GENETIC MARKERS, TRANSLOCATIONS AND SEXING GENES ON CHROMOSOME 2 OF *Ceratitis capitata*

J.L. CLADERA
Instituto de Genética Ewald A. Favret,
Instituto Nacional de Tecnología Agropecuaria,
Castelar, Argentina

Abstract

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A review is presented of results obtained in a search for genetic markers, translocations and selectable genes obtained at the Instituto de Genética, Castelar, Argentina, with special reference to chromosome 2 linked mutations and genes useful for developing self-sexing strains in *Ceratitis capitata.*

1. INTRODUCTION

In any attempt to improve the sterile insect technique (SIT) by means of genetic sexing systems a fair amount of basic knowledge on genetic markers and chromosomal morphology (besides some selectable genes) is required. The linkage group associated with chromosome 2 of the Mediterranean fruit fly, *Ceratitis capitata,* is not as well studied as others. Rössler and Rosenthal [1] did not identify any morphological markers and Malacrida et al. [2] questioned the linkage of nine biochemical variants to any morphological marker of linkage group 2. A number of reports have been produced at the Instituto Nacional de Tecnología Agropecuaria (INTA) in Castelar but they remain unpublished (INTA Yearly Reports). The description of these markers and some information obtained on chromosome 2 will probably be useful in the general context of the genetics of the medfly and its application to the improvement of the SIT.

The purpose of this paper is also to clarify some lack of uniformity in the nomenclature of published and unpublished genetic variants and translocations in this linkage group and a first attempt is made to put them on the genetic map. Genes affecting the rate of development have been isolated within this linkage group. How they are now used for the construction of new genetic sexing strains is briefly described.

2. HISTORICAL PERSPECTIVE

Research on the biology and genetics of *C. capitata* started at Castelar in the mid-1970s, well before any institutional support was assured. At that time only a small number of papers were known on the existence of genetic variants in this species. Starting in November 1981, the author's laboratory entered a period of interaction with the International Atomic Energy Agency under Research Contract No. 2973/R3/RB. The title of the contract was "Induction and utilization of mutations to delineate the complexities of sex determination and the stability of Y–autosome translocations in *Ceratitis capitata*". This project was acknowledged by INTA as an institutional plan with the title "Determinación de métodos genéticos para el control de poblaciones de *Ceratitis capitata*" in December 1982. A good deal of knowledge has been gained in the last two decades, particularly with relevance to the development of genetic sexing strains.

3. MUTATIONS

The mutants used in this laboratory are listed in Table I. A condensed description of their phenotypes follows.

3.1. Niger

nig: black adult, black pupa and black larval spiracles. There are two alleles: nig^1, originally found at Castelar, and black fly (proposed symbol nig^{bf}), found at the Agency's Laboratories in Seibersdorf, near Vienna. Origins: nig^1: ethylmethane sulphonate (EMS); nig^{bf}: spontaneous [3, 4].

TABLE I. MUTATIONS OFTEN USED AT INTA, CASTELAR

Name	Symbol	Dominance	First cited	Refs
(a) On chromosome 2				
Niger	*nig*	Recessive	1979	[3, 4]
Pupal esterase	*Est-1*	Codominant	1981	[4, 5]
Long pupa	*lp-1*	Recessive	1985	[6]
Dark dorsal abdomen	*d*	Recessive	1985	[7]
Slow	*sw*	Recessive	1990	[8, 9]
(b) Not on chromosome 2				
Black pupa	See text	Semidominant	1985	[7]

3.2. Pupal esterase

Est-1: this gene is expressed only in pupae 24–48 h after pupariation. The preferred substrate for it is 2-naphthyl acetate. There are three alleles: $Est\text{-}1^c$ is found only in the field; the other two, $Est\text{-}1^a$ and $Est\text{-}1^b$, have been studied in the laboratory and found in the field. Origin: naturally occurring variation.

The same symbol has also been used for a gene active in adult flies. It would be possible to distinguish them simply by referring to pupal esterase 1 (*pEst-1*) and adult esterase 1 (*aEst-1*). Variation in pupal esterase does not show a correlation with any band observed in the adult [4, 5].

3.3. Long pupa

lp-1: pupal case 10–20% longer than in the wild type. The shape is not ovoid but rather club-like, half-way between the shapes of a larva and a pupa. There is only one allele.

lp-2: linked but not allelic; because of its variable and milder effect, not analysed in detail. Origin: X rays [6].

3.4. Dark dorsal abdomen

d: dark adult body with no effect on pupa. There are three alleles, identifiable by the growing intensities of their effects: $d^i > d^d > d^a$. The most commonly used allele is d^d. Origin: EMS [7].

3.5. Slow

sw: mutations at this locus cause a visible effect on eye iridescence and a more purple-reddish pigmentation than in the wild type. They also cause a slowing down of the rate of development. There are two alleles: slow-mazzard (sw^z) and slow-mulberry (sw^y), the latter having a stronger effect on development than the former. Origin: EMS [8, 9].

3.6. Black pupa

Although not linked to previous markers, black pupa is included here because, in the homozygous condition, it produces a phenotype indistinguishable from *nig* on the pupa. However, the heterozygous form is intermediate in colour. In addition, it causes very little, if any, effect on the adult. Three alleles have been isolated: *Bp*, *Bn* and *Bs*. Later they proved to be alleles of a gene previously described as *dp*. Therefore, it is proposed here to unify nomenclature as

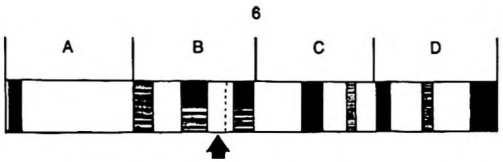

FIG. 1. Schematic representation of region 6 on the polytene banding pattern of chromosome 2, in translocation strain T5038, showing the site where the sex chromosome complex is associated to band 6B.

follows: dp^{1}, dp^{Bp}, dp^{Bn}, dp^{Bs}. Origins: dp^{1}: spontaneous; dp^{Bp}, dp^{Bn}, dp^{Bs}: EMS [7].

4. TRANSLOCATIONS

The first evidence associating the linkage group of *nig* to chromosome 2 came from a translocation between this chromosome and chromosome 4 induced by X rays, selected by its property to reduce hatchability, and identified just by cytological observations. The linkage of the inheritance of *nig* to the marker carmine eye indicated the association of *nig* and *ca* to chromosomes 2 and 4 (E. Lifschitz and F.C. Manso, unpublished observations). After a number of generations the strain carrying this translocation was lost.

Another translocation was isolated by its property to link *nig* to the sex: T(Y,nig^{+})5038. As it happened to be a translocation not visible in mitosis, the final proof for its association to chromosome 2 has only recently been obtained using

TABLE II. HOLANDRIC LINES DERIVED FROM T5038

Male		Female	
Phenotype	Genotype	Phenotype	Genotype
+	Y+/*nig*	*nig*	*nig/nig*
+	Y+/*lp*	*lp*	*lp/lp*
+	Y+/*d*	*d*	*d/d*
+	Y++/*lp d*	*lp d*	*lp d/lp d*
+	Y+++/*lp d sw*	*lp d sw*	*lp d sw/lp d sw*
sw	Y++*sw*/*lp d sw*	*lp d sw*	*lp d sw/lp d sw*
d sw	Y+*d sw*/*lp d sw*	*lp d sw*	*lp d sw/lp d sw*

polytene chromosome preparations (Fig. 1). The breakpoint has been localized at region 6B in the polytene chromosome map of orbital bristle trichogenic cells [10].

By means of T5038, a number of strains have been obtained, linking the inheritance of different genes to the sex (wild type allele linked to males and mutant alleles to females). Some of them are listed in Table II. The all wild type male strains were obtained simply by crossing T5038 males to the appropriate mutant female and backcrossing F_1 males to females of the same mutant strain. The two male marked strains (*sw* and *d sw*) were obtained by crossing two single individuals produced by male recombination events.

5. MALE RECOMBINATION

Early cytological observations of meiosis were not conclusive as to whether chiasmata were present or not in *C. capitata* males. Therefore, finding two linked genes, *Est-1* and *nig*, was very useful for performing a genetic test. The conclusion was that recombination does not occur in males to the same extent as in females [4]. Other observations followed, working with larger numbers and using morphological markers, the result of which was the demonstration of exceptions to that rule, usually occurring in the order of 10^{-2} [1].

A large scale experiment was then set up in the laboratory to find the magnitude of this phenomenon. Two morphological markers, *lp* and d^d, were chosen which are located far apart in the female linkage map. Overall 44 639 flies were scored, among which 49 progeny tested individuals proved to be true cases of male recombination (Table III). Therefore, the frequency of male crossing-over between two markers far apart on chromosome 2 was $(1.10 \pm 0.16) \times 10^{-3}$, remarkably low for two genes that distance apart [11].

6. CONDITIONAL LETHALS

A project directed towards the isolation of temperature sensitive lethal mutations in the medfly was initiated in Castelar around 1985. Information was obtained on the thermal tolerance of the embryo and the conditions required to perform a screening for such mutations [12]. Some strains were actually obtained [13] but this project had to be discontinued in 1988 for lack of support.

A more promising line of research then appeared: eye colour mutants with a conspicuously longer time of development. The first mutant, named slow-mazzard (sw^z) [9], requires about two days more than the wild type to reach the pupal stage at normal temperature (Fig. 2(a)). When a holandric line was built with T5038 (wild type male, mutant female), and temperature was reduced by 2°C, an automatic sexing

TABLE III. PROGENY TESTED CASES OF MALE RECOMBINATION AMONG 44 639 PROGENIES OF ++/*lp d* MALES

	(+) *d*	*lp* (+)	Total
Male	11	10	21
Female	14	14	28
Total	25	24	49

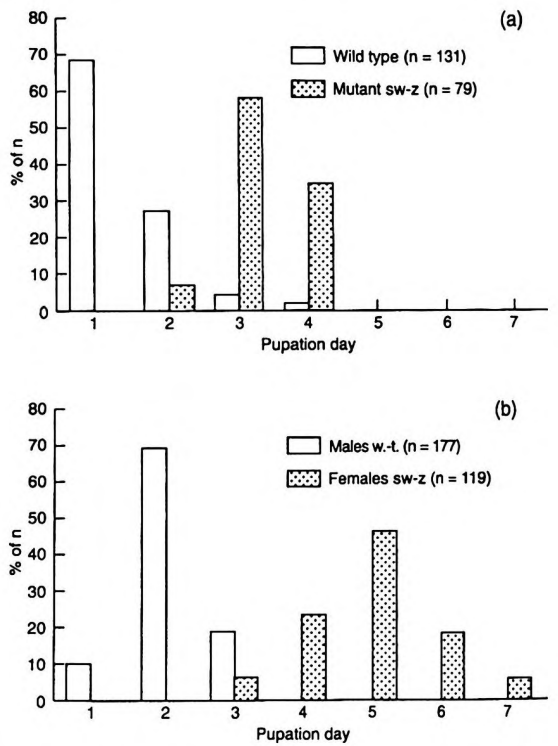

FIG. 2. Percentage of wild type and sw^z *flies arising from successive days of pupation in (a) the two original strains (temperature 26°C) and (b) a strain with a Y–2(+/sw) translocation (temperature 24°C).*

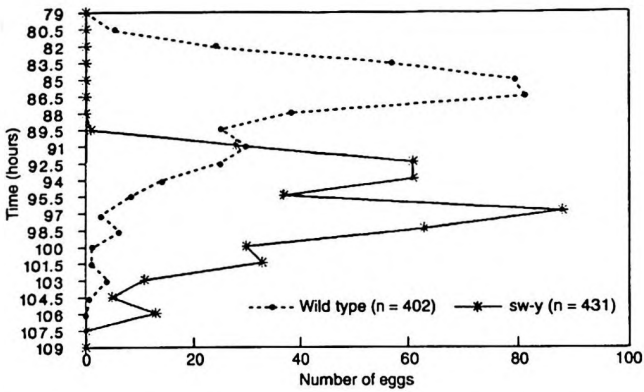

FIG. 3. Number of wild type and sw^y *flies arising from eggs hatching at different times after laying (temperature 22°C).*

strain was obtained in which the larvae jumping out of the food during the first days are the males and those jumping later are the females (Fig. 2(b)).

The embryonic development was also longer in this mutant than in the wild type, but not so much as to allow separation at that stage in the conditions of this experiment. A second mutant, named slow-mulberry (sw^y) [8], was also obtained; this was associated with a stronger reduction in the rate of development during both embryonic and larval development. Using this strain and lowering the temperature by another 2°C, a good separation of the two populations of embryos was obtained at the time of hatching (Fig. 3). It has been shown that both mutations are alleles of one

TABLE IV. LINKAGE MAP OF GENES LOCATED ON CHROMOSOME 2

Gene	T5038	*lp*	*nig*	*Est*	*d*	*sw*
Distance (c.o.)		15(18)	c. 50	20	33	
Polytene	1A 6B		15D–16A			24D
			?			
Refs	[9, 10]	[6],[a]	[6, 9, 15]	[15]	[9],[a]	

[a] Unpublished.

gene [14]. Research is in progress aimed at the use of this gene in a sexing strain at a mass rearing facility.

7. MAP OF CHROMOSOME 2

Most of the information available on the relative locations on the recombination map of the markers mentioned in this paper has been brought together in Table IV. Although some genetic distances have been included in the picture, they must be taken as approximate. The gene order, however, is reasonably certain.

What has been learned from working with the linkage group associated to chromosome 2 is interesting and contributes to the growing body of genetic knowledge that is accumulating on this species. It is hoped that this will be useful in the near future not only in helping to improve the SIT but also in developing newer and more sophisticated strategies for the control of this and other insect pests.

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