. by crossing over between the new (5L.4L) half-translocation (Fig. 8) and a normal chromosome 5, any desired genetic material or appropriate rearrangement can be inserted into a C(5L) without irradiation.

Besides allowing transfer of genetic material between CC and wild strains, the rearrangements used in the bridging systems provide tools for the study of segregation of translocations and compound chromosomes. For example, it is evident that both 2 : 2 and 3 : 1 segregations occur frequently in C(5L);(4L.5R);(4L.4R) females (Table 1). The recovery of copper-coloured progeny in the crosses with T(Y;5)23 could imply the occurrence of adjacent-2 segregations (Fig. 3), but could also result from 3 : 1 segregation in the male. In *D. melanogaster* males Lindsley et al. (1972) formed the impression that adjacent-2 segregation was no more frequent than 3 : 1 segregation, and that both of these were much less frequent than alternate and adjacent-1 segregation. Van Heemert (1974) observed 3 : 1 segregations in an X-autosome translocation of *Hylemya antiqua* (Meigen) but no adjacent-2 segregations. These observations and several other questions concerning segregation arising from the data, warrant further investigation.

Foster et al. (1980b) discussed the possibility that the coppery-brown body colour phenotype associated with most 5L duplications was due to trisomy for a locus in region 67A–69A. Consistent with this hypothesis is the observation that trisomy for region 71A/B–73C does not result in this phenotype. Moreover, since the bz locus lies in region 71A/B–73C, trisomy for this locus cannot be responsible for the copper-body phenotype.

Although the present paper describes a workable solution to the problem of inbreeding in the non-compound portion of the genome of CC strains, the problem of inbreeding from crossing over within compound arms remains unsolved. The options for overcoming this problem are varied, including (for example) selection of homozygous arms which do not lead to reduced fitness, or prevention of exchange within CC arms through either structural heterozygosity or other mechanisms. A compound chromosome which contained several lethal mutations on each homologous arm could be expected to persist as a heterozygote, because of elimination of lethal products of recombination, but in order to persist indefinitely in a mixed population the advantage of being heterozygous would have to outweigh the low fecundity of females carrying such a chromosome. In practical terms, a CC strain deliberately constructed to contain this type of chromosome may be too infertile to mass-rear. Considerable research into the genetics of the CC strains and the practical question of field fitness remains to be done.

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