Spontaneous recombination between wp^+ and the translocation breakpoint in the T:Y(wp^+)30C genetic sexing strain of *Ceratitis capitata* (Wied.)

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The frequency of male recombination (r) between the translocation breakpoint and the wp^+ gene, and the selection coefficient (s) against wp/wp , in the genetic sexing strain T:Y(wp^+)30C of the Mediterranean fruit fly *Ceratitis capitata* (Wied.) were measured in population experiments over 11 generations. The population was 50 females and 50 males in the first generation, after which 150 pupae were used to start each subsequent generation. A computer program was used to estimate values of r and s . The best fit to the observed data was obtained when $r = 0.14 \pm 0.04$ per cent and $s = 26.0 \pm 2.7$ per cent. Using these estimated values, predictions were made on the frequency of brown females and white males for up to 100 generations. The frequency of white males was predicted never to exceed 0.5 per cent, to be reached after 25 generations, while the frequency of brown females was expected to exceed 33 per cent after 25 generations. Published data on the mass reared T:Y(wp^+)30C strain, maintained with a population size of 240,000 adult flies, were subjected to the same analysis, indicating a higher value of s (38.0 ± 3.2 – 52.0 ± 0.3 per cent) under these conditions, but a similar value of recombination. Again, the frequency of white males was predicted never to exceed 0.5 per cent as a result of recombination. When analysing published data on T:Y(wp^+)101, another genetic sexing strain, a lower stability was indicated, i.e. a more rapid generation of brown females and white males than in T:Y(wp^+)30C. No suitable values of s and r could be calculated from the T:Y(wp^+)101 data. It is concluded that outside contamination had probably occurred in the latter strain during both periods when it was mass reared.

Keywords: *Ceratitis capitata*, genetic sexing, male recombination, translocation stability

Introduction

The efficiency of genetic sexing strains in generating males for the sterile insect technique depends on their stability under mass rearing conditions. Strains based on Y chromosome translocations of a 'sexing' gene may become unstable through contamination by introduced foreign genotypes, because of recombination between the translocation breakpoint and the 'sexing' gene (Hooper *et al.*, 1987; Busch-Petersen & Kafu, 1989) or the disruption of the translocation and reversion to the normal karyotype, as observed in the sheep blow fly, *Lucilia cuprina* (Wied.) (Foster *et al.*, 1980).

The Y-chromosome translocation strains in the medfly, T:Y(wp^+)101 and T:Y(wp^+)30C, both produce a sexual colour difference in the pupae: brown (Y- $wp^+/-wp$) males and white (wp/wp) females, due to a Y-translocation of the autosomal wp^+ (brown pupae) allele. The occasional recombinants observed have the genotypes Y- wp/wp (white males) or wp^+/wp (brown females) (Busch-Petersen, 1989).

The frequency of recombination between wp^+ and the translocation breakpoint has been measured in T:Y(wp^+)101 and shown to be 0.24 per cent (Busch-Petersen, 1989). The level of instability observed by Hooper *et al.* (1987) in this strain was so high that it led them to postulate a fitness disadvantage of 30–40 per cent in white pupae. In this study we present evidence

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on the frequency of spontaneous recombination in T:Y(wp^+)30C and also on the coefficient of selection against wp .

Data on the frequency of the two recombinant classes, white (Y- wp/wp) males and brown (wp^+/wp) females, were obtained during population experiments in which the recombinants were recorded in each generation (Kafu, 1992). The study was performed over 11 generations and provided evidence not only of recombination between the translocation breakpoint and the 'sexing' gene but also on the relative fitness of the two recombinant classes. These data were used to make predictions on the level of breakdown over 100 generations.

Materials and methods

T:Y(wp^+)30C is a genetic sexing strain in which the wild type (wp^+) allele of the white pupae (wp) gene on chromosome 5 has been translocated onto the Y-chromosome while the recessive mutant allele is carried on the autosome. It was isolated in 1985 by Busch-Petersen *et al.* (1988).

T:Y(wp)/ wp is a strain produced by Kafu (1989) by crossing recombinant white pupae males taken from the mass reared T:Y(wp^+)30C strain to homozygous white females from the same strain.

The population experiments on the T:Y(wp^+)30C strain described here were carried out in laboratory rearing cages (15.5 × 11.0 × 11.0 cm). The eggs were collected in water and the larvae reared on a carrot diet (Kafu, 1989). The adults were fed on a mixture of sugar and hydrolysed yeast. The ambient conditions were 26 ± 1°C and 65 ± 5 per cent r.h.

The data reported were obtained from experiments carried out primarily to investigate the effect of artificially adding a minority of recombinants. Male recombinants were taken directly from strain T:Y(wp^-/wp); female recombinants were derived from the progeny of the cross EgII × T:Y(wp/wp) (Kafu, 1992). Once the experiments were under way, it was clear that they were revealing evidence of spontaneous recombination, in addition to the recombinants added, and a difference in frequency of the two recombinant classes. In each experiment, one class of fly was not expected as a result of the experimental procedure, and could only arise by spontaneous recombination. It is this spontaneous recombination which is reported here. The frequencies of the two recombinant classes (i.e. white pupae males and brown pupae (wild type) females) were recorded in separate but concurrent experiments. The first series of experiments were set up as follows:

1 To measure the frequency of recombinant (Y- wp/wp) males.

Experiments K3 and R3: 49 wp/wp , 1 wp^+/wp , 50 Y- wp^+/wp .

2 To measure the frequency of recombinant (wp^+/wp) females.

Experiments K1 and R1: 50 wp/wp , 49 Y- wp^+/wp , 1 Y- wp/wp .

Each experiment was replicated three times. The replicates were each set up with 100 flies (generation P) and every generation from then on (a total of 10 generations) was started with 150 pupae. These were taken at random except that in experiment R3 one wp^+/wp female, and in experiment R1 one Y- wp/wp male were added in each generation. In experiments K3 and K1, the same flies were added only in generation P.

A second series of experiments was carried out, in precisely the same way as K1, K3, R1 and R3, except that five white (Y- wp/wp) males (K2 and R2) or five brown (wp^+/wp) females (K4 and R4) were artificially added.

Although the experiments were not designed primarily to measure spontaneous recombination, the data are sufficiently clear and consistent to allow us to do so. The presence of the added wp^+/wp female or Y- wp/wp males, will be expected to cause recombination to be slightly underestimated. Their effect will be least when the number added is low. The results are interpreted accordingly.

The frequency of recombination between wp^+ and the translocation breakpoint on the Y-chromosome (r) and the coefficient of selection against wp/wp homozygotes (s), were estimated using a computer programme designed to produce the best fit to the observed data (see Appendix).

Results

The percentage of recombinant (white) male pupae (experiments K3 and R3 combined) is shown in Fig. 1 (A1) where the mean sample numbers ± s.d. of K3 and R3 (each based on 3 + 3 = 6 replicates) varied between 141 ± 36.0 and 187 ± 8.5, respectively. The percentage of recombinant (brown) female pupae (experiments K1 and R1 combined) is shown in Fig. 1 (A2), where the mean sample number ± standard deviation of K1 and R1 (6 replicates) for each generation varied between 140 ± 23.4 and 184 ± 9.2, respectively. White males were substantially less abundant than brown females, being, it is assumed, less fit. Using the computer program on both brown females and white males (see Appendix), values of r (recombination) and s (selection against wp) were calculated (Table 1). The value of r was the same (0.0014) whether a white male/brown female had been artificially added in one generation (K1 and K3) or in every generation (R1 and R3). When

the four experiments were combined, an overall value for r of 0.14 ± 0.04 per cent and for s of 26 ± 2.7 per cent was obtained (Table 1).

The results of the second series of experiments (K2, K4, R2, R4) are shown in Fig. 1 for males (C1) and females (C2). Lower overall values of r (0.08 ± 0.05 per cent) and of s (22 ± 9 per cent) were calculated (Table 1), the value for r , but not for s , being significantly lower than in the first series of experiments ($t = 2.93$, $P < 0.01$).

Using the calculated values for s and r , it is possible to predict the frequency of brown females and white males arising from spontaneous recombination over a period of 100 generations. The result of doing this, using the data from experiments K1, K3, R1 and R3 is shown in Fig. 1 (B1 and B2). The frequency of white

Table 1 Values calculated for s (selection coefficient against wp), and r (recombinant between wp^+ and Y) in the T:Y(wp^+)30C genetic sexing strain

Experiment	$r \pm \text{s.d.}$	$s \pm \text{s.d.}$
K1, K3	0.0014	0.28
R1, R3	0.0014	0.22
K1, K3, R1, R3	0.0014 ± 0.0004	0.26 ± 0.027
K2, K4	0.0004	0.38
R2, R4	0.0008	0.10
K2, K4, R2, R4	0.0008 ± 0.0005	0.22 ± 0.09

Fig. 1. Spontaneous recombination observed in laboratory cage cultures of the T:Y(wp^+)30C strain during 10 generations (A, C), with corresponding computer simulations over 100 generations (B, D). In A and B, lines are fitted on the basis of $r = 0.14 \pm 0.04$ per cent and $s = 26 \pm 2.7$ per cent, and in C and D, on the basis of $r = 0.08 \pm 0.05$ per cent and $s = 22 \pm 8.5$ per cent, where r is the recombination frequency between wp^+ and the translocation breakpoint on the Y-chromosome, and s is the selection coefficient against wp/wp . Standard errors are indicated by dotted lines. The scale on the lower right y-axis applies only to B2 and D2. A1 and B1: Y- wp/wp (white) males in experiments K3 and R3 combined, A2 and B2: wp^+/wp (brown) females in experiments K1 and R1 combined, C1 and D1: Y- wp/wp (white) in experiments K4 and R4 combined, C2 and D2: wp^+/wp (brown) females in experiments K2 and R2 combined.

males never exceeds 0.5 per cent, reached after 25 generations, whereas the frequency of brown females exceeds 33 per cent after 25 generations. After 100 generations, all pupae, except 0.2 per cent of the males, are brown. Virtually identical predictions were obtained using the data from K2, K4, R2 and R4 (Fig. 1 (D1 and D2)).

Discussion

The two estimates made of spontaneous recombination between wp^+ and the translocation breakpoint on chromosome 5 in T:Y(wp^+)30C, are 0.08 ± 0.05 per cent and 0.14 ± 0.04 per cent. This compares with 0.24 per cent measured in T:Y(wp^+)101 by Busch-Petersen (1989). The difference between the two estimates for the T:Y(wp^+)30C strain, probably reflects the different proportions of artificially added recombinants. The higher value r of 0.14 ± 0.04 per cent is expected to be the more accurate estimate (because fewer artificial contaminants were added) but, even so, it is lower than that observed by Busch-Petersen (1989) in strain T:Y(wp^+)101. Since Kerremans *et al.* (1990) found the translocation breakpoints on the autosomes in T:Y(wp^+)30C and T:Y(wp^+)101 to be probably identical, the difference between our estimate of spontaneous recombination and that of Busch-Petersen (1989) may result from differences in experimental design. However, a difference in recombination rate would fit in with the observation that the T:Y(wp^+)30C sexing strain is more stable than the T:Y(wp^+)101 strain under mass rearing conditions (Busch-Petersen & Kafu, 1989). At the same time, note must be taken of the fact that in computer simulations Hooper *et al.* (1987) and Busch-Petersen & Baumgartner (1991) showed that the rate of breakdown in

genetic sexing strains is heavily influenced by the viability of the various genotypes and karyotypes. A differential influence of such factors in these two strains may also be the cause of the observed differences.

Data on the rates of breakdown of T:Y(*wp*⁺)101 and T:Y(*wp*⁺)30C in large rearing cages, containing 240,000 flies each, were reported by Hooper *et al.* (1987) and Busch-Petersen & Kafu (1989). In these cases, the possibility of outside contamination has also to be borne in mind. We subjected their data to our analysis and compared them with the present results. Using the data from T:Y(*wp*⁺)30C, it is possible to calculate recombination rates and selection coefficients, just as we have done with our small cage experiments, ignoring the possibility of outside contamination. When both *r* and *s* are allowed to vary freely, the best fit to the total data (excellent for females but poor for males) (Fig. 2: A1 and A2) is obtained when *r* is 0.04 ± 0.012 per cent and *s* is 52 ± 0.3 per cent, giving a low value for recombination and a very high value for selection against white males. When *r* was fixed at 0.14 per cent (Fig. 2: C1 and C2) (the value of *r* obtained in experiments K1, K3, R1 and R3 combined), *s* comes out at 38 ± 3.2 per cent and the fit is still satisfactory. However, when *s* is set at 26 per cent and *r* at 0.14 per cent (both values obtained in experiments K1, K3, R1 and R3 combined) the fit is very poor. It therefore appears that selection against white males was somewhat more stringent in the mass rearing cages than in our laboratory cages.

The selection against white males (10–38 per cent in laboratory cages, 38–52 per cent in mass rearing cages) was very high in both cases, compared with the 8.5 per cent fitness disadvantage of *wp* found experimentally by Busch-Petersen (unpublished). It must be kept in mind, however, that the lower fitness in a T:Y(*wp*⁺)

sexing strain is potentially due not only to the mutant *wp* allele, but also to the fact that it is associated with a Y-chromosome translocation. The estimated value of *s* was comparable with the value of 30–40 per cent calculated by Hooper *et al.* (1987) to be required to account for the rapid breakdown of the T:Y(*wp*⁺)101 strain in mass rearing cages.

The results of Hooper *et al.* (1987) and of Busch-Petersen & Kafu (1989) on T:Y(*wp*⁺)101 breakdown in mass rearing cages were each subjected to our analysis, but no sensible values of *s* and *r* could be calculated. In both strains under mass culture breakdown was very rapid, with the appearance of a high proportion of white males as well as brown females. It seems likely, therefore, that outside contamination had occurred in these cases.

The data and simulations presented in this paper indicate that spontaneous recombination in the T:Y(*wp*⁺)30C strain is unlikely to result in the frequency of white males exceeding 0.5 per cent. The actual rate of breakdown observed in the same strain in mass rearing cages (Busch-Petersen & Kafu, 1989), confirms this suggestion (Fig. 2: A1, C1). It follows that if the frequency of white males consistently exceeds 1–2 per cent in this strain, it is reasonable to look for an alternative explanation; probably contamination with untranslocated Y-chromosomes will have occurred.

In other genetic sexing strains, based on Y-5 translocations, the theory of a differential rate of increase of brown and white pupae recombinants appears to be supported. The most complete evidence available to us comes from Dr M. C. Zapater, University of Buenos Aires (personal communication). He has recorded the incidence of aberrant pupae (white females, brown males) in strain T:(Y-5)122 kept under “simulated

Fig. 2 Spontaneous recombination, observed in the T:Y(*wp*⁺)30C sexing strain maintained for 10 generations in a mass rearing cage. For A1 and A2, computer fits are based on $r = 0.04 \pm 0.012$ per cent, $s = 52 \pm 0.3$ per cent; for C1 and C2, the fits are based on $r = 0.14$, $s = 38 \pm 3.2$ per cent. Predicted frequencies up to 100 generations are also given, where B1 is compared with A1, B2 with A2, D1 with C1 and D2 with C2. The scale on the lower right y-axis applies only to B2 and D2. A1–D1: Y-*wp*/*wp* (white) males. A2–D2: *wp*⁺/*wp* (brown) females.

mass rearing conditions". During 17 generations of observation, the brown female frequency was about twenty times the average white male frequency, which was 0.02 per cent. This strain was clearly more stable than T:Y(wp⁺)30C.

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References

- BUSCH-PETERSEN, E. AND BAUMGARTNER, H. 1991. Simulation of instability in genetic sexing strains: effects of male recombination in association with other biological parameters. *Bull. Ent. Res.* **81**, 11–20.
- BUSCH-PETERSEN, E. 1989. Male recombination in a genetic sexing strain of *Ceratitis capitata* (Diptera: Tephritidae) and its effect on stability. *Ann. Entomol. Soc. Am.*, **82**, 778–784.
- BUSCH-PETERSEN, E. AND KAFU, A. 1989. Stability of two mass-reared genetic sexing strains of *Ceratitis capitata* (Diptera: Tephritidae) based on pupal colour dimorphisms. *Environ. Entomol.*, **18**, 315–322.
- BUSCH-PETERSEN, E., RIPFEL, J., PYREK, A. AND KAFU, A. 1988. Isolation and mass rearing of a pupal genetic sexing strain of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). In IAEA (ed.): *Modern Insect Control: Nuclear Techniques and Biotechnology. Proceedings of an International Symposium*, 16–20 Nov. 1987. Vienna, Austria: International Atomic Energy Agency, pp. 211–219.
- FOSTER, G. G., MADDERN, R. H. AND MILLS, A. T. 1980. Genetic instability in mass-rearing colonies of a sex-linked translocation strain of *Lucilia cuprina* (Wied.) (Diptera: Calliphoridae) during a field trial of genetic control. *Theor. Appl. Genet.*, **58**, 169–175.
- HOOVER, G. H. S., ROBINSON, A. S. AND MARCHAND, R. P. 1987. Behaviour of genetic sexing strain of Mediterranean fruit fly, *Ceratitis capitata*, during large scale rearing. In Economopoulos, A. P. (ed.) *Fruit Flies. Proceedings of the Second International Symposium*, Colymbari, Crete, 1986. Elsevier Science Publishers, Amsterdam, the Netherlands, pp. 349–362.
- KAFU, A. 1989. *Effect of contamination with wild type and "recombinant" genotypes on the stability of the T:Y(wp⁺)30C genetic sexing strain of the Mediterranean fruit fly, Ceratitis capitata (Wied.)*. M.Sc. Thesis, University of Manchester, United Kingdom.
- KAFU, A. 1992. *Laboratory study of factors affecting the stability of a Y-autosome translocation in the Mediterranean fruit fly, Ceratitis capitata (Wied.) in relation to the development of a genetic sexing technique based on pupal colour*. Ph.D. Thesis, University of Manchester, United Kingdom.
- KERREMANS, PH., BOURTZIS, K. AND ZACHAROPOULOU, A. 1990. Cytogenetic analysis of three genetic sexing strains of *Ceratitis capitata*. *Theor. Appl. Genet.*, **80**, 177–182.

Appendix

The equations pertaining to the model are detailed in this Appendix. The dominant brown allele (wp⁺) and the recessive white allele (wp) are denoted by subscripts 1 and 2 respectively. Then the genotype frequencies (P_{ij}^m) and (P_{ij}^f) in terms of the gene frequencies for males and females respectively in generation n are given by:

$$\text{Males: } P_{ij}^m(n) = p_i^{mY}(n) p_j^{ma}(n);$$

$$P_{ij}^m \neq P_{ij}^f, \sum_{i=1}^2 p_i^{mY} = \sum_{i=1}^2 p_i^{ma} = 1 \quad (1)$$

$$\text{Females: } P_{ij}^f(n) = p_i^f(n) p_j^f(n); \sum_{i=1}^2 p_i^f = 1. \quad (2)$$

The superscripts m and f denote male and female, respectively. Let r denote the recombination rate between the translocation breakpoint and wp⁺ in the male. The selection is against the white homozygote. Thus the elements of the selection matrix [W_{ij}] are all unity, except W_{22} which has a value $(1-s)$ where $0 \leq s \leq 1$. If the dominance is not complete, we can introduce a penetrance factor P such that the fraction $(1-P)$ of the heterozygotes are brown and P are white.

The frequencies in the $(n+1)$ th generation are related to the frequencies in the n th generation through the following equations (D is the normalization factor for genotypic frequencies):

Males

$$\begin{aligned} P_{ij}^m(n+1) &= p_i^{mY}(n+1) p_j^{ma}(n+1) \\ &= \sum_k [(1-r) W_{ik} P_{ik}^m(n) \\ &\quad + r W_{ki}(n)] \left[\sum_k W_{jk} P_{jk}^f(n) \right] / D \end{aligned} \quad (3)$$

which yields

$$p_1^{mY}(n+1) = [(1-r)p_1^{mY}(n) + rp_1^{ma}(n)][1 - s\{p_2^f\}^2]/\Delta \quad (4)$$

$$p_2^{mY}(n+1) = [(1-r)p_2^{mY}(n) + rp_2^{ma}(n) - sp_2^{mY}(n)p_2^{ma}(n)] \times [1 - s\{p_2^f\}^2]/\Delta \quad (5)$$

$$p_1^{ma}(n+1) = [1 - sp_2^{mY}(n)p_2^{ma}(n)]p_1^f(n)/\Delta \quad (6)$$

$$p_2^{ma}(n+1) = [1 - sp_2^{mY}(n)p_2^{ma}(n)]p_2^f(n)[1 - sp_2^f(n)]/\Delta, \quad (7)$$

where the normalization factor for gene frequencies, Δ , is given by

$$\Delta = [1 - sp_2^{mY}(n)p_2^{ma}(n)][1 - s\{p_2^f(n)\}^2]. \quad (8)$$

Females

$$\begin{aligned} P_{ij}^f(n+1) &= p_i^f(n+1)p_j^f(n+1) \\ &= \frac{1}{2} \left\{ \sum_k [(1-r)W_{kj}P_{ki}^m(n) + rW_{jk}P_{ik}^m(n)] \right\} \\ &\quad \times \left\{ \sum_k W_{ki}P_{ki}^f(n) \right\} \\ &\quad \times \frac{1}{2} \left\{ \sum_k [(1-r)W_{ki}P_{ki}^m(n) + rW_{ik}P_{ik}^m(n)] \right\} \\ &\quad \times \left\{ \sum_k W_{kj}P_{kj}^f(n) \right\} / D \end{aligned} \quad (9)$$

which yields

$$p_1^f(n+1) = \{ \frac{1}{2}[(1-r)p_1^{ma}(n) + rp_1^{mY}(n)][1 - s\{p_2^f\}^2] + \frac{1}{2}[1 - sp_2^{mY}(n)p_2^{ma}(n)]p_1^f(n) \} / \Delta \quad (10)$$

$$\begin{aligned} p_2^f(n+1) &= \{ \frac{1}{2}[(1-r)p_2^{ma}(n) + rp_2^{mY}(n) - sp_2^{mY}(n)p_2^{ma}(n)] \\ &\quad \times [1 - s\{p_2^f\}^2] \\ &\quad + \frac{1}{2}[1 - sp_2^{mY}(n)p_2^{ma}(n)]p_2^f(n)[1 - sp_2^f(n)] \} / \Delta, \end{aligned} \quad (11)$$

where the normalization Δ is given by (8). Finally, the frequencies of brown females ($p_b^f(n)$) and white males ($p_w^m(n)$) in generation n are given by

$$p_b^f(n) = [p_1^f(n)]^2 + 2(1-P)p_1^f(n)p_2^f(n) \quad (12)$$

$$\begin{aligned} p_w^m(n) &= p_2^{mY}(n)p_2^{ma}(n) \\ &\quad + P[p_1^{mY}(n)p_2^{ma}(n) + p_2^{mY}(n)p_1^{ma}(n)]. \end{aligned} \quad (13)$$

Calculations indicate that $P \approx 0$. Even a small value of $P \approx 0.0001$ produces a very large rate of increase in both the brown males and white females that is incompatible with the data. Thus P was set to zero in all subsequent calculations. The best values of the selection coefficient s and the recombination factor r were obtained by searching in the $[s, r]$ space to obtain the minimum value for the function

$$F = \sum_n [(E_b^f(n) - T_b^f(n))^2 + (E_w^m(n) - T_w^m(n))^2], \quad (14)$$

where $E_b^f(n)$ and $E_w^m(n)$ are the experimental values and $T_b^f(n)$ and $T_w^m(n)$ are the theoretical values for frequencies of the brown females and white males in generation n . The standard errors for s and r were then computed from the covariance matrix at the best fit values of s and r using the standard errors of the experimental data.