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Isolation and cytogenetic analyses of genetic sexing strains for the medfly, *Ceratitis capitata*

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Abstract Over the last 10 years, several genetic sexing strains have been isolated for the Mediterranean fruit fly, *Ceratitis capitata*, with the aim of improving the Sterile Insect Technique. However, a major problem with the currently used genetic sexing system, which is based on translocations, is their potential genetic instability. Therefore, careful monitoring and chromosome analyses are necessary when new genetic sexing strains are developed. Instability of a genetic sexing strain can be the consequence of recombination or the survival of aneuploid individuals occurring as a consequence of adjacent-1 segregation in the meiosis of males with Y-autosome translocations. Recently, genetic sexing strains have been isolated that show only low levels of recombination. However, many aneuploid flies are produced by these strains. Therefore, we have made an attempt to isolate new genetic sexing strains that show a low percentage of recombination and no survival of aneuploid individuals. We report their genetic behaviour and the polytene chromosome structure of these new strains.

Key words Genetic sexing · *Ceratitis capitata* · Polytene chromosomes · Y-autosome translocations · Sterile Insect Technique

Introduction

The efficacy of the Sterile Insect Technique (SIT) for the control or eradication of the Mediterranean fruit fly can be improved by using genetic sexing (GS) strains (Whitten and Foster 1975; Robinson et al. 1986). The development of GS strains facilitates the release of only males. Several GS strains have been developed over the last few years, but all have certain features that make them unattractive for use in a mass-rearing factory. The first GS strains were based on pupal colour mutations (*dp*: Rössler 1979; *wp*: Robinson and Van Heemert 1982) with males emerging from wild-type pupae and females emerging from mutant pupae. These GS systems require the use of expensive pupal separation machines that are too slow for an operational mass-rearing factory, and the extra handling lowers the quality of the released males. Moreover, this method of sex-separation does not reduce the cost of rearing the flies, although it does reduce the costs of several down-line procedures.

Recently, we have developed new GS strains based on a temperature-sensitive lethal (*tsl*) mutation (Franz et al. 1994) so that female zygotes can be killed following a high-temperature treatment. This was done by linking the wild-type allele of a *tsl* mutation to the male-determining chromosome, the Y chromosome (Whitten et al. 1977). The structure of these, where the males are heterozygous for the selectable marker and carry a Y-autosome translocation, can lead to instability, either due to recombination in the chromosomal region between the translocation breakpoint and the selectable marker or due to the survival of adjacent-1 segregants (Franz et al. 1994). Therefore, we have defined three criteria for the identification of superior GS strains.

1) The autosomal translocation breakpoint should be very close to the mutation used for sexing. This will reduce the probability of the occurrence of recombination. The two mutations used currently for the construction of GS strains are *white pupa* (*wp*) and a temperature-sensitive lethal (*tsl*) mutation. The loci are closely linked (Kerremans and Franz 1994). This means that tests for stability relative to *wp* can be used as a good indicator for strain stability relative to *tsl*.
2) The strain should produce no or only low levels of offspring that are the consequence of adjacent-1 segregation in the male meiosis.
3) Simple translocations, involving only a single autosome

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and the Y chromosome, are optimal, since fertility is higher in such strains. These do not enhance the stability but do assist fly production during mass-rearing.

With these three criteria in mind, we have isolated 13 new Y-autosome translocation strains. The polytene chromosomes of the strains were analysed together with their segregation characteristics. Based on these two parameters a choice could be made as to their suitability as practical GS strains.

Materials and methods

Strains and crosses

Mutant strains used in these experiments are described by Rössler and Rosenthal (1988, 1992). Briefly, a strain containing the *white pupae* (*wp*) and the *white eye* (*we*) mutations was used. These mutations are located on chromosome 5 at position 59B and 46A-50A of the trichogen map, respectively (Kerremans and Franz 1994). The temperature-sensitive lethal (*tsl*) mutation is located at position 59C-61D (Kerremans and Franz 1994). The mutation *black fly* (*bf*, Kerremans et al. 1992) is on chromosome 2, and the mutations *dark pupae* and *apricot eye* (*dp*, *ap*, Rössler and Koltin 1976) are located on chromosome 3 and 4, respectively. EgII is a wild-type strain collected in Egypt in March 1983.

EgII pupae, 1 day before adult emergence, were irradiated in a [^{60}Co] irradiator with 40 Gray (Gy). Male flies were placed with non-irradiated virgin *wp we* females in large cages each fitted with an oviposition net. Individual F_1 males were crossed with *wp we* females. Further information about crosses and irradiation protocols are described by Kerremans et al. (1992) or references therein.

Chromosome preparations

Orbital bristle trichogen cell polytene chromosomes were prepared from 5- to 6-day-old pupae using the method reported by Bedo (1986, 1987). The chromosomes were interpreted using polytene maps of the orbital bristle cells (Bedo 1987).

Terminology

GS strain: A genetic sexing (GS) strain contains two components; a Y-autosome translocation and a mutation that can be used as a selectable marker. The wild-type allele of this locus is on the translocated autosome, while the mutant allele is on the free autosome. [Refer to Franz et al. (1994) and Foster et al. (1985) for further information.]

Aberrant phenotypes: All phenotypes, excluding wild-type males (wp^+ we^+) and mutant females (*wp we*), are called aberrant phenotypes. These phenotypes can occur after recombination or adjacent-1 segregation. Other types of segregation (adjacent-2, non-disjunction) have not yet been observed.

Strain instability: GS strains that produce aberrant phenotypes (more than 0.5%) are called unstable strains. If there is a gradual increase of aberrant phenotypes in each generation, there is strain breakdown.

Results

Induction and isolation of Y-autosome 5 translocations

From 300 F_1 males tested, 13 single-pair families were recovered that showed linkage between sex and the two markers *we* and *wp* located on chromosome 5 (Table 1). To determine whether other autosomes are involved in the T(Y;A) translocations, males of these 13 strains were crossed with females homozygous for *bf*, *dp* or *ap*. These mutations are on chromosomes 2, 3 and 4, respectively. The F_1 males were again crossed with the respective mutant females. All strains were also analysed cytogenetically (Table 2, Figs. 2 and 3). In all cases, the results of the cytogenetic analyses were in agreement with the genetic data. For chromosome 6, no morphological marker was available, and the data are based on cytogenetic observations.

Table 1 Genetic behaviour of different T(Y;A) strains. The table represents the summary of data obtained by maintaining the translocation strains for several generations. Each generation was set up with females from a *wp we* strain and translocation males from the previous generation

	Brown pupae	Brown pupae	Brown pupae	Brown pupae	White pupae	White pupae	White pupae	White pupae
	White eyes	White eyes	Wild-type eyes	Wild-type eyes	White eyes	White eyes	Wild-type eyes	Wild-type eyes
	Female	Male	Female	Male	Female	Male	Female	Male
3–4	0	0	0	838	879	0	1	0
3–41	15	0	3	1741	1560	0	1	6
3–86	0	133	0	2693	2366	0	1	0
3–128	0	1	162	359	333	309	0	0
3–129	15	18	1	6585	6065	5	0	0
3–136	0	74	0	273	285	194	0	0
3–139	2	3	0	1875	1637	0	34	0
3–179	0	746	0	2166	1927	0	2	0
3–182	0	0	0	773	645	468	0	121
3–192	0	0	0	1195	1160	0	0	186
3–204	1	1	0	1046	1018	0	4	2
3–245	8	0	10	7710	7693	2	10	1
3–263	0	119	1	603	546	0	0	0

Table 2 Chromosome structure of T(Y;A) strains. The second column shows the location of the chromosome 5 breakpoint. If more than one breakpoint is present in chromosome 5, only the breakpoint relevant for the potential stability of strain, relative to *wp* and *tsl*, is given. The third column gives the new order of the chromosomes involved in the translocation. The centromere-containing chromosome is underlined. For the breakpoints of strains T(Y;5)3–179, T(Y;5)3–192 and T(Y;5)3–263, see also Kerremans and Franz (1994)

Strain	Breakpoint chromosome 5	New chromosome order (autosome(s): order)
3–4	59C	*2*,Y: 1A..16C/Y *4,2*: 100D..89A-E/19A-E..24D *5,4*: 44A..59C/85A..82A *Y*,2,4,5: Y/16C..19A-E/89A-E..85A/59C..62D
3–41	57A	*5,2*: 44A..57A/11A..1A *2*,Y: 24D..11A/Y *Y,5*: Y/57A..62D
3–86	55B	*Y,5*: Y/55B..62D *5,Y*: 44A..55B/Y
3–128	56C-D	*5*,Y: Y/44A..56C-D *Y,5*: Y/56C-D..62D
3–129	58B	*Y,5*: Y/58B..62D *5*,Y: 44A..58B/Y
3–136	58A	*3*,Y: 43D..41A/Y *5,3*: 44A..58A/41A..25A *5,6*: 62D..58A/64B..81E *6,Y*: 63A..64B/Y
3–139	60D	Y,5,3: Y/51D..60D/42A-B..43D *3,5*: 25A..42A-B/60D..62D *Y,5*: Y/51D..44A
3–179	57C	*Y,5*: Y/57C..62D *5*,Y: 44A..57C/Y
3–182	53B	*Y,3*: Y/25A..28D *5*,Y: Y/53B..62D *3,5*: 44A..53B/28D..43D
3–192	52C	Y,5: Y/52C..62D 5,*Y*: 44A..52C/Y
3–204	52B	*6*,5: 81E..67D/50D..44A *Y*,5,6: Y/52B..50D/67D..63A *5*,Y: Y/52B..62D
3–245	61B	*Y,5*: Y/61B..62D *5*,Y: 44A..61B/Y
3–263	56B	*Y,5*: Y/56B..62D *5*,Y: 44A..56B/Y

In previous experiments in which a dose of 50 Gy for irradiation was used (Franz and Kerremans 1993), many strains with multiple translocations were recovered. In the present experiment the irradiation dose was lowered to 40 Gy, with the result that more strains with simple translocations, involving only chromosome 5 and the Y chromosome, were isolated (Fig. 1). A disadvantage of using a lower dose is that fewer Y-autosome translocation strains were found (13/300, 4.3%) compared to 15 strains among 211 single pairs (7.1%) in the previous experiments using 50 Gy.

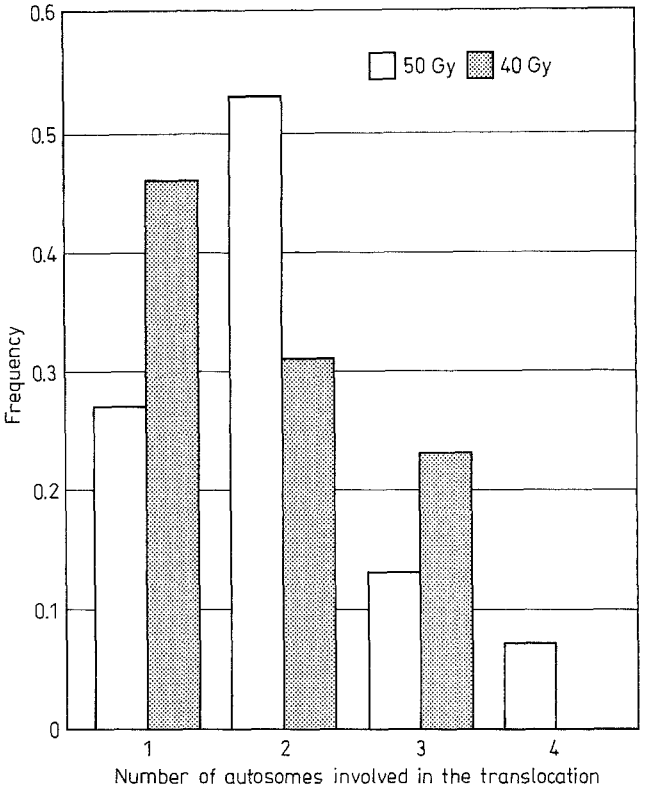


Fig. 1 The complexity of the Y-A translocations using either 50 Gy (Franz et al. 1994) or 40 Gy (this report) for induction. The *horizontal axis* shows the number of autosomes involved in the translocation; the *vertical axis* gives the frequency of the GS strains of a particular complexity

Cytogenetic and genetic analysis of the new strains

The polytene chromosomes from trichogen cells were analysed using the corresponding chromosome map (Bedo 1986, 1987). Figures 2 and 3 show the breakpoints in chromosome 5 of the 13 strains analysed. The translocations shown are either autosome-autosome or Y-autosome translocations. The Y chromosome is visible as a large heterochromatic body and nucleolus (Bedo 1987).

A total of seven translocation strains were recovered with a simple translocation between chromosome 5 and the Y chromosome, i.e. T(Y;5)3–86, T(Y;5)3–128, T(Y;5)3–129, T(Y;5)3–179, T(Y;5)3–192, T(Y;5)3–245 and T(Y;5)3–263. Their chromosome structure is given in Table 2 and the data on segregation in Table 1. Two of these translocations have an autosomal breakpoint in the left arm of chromosome 5, i.e. T(Y;5)3–86 and T(Y;5)3–192 (Table 2, Figs. 2 and 3). Because the distance between the translocation breakpoint and the *wp* locus is quite large, a relatively high level of recombination is expected. The remaining five translocation strains have their breakpoint in the right arm (Table 2), closer to the *wp* locus, and are expected to show a lower level of recombination (criterium 1). High levels of aberrant phenotypes were found in strains T(Y;5)3–86, T(Y;5)3–179, T(Y;5)3–192 and T(Y;5)3–263. They are, however, not the consequence of recombination

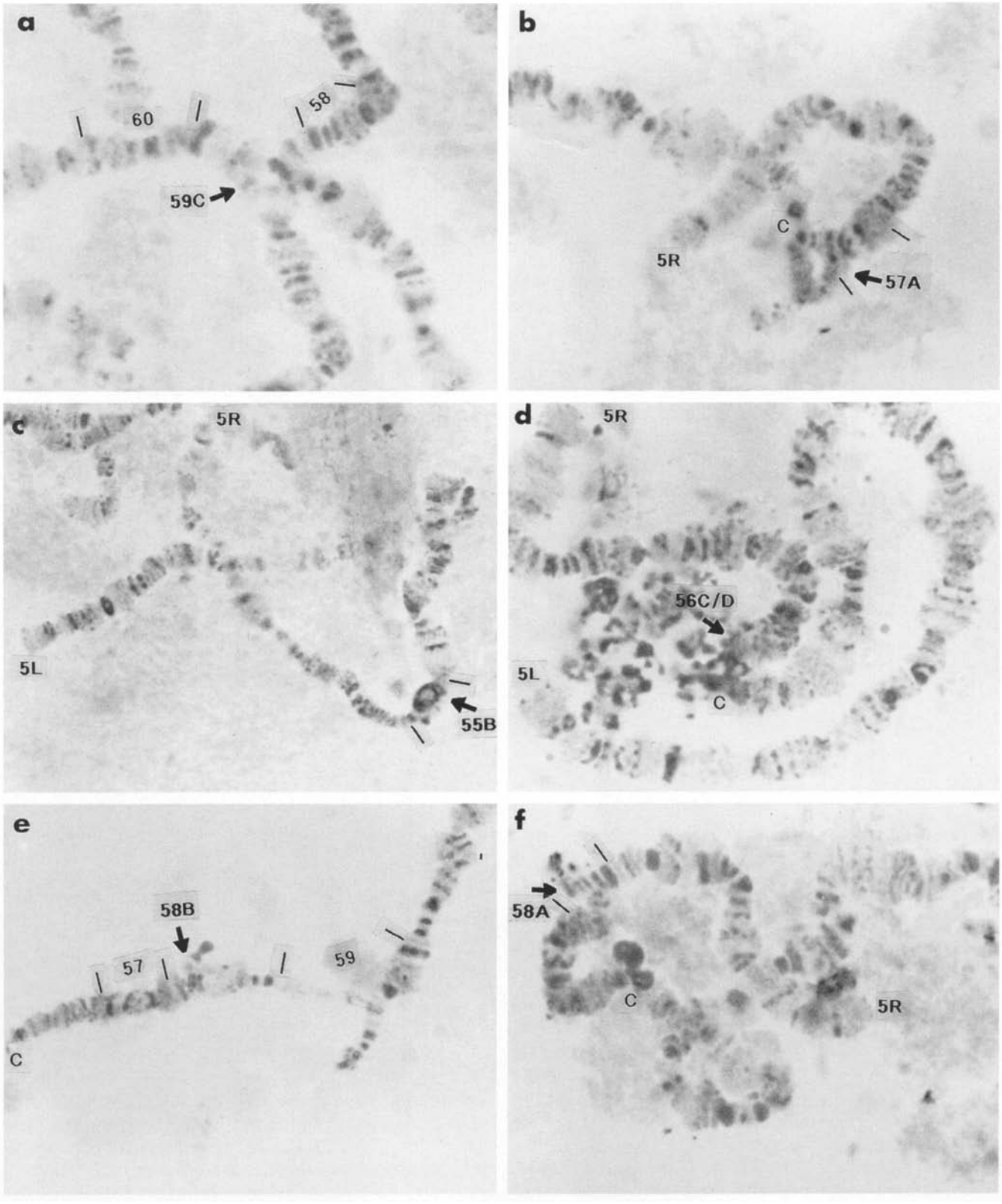
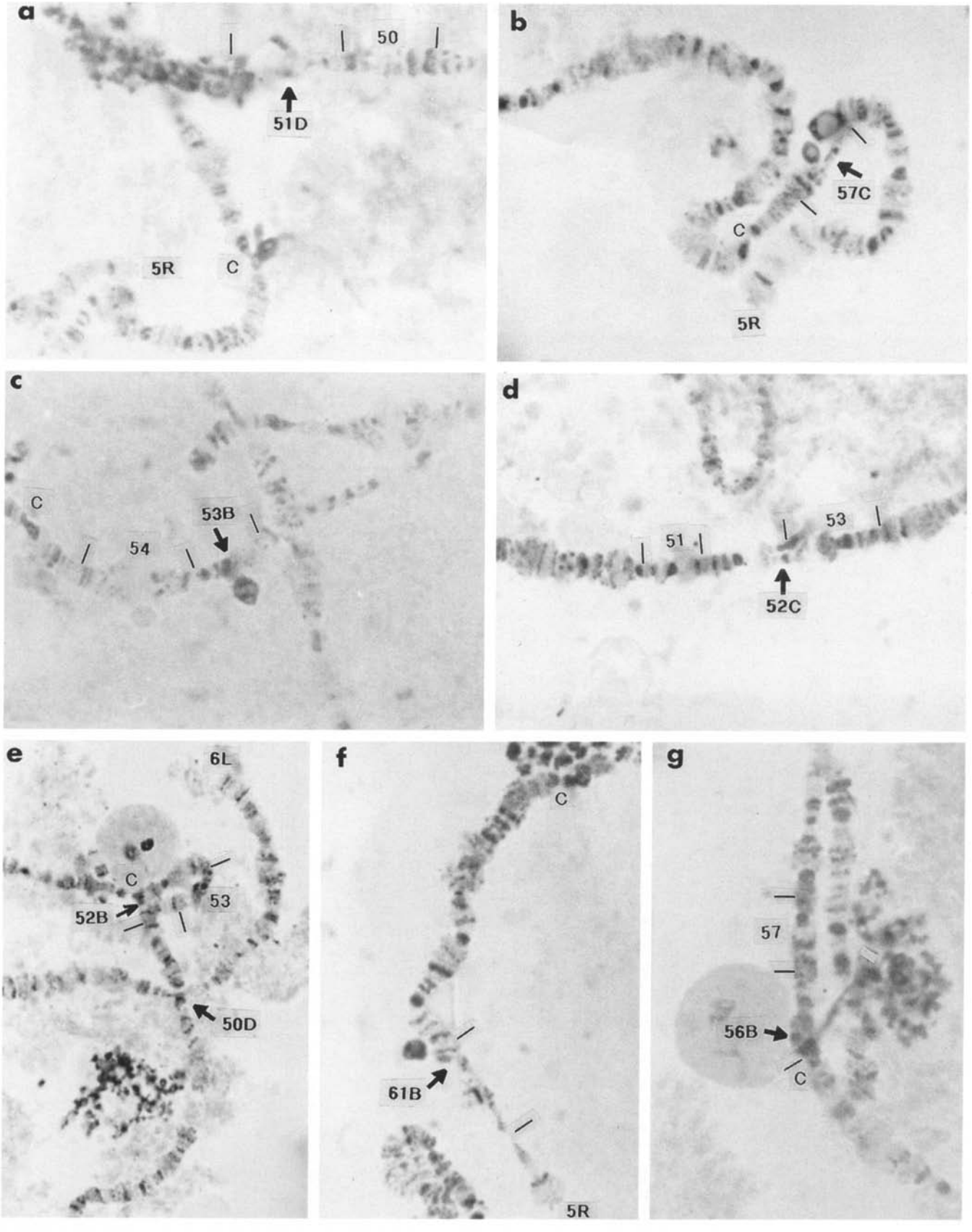


Fig. 2 The translocation breakpoints on chromosome 5 (*arrows*) are shown for strains T(Y;2;4;5)3–4 (**a**), T(Y;2;5)3–41 (**b**), T(Y;5)3–86 (**c**), T(Y;5)3–128 (**d**), T(Y;5)3–129 (**e**) and T(Y;3;5;6)3–136 (**f**). Where appropriate, the chromosome tips (*5L* and *5R*) and the centromere (*c*) are labelled

Fig. 3 The translocation breakpoints on chromosome 5 (*arrows*) are shown for strains T(Y;3;5)3–139 (**a**), T(Y;5)3–179 (**b**), T(Y;3;5)3–182 (**c**), T(Y;5)3–192 (**d**), T(Y;5;6)3–204 (**e**) and T(Y;5)3–245 (**f**) and T(Y;5)3–263 (**g**). Where appropriate, the chromosome tips (*5L* and *5R*) and the centromere (*c*) are labelled


a
50
51D
5R
C
b
57C
C
5R
c
C
53B
54
d
51
53
52C
e
6L
C
53
52B
50D
f
C
61B
5R
g
57
56B
C

but represent unbalanced genotypes caused by adjacent-1 segregation during male meiosis (Willhoeft et al., in preparation). Two strains, T(Y;5)3–129 and T(Y:5)3–245, have the autosomal breakpoint closest to the *wp* locus, and only a few aberrant flies were detected (Figs. 2 and 3).

After screening 12 689 F_1 flies of crosses between T(Y;5)3–129 translocation males and females from a homozygous *wp we* strain, males and females with the wp^+ *we* phenotype were recovered at a frequency of 0.19% and 0.14%, respectively (Table 1). Other aberrant flies found were *wp we* males (0.04%) and 1 wild-type female (0.01%).

Among the 15 434 F_1 flies, screened from crosses between T(Y;5)3–245 males and *we wp* females, 31 recombinants were detected (Table 1). This gives a recombination frequency between the translocation breakpoint and *wp* of 0.084%. The recombination frequency relative to *we* is 0.194%. These values are in agreement with the linear order of the two mutations and the translocation breakpoint. Only 1 double recombinant was recovered, giving a frequency of 0.006%.

The remaining strains have multiple translocations:

1) In strain T(Y;2;4;5)3–4, autosomes 2, 4 and 5 are involved in the translocation (Table 2 and Fig. 2). The Y chromosome is translocated to chromosome 2. Chromosome 2 shows a reciprocal translocation with chromosome 4, and chromosome 4 has a reciprocal translocation with chromosome 5. The breakpoint in chromosome 5 is at position 59C (Fig. 2). This strain is very stable (Table 1), but cannot be used as a GS strain (criterium 3 not satisfied).

2) Strain T(Y;3;5;6)3–136 contains a complex translocation between the Y chromosome and chromosomes 3, 5 and 6. Chromosome 5 translocated to chromosome 3 at position 58A (Fig. 2). The new polytene chromosome order is given in Table 2. In contrast to strain T(Y;2;4;5)3–4, high levels of aberrant phenotypes were found. Two types were observed, wp^+ *we* males and *wp we* males at frequencies of 9.0% and 23.5%, respectively.

3) Four strains have two autosomes involved in the translocation, i.e. strains T(Y;2;5)3–41, T(Y;3;5)3–139, T(Y;3;5)3–182 and T(Y;5;6)3–204 (Table 2, Figs. 2 and 3). Aberrant flies occur at frequencies ranging from 0.4% to 29% (Table 1).

Distribution of translocation breakpoints in chromosome 5

Figure 4 shows the distribution of all observed breakpoints, autosome-autosome as well as Y-autosome, detected in chromosome 5 in a total of 28 translocation strains; 15 strains are described elsewhere (Franz et al. 1994; Kerremans and Franz 1994), and 13 strains are described in this report. Slightly more breakpoints are found in the middle of the right arm. This result might be the consequence of non-random sensitivity of chromosome 5 to chromosome breaks or to the method used for selecting Y-autosome 5 translocations.

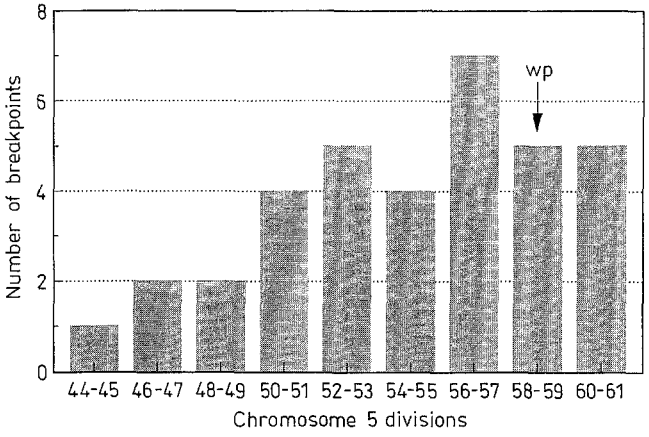


Fig. 4 The distribution of the translocation breakpoints on chromosome 5 (in total 28 translocations) is shown as nine classes, each corresponding to two divisions of the TC chromosome map (no breakpoints were detected in division 62)

Discussion

We attempted to isolate new translocation strains that (1) show very low levels of recombination (criterium 1), (2) produce few aneuploid offspring (criterium 2) and (3) have simple translocations (criterium 3).

The following new translocations satisfy the first criterium, i.e. their breakpoint is close to *wp*: T(Y;2;4;5)3–4, T(Y;2;5)3–41, T(Y;5)3–129, T(Y;3;5;6)3–136, T(Y;5)3–179, T(Y;5)3–245. These strains should theoretically show a lower or equal level of recombination with *wp* when compared with T(Y;5)2–22 or T(Y;5)1–61 (Franz et al. 1994). The recombination frequencies in these strains are around 0.03–0.06% (Franz et al. 1994). Two strains, T(Y;3;4)3–4 and T(Y;5)3–179, have a comparable or lower recombination frequency, while others have a higher recombination frequency. Strain T(Y;5)3–129 has a higher recombination frequency than T(Y;5)2–22, although its breakpoint is closer to the *wp* locus. It is possible that other factors, such as the position of the breakpoint on the Y chromosome or recombination suppressors/enhancers, also influence the recovery of these aberrant phenotypes (Kerremans et al. 1990). There are some indications that exchanging the genetic background of the GS strain can influence the recombination frequency between the translocation breakpoint and the *wp* locus (unpublished data).

Ideally, the autosomal breakpoint should be very close to *tsl* (used as genetic sexing gene) and *wp* (an easy marker for monitoring strain breakdown during mass-rearing). From Fig. 4, we can deduce that with our isolation method, we recover many translocations in the region 56–57, between the centromere and the *wp* locus. Slightly fewer translocations were recovered in the regions 58–59 and 60–61, i.e. close to the *wp* and *tsl* locus. This might either be the consequence of our screening procedure or could mean that translocations are more easily induced in certain chromosome regions.

The second criterium, absence of viable aneuploid offspring, is satisfied for strains T(Y;2;4;5)3–4, T(Y;2;5)3–41, T(Y;5)3–129 and T(Y;5;6)3–204. In comparison, the strains T(Y;5)1–61 and T(Y;5)2–22 produce 15% and 21% aneuploid offspring, respectively (Franz et al. 1994). Therefore, strain T(Y;5)3–129 is clearly an improvement. Strain T(Y;5)3–245 is not included in the list of strains that satisfy criterium 2 because there is some evidence that a certain proportion of the *wp we* females are aneuploid (unpublished data).

The strains T(Y;5)3–86, T(Y;5)3–128, T(Y;5)3–129, T(Y;5)3–179, T(Y;5)3–192, T(Y;5)3–245 and T(Y;5)3–263 have only chromosome 5 involved in the translocation (criterium 3). It is well-known from *Drosophila melanogaster* that the complexity of a translocation influences fertility (Ashburner 1989); this is also observed in *Ceratitis capitata* (Franz and Kerremans 1993). This is the consequence of the production of unbalanced gametes that do not produce viable zygotes. If strains with lower fertility are used in a mass-rearing factory more breeding cages have to be set up to produce the same number of male offspring as strains with normal fertility. For this reason, only strains with a simple Y-5 translocation are considered for further analyses.

In previous experiments (Franz et al. 1994) we have isolated two GS strains [T(Y;5)1–61 and T(Y;5)2–22] that are very stable, i.e. no breakdown of the sexing system was observed after more than ten generations of simulated mass-rearing. These strains satisfy criteria 1 and 3, i.e. the autosomal breakpoint is relatively close to *wp* and *tsl*, and only autosome 5 is involved in the translocation. However, both strains produce viable, aneuploid offspring and, therefore, criterium 2 is not met. Because these flies survive only a few days and are most probably not competitive in the field, the effectiveness of the sterile-male release would be reduced.

However, these aneuploid flies are sensitive to elevated temperatures (unpublished results). Because we use the *tsl* mutation for genetic sexing, not only the homozygous *tsl* females can be eliminated at the egg stage, but also a major proportion of the aneuploid genotypes. The application of a temperature treatment for sex-separation therefore avoids the problem mentioned above. As a result, however, the overall production of viable eggs is reduced, a crucial parameter for mass-rearing. Strain T(Y;5)3–129 is the only one that satisfies all three criteria. The only troubling factor is that the recombination frequency is slightly higher than expected. However, it is possible that changes to the genetic background or modifications of certain environmental factors (temperature, food composition, population density, etc.) could be effective in lowering the recombination frequency.

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