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Anastrepha obliqua (Diptera: Tephritidae) mass-rearing: effect of relaxed colony management

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Abstract. In this study, the effects of relaxed mass-rearing conditions on *Anastrepha obliqua* (Macquart) production and quality were determined. Relaxed rearing conditions were defined by a reduction in the density of adult flies, from 80,000 to 60,000 flies per mass-rearing cage, and a reduction in the density of larvae, from 6.18 to 3.70 eggs/g of diet. In the parental generation, flies reared under relaxed conditions exhibited significant and a few non-significant changes – increased daily fecundity from 37 to 42 eggs per female, larval recovery from 80 to 91%, larval weight from 18.5 to 19.5 mg, pupation at 24 h from 92 to 96%, pupal weight from 13.5 to 14.3 mg, adult emergence from 92 to 94% and percentage of fliers from 89 to 90%. During the following 12 generations, non-significant differences were observed, but comparisons between relaxed and non-relaxed colonies were significant. The sexual competitiveness of males produced under relaxed conditions was similar to that of wild males.

Key words: *Anastrepha obliqua*, Tephritidae, mass-rearing, relaxed colony management, México

Introduction

The West Indian fruit fly *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) is the main fruit fly species that attacks mango fruits in commercial orchards at low altitudes (Aluja *et al.*, 1987, 1996). In Mexico, this fly has the second greatest economic impact among all species belonging to the *Anastrepha* genus (Reyes *et al.*, 2000). An integrated programme utilizing the sterile insect technique has been implemented to control this pest in northern Mexico since 2001 (Rull Gabayet *et al.*, 1996).

The adaptation of fruit flies to mass-rearing conditions, or colonization, may be a difficult and resource-consuming effort. This was the case for *A. obliqua*. Throughout the process, there is a tendency to maintain strains adapted for mass-production that have been selected for fast and uniform development, the ability to reach sexual maturity at nearly the same time, high fecundity and homogeneity in quality parameters.

However, this long-term selection under mass-rearing conditions could adversely affect the mating performance of sterile flies (Leppla *et al.*, 1983). An improvement in the general vigour of a colony can be achieved by reducing the stress of the rearing regimen of the mother colony (Calkins and Parker, 2005).

The filter rearing system (FRS) is a recent development for mass-rearing genetic-sexing strains of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Fisher and Caceres, 2000). The FRS has a potential application in colony management in almost any insect-rearing programme (Parker, 2005). The concept involves maintaining a small colony under minimal stress conditions, e.g. circadian rhythm of the light–dark

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cycle, low density, male-biased sex ratio and optimal temperature. Care must be taken to maintain desirable adaptations such as oviposition behaviour, high fecundity in the colony, and survival ability and appropriate mating behaviour under field conditions. Selection pressure must be reduced in the colony to maintain traits for successful performance in the field.

In the mass-rearing of *A. obliqua* at the Moscafrut facility in Metapa, Chiapas, Mexico, the relaxed rearing method is used for colony management to prevent the accumulation of deleterious traits from high-density rearing. Flies are reared at a low density to increase their vigour and size. The egging cages have been designed in such a way that insects must fly to access food and drink; this selects against non-flyers. Liedo *et al.* (2007) have shown that the use of inserts to increase the surface resting area within adult cages for the mother colony results in strains with higher mating competitiveness. They recommended investigating the introduction of inserts for already adapted colonies to determine whether flies would then recover their mating competitiveness (a reverse effect).

The objective of this study was to assess the effect of relaxed mother-colony rearing conditions on the production and sexual performance of *A. obliqua*. Relaxed conditions were characterized by a lower adult density in reproduction cages and a lower egg density per gram of larval diet.

Materials and methods

Insects

Flies as pupae were obtained from the mass-rearing colony maintained in the Moscafrut Facility in Metapa de Domínguez, Chiapas, Mexico. This strain was initially obtained from an experimental colony at the Subtropical Agricultural Research Laboratory (ARS-USDA, Weslaco, Texas, USA), which was established from flies collected from *Spondias mombin* L. fruits in the State of Veracruz, Mexico (Moreno *et al.*, 1997). This strain had been reared on an artificial diet (Artiaga-López *et al.*, 2004) for at least 150 generations before the present study. The last introduction of wild material was done 8 years before the study.

Mother colony under relaxed conditions

Relaxed handling conditions consisted of a low adult density in reproduction cages (60,000 *vs.* 88,000 pupae per cage) and a low larval density in the larval rearing diet (3.7 *vs.* 6.18 eggs/g of diet).

Mass-rearing management

Two days before emergence, the pupae from each group were separated from the pupation substrate using a sieve (mesh 18) and placed at the densities specified above in Mission-type mass-rearing egging cages consisting of an aluminium frame (215 × 180 × 30 cm) with a front chamber where the oviposition substrate panel is installed (Fig. 1). Adults were fed *ad libitum* with a mixture of sucrose and enzymatic yeast hydrolysate (ICN Biomedicals, Costa Mesa, California, USA) at a 3:1 ratio, and water was provided in a plastic pipe with a horizontal strip of filter paper (Schwarz *et al.*, 1985; Message and Zucoloto, 1989). The adult colonies were kept at 26–27 °C, 70 ± 5% relative humidity (RH) and 13 h light–11 h dark photoperiod. Light was provided by white 75 W fluorescent tubes placed 60 cm above the cages; light intensity inside a cage was approximately 700 lux.

The eggs were collected using gauze oviposition panels placed on the cage wall. These panels consisted of white gauze covering a thin layer of transparent silicon (Dow Corning Corporation, Midland, Michigan, USA). To avoid dehydration of the eggs, a uniform layer of 1% furcellaran (a gelling agent) was sprayed on the external surface of the panel three times a day. Furcellaran was prepared by mixing 24 g of furcellaran in 1 litre of tap water. The mixture was boiled, stirred and allowed to cool. Before use, furcellaran was blended and poured into a compressed-air spray tank at 60 psi. Eggs were collected daily at 16.00 h by spraying tap water onto the panel, using a flat-fan spray nozzle directed at a downward angle of 45 deg. Eggs were washed with tap water and then 1 ml of eggs per 149 ml of water chlorinated at 5 ppm was transferred into 9-litre polycarbonate plastic bottles (Nalgene™). For egg incubation, a bubbling system was used by injecting air (airflow of 3.0 l/min) through the egg solution (Schwarz *et al.*, 1985).

For larval development, a modified diet based on the formulation of Artiaga-López *et al.* (2004)

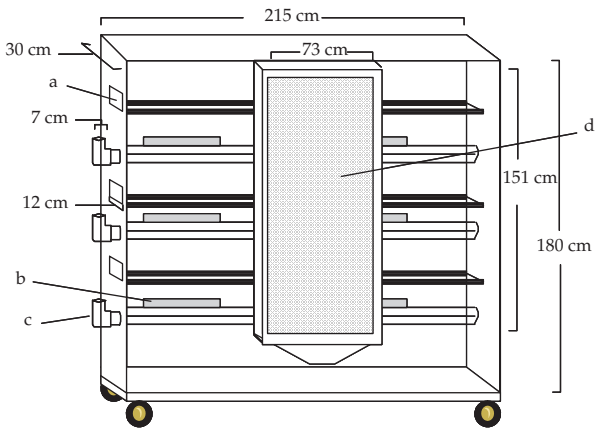

Fig. 1. (colour online) Drawing of a Mission-type mass-rearing cage for *Anastrepha obliqua*: (a) door for food trays, (b) horizontal strip of filter paper to provide water, (c) plastic pipe, and (d) screen oviposition panel.

was used. It consisted (by weight) of 16.33% corncob fractions (Corn cob fractions 100; Mt Pulaski Products, Inc., Chicago, Illinois, USA), 9% sugar (Ingenio Huixtla, Chiapas, Mexico), 6.33% torula yeast (Lake States Div. Rhinelander Paper Co., Rhinelander, Wisconsin, USA), 8.66% corn flour (Maíz Industrializado del Sureste, S.A. de C.V., Arriaga, Chiapas, Mexico), 0.18% Nipagin (methyl parahydroxybenzoate) (Mallinckrodt Speciality Chemicals Co., St Louis, Missouri, USA), 0.33% sodium benzoate (Cia. Universal de Industrias, S.A. de C.V., Mexico), 0.1% guar gum (Tic Gums, Inc., Belcamp, Maryland, USA), 0.44% citric acid (Anhidro acidulante FNEUM, Mexana; S.A. de C.V., Morelos, Mexico) and 58.63% tap water. Six kilograms of diet were placed in each tray (772 mm length, 397 mm width and 73 mm depth) (Model 805208; Molded Fiber Glass Tray Company, Linesville, Pennsylvania, USA). Developing larvae were kept at 26 ± 1 °C and 80% RH for 10 days until maturity, after which the larvae were washed out of the medium. To eliminate excess water, larvae were mixed with vermiculite and separated from it with a sieve for pupation without substrate at 21 ± 1 °C and 70% RH. After 24 h, the pupae were covered with vermiculite and transferred to a second room at 25 ± 1 °C and 80% RH for maturation for 14 days. One day before adult emergence, the pupae were separated from the vermiculite using a sieve (mesh 18) and then counted, weighed and placed inside the egging cages for adult emergence.

Generational rearing parameters

Rearing parameters were estimated during the parental generation and the next 12 generations. Fecundity was estimated using the daily volume of eggs collected and expressed as the number of eggs/female per day, estimating the initial number of females per cage. Fertility was expressed as the mean percentage of egg hatch and was estimated from the maximum number of hatched eggs by counting the number of empty chorions in three samples of 100 eggs incubated for 120 h. Larval recovery was defined as the percentage of eggs transferred to the diet that developed into mature larvae. Pupal recovery was defined as the percentage of larvae that underwent transformation to pupae. Both recovery values were calculated according to the method described by Rivera *et al.* (2007). Average weights of both larvae and pupae were estimated from counts of the total number of insects in three samples of 7.5 g. Adult emergence was based on eight samples of 50 pupae in containers (140 mm high and 80 mm diameter), counting only the normal adults that had emerged at 5 days. Flight ability was estimated using three samples of 150 pupae and counting the number of fliers among the emerged flies (FAO/IAEA/USDA, 2003; Hernández *et al.*, 2005).

Mating performance

Six field cages (3 m diameter and 2 m high) supported by a metal frame were set up in a mango orchard in Tapachula, Chiapas, Mexico, at an altitude of 137 m above sea level. Potted host mango and citrus trees were placed alternately around the inside circumference and at the centre of each field cage. In each cage, 20 males and 20 females of the tested wild populations collected from mango fruits and 20 sterile males and 20 sterile females of the mass-reared strain were released. Wild flies were 15–17 days old and sterile flies 8–10 days old. To identify individual flies, a small numbered piece of paper was glued onto the dorsal side of each fly's thorax using white glue. Throughout the observation period, the number and type of matings were recorded – wild male and female, sterile male and female, wild male and sterile female or sterile male and wild female. Each cage was regarded as a replicate. The indices used to compare the behaviour of relaxed and non-relaxed colonies were strain isolation index (ISI), female relative performance index (FRPI), male relative performance index (MRPI) and relative sterility index (RSI) (Cayol *et al.*, 1999; FAO/IAEA/USDA, 2003). Male calling was recorded during 30-min periods based on vigorous wing fanning, everted prostiger and puffed pleural glands. Observations were made in the morning (07.00–12.00 h).

Data analysis

The data on egg hatch, larval and pupal recovery, adult emergence and flight ability were arcsine-transformed using the following formula:

$$Y = \sin^{-1}\sqrt{(Y/100)},$$

where Y values are considered to be a percentage (Underwood, 2005), and then subjected to ANOVA and mean separation using the Tukey honestly significant difference procedure for generational comparisons ($P \leq 0.05$) (SAS Institute, 1999).

Results

Relfaxed rearing conditions improved the quality of the mother colony. Daily egg production, expressed as millions of eggs collected/cage per day, decreased significantly under relaxed conditions compared with that of the control ($F = 349.47$; $\mathrm{df} = 1,\ 208$; $P < 0.0001$) (Figs 2A and 3A). However, daily fecundity, expressed as the number of eggs/female per day, increased

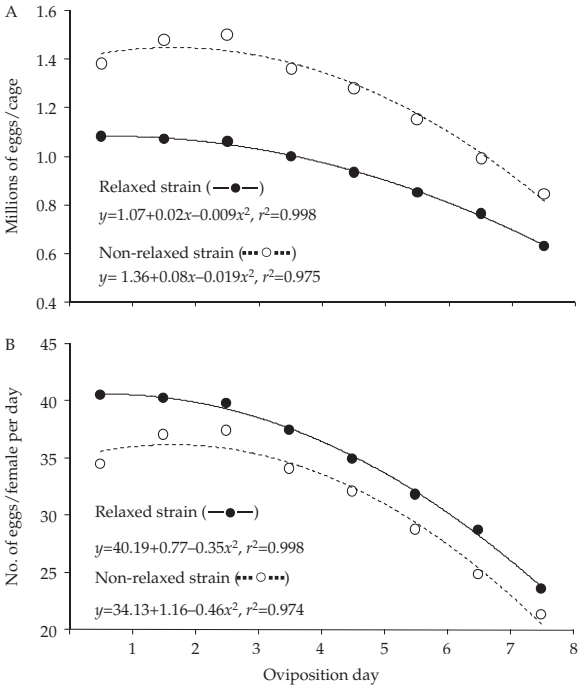

Fig. 2. Eggs per cage and daily fecundity during the first few oviposition days in *Anastrepha obliqua*.

significantly under relaxed conditions ($F = 20.52$; df = 1, 208; $P < 0.0001$) (Fig. 3B).

Individual fecundity rates were highest during the first two or three oviposition days and then decreased with age. Individual fecundity was always higher for the relaxed system during the eight oviposition days. Differences among the oviposition days were highly significant for the relaxed colony ($F = 11.23$; df = 7, 104; $P < 0.0001$) and also for the non-relaxed colony as the control ($F = 33.24$; df = 7, 104; $P < 0.0001$). The daily individual fecundity was described by $y = -0.46x^2 + 1.96x + 34.13$, $r^2 = 0.974$ ($F = 21.25$; df = 1, 6; $P = 0.0037$), for the relaxed colony and by $y = -0.35x^2 + 0.77x + 40.19$, $r^2 = 0.997$ ($F = 25.90$; df = 1, 6; $P = 0.0022$), for the non-relaxed colony.

Differences in fecundity among 12 generations were significant for the relaxed colony ($F = 6.65$; df = 12; $P < 0.0001$), but not for the non-relaxed colony ($F = 0.23$; df = 12, 143; $P = 0.9963$). Fertility, expressed as the percentage of egg hatch, was not significantly different among the generations for the relaxed colony ($F = 1.16$; df = 12; $P = 0.3162$), but significant among the generations for the not-relaxed colony ($F = 2.54$; df = 12, 169; $P = 0.0042$) (Table 1). The interaction between generations and colony type was not significant for fecundity ($F = 0.31$; df = 12, 286; $P = 0.9885$).

Larval survival, expressed as the percentage of larval recovery, was significantly greater in the relaxed colony than in the non-relaxed colony ($F = 4.31$; df = 12, 286; $P < 0.0001$) in the 12 generations tested (Fig. 4). The interaction between generations and colony type was not significant ($F = 1.04$; df = 12, 286; $P = 0.4072$).

Pupation was significantly greater in the relaxed strain than in the non-relaxed strain in the parental, third, fourth, eighth and eleventh generations. In the other generations, differences between the rearing systems were not significant ($F = 4.65$; df = 12, 286; $P < 0.0001$) (Table 2). The interaction between generations and colony type was significant ($F = 2.61$; df = 12, 286; $P = 0.0026$).

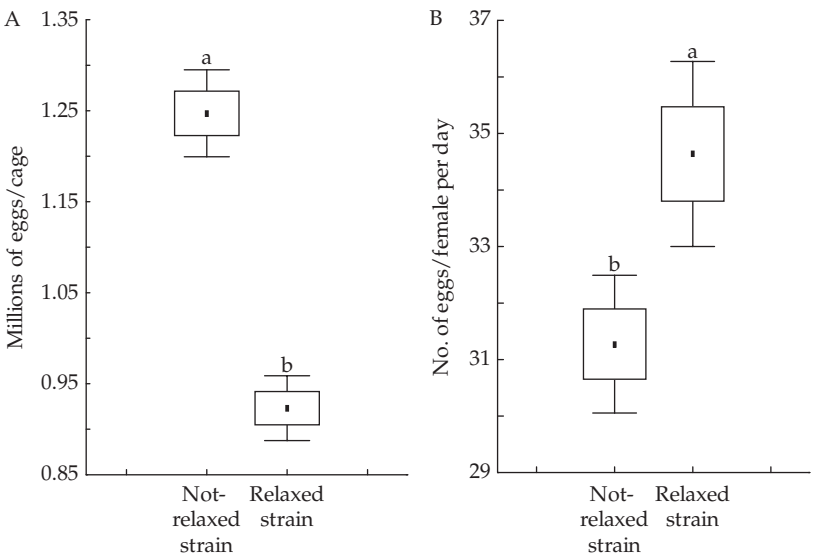

Fig. 3. Eggs per cage (A) ($F = 349.47$; df = 1, 208; P, 0.0001) and individual fecundity (B) ($F = 20.52$; df = 1, 208; P, 0.0001) of the relaxed and non-relaxed strains of *Anastrepha obliqua*.

Table 1. Fecundity and fertility of *Anastrepha obliqua* under relaxed conditions in the Moscafrut facility at Metapa de Domínguez, Chiapas

Generations	Fecundity (no. of eggs/female per day)		Fertility (% egg hatch)	
	Non-relaxed strain	Relaxed strain	Non-relaxed strain	Relaxed strain
P	37.93 ± 8.57a	42.46 ± 1.13a,b,c	89.07 ± 0.73a,b	88.57 ± 0.98a
1	34.61 ± 9.98a	36.88 ± 2.06c	90.21 ± 0.76a,b	89.36 ± 0.65a
2	27.22 ± 7.17a	35.32 ± 2.08c	91.29 ± 0.62a	90.86 ± 0.82a
3	33.83 ± 7.50a	46.65 ± 2.65a,b	88.71 ± 1.35a,b	90.43 ± 0.55a
4	38.63 ± 9.91a	48.79 ± 0.98a	90.00 ± 1.18a,b	90.86 ± 0.84a
5	32.64 ± 6.35a	36.32 ± 2.26c	88.64 ± 1.23a,b	89.14 ± 0.81a
6	36.46 ± 9.18a	34.41 ± 1.14c	88.43 ± 0.86a,b	89.79 ± 1.11a
7	29.96 ± 4.47a	36.73 ± 1.45c	90.29 ± 0.53a,b	90.07 ± 0.67a
8	43.47 ± 9.98a	39.81 ± 2.02b,c	88.29 ± 1.13a,b	90.07 ± 0.76a
9	33.30 ± 7.72a	37.11 ± 1.41c	91.43 ± 0.68a	91.07 ± 0.79a
10	36.37 ± 9.69a	35.31 ± 1.11c	88.93 ± 0.91a,b	88.86 ± 1.01a
11	36.95 ± 5.61a	39.73 ± 1.92b,c	88.57 ± 0.46a,b	89.07 ± 0.92a
12	34.01 ± 9.42a	36.70 ± 1.41c	85.86 ± 0.96b	88.21 ± 1.10a

P, parental.
Means within columns followed by the same letter do not differ significantly according to the Tukey honestly significant difference test ($P < 0.05$). Significant differences between relaxed and non-relaxed strains are indicated in the text.

Larval and pupal weights of the relaxed strain were always greater than those of the non-relaxed strain. Differences between the two rearing systems were highly significant ($F = 10.13$; df = 12, 286; $P < 0.0001$ for larval weight; $F = 5.03$; df = 12, 286; $P < 0.0001$ for pupal weight) (Fig. 5A and B). The interaction between generations and colony type was not significant for both larval and pupal weights (larvae: $F = 1.31$; df = 12, 286; $P = 0.2087$) (pupae: $F = 1.27$; df = 12, 286; $P = 0.2347$). Larval weight (Fig. 5A) tended to decrease over the generations, and when fitted to the regression linear model ($y = 19.56 - 0.05x$, $r^2 = 0.46$), it was found to be significant for the relaxed colony ($F = 9.25$; df = 1, 11; $P = 0.0112$) and not significant for the non-relaxed colony ($y = 18.72 - 0.06x$, $r^2 = 0.25$) ($F = 3.76$; df = 1, 11; $P = 0.0786$). Pupal weight (Fig. 5B) remained rather constant over the generations, and when fitted to the regression linear model ($y = 14.29 - 0.0099x$, $r^2 = 0.033$), it was found to be not significant for the relaxed colony ($F = 0.38$; df = 1, 11; $P = 0.5496$) and the not-relaxed colony ($y = 13.47 + 0.001x$, $r^2 = 0.001$) ($F = 0.0008$; df = 1, 11; $P = 0.9775$).

There were significant differences in adult emergence in the fourth, ninth and tenth generations ($F = 4.54$; df = 12, 286; $P < 0.0001$) (Table 3). The interaction between generations and colony

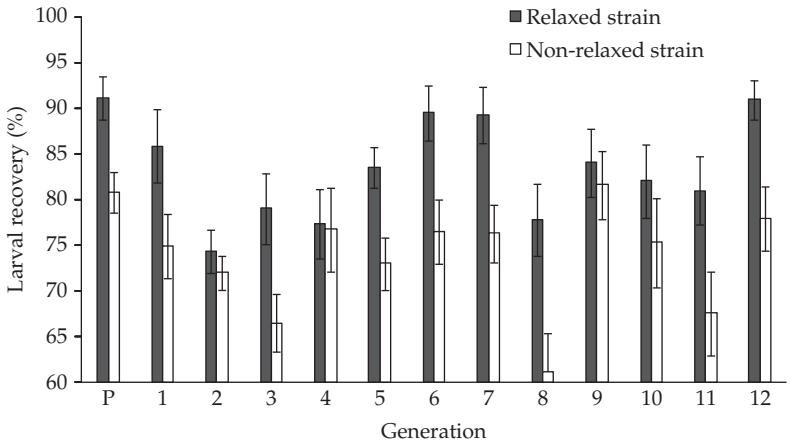

Fig. 4. Larval recovery (%) per generation of the relaxed and non-relaxed strains of *Anastrepha obliqua*.

Table 2. Pupation (%) at 24 h of *Anastrepha obliqua* under relaxed conditions in the Moscafrut facility at Metapa de Domínguez, Chiapas

Generations	Pupation (%) at 24 h	
	Non-relaxed strain	Relaxed strain
P	92.15 ± 0.66B,a	96.36 ± 0.42A,a
1	92.45 ± 0.96A,a	91.90 ± 1.36A,b,c
2	91.88 ± 0.94A,a	94.01 ± 0.62A,a,b
3	88.04 ± 1.41B,a,b	93.15 ± 0.68A,a,b,c
4	81.01 ± 3.60B,b	91.86 ± 1.05A,b,c
5	90.15 ± 1.29A,a	92.71 ± 1.25A,a,b,c
6	93.08 ± 0.81A,a	94.93 ± 0.42A,a,b
7	90.30 ± 2.88A,a	94.22 ± 0.77A,a,b
8	90.87 ± 1.37B,a	94.76 ± 0.44A,a,b
9	89.79 ± 1.34A,a	89.77 ± 1.26A,c
10	91.88 ± 0.99A,a	91.94 ± 1.22A,b,c
11	88.08 ± 2.68B,a,b	94.63 ± 0.57A,a,b,c
12	94.34 ± 0.98A,a	94.37 ± 0.67A,a,b

P, parental.
Means within columns followed by the same letter do not differ significantly according to the Tukey honestly significant difference (HSD) test ($P < 0.05$). Means within rows followed by the same capital letter do not differ significantly according to the Tukey HSD test ($P < 0.05$).

type was not significant ($F = 1.56$; df = 12, 286; $P = 0.1017$).

With only two exceptions, there were no significant differences in flight ability. The two cases where flight ability was significantly higher for the relaxed colony were the ninth and tenth generations ($F = 5.93$; df = 12, 286; $P < 0.0001$) (Table 3). The interaction between generations and colony type was not significant ($F = 0.79$; df = 12, 286; $P = 0.6555$).

Regarding mating performance, the ISI indicated 25% incompatibility for sterile males. The FRPI indicated a mean close to a 50% preference for sterile males. The MRPI indicated a mean close to a 5% preference for sterile males. The RSI indicated a mean less than 25% preference for the wild males (Fig. 6).

Figure 7A shows the percentage of males calling against time. In general, sterile males were calling more frequently than wild males. Differences in the percentage of calling males between the two rearing systems was significant ($F = 5.04$; df = 25, 286; $P < 0.0001$). The peak of calling behaviour for sterile males was at 09.00 h. Differences in sterile male calling frequency at different times of the day were significant ($F = 2.48$; df = 12, 143; $P < 0.0056$). Wild male calling frequency peaked at 10.00 h; differences in wild male calling frequency among the times of day were highly significant ($F = 27.27$; df = 12, 143; $P < 0.0001$). However, wild males exhibited a more consistent calling behaviour between 08.00 and 10.30 h. A paired comparison of calling frequency at 30-min intervals indicated no significant difference ($F = 2.22$; df = 1, 22; $P = 0.05$).

The number of mating males was recorded every 30 min from 06.00 to 12.00 h. The percentage of mating for each strain in every 30-min interval is shown in Fig. 7B. Sterile males achieved more matings than wild males from 06.30 to 07.30 h, but before 08.30 h, the inverse trend was observed. The sterile and wild males exhibited a significant difference in the number of matings in the morning ($F = 4.92$; df = 25, 286; $P < 0.0001$). The sterile males exhibited the highest mean mating at 10.00 and 10.30 h ($F = 4.25$; df = 12, 143; $P < 0.0056$), while the wild males exhibited the highest mean mating from 08.00 to 11.30 h ($F = 6.32$; df = 12, 143; $P < 0.0001$).

Discussion

The relaxed conditions of low density permitted the recovery of fecundity in adults and low-density larvae in artificial diets generated big flies. Low density prevented the accumulation of the deleterious effects of high density and revealed the

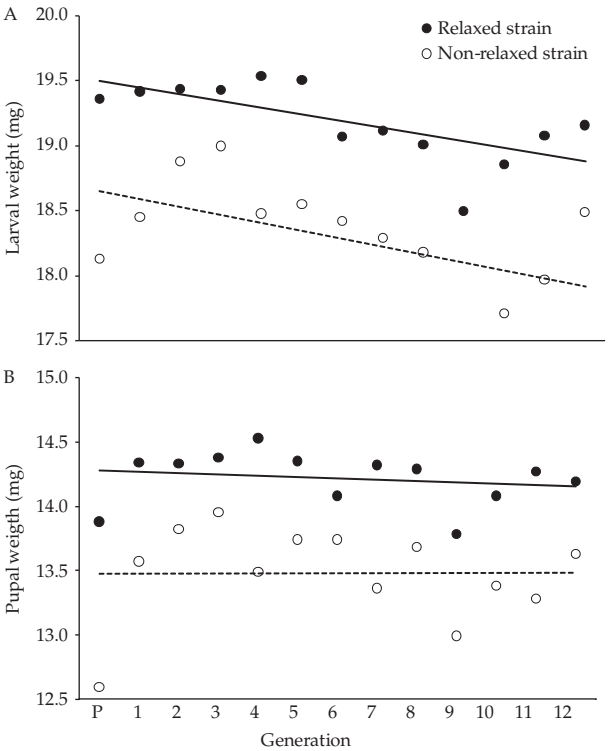

Fig. 5. Larval (A) and pupal (B) weight (mg) per generation of the relaxed and non-relaxed strains of *Anastrepha obliqua*. P, parental generation.

Table 3. Adult emergence (%) and flyers (%) of *Anastrepha obliqua* under relaxed conditions in the Moscafrut facility at Metapa de Domínguez, Chiapas

	Adult emergence (%)		Flyers (%)	
Generations	Non-relaxed strain	Relaxed strain	Non-relaxed strain	Relaxed strain
P	92.39 ± 0.96A,d	94.08 ± 0.69A,a,b	89.20 ± 0.93A,b,c	90.41 ± 0.74A,a,b
1	94.80 ± 0.48A,a,b,c,d	95.14 ± 0.22A,a,b	91.11 ± 0.67A,a,b,c	92.03 ± 0.28A,a,b
2	95.44 ± 0.45A,a,b	94.22 ± 0.58A,a,b	91.61 ± 0.59A,a,b	91.24 ± 0.59A,a,b
3	93.92 ± 0.54A,a,b,c,d	95.25 ± 0.49A,a,b	90.00 ± 0.67A,a,b,c	91.64 ± 0.79A,a,b
4	93.83 ± 0.50B,a,b,c,d	95.39 ± 0.47A,a,b	90.97 ± 0.72A,a,b,c	92.75 ± 0.61A,a
5	95.83 ± 0.51A,a	95.50 ± 0.65A,a,b	92.55 ± 0.64A,a	92.44 ± 0.77A,a
6	94.77 ± 0.57A,a,b,c,d	95.49 ± 0.29A,a,b	91.05 ± 0.72A,a,b,c	92.22 ± 0.37A,a,b
7	94.11 ± 0.63A,a,b,c,d	94.69 ± 0.58A,a,b	89.22 ± 0.86A,b,c	90.86 ± 0.56A,a,b
8	92.86 ± 0.55A,b,c,d	93.67 ± 0.77A,a,b	88.89 ± 0.60A,b,c	89.72 ± 0.96A,a,b
9	93.14 ± 0.43B,b,c,d	94.94 ± 0.60A,a,b	88.44 ± 0.61B,b,c	90.78 ± 0.66A,a,b
10	92.58 ± 0.56B,c,d	94.89 ± 0.67A,a,b	88.28 ± 0.71B,c	90.97 ± 0.80A,a,b
11	93.33 ± 0.47A,a,b,c,d	92.99 ± 0.68A,b	88.50 ± 0.52A,b,c	89.22 ± 0.79A,b
12	95.08 ± 0.43A,ab,c	95.86 ± 0.28A,a	91.05 ± 0.69A,a,b,c	92.22 ± 0.33A,a,b

P, parental.
Means within columns followed by the same letter do not differ significantly according to the Tukey honestly significant difference (HSD) test ($P < 0.05$). Means within rows but from either 'Adult emergence' or 'Flyers' followed by the same capital letter do not differ significantly according to the Tukey HSD test ($P < 0.05$).

existence of a reverse effect as suggested by Liedo *et al.* (2007) for sexual competitiveness. These authors showed that an increase in the surface resting area within adult cages of the mother colony, as well as the use of low adult cage density during colonization, resulted in strains with high mating competitiveness. This suggests that adaptations such as oviposition behaviour and appropriate mating behaviour should be maintained, and therefore assumedly a low selection pressure will make large production lines function efficiently and, without changes in field capability, increase the efficacy of sterile males (Fisher and Caceres, 2000).

Colonization for mass-rearing is a selection process in which insects adapt to the new rearing conditions (Leppla *et al.*, 1983; Leppla, 1989). The necessary high density used under mass-rearing favours the traits conferring enhanced competitive ability for limited resources (Joshi and Mueller, 1988). This selection can affect the characteristics of the laboratory population, selecting for fast development, small size, and reduced courtship and sexual competitiveness (Liedo *et al.*, 2007, Hernández *et al.*, 2009).

It is expected that a low density will reduce competition stress and favour food consumption by larvae. According to Orozco-Dávila *et al.* (2006), larvae could be maintained at a low density and thus could have a greater chance for weight increase, which directly influences pupal weight. According to Meza *et al.* (2005), mating ability is greatly influenced by the size of males.

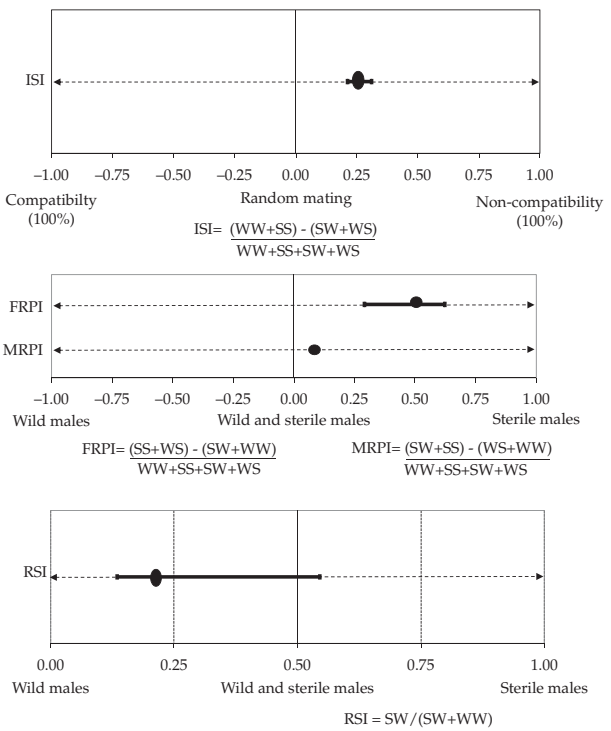

Fig. 6. Sexual index of the wild and relaxed males of *Anastrepha obliqua*. WW, wild male and female; SS, sterile male and female; WS, wild male and sterile female; SW, sterile male and wild female; ISI, strain isolation index; FRPI, female relative performance index; MRPI, male relative performance index; RSI, relative sterility index.

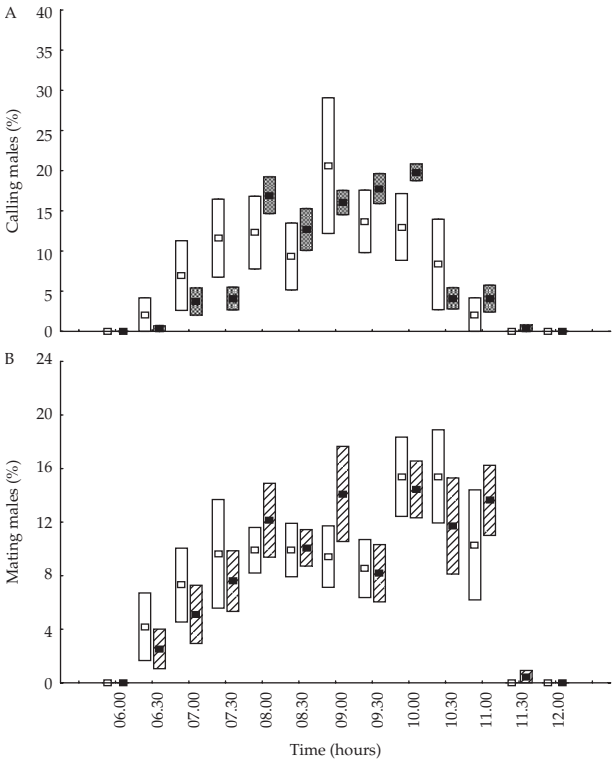

Fig. 7. (A) Calling and (B) mating males of relaxed and non-relaxed strains of *Anastrepha obliqua*.

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