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Male–female separation with a genetic sexing strain of Medfly, *Ceratitis capitata,* based on the gene *sw* (*slow*)

M.M. Viscarret, C.E. Stolar & J.L. Cladera*
Instituto de Genética, INTA, C.C. 25 (1712) Castelar, Buenos Aires, Argentina

The rate of development of the Medfly, *Ceratitis capitata,* slows down in the presence of mutation *slow, sw*. Separation of fast-developing males from slow-developing females is possible when this locus on chromosome 2 is linked by a translocation to the male determining chromosome Y. We report here the results obtained in an experiment aimed at improving male–female separation by lowering the rearing temperature. Low temperature treatments were applied to first and second larval stages. This resulted in an increase in the separation in the median time of popping of female and male larvae. The proportion of males accumulated when the females start popping was also affected but to a lesser extent. Experiments on the application of pulses of cold temperature later during larval development are in progress to further improve the separation between sexes using the genetic sexing strain based on the gene *sw*.

INTRODUCTION

The rate of development of the Medfly, *Ceratitis capitata,* slows down in the presence of mutation *slow, sw* (Manso & Lifschitz 1992); mutants at this locus also show changes in the eye colour and iridescence. At the beginning of the embryonic development there is no difference in the rate of development between *sw*+ and sw– but at the transition between embryo and larva the separation between mutant and wild-type individuals is possible by setting aside hatched from unhatched eggs (J.L.C., unpubl. data, Report of a Scientific Visit, IAEA, 1995). Comparing the three alleles that have been described (Cladera 1997), *sw-y* and *sw-x* are better than *sw-z* for separation at the egg-to-larva transition (Pizarro *et al*. 1997). In either case, the separation is always better at the larva-to-pupa transition.

The locus *sw,* on chromosome 2, linked by a translocation to the male-determining chromosome Y (Cladera & Delprat 1995) allows separation of fast-developing males from slow-developing females on small scale (Cladera 1995). To improve the linkage stability between the trait *sw,* and the inheritance of the male factor, new Y-2 translocations were induced. Strains showing a tighter linkage between *sw* and the male sex were screened (Delprat *et al*. 2002).

The strain Cast191 was selected among 22 new genetic sexing strains produced. The performance of males of this strain was tested in field cages and showed that irradiated Cast191 males were as successful as wild males in mating with wild females (Cladera *et al*. 2002). Cast191 is now being reared under small factory conditions.

We report here the results obtained in an experiment aimed at improving male–female separation by lowering the rearing temperature. At the beginning of the embryonic development, low temperatures sometimes result in reduced egg-to-adult survival (Stolar *et al*. 2000); also some maternal effect is conferred onto the *sw*+ embryos. Consequently, in this study, the low temperature treatments were applied to first and second larval stages.

*To whom correspondence should be addressed.
E-mail: jcladera@cnia.inta.gov.ar

MATERIALS AND METHODS

Medfly eggs from seven-hour collections were seeded on a carrot-based larval diet (Terán 1977) at a ratio 1 ml of eggs :1 kg of diet. Individual plastic vials were set for each of four replicates with approximately the same number of eggs for each treatment. Either a fix number of eggs was counted with an insect pin before placing them on the diet ('counting' method), or a drop of eggs was dropped with a pipette and the resulting number of eggs was counted after hatching ('dropping' method).

The experimental protocol followed is shown in Table 1. The entire experiment was replicated twice: 'counting' the eggs, and 'dropping' the eggs. Larvae were kept in the dark until they popped. Afterwards, the light was left on. Pupation took place in vermiculite and pupae were collected twice a day, recording the number of pupae collected. Pupae were then placed in individual vials to await observation as adults. On the seventh day after the first collection all the remaining pupae were placed in the same bottle, as previous assays had shown that >70% pupae 'popped' (left the diet) by the seventh day.

The number of eggs seeded, pupae obtained, and emerged adults of each sex were recorded.

Table 1. Temperatures and durations of the cold treatments applied to early stages of the Medfly genetic sexing strain, *sw.*

Treatment	Embryo	Larva (first and second instar)	Larva (third instar)	Pupa
1	26°C	24 h at 15°C	21°C	26°C
2	26°C	48 h at 15°C	21°C	26°C
3	26°C	24 h at 15°C	26°C	26°C
4	26°C	48 h at 15°C	26°C	26°C
5	26°C	(21°C)	21°C	26°C
6	26°C	(26°C)	26°C	26°C

Table 2. Difference (days) between Medfly female and male median popping time (DJ50) and percentage of males accumulated when 5% or less females have accumulated (PAM), obtained for the treatments described in Table 1 (mean ± S.E.), by either counting or dropping the eggs (see Materials and Methods).

Treatment	DJ50		PAM	
	Counting	Dropping	Counting	Dropping
1	3.63 ± 0.23	4.29 ± 0.10	95.04 ± 0.89	75.62 ± 21.69
2	3.77 ± 0.24	4.73 ± 0.05	82.60 ± 12.92	97.67 ± 0.81
3	1.75 ± 0.12	2.19 ± 0.19	68.83 ± 7.72	83.26 ± 2.66
4	1.53 ± 0.16	1.57 ± 0.11	45.09 ± 16.60	64.83 ± 10.15
5	3.34 ± 0.13	4.63 ± 0.07	88.46 ± 2.43	97.33 ± 1.43
6	1.59 ± 0.08	1.77 ± 0.08	42.39 ± 14.96	39.72 ± 22.98

The following variables were then calculated:

1: DJ50 = difference in days between the median times for popping (pupating) of females and males.

2: PAM = percentage of males accumulated when 5% or less females accumulated.

3: L_{e-p}, Survival from egg to pupa = (number of pupae obtained/number of eggs seeded) × 100

4: L_{p-a}, Survival from pupa to adult = (number of adults emerged/number of pupa obtained) × 100

ANOVA from STATISTICA for Windows (StatSoft Inc. 2000) was performed in order to establish possible differences between treatments for the variables 1, 3, and 4. Tukey's analysis was used in comparing means. Variable 2 was not normal; it was analysed with a non-parametric Kruskal-Wallis test using Statistix 7.0 (Analytical Software 1998), separately for the 'counting' and 'dropping' assays.

RESULTS

The treatments applied to the early Medfly larval stages produced significant differences for variable DJ50 (Table 2) resulting from temperature treatments ($P < 0.001$), seeding method ($P < 0.001$), and the interaction between both factors ($P < 0.001$), but single-effects analysis gave $P < 0.001$ for the differences between treatments, and $P = 0.07$ for seeding. Tukey's analysis revealed two groups of means: treatments 1, 2 and 5 (21°C until popping) showed a higher DJ50 than treatments 3, 4 and 6 (26°C throughout).

For the variable PAM (Table 2), in the 'counting' method, we found differences between treatments ($P = 0.03$); differences between the control at 26°C and treatment 1 reached a significance level (alpha = 0.15, $Z = 2.58$). In the 'dropping' method, the differences between treatments were even larger ($P = 0.01$); in this case the 26°C control was significantly different from treatments 2 and 5 ($alpha = 0.1, Z = 2.71$).

In the survival from egg to pupa (Table 3), there were significant differences between the 'counting' and the 'dropping' methods but not between temperature treatments nor the interaction between these factors (ANOVA, $P = 0.001$, $P = 0.13$, and $P = 0.11$, respectively). Pupa-to-adult survival showed highly significant differences between temperature treatments, method of seeding, and the interaction between these factors ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively), but the analysis of single effects showed differences only between the two methods of

Table 3. Medfly survival from egg to pupa (L_{e-p}) and survival from pupa to adult (L_{p-a}) after the treatments described in Table 1 (mean ± S.E.), by either counting or dropping the eggs (see Materials and Methods).

Treatment	L_{e-p}		L_{p-a}	
	Counting	Dropping	Counting	Dropping
1	29.07 ± 4.64	35.15 ± 1.00	91.21 ± 1.67	93.53 ± 1.66
2	21.45 ± 1.40	31.09 ± 1.76	82.81 ± 1.86	92.85 ± 1.23
3	21.65 ± 1.98	36.09 ± 1.84	78.05 ± 2.50	94.64 ± 1.70
4	23.00 ± 2.98	40.54 ± 1.80	76.27 ± 2.32	90.25 ± 0.32
5	24.10 ± 2.10	35.85 ± 1.55	89.99 ± 2.73	90.79 ± 1.41
6	23.20 ± 3.22	39.68 ± 2.31	81.37 ± 0.79	93.87 ± 0.41

seeding the eggs ($P < 0.001$) and not between temperature treatments ($P = 0.15$).

DISCUSSION

In order to get the best out of the genetic sexing strain Cast191, a compromise must be reached between small male–female overlapping, high egg-to-adult survival, short overall time for the life cycle, and certain other quality parameters. In this paper male–female separation is compared using two criteria – the distance between the median point of male and female populations (DJ50), and the proportion of males accumulated when the females start popping (PAM). Also, survival was split into two periods, egg to pupa, and pupa to adult.

Low survival occurred in a previous experiment (Stolar *et al.* 2000). It was assumed that it was caused by the temperature treatments, but some artifact was also suspected. We showed here that survival with the 'dropping' method was higher than with the 'counting' method, regardless of the temperature treatment applied. This is probably explained by some mortality of the immature stages caused by the handling of embryos during the egg counting process.

We showed that a better separation between male and female populations (variable DJ50) is achieved by rearing larvae at 21°C (treatments 1, 2 and 5) than by rearing them at 26°C, but under the conditions of this study the application of initial pulses of colder temperature did not produce significant differences. It should be noted, however, that treatment 2 (48 h at 15°C, followed by 21°C) gave consistently better male– female separation than any other treatment.

When the percentage of males is considered (variable PAM), the results are less clear cut. Considering only the 'dropping' assay, treatment 2 (48 h at 15°C, followed by 21°C) and treatment 5 (21°C) gave a higher percentage of males than the control at 26°C (treatment 6) but not higher than treatments 3 and 4.

In conclusion, the variable PAM is less treatment-sensitive than the variable DJ50. But, as variable PAM has more practical value than variable DJ50, it would be worthwhile to keep on using both. In future experiments the number of replicates and the volume of eggs should be increased in order to reduce the variability within treatments.

Assays are in progress to further improve the separation between sexes using the genetic sexing strain based on the gene *sw*. Pulses of cold temperature later on during larval development have already produced encouraging results (unpubl. data).

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