GENES AND CHROMOSOME ARRANGEMENTS AFFECTING SEX RATIO IN THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wied.)

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Abstract

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The *MP* (male producing) factor, which shows temperature sensitive meiotic drive favouring the Y chromosome, proved to be highly variable in spermatozoal deficiency in different cysts within a single testis. However, the overall loss of sperm corresponded almost precisely with the loss of females. The minimum proportion of females consistently obtained in inbred lines was about 30–35%. On the basis of parallel studies with the mosquito *Aedes aegypti*, variability between cysts is open to interpretation in terms of different rates of senescence. The T:Y(wp^+)30C genetic sexing strain, which is designed to generate males with brown (wild type) puparia and females with white puparia, was contaminated artificially in a series of population experiments to investigate the pattern of breakdown. Wild type contamination with either sex caused an increase of brown pupae. The sex ratio became progressively distorted in favour of females after contamination with females, mated or unmated, but not after male contamination. The experiments revealed evidence of a low frequency of natural recombination between wp^+ and the translocation breakpoint on the Y chromosome, shown by the appearance of *wp* males. The frequency of male recombination (r) and the selection coefficient (s) against *wp*/*wp* were measured over 11 generations. The best fit to the observed data was obtained with $r = (0.14 \pm 0.04)\%$ and $s = (26.0 \pm 2.7)\%$. Using these estimates to predict the frequency of wp^+ females and *wp* males for up to 100 generations, it was concluded that white males would never exceed 0.5% whereas the frequency of brown females was expected to exceed 33% after 25 generations. Published data on the mass reared strain, maintained with a population size of 240 000 adult flies, were subjected to the same analysis. A

higher value of s (between (38.0 ± 3.2)% and (52.0 ± 0.3)%) was obtained under these conditions. Electrophoretic studies on esterases revealed a significantly higher activity in a recently colonized strain from Morocco than in two laboratory strains (H1 and TY4). No change in activity was observed in this strain during the first three generations of laboratory culture.

1. INTRODUCTION

The technical contract (5261/TC) of the International Atomic Energy Agency under which this work on genetic sexing of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), was carried out extended over the period 1989–1994. The aspect of the work relating to sex ratio distortion by meiotic drive at the *MP* (male producing) locus was already well ahead by then [1–4]. Our aim as we began the contract extension was to enhance the action of *MP*, with the hope of producing a greater reduction of females. To this end we engaged in a programme of selective inbreeding, and we also experimented with manipulating the parental rearing temperature, having demonstrated that the degree of distortion was temperature dependent. Temperature treatments were eventually abandoned as unproductive (see below). Selection experiments produced lines generating around 30–35% females in continuous culture for ten or so generations, without further selection [5, 6]. These strains were used for investigating the mechanism of distortion.

This evidence located *MP* (or a regulator of *MP*) on the Y chromosome, and indicated resistance to *MP* to be either X linked or autosomal. Cytological investigations revealed changes during spermiogenesis, to be reported below.

The second major component of our work arose out of a series of population experiments and computer simulations to investigate the process of breakdown of Y translocation based genetic sexing strains, carried out at the Agency's Laboratories in Seibersdorf [7–9]. Here we report the results of our joint studies on two aspects of work on strain T:Y(wp^+)30C: (1) the effect of adding wild type contaminants to the strain; and (2) measurements of recombination between *wp* and the Y chromosome. We have shown that these two effects have a different outcome in terms of the pattern of strain breakdown.

Recent work has centred upon investigating the impact of unconscious laboratory selection on sexing strains, with respect to which we shall report preliminary work on esterase activity.

2. MATERIALS AND METHODS

T:Y(wp^+)30C [= TY30C], isolated in 1985 by Busch-Peterson et al. [10], is a strain in which the wp^+ allele has been translocated on to the Y chromosome in a *wp*

background, so that males are T:Y-*wp*$^+$/*wp* (brown puparia) and females *wp/wp* (white puparia).

T:Y(*wp*$^+$)4 [= TY4] is a strain based on the same genotype as T:Y(*wp*$^+$)30C, supplied by G. Franz of the Agency's Laboratories, Seibersdorf.

MP comprises A425 and various selected substrains. A425 originated from a single X ray exposed male [1]. The substrain investigated for esterases was H1 [11]; the substrain investigated cytologically was H1-18-27/9 [6]. Both lines supported a significant excess of males.

CHIOS was recently collected on the Greek island of that name, and was obtained from G. Franz.

DOUBLE-CHAETA, obtained in 1979 from Y. Rössler in Israel, was originally homozygous for the mutant double chaeta (*dc*) although the mutant phenotype was no longer evident.

SEIBERSDORF was a long standing laboratory colony.

MOROCCO was obtained from P. Howse, of Southampton University, straight from the field, and was collected from Argon trees.

Most of the experimental methods have been published [12–14] or reported in University of Manchester postgraduate theses [4–6, 15]. Others are described briefly in the appropriate section.

3. DISCUSSION OF RESULTS

3.1. Strains with excess of males due to meiotic driving *MP* factor

The proportion of females in progenies of *MP* males was found to decrease significantly when such males were exposed as pupae to a reduction in temperature from (26 ± 2)°C to (18 ± 1.5)°C for 24 h from the end of day 3 of pupal development [1–4]. More recent studies have shown, however, that this only happens when the proportion of females is initially relatively high (around 45%). In genetically selected lines, yielding a lower proportion of females (30–35%), temperature treatments of 18°C were found not to reduce the proportion of females any further.

The holandric pattern of inheritance of *MP* [4] resembles that found in the better known *MD* (meiotic drive) system of *Aedes aegypti* [16]. The *MD* haplotype causes X chromosomes to fragment into two or more pieces. The result is a reduction in the number of spermatozoa and gross abnormalities in some of the remainder [16]. The extent of X chromosome fragmentation is more than enough to explain the deficiency of spermatozoa and almost sufficient to explain the loss of females (Table I). The reduction of sperm density in *MD* males is, however, insufficient to explain the depletion of females observed in the progeny of such males (Table I). The

proportion of abnormal sperm cannot be defined precisely in *Ae. aegypti*, which probably accounts for the disparity.

In *MP* males of *C. capitata* we have found a comparable depletion of sperm and similar types of abnormality: compound spermatozoa with multiple axial filaments and extra mitochondrial derivatives. However, the possibility of investigating the precise extent of spermatozoal loss and abnormality is more favourable in this species. This is because in the young testis of *C. capitata*, spermatozoa are arranged in defined cysts, those in the same cyst being the product of a single primordial germ cell [6]. The normal number per cyst in *C. capitata* is 256, the result of six mitoses plus meiosis. By counting the number of normal spermatozoa per cyst in *MP* males, it can readily be shown that the average depletion corresponds almost precisely with the loss to be expected from the sex ratio distortion observed in the progeny of such males (Table I). The correspondence confirms that it is mainly X bearing spermatozoa that are lost or abnormal.

One observation to be noted [6] is the wide variation between cysts within the same *MP* testis. Some show 50% spermatozoa missing or abnormal (100% expression of *MP*); others show the full complement of apparently normal spermatozoa (zero expression of *MP*, Fig. 1). Because the spermatozoa from different cysts become mixed together, it is the average content of spermatozoa per cyst which is reflected in the sex ratio (Table I).

TABLE I. PERCENTAGE OF FEMALES OBSERVED IN THE PROGENY OF *Ae. aegypti* MALES CARRYING THE Y LINKED *D* GENE AND *C. capitata* MALES CARRYING THE Y LINKED *MP* GENE, COMPARED WITH PERCENTAGE OF FEMALES EXPECTED ON THE BASIS OF (1) OBSERVED FRAGMENTATION OF X AND Y CHROMOSOMES AT SPERMATOGENESIS, (2) SPERM DEPLETION (DENSITY COMPARED WITH MALES NOT CARRYING THESE GENES), (3) SPERM DEPLETION PLUS SPERM ABNORMALITY

Species	Meiotic drive gene	Percentage female pupae or newly emerged adults				Ref.
		Predicted by X or Y breakage at spermatogenesis	Predicted by sperm density (including abnormal sperm)	Predicted by density of normal sperm	Observed	
Ae. aegypti	*D*	8.6	21	<21	3.8	[17]
C. capitata	*MP*	—	42.9	37.7	37.6	[6]

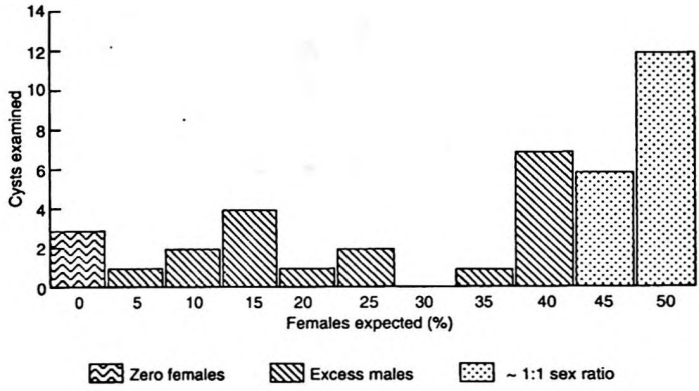

FIG. 1. Percentage females expected from the gametes present in 39 mature cysts taken from two testes of the MP *strain of* C. capitata.

To be of practical use as a genetic sexing technique, a meiotic drive system, such as *MD* or *MP*, needs to be conditional in its action, in a way that can be easily controlled. To breed such a strain in large numbers demands culture conditions in which the gene is not expressed. Then, with an appropriate stimulus applied, the strain can be transformed to provide only males. A possible approach to making *MP* conditional in its action could lie in exploiting the cause of the variation between cysts of the same genotype. The possibility of some definable stimulus which switches on the *MP* genes in some cysts but not others is intriguing and seems worth investigation. Evidence so far indicates that the variation is not defined by the age of the cyst. When spermatozoa of the first cysts produced were sampled by mating newly emerged males, the sex ratio in the progeny corresponded with the sex ratio predicted from the average degree of sperm loss/abnormality in the cysts examined from the testes of unmated sibs (Table I). Variation between cysts is a subject for our future attention.

Progress in enhancing the potential of meiotic drive genes must surely benefit from future developments at the molecular level. With respect to *MD*, we already have information about the map position of *D* in relation to *M* and to a closely linked band of guanine–cytosine enriched DNA, identified by Hoechst 33258 fluorescence [16]. With the *D* gene cloned, which must be the primary aim, it will be possible to examine its structure with the hope of gaining information on its mode of action. It may also be possible to use it as a probe for the *MP* factor.

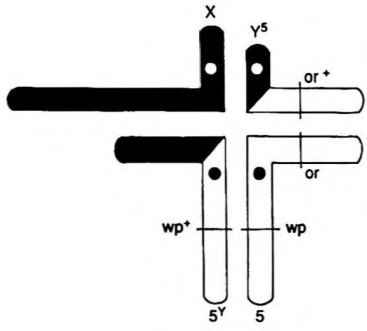

FIG. 2. Diagrammatic representation of the male karyotype of the T:Y(wp⁺)30C sexing strain of C. capitata *(or: orange eye; wp: white pupa).*

Many questions about *MP* remain unanswered: do X chromosomes fragment as in *Ae. aegypti*? If they do, what causes it and what protects the Y chromosome from fragmentation? In both species, questions arise which can only be answered by molecular study.

The *D* gene of the *Drosophila SD* system has recently been cloned and sequenced [18]. It may prove an effective probe for locating *D* in *Ae. aegypti* or *MP* in *C. capitata*. Also investigated in *Drosophila* has been the locus *R* which controls response to *D*. Wu and his colleagues have shown *R* to be a satellite DNA array with sensitivity related to copy number [18]. Clearly the potential for genetic manipulation of meiotic drive, as an approach to genetic sexing, is hardly yet exploited. While we may be sure that there is no 'quick-fix' solution to the problem, we feel bound to explore its possibilities.

3.2. Studies on stability of T:Y(*wp*⁺)30C sexing strain

Research on the sexing strain T:Y(*wp*⁺)30C (Fig. 2) by Kafu et al. [12, 13] was directed towards analysing the phenotypic consequences of strain breakdown arising from (a) recombination between *wp* and the Y–5 translocation junction, and (b) outside contamination with *wp*⁺ flies. Key changes in sexual pupal colour and sex ratio, which could be monitored in factory populations in order to identify the cause of breakdown and regulate strain replacement, were identified.

3.2.1. *Recombination*

Laboratory population experiments revealed a low frequency of natural recombination in males between wp^+ and the translocation junction on the Y chromosome, shown by the appearance of *wp* males and wp^+ females. The frequency of recombination (r) and the selection coefficient (s) against *wp/wp* were measured over 11 generations. The best fit to the observed data was found with $r = (0.14 \pm 0.04)\%$ and $s = (26.0 \pm 2.7)\%$. Using these estimates to predict the frequency of wp^+ females and *wp* males for up to 100 generations, it was concluded that *wp* males would never exceed 0.5% after 25 generations even in the absence of outside contamination. Published data on the strain, under conditions of mass rearing, maintained with a population size of 240 000 adult flies, were subjected to the same analysis. A higher value of s (between $(38.0 \pm 3.2)\%$ and $(52.0 \pm 0.3)\%$) was indicated under these conditions. The higher value of s implies greater selection against *wp/wp*, and therefore a more rapid increase in brown females under factory conditions [13].

3.2.2. *Outside contamination by wild type flies*

In laboratory population experiments [12], we showed that the effect of wild type contamination differed according to whether the contaminants were male or female, and if female, whether they had mated. Females became more in excess after female contamination, mated or unmated, but not after male contamination. The changes observed in brown and white frequencies under the three regimes are summarized in Table II. Outside contamination was clearly capable of producing a substantial increase in white male pupae, something that does not occur by recombination. This then is a sign to be looked for as an early warning that a factory strain of T:Y(wp^+)30C has been contaminated.

TABLE II. SUMMARY OF EFFECTS OF CONTAMINATING THE T:Y(wp^+)30C GENETIC SEXING STRAIN ON ONE OCCASION WITH WILD TYPE FLIES

Wild type flies added	Frequency of white male pupae	Frequency of brown female pupae	Percentage males	Frequency of brown pupae
Males	Increase	Increase	No change	(Increase)[a]
Virgin females	No change or slight increase	Increase	Decrease	Increase
Mated females	(Increase)[a]	Increase	Decrease	Increase

[a] Only at high levels of contamination.

3.3. Electrophoretic studies on esterases

Work on esterases in mosquitoes has shown that insect stocks remain highly polymorphic after many years of culture. The pattern of allele frequency distribution observed in *Ae. aegypti* [19] makes it unlikely that esterase variants are entirely neutral. Yet, different stocks are polymorphic for different combinations of alleles. If selection is to be invoked to account for this variability, the question naturally arises, what kind of selection could protect different sets of alleles in different stocks living under the same laboratory conditions in Manchester? Experiments to look for associations of esterase heterozygosity with fitness factors showed heterozygous larvae to be significantly larger and slower growing than homozygotes [20]. Recent work on *Culex quinquefasciatus* showed a comparable degree of variability in this species [21, 22]. It also revealed changes taking place upon laboratory colonization, including marked reductions in band density (i.e. esterase activity) during the first 30 generations of laboratory culture; these were associated with reduced resistance to organophosphorus insecticides.

Because these observations in mosquitoes have implications with respect to laboratory adaptation and quality control for mass sterilized release in general, we decided to investigate esterases in the medfly. The number and density of esterase isozymes active against α-naphthyl acetate were investigated in two laboratory adapted medfly strains, TY4 and H1, and compared with a wild caught strain from Morocco, tested over the first three generations of laboratory culture (Mor P, Mor F_1, Mor F_2). The aim was to answer two questions: (1) whether changes in esterase

TABLE III. DENSITY (ARBITRARY UNITS) OF ESTERASE ISOZYMES ON POLYACRYLAMIDE GELS OF THIRD INSTAR LARVAE OF THREE STRAINS OF *C. capitata*, ONE OF WHICH WAS INVESTIGATED DURING THE FIRST THREE GENERATIONS OF LABORATORY CULTURE

Strain/Generation	Est-2A			All isozymes		
	N	Mean	SD	*N*	Mean	SD
Mor P	24	1.03^{ac}	0.40	81	0.83^{a}	0.49
Mor F_1	21	0.93^{ac}	0.95	75	0.72^{ab}	0.69
Mor F_2	19	1.48^{a}	1.17	79	0.93^{a}	0.82
H1	32	0.62^{bc}	0.26	105	0.52^{bc}	0.27
TY4	18	0.56^{bc}	0.14	83	0.46^{c}	0.34

Notes: (1) Thirty-two larvae in four replicates were investigated in each strain/generation.
(2) Means followed by the same letter are not significantly different.

production can be used as an index of laboratory adaptation; and (2) whether inbreeding by sib mating (single family selection) for several generations, which had taken place extensively in the H1 strain, would affect esterase production.

The electrophoresis was carried out on vertical polyacrylamide gels, relative mobility being measured against bromophenol blue in 50% sucrose solution. The method is described by Khayrandish and Wood [211. Band density was measured with an LKB 2202 Ultrascan Laser densitometer. Thirty-two 'jumping' (late third instar) larvae were investigated in each strain/generation on the basis of four replicates (gels) each. The replicates were derived from independent larval cultures. Nine isozymes were identified on the basis of relative mobility with bromophenol blue. The single most frequent isozyme present in a large population of larvae of all strains was the middle band of the nine, designated Est-2A (relative mobility RM = 0.26–0.27). The mean densities of this band and those of all bands are compared in Table III. The two laboratory strains show lower esterase activity than the MOROCCO strain. Statistical analysis using data on all nine isozymes revealed the greatest difference to be between MOROCCO and the two laboratory strains (Meddis test, $Z = 3.25$, $P < 0.001$). The variation between samples of MOROCCO revealed no progressive trend except an increase in variance. The data from Est-2A were broadly characteristic of the total picture.

Thus the data collected so far indicate the possibility that a reduction in esterase activity occurs upon laboratory colonization of *Ceratitis capitata*, similar to that observed in *Culex quinquefasciatus* [21, 22]. They also indicate that any reduction which does take place is unlikely to be apparent within the first three generations of laboratory culture. However, we need to look at more generations and more strains. Clearly, the variation between strains may have a different cause.

ACKNOWLEDGEMENTS

Thanks are due to those who co-operated in building the foundations of this research programme, especially S.S. Saaid, R.M. Shahjahan, A. Galloway and J.R.S. Wood. Thanks are also owed to those who supplied us with medfly strains and to J.B. Cosgrove for help with statistical analysis.

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