EFFORTS TO SYNTHESIZE 'BALANCER' STRAINS FOR CHROMOSOME 5 OF *Ceratitis capitata* AND CYTOLOGICAL MAPPING OF THE *Cy* MUTATION

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Abstract

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The authors describe their first efforts to construct 'balancer' chromosomes for the Mediterranean fruit fly, *Ceratitis capitata*. The experiments were focused on chromosome 5, which is genetically the best characterized medfly chromosome. Following the synthesis of a multiple marker strain for chromosome 5, bearing the *Cy* mutation as a selectable marker, attempts were made to induce chromosome rearrangements by gamma irradiation and select those chromosomes showing crossing-over suppression. During this process the cytological mapping of the *Cy* mutation was achieved.

1. INTRODUCTION

'Balancer' chromosomes are special chromosome rearrangements which suppress exchange between homologous chromosomes owing to their complex structures. Such chromosomes, in addition to several chromosome rearrangements, usually bear at least one visible dominant mutation as well as one or more morphological, recessive mutations for identifying the heterozygotes and for easy chromosome manipulation. Balancer strains have proved very useful for genetic analysis in *Drosophila* and are expected to be important in the Mediterranean fruit fly, *Ceratitis capitata*, especially for the synthesis of new genetic sexing strains and/or to improve the existing one. So, having as a model the balancer *Drosophila* strains [1], we have undertaken the synthesis of analogous strains in *C. capitata*.

2. MATERIALS AND METHODS

2.1. Medfly strains

Benakeion is a mass reared strain which is considered as having the standard chromosome arrangement.

Cy we bb wp is a multiple marker strain having the dominant mutation *Cy* (curly, lethal as a homozygote) and the three recessives *we* (white eye), *bb* (blond body) and *wp* (white pupa) all mapped on chromosome 5 by Rössler and Rosenthal [2]. The *bb* mutation is homologous to *ye* (yellow body), characterized and mapped by Rössler and Rosenthal [2].

we bb wp is the multiple marker strain.

Both multiple strains were synthesized by crossing an initial *Cy wp* strain provided by Rössler to appropriate strains and selecting the respective genotypes and phenotypes.

2.2. Induction and selection of chromosome rearrangements

Cy we bb wp males, one to two days old, were gamma irradiated with a dose ranging from 30 to 40 Gy in five different experiments. The following crossing scheme was used for the isolation and maintenance of induced chromosome rearrangements:

P gen *Cy we bb wp*(*irrad* ♂) × Benakeion(♀) (mass culture)

F_1 gen *Cy*(♀) × *we bb wp*(♂) and *Cy*(♂) × *we bb wp*(♀) (single pairs)

↓ ↓

Genetic analysis in F_2 adults (screen for recombinants)

Cytological analysis in F_2 larvae

We maintained for detailed cytogenetic analysis those F_2 families either showing absence of recombination or bearing any chromosome rearrangement, by outcrossing *Cy* flies to the *we bb wp* strain. We considered the outcrossing necessary in order to improve the low fertility and viability associated with the irradiated chromosomes.

3. RESULTS AND DISCUSSION

3.1. Characterization of *Cy* mutant

Following a pilot experiment it became clear that *Cy* strains are always associated with a heterozygous translocation in which chromosomes 4 and 5 are involved (Fig. 1). At first it was considered a spontaneous chromosome mutation in a multiple marked *Cy* strain constructed for use in irradiation experiments. However, more detailed cytological analysis performed in all strains having the *Cy* phenotype revealed that the translocation is always present with *Cy*. It should be pointed out that *Cy* is an induced mutation isolated by Rössler (personal communication) in 1987 following irradiation. Since then, all the existing *Cy* strains have been derived from

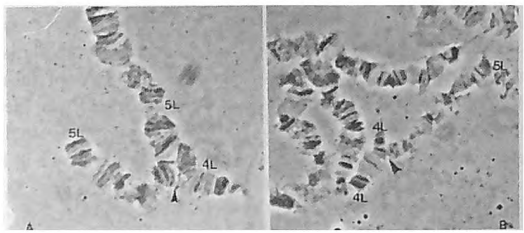

FIG. 1. Salivary gland polytene chromosome from Cy *strains. (A) The pairing cross of the heterozygous T(4;5)*Cy. *(B) The two homologous chromosomes 4, one normal and one translocated. The breakpoints are 41B and 63C for chromosomes 4 and 5, respectively.*

this induced mutation and maintained by crossing *Cy* flies in each generation. Thus, the mutation is not associated with the translocation but with one of the breaks. In view of these data the chromosome position of *Cy* had to be redetermined to find whether it was on chromosome 4 or 5.

Figure 2 shows the karyotype as well as the meiotic segregation of the heterozygous translocation T(4;5)*Cy*. According to this scheme we expect, in addition to the balanced gametes (1, 2) resulting from alternate segregation, two unbalanced gametes (3, 4) from adjacent-1 segregation. The contribution of these last gametes to the viable zygotes could give an answer regarding the position of the *Cy* mutation.

In trying to determine the chromosomal position of *Cy*, three types of single-pair crosses were set up (ten crosses in each case) and the genotypes of their F_1 progeny were examined during the larval stage. Figure 3 shows the crosses as well as the observed karyotypes following polytene chromosome analysis in F_1 larvae. Four classes of karyotypes were observed among the progeny in crosses of type A, where both mated flies had the *Cy* phenotype. Class 1 consists of the normal genotype while

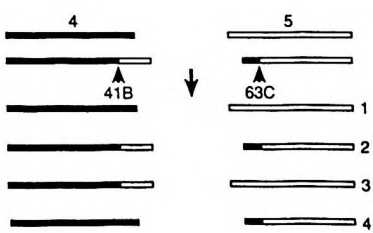

*FIG. 2. The karyotype and meiotic segregation of the translocation T(4;5)*Cy.

class 2 is heterozygous for the translocation T(4;5)*Cy*. The other two classes comprise individuals with an unbalanced chromosome constitution. Both are partially trisomic and monosomic for chromosomes 5 and 4, respectively. One of these has three complete chromosomes (two 5 and one 4) and one translocated, and the second has only one complete chromosome (chromosome 5) and three translocated (two 4 and one 5). Polytene chromosomes from those two aneuploids are shown in Fig. 4.

Three classes of genotypes were found in crosses of type B (Fig. 3), where only one of the mated flies had the *Cy* phenotype. A different situation was observed in

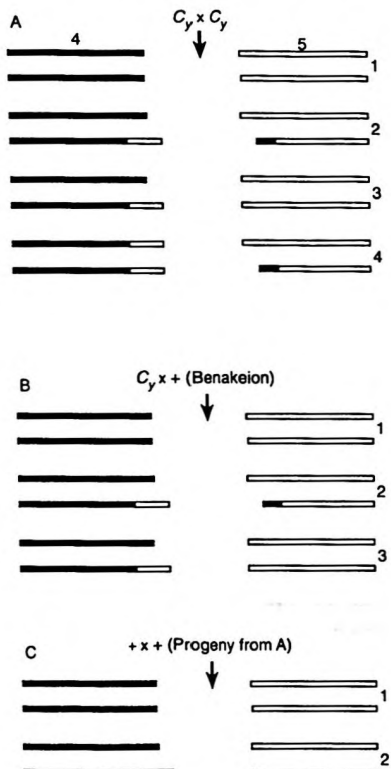

FIG. 3. Types of crosses and the karyotypes observed among their progeny (see text for a detailed description).

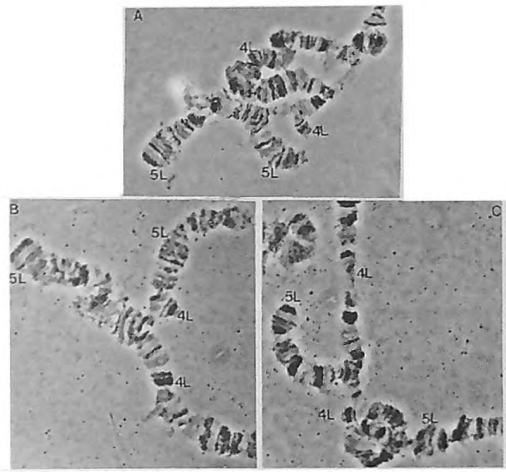

FIG. 4. Aneuploid larvae bearing a triplication for 5L tip and a deficiency for 4L tip. (A) One normal chromosome 4. (B, C) Both chromosomes 4 translocated.

crosses of type C (Fig. 3), where mated flies had the wild phenotype and both were progeny from crosses of type A. Some crosses produced only one normal genotype, while in others two classes of progeny were observed, one showing the normal karyotype and the second an aneuploid one.

These data clearly show that only one of the unbalanced gametes (class 3, Fig. 2) contributes to the viable zygotes at least up to the larval stage. The second (class 4, Fig. 2) may be due to the deleted part of chromosome 5, which does not contribute to the zygotes. In addition to the cytological analysis in the larval stage, F_1 progeny from all three crosses were also checked for the presence of the *Cy* phenotype. Flies showing the *Cy* phenotype were always observed in the first two crosses (A, B) but never in the third (C).

This observation, in combination with the genotypes found in F_1 larvae as well as with the recessive lethal condition of *Cy* mutation, clearly shows that *Cy* maps to chromosome 5 (supporting earlier genetic data [2]) and also to the translocation breakpoint. This position favours the stability of *Cy* strains. The *Cy* phenotype is always associated with the translocation T(4;5)*Cy*.

It is interesting to note that *Cy* in *Drosophila* is also mapped to an inversion breakpoint, although at the moment there is no evidence concerning homology between the two mutations.

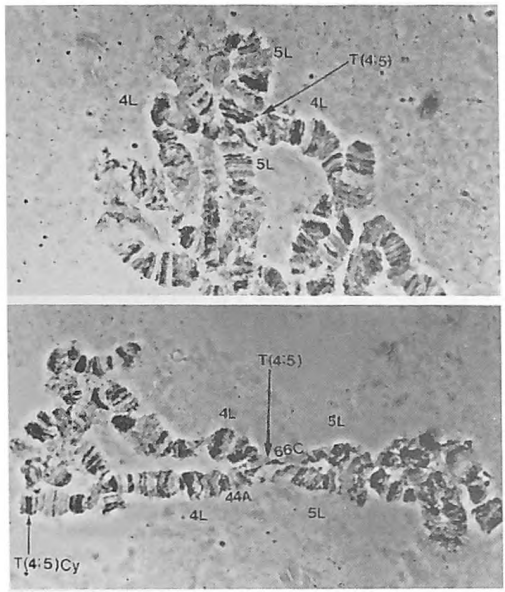

FIG. 5. An induced translocation between chromosomes 4 and 5, in addition to the T(4;5)Cy. Arrows indicate the breakpoints 44A and 65C for chromosomes 4 and 5, respectively.

3.2. Inversion induction

During two years of effort on the synthesis of a balancer strain for medfly chromosome 5, 450 single-pair crosses were set up in five irradiation experiments. However, most of these crosses exhibited low viability, and in others no progeny were produced, a fact that can be attributed to the induced mutations in the genome of the irradiated males. This of course is a problem in cytogenetic analysis, where a large number of progeny are necessary for a detailed analysis. To overcome these difficulties the *Cy we bb wp* flies were subsequently outcrossed to the *we bb wp* strain until the viability was improved. It should be emphasized here that cytological analysis in *C. capitata* is not as easy as in other Diptera, owing mainly to chromosome quality: chromosomes often break and usually show extensive ectopic pairing, so a large number of chromosome slides are needed for a complete analysis.

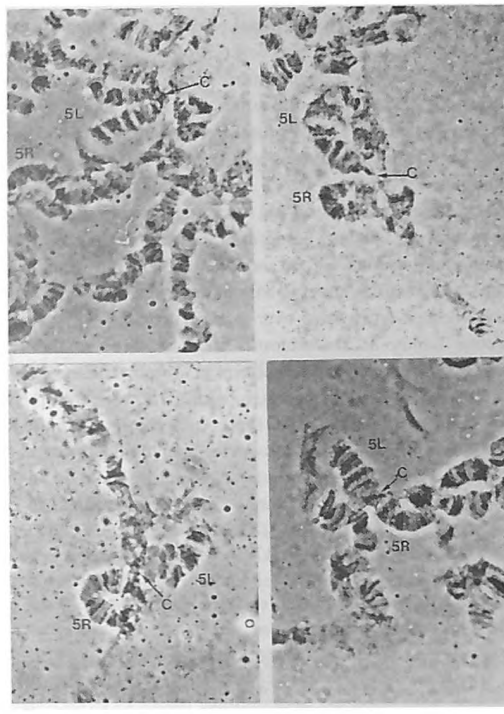

FIG. 6. Pairing configuration of the chromosome 5 centromeric region in the progeny of the F_2 family showed an absence of recombinants.

This condition, in combination with the low viability observed in most families, added to the difficulties. The genetic and cytological analysis could not be completed at the F_2 generation in most of the families.

The results of the first four experiments were disappointing, in that no crossover suppressor in chromosome 5 was found. Although a number of rearrangements, mostly translocations, were observed, none involved chromosome 5, a fact that is in very good agreement with the genetic analysis.

In the last experiment, we raised the irradiation dose to 40 Gy, hoping to increase the frequency of the induced chromosome mutations. A total of 150 F_1 *Cy* individuals (80 males and 70 females) were crossed to the *we bb wp* strain. However,

as a result of both low egg hatch and pupal eclosion, only 25% of them produced progeny, which in some cases were very few. Cytological analysis in these families, where it was possible, revealed a large number of chromosome rearrangements in all families examined, but owing to the difficulties mentioned above, it was not possible to identify the rearrangements. Therefore, we maintained most of the families by outcrossing to analyse them in subsequent generations.

In one family derived from F_1 *Cy* males during the fifth generation a new translocation between chromosomes 4 and 5 was identified in addition to the existing one. It is clear from Fig. 5 that both new breaks are in about the middle of the two left arms. The viability of the strain is good and it is maintained by mating only *Cy* flies. Genetic analysis to assess the effect of this induced rearrangement in combination with the *Cy* translocation on crossing-over is not yet complete.

In a second family no recombinants were observed in the F_2 generation. Cytological analysis in the next and subsequent generations did not show an obvious rearrangement, except a kind of unusual pairing in the centromeric region of chromosome 5. It must be pointed out that this pairing configuration (Fig. 6) is always present with the *Cy* translocation and also in the aneuploid karyotype (Fig. 3, cross A, class 4). If this is a real chromosome rearrangement it should be an inversion with the breaks close to the centromere. Further studies are in progress to confirm these observations.

4. CONCLUSIONS

The results clearly show that the synthesis of a balancer strain, at least for medfly chromosome 5, is not as easy as was first considered. It is possible that the strain used in these experiments is itself one of the reasons for this. However, other reasons related to the dose or the type of irradiator cannot be ruled out. Therefore, our next step will be, in addition to a complete cytogenetic analysis of all families maintained, to try a new irradiation experiment, using this time a different irradiator and possibly a different dose, in addition to a new multiple marker strain for chromosome 5.

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[2] RÖSSLER, Y., ROSENTHAL, H., Genetics of the Mediterranean fruit fly (Diptera: Tephritidae): Morphological mutants on chromosome 5, Ann. Entomol. Soc. Am. **85** (1992) 525–531.