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Viability and behaviour of translocations that suppress female recombination in the Mediterranean fruit fly, *Ceratitis capitata* (Wied.)*

E. Busch-Petersen, A. Pyrek and J. Ripfel

Entomology Unit, IAEA Laboratories, A-2444 Seibersdorf, Austria

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Summary. The viability of a series of recombination suppressor (RS) strains in *Ceratitis capitata*, all previously found to contain a reciprocal autosomal translocation, was assessed for egg hatchability and adult emergence in both the homozygous and heterozygous state. Except in T 30C, which contains a Y-autosome translocation in addition to the A-A translocation, egg hatch was significantly reduced in all heterozygous translocation strains, and ranged from 42.4% to 58.5% in seeded eggs compared to a control value of 82.8%. Adult emergence from hatched eggs was affected to a lesser extent, but with a range of 59.5% to 84.2%, compared to the control value of 83.1%, remained significantly reduced in 4 of the 6 translocation strains, as well as in the male line of T 30C. In the homozygous configuration all strains, except T 19 and T 109, showed a significant reduction in egg hatchability, whereas adult emergence was not adversely affected. A significant reduction in the egg hatchability of the translocation heterozygotes compared to that of the homozygotes was observed in 5 of the 7 strains, the observed reduction in T 55/109 being non-significant while that of T 30C was significantly increased. The behaviour of translocations as recombination suppressors and their suitability for inclusion in breeding schemes for the isolation of induced recessive mutations is discussed.

Key words: *Ceratitis capitata* – Recombination suppression – Viability – Translocations – Genetic sexing

Introduction

The ability to prevent the occurrence of recombination between marker genes or to prevent the survival of such recombinants is an integral part of any breeding scheme aimed at the efficient induction and isolation of recessive genetic mutations. Such an inhibition of recombination may generally be observed in paracentric inversion heterozygotes, where recombinant events within the inverted chromosome segment result in the production of dicentric and acentric chromosomes, and consequently in unbalanced gametes and inviable zygotes. Alternatively, it can also be observed in translocation heterozygotes when crossing-over occurs in the interstitial segment between the centromere and the translocation breakpoint. In this case, recombinant events result in the transfer of nonhomologous segments, leading to the formation of unbalanced gametes in all alternately segregating centromeres (for a more detailed description, see Strickberger 1976).

As part of a programme to develop genetic sexing mechanisms for the medfly, *Ceratitis capitata* (Wied.), a series of recombination suppression (RS) factors were induced using gamma irradiation (Busch-Petersen and Southern in press). All RS lines were shown cytologically to contain a reciprocal autosomal translocation, in all cases involving the long arm of the submetacentric chromosome 3 plus one other autosome. Female recombination between the two markers, apricot eye (*ap*) and double chaetae (*dc*) (Rössler and Koltin 1976), was reduced by 77.6% to 99.1% (Busch-Petersen and Southern in press), male recombination being virtually absent in the medfly (Rössler 1982). In the present paper the viability of the isolated RS strains and their behaviour and suitability in the isolation of induced recessive mutations is reported.

* This work forms part of a Joint FAO/IAEA research programme on the development of genetic sexing mechanisms for the Mediterranean fruit fly, *Ceratitis capitata* (Wied.)

Table 1. Mean viability and standard deviation in a series of translocation strains measured as egg hatch from total eggs and as adult emergence from hatched eggs in the homozygous and heterozygous configurations

Strain	Homozygote				Heterozygote			
	Egg hatch		Adult emergence		Egg hatch		Adult emergence	
	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD
T 19	72.1 a	3.8	79.2 abcd	6.6	45.4 cd	8.3	77.3 ab	13.0
T 30B	55.0 b	8.0	81.9 abc	4.9	42.4 d	1.6	64.6 cd	10.3
T 30C (♀)	55.7 b	1.8	86.1 ab	2.1	74.7 a	1.4	79.6 ab	7.8
T 30C (♂)	35.8 c	2.1	68.4 d	4.1	54.3 b	3.5	63.0 d	3.5
T 109	80.7 ab	10.9	86.2 ab	1.1	58.5 b	3.9	59.5 d	5.5
T 147	55.5 b	5.0	88.3 a	2.7	45.7 c	1.7	84.2 a	8.8
T 30/55	61.0 b	2.4	73.9 cd	5.2	44.2 c	1.6	71.0 bc	5.4
T 55/109	56.6 b	5.3	77.3 cd	0.9	51.6 bc	7.2	60.0 cd	8.9
dc	77.2 a	2.6	78.2 bcd	5.5	82.8 a	5.9	83.1 a	3.1

[a] Same letter after means indicates no significant difference (*t*-test) within columns

Material and methods

The viability of seven translocation strains was determined in the homozygous and heterozygous chromosomal configurations. Viability of the homozygous translocations was measured by crossing homozygous males and females to normal untranslocated females and males, respectively, while that of the heterozygous translocations was measured by backcrossing the F1 flies to the untranslocated strain. All four reciprocal backcrosses were tested. The same combination of crosses and backcrosses, but utilising the phenotypically similar double chaetae (*dc*) strain, was employed as the control. Viability was calculated both as mean egg hatch from the total number of eggs collected and as adult emergence from hatched eggs.

The sexes of the newly emerged adult flies were separated within 16 hours of emergence to ensure adult virginity (Rössler 1975), and subsequently crossed in lots of 25 flies per sex. Two to four replicates were set up in the determination of homozygous viability, while the heterozygous viability was determined from four to ten replicates. Eggs were collected three times at 2-day intervals on black carbon filter paper, counted, and placed on a carrot-based larval diet. The number of hatched eggs was counted 3 days after collection and the resultant sex ratio was determined upon adult emergence. All developmental stages were reared at 25.0 ± 0.8 °C and 60 ± 5% RH.

Results

Significant differences were observed between the viability of reciprocal crosses in T 30C, both in the egg hatch of the homozygous ($t = 10.0$; $P < 0.01$) and heterozygous ($t = 12.2$; $P < 0.001$) configurations, and in adult emergence ($t = 5.44$; $P < 0.05$, and $t = 4.71$; $P < 0.01$, respectively), the viability being consistently higher in the female translocation carrier (Table 1). No such difference was observed in the other strains, nor was there any significant difference in the adult sex-ratio. Reciprocal crosses of all strains, except T 30C, were therefore combined for statistical purposes.

The observed mean egg hatch and adult emergence of the homozygous and heterozygous configurations and of the corresponding *dc* strain are shown in Table 1. No significant difference was observed in mean egg hatch of the homozygous T 19 and T 109 strains, as compared to the control, whereas all other lines showed a significant reduction in the mean number of hatching eggs. Egg hatch and adult emergence in T 30C(♂) was significantly below that of all other lines. The highest rate of 88.3% adult emergence was observed in T 147. This was significantly higher than the control ($t = 2.85$; $P < 0.05$), whereas emergence in all remaining strains was statistically similar to the control (Table 1).

A larger degree of variation, both in egg hatch and in adult emergence, was observed in the heterozygous configuration of the translocation strains (Table 1). Here egg hatch was similar to the control only in T 30C(♀) ($t = 2.36$; $P < 0.05$), whereas it was significantly reduced in all other strains. Adult emergence was significantly reduced in T 30B, 30C(♂), 109, 30/55, and 55/109, but similar to the control in T 19, 30C(♀), and 147 (Table 1).

The heterozygous viability of all translocation strains, as compared to that of the respective homozygous configurations and taking into account the control mortality of the phenotypically similar *dc* strain, are shown in Table 2. T 19, 30B, 109, 147, and 30/55 showed a significantly decreased egg hatch, whereas an increased level was observed both in the female and male lines of T 30C (Table 2). No significant reduction was found in the mean egg hatch of T 55/109 ($t = 0.97$, $P > 0.05$).

Significant differences in adult emergence between the homozygous and heterozygous configurations were

Table 2. Viability of a series of translocation heterozygotes compared to the viability of the respective homozygotes. Corrections made for control mortality (viability of *dc* = 100)

Strain	Corrected heterozygous viability[a]	
	Egg hatch (%)	Adult emergence (%)
T 19	58.7 b	91.8 a
T 30B	71.9 b	74.2 b
T 30C (♀)	124.9 b	87.0 a
T 30C (♂)	140.5 b	86.6 a
T 109	67.7 b	65.0 b
T 147	76.8 b	89.7 a
T 30/55	67.6 b	90.4 a
T 55/109	85.0 a	73.1 b

[a] The viability of each homozygous translocation strain was allocated the letter a; the same letter after the corresponding heterozygous values indicates no significant difference (*t*-test) within strains

Table 3. Female recombination observed between the mutant markers, *ap* and *dc*, in a series of translocation strains and in the standard *ap dc* strain

Strain	Recombination (%)
T 19	4.09
T 30B	0.17
T 30C	42.9
T 109	0.85
T 147	2.81
T 30/55	0.35
T 55/109	0.44
ap dc	18.25

restricted to T 30B, 109, and 55/109 (Table 2). Although adult emergence was consistently lower in the remaining heterozygotes, this was never significant at the 95% level.

Discussion

The viability of strains carrying a heterozygous translocation is commonly lower than that of standard wild-type strains because of the production of aneuploid gametes during adjacent-1 and adjacent-2 segregation of the translocated chromosomes during meiotic division. In animals, where gametic nuclei are rarely functional until after fertilization, unbalanced gametes usually produce inviable zygotes rather than inviable gametes (Strickberger 1976). As a result, viable zygotes must necessarily arise through alternate meiotic segregation, thus producing the distinct reduction in egg hatch characteristically associated with translocation heterozygotes. The resultant degree of sterility varies widely but is commonly expected to average about 50% (Robinson 1976; Roberts 1975). The mean egg hatch of the translocation heterozygotes ranged from 42.4% in T 30B to 74.7% in T 30C(♀). This compares to the 54.4% to 64.6% found by Kaiser et al. (1983) in *Anopheles albimanus*, and to the 26.1% to 54.6% found by Steffens (1983) in *C. capitata*. It may be noted that the translocation heterozygotes produced by Steffens (1983) were isolated by screening for reduced egg hatch and would, therefore, not have included translocations associated with a higher viability.

Suppression of recombination in heterozygous translocation strains may arise from crossing-over in the interstitial segment between the translocation breakpoint and the centromere. Such crossing-over results in the production of unbalanced gametes when alternate and adjacent-2 segregation takes place, while adjacent-1 segregation now produces all balanced gametes. Thus, assuming that 50% of meiotic segregants arise through alternate segregation (Roberts 1975), and that the last 50% is divided about equally between adjacent-1 and adjacent-2 segregation, the normal degree of 18.25% recombination between *ap* and *dc* would be expected to be reduced to approximately 4.5%, when both markers are located in the interstitial segment of the translocation heterozygote. Excluding T 30C, this level of recombination was approached only by T 19 (Table 3), whereas all other strains showed a very much higher level of recombination suppression. This added level of recombination suppression would suggest that translocation heterozygosity somehow interferes with the initiation or maintenance of cross-over synapsis rather than merely eliminating cross-over chromatids as aneuploid zygotes. Indeed, in a similar study in *Drosophila melanogaster*, Roberts (1970) found that a tip-base translocation lowered crossing-over in the arm broken near the tip, from a control value of approximately 40% to less than 1%. It was therefore concluded that recombination suppression was not caused solely by elimination of cross-over strands in aneuploid segregants but also by a prevention of synapsis in certain arms heterozygous for distal breakpoints. This conclusion was reinforced by Roberts' (1970) failure to recover double cross-over products in long interstitial regions, hence suggesting that cross-overs in these regions were simply not occurring.

In T 30C(♀) and T 30C(♂) the corrected viability of the heterozygotes was higher than that of the homozygotes, when measured as egg hatch. In fact, the homozygous viability of T 30C(♂) was significantly lower than that of any of the other strains. It thus appears that the translocation breakpoints induced in T 30C are heavily detrimental in the homozygous condition, or that lethal genes have been selected in these lines, which affect adult fertility or subsequent gametogenesis. The effects of such factors would be largely eliminated in the heterozygous condition, thus resulting in the lethality of the homozygote being higher than that of the heterozygote. This also explains the difficulties encountered in the isolation of T 30C in the homozygous condition; this strain required 13 generations of single-pair inbreeding

as compared to 4–5 generations for the other strains. The observed differences in the egg hatch of reciprocal crosses of T 30C are most likely due to the confirmed presence of a Y-autosome translocation in this strain in addition to the A-A translocation (Busch-Petersen and Southern, in press).

Assessment of the suitability of the various isolated RS strains for inclusion in breeding schemes requiring the suppression of female recombination must take into consideration the degree of recombination suppression conferred by each respective strain, as well as the viability of these strains. T 30B, 30/55, and 55/109 all reduce recombination between the *ap* and *dc* marker genes to below 0.5% and would thus appear to be the more suitable. Of these three strains, heterozygous egg hatch was significantly reduced in T 30B while no significant difference was observed in T 30/55 and T 55/109. The latter two strains therefore appear the most suitable. T 30/55 is presently being employed in an induction programme aimed at isolating recessive temperature sensitive lethal factors for subsequent use in the genetic separation of male and female medflies mass reared for release in sterile insect release programmes.

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