El Colegio de la Frontera Sur

La selección como estrategia para mejorar la competitividad sexual de los machos de cría masiva de *Anastrepha ludens* (Díptera: Tephritidae).

TESIS
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Dedicatoria

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Contenido

I. Introducción

Las moscas de la fruta (Diptera: Tephritidae) constituyen una de las plagas de mayor importancia en todo el mundo debido a su impacto económico directo y a las estrictas restricciones cuarentenarias impuestas por muchos países (Aluja y Mangan 2008). Las especies que mayor atención han recibido por el impacto negativo que ocasionan en la producción y comercialización de frutos, se encuentran en los géneros *Anastrepha, Ragholetis, Batrocera, Toxotrypana, Dacus* y *Ceratitis* (Hernández-Ortiz y Aluja 1993).

El género *Anastrepha* (Schiner) es considerado el grupo más diverso de la familia Tephritidae y el de mayor importancia en América (Hernández-Ortíz et al. 2010). En México se reportan 37 especies del género *Anastrepha*, de las cuales solamente cuatro son de importancia agrícola: *Anastrepha ludens, A. obliqua, A. striata* y *A. serpentina* (Hernández-Ortíz 2007). La mosca Mexicana de la fruta, *Anastrepha ludens* (Loew), es considerada una de las plagas más severas tanto de los cítricos (*Citrus spp.*) como del mango (Aluja et al. 2014).

Debido a su importancia, en 1992 las moscas de la fruta fueron declaradas plagas de interés público (Rull et al. 1996, Reyes et al. 2000) por lo que se implementó la Campaña Nacional Contra Moscas de la Fruta (CNCMF) (NOM-023-FITO-1995, NOM-075-FITO-1997). Esta Campaña aplica un manejo integrado de plagas con enfoque en áreas grandes donde la Técnica del Insecto Estéril (TIE) ha jugado un papel clave, logrando establecer zonas libres y/o de áreas de baja prevalencia de moscas de la fruta en el norte del país (Hendrichs et al. 2007, Liedo y Toledo 2007, Gutiérrez y Santiago 2008, Gutiérrez 2010).

La TIE es un método de control de plagas amigable con el ambiente, ampliamente utilizado en diversas partes del mundo, y de gran relevancia en el control de varias especies de moscas de la fruta donde se ha aplicado con éxito (Enkerlin 2005). La aplicación de esta técnica requiere de la cría, esterilización y liberación sistemática de un gran número insectos de la especie plaga, con la finalidad de que los machos estériles compitan exitosamente con los machos silvestres por aparearse con hembras silvestres. Por lo tanto, la eficiencia de la TIE depende críticamente de la capacidad de los machos estériles para copular a las hembras silvestres, induciendo esterilidad y en última instancia reducir la tasa de crecimiento poblacional de la plaga (Knipling 1955, Hendrichs et al. 2002, Liedo et al. 2010).

Los insectos de cría masiva experimentan ambientes bióticos y abióticos muy diferentes de aquellos en los que se desarrollan los insectos silvestres. Estas diferencias ambientales tales como la densidad, la dieta artificial de las larvas y adultos, intensidad de luz entre otras, pueden afectar el desempeño de los machos e influir en su capacidad de apareamiento (Cayol 2000, Lux et al. 2002, Lance y McInnis 2005, Rull et al. 2005). Lo anterior ha propiciado que diversas líneas de investigación se orienten al desarrollado de estrategias que permitan equilibrar los efectos negativos de la cría masiva sin comprometer los atributos favorables de la producción masiva (Cayol 2000, Sørensen et al. 2012). Algunos de los métodos implementados para mejorar el desempeño de los machos de cría masiva se han basado en uso de mejores dietas, uso de hormonas, aromaterapia, desarrollo de cepas de sexado genético, manejo de las colonias madre,

modificaciones enfocadas al proceso de colonización, entre otras (Robinson y Hendrichs 2005, Liedo et al. 2007, Pereira et al. 2013).

Por otro lado, las investigaciones orientadas a la ecología evolutiva del comportamiento de moscas de la fruta han permitido desentrañar la dinámica de elección de pareja (Burk 1981, Calkins 1984, Arita y Kaneshiro 1989, Whittier et al. 1995, Eberhard 1996, Benelli et al. 2012), lo que contribuye al conocimiento sobre los rasgos y aptitudes primordiales para el éxito reproductivo de los machos estériles. Por tal motivo, la selección artificial de los rasgos o atributos considerados como buenos indicadores de la aptitud del macho representan una promisoria alternativa para mejorar la eficiencia de la TIE (Whittier et al. 1995, Boake 1996, Benelli et al. 2014).

Estudios relacionados con la selección de machos por su propensión a copular mostraron ser efectivos (Harris et al. 1986, 1988). Sin embargo, estos fueron realizados en condiciones de laboratorio. El conocimiento sobre el sistema de apareamiento lek-polígamo de varias especies de moscas de la fruta (Prokopy y Hendrichs 1979, Burk 1981) mostró que la mayor propensión a copular en condiciones de laboratorio estaba asociada a una menor selectividad de las hembras y por consecuencia un cortejo menos elaborado por parte de los machos. Esto resultó que bajo condiciones de campo las hembras silvestres rechazaban a esos machos (Calkins et al 1984). Estudios más recientes han documento una mejora en el desempeño de los machos de cría masiva mediante distintos sistemas de manejo de colonia. Rull y Barreda-Landa (2007) demostraron que es posible mejorar el desempeño de los machos estériles de *A. ludens* mediante la hibridación de machos silvestres con hembras de cría masiva. McInnis et al. (2002) mejoraron el desempeño de los machos estériles de *Ceratitis capitata*, a través

de un proceso combinado de selección e hibridación. Aunque estos estudios han confirmado que es posible mejorar el desempeño de los machos de laboratorio mediante cruzamientos con cepas silvestres o con determinadas características deseables, los procesos de hibridación representan dificultades para su implementación en la cría masiva y pueden resultar complicados cuando se crían cepas de sexado genético o genéticamente modificadas. Ante estas dificultades, la selección de genotipos con alto éxito reproductivo adquiere mayor relevancia. Recientemente se ha comprobado el efecto positivo de la selección en *A. ludens* (Bosa et al. 2016, Quintero et al. 2016).

Aunque los resultados de la selección son promisorios, aun se requiere fortalecer el conocimiento respecto a los efectos de los procesos de selección en múltiples atributos biológicos clave para la producción masiva. Debido a que la selección representa una posibilidad para mejorar el desempeño de los insectos estériles y por lo tanto la efectividad de la TIE, el presente trabajo tuvo como objetivo comparar el efecto de un proceso de selección en parentales, y de selección continúa a lo largo de cuatro generaciones, en la competitividad sexual de machos de *A. ludens* y su efecto en algunos atributos biológicos (sobrevivencia y reproducción).

En el siguiente capítulo se presentan los resultados de esta investigación de acuerdo al manuscrito que fue sometido para publicación en la revista Entomologia Experimentalis et Applicata. En el capítulo III se presenta una discusión general y las principales conclusiones del trabajo y en el capítulo IV la literatura citada en esta introducción y en el capítulo III.

II. Effect of artificial selection on mating competitiveness and demography of *Anastrepha ludens* (Diptera: Tephritidae) for sterile insect technique application

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Effect of artificial selection on mating competitiveness and demography of *Anastrepha ludens* (Diptera: Tephritidae) for sterile insect technique application

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Running head: Selection on mating competitiveness for SIT

Key words: Sexual selection, Mass-rearing, Colony management, Demography, Mexican fruit fly

Abstract

The sterile insect technique (SIT) has been applied successfully for the control of several fruit fly species of economic importance. However, mass-rearing conditions have resulted in selection of sexual behavioral traits that differ from wild flies reducing mating competitiveness of sterile flies and SIT efficiency. Artificial selection in mass-rearing colonies represents an alternative to improve the sexual performance of sterile flies. Here, we evaluated the effect of selection of *Anastrepha ludens* (Loew) (Diptera: Tephritidae) mass-reared males based on their mating competitiveness. Two modes of selection were compared, one single selection event on parental flies, and continuous selection along four consecutive generations. Field cage mating tests showed that wild males were more competitive than mass-reared males. Although no significant differences were found between mass-reared selected and non-selected males, in the fourth generation, males from the selected colonies performed better and were closer to the wild males than males from the non-selected colonies. Mating latency tended to be longer for wild males, and males form selected colonies tended to take longer to mate, but significant differences were only found in the second generation. No consistent differences or patterns were observed in copula duration or female mating inhibition. Wild females had greater life expectancy and lower reproductive rates than mass-reared females. Survival and fecundity decreased with increasing rearing generations, except in the colony with continuous selection. Our results suggest a trade-off between mating competitiveness and survival and reproductive rates. Reasons for disparity in results on selection of sexual performance, and reduction in survival and reproductive rates through rearing generations are discussed.

63 **Introduction**

64 The sterile insect technique (SIT) is an environmentally friendly pest control method that has been
65 used successfully against some species of tephritid fruit flies (Enkerlin, 2005; Robinson &
66 Hendrichs, 2005; Gilchrist & Meats, 2014). This technique requires the mass production,
67 sterilization and release of the target species with the aim that sterile males will mate with wild
68 females inducing sterility and reducing the wild population growth rate (Knipling, 1955; Hendrichs
69 et al., 2002; Liedo et al, 2010).

70 The loss of sexual competitiveness of the mass-reared sterile males could be a limiting factor for
71 the successful application of the SIT (Calkins, 1984). This loss could be attributed to the effects
72 of irradiation and/or the artificial conditions used for mass-rearing that inadvertently affect the
73 physiology and behavior of the sterile insects. The time for initiation of sexual activities, male
74 ability to inhibit female remating, male courtship patterns, and acoustic, visual and chemical
75 signals are examples that affect female mating choice and the effectiveness of SIT (Liedo & Carey,
76 1996; McInnis et al., 1996; Cayol, 2000; Vera et al., 2003; Lance & McInnis, 2005; Lux et al.,
77 2007ab).

78 Differences in biological traits of wild and mass-reared insects are attributed to different selection
79 factors under mass-rearing conditions and the natural environment. Mass-reared insects are
80 characterized for their high fecundity and short developmental time (Greanny & Szentesi, 1979;
81 Vargas & Carey, 1989; Meats et al., 2004; Hernández et al., 2009), which are favorable adaptations
82 for mass production. However, differences between the sexual behavior of mass reared flies
83 compared to wild flies can have adverse effects on the performance of sterile insects (McInnis et
84 al., 1996; Lance et al., 2000; Rull et al., 2005; Lux et al., 2007, Matsuyama & Kuba, 2009; Meza
85 et al., 2014). Strategies trying to balance mass-rearing efficiency and field sexual performance

86 have been investigated (Cayol, 2000; Liedo et al., 2007; Sørensen et al., 2012). For example, post-
87 teneral diets, hormone analogues and aromatherapy have improved the field performance of sterile
88 males (Pereira et al., 2013).
89 Colonization process or colony management methods have been proposed to improve the mating
90 competitiveness of sterile insects under natural conditions. Rull & Barreda-Landa (2007) showed
91 that it was feasible to improve the performance of *Anastrepha ludens* (Loew) sterile males through
92 hybridization of wild males with mass-reared adapted females, without affecting the productivity
93 required for mass production. Gilchrist & Meats (2014) found that outcrossing *Bactocera tryoni*
94 (Froggatt) strains partially adapted to laboratory conditions with the mass-reared adapted strain
95 produced heat tolerant flies with high egg production. McInnis et al. (2002) were able to improve
96 the performance of mass-reared *Ceratitis capitata* (Wiedemann) males through a combination of
97 selection and hybridization. However, hybridization process might be complicated to implement
98 in mass-rearing protocols, particularly when genetic sexing strains or genetic modified strains are
99 produced.
100 The Mexican fruit fly, *Anastrepha ludens*, is a major pest of some citrus fruits (*Citrus* spp.) and
101 mango (*Mangifera indica* L.) in Mexico and Central America (Aluja, 1994; Thomas, 2003). As
102 part of a national area-wide integrated pest management program, the use of the SIT to control this
103 pest species has been implemented. Our aim here was to study the effect of artificial selection
104 based on sexual competitiveness on the mating performance of mass-reared flies and their life
105 history traits. Two modes of selection were compared, selection on the parental generation only,
106 and selection in each generation through four consecutive generations.
107

Materials and Methods

Study site

The study was carried out under laboratory and field cage conditions. Standard *A. ludens* rearing and experiments to test mating inhibition and determination of demographic parameters were done under laboratory conditions at El Colegio de la Frontera Sur, in Tapachula, Chiapas, México. Laboratory conditions were 25±2°C, temperature, 65±5% relative humidity, and 12L:12D photoperiod. The selection process and tests to assess mating competitiveness were done in field cages located at the experimental orchard of the Moscafrut facility (SAGARPA-IICA) located in Metapa, Chiapas (14°49'33.8"N 92°11'46.1"W), and at 100 m above sea level (masl). Environmental conditions were 25-30°C temperature, 70-90% relative humidity and 3,500-0 Lux light intensity. Field cages were 3 m in diameter by 2 m high (Calkins & Webb, 1983) with a ca. 1.8 m in height mango tree placed inside, at the center of the cage.

Insects

Parental mass-reared flies were obtained as pupae from a production batch selected at random from the standard production at the Moscafrut facility with approximately 80 generations. Wild flies were obtained as larvae from infested sour oranges (*Citrus aurantium* L.), grapefruits (*C. paradise* L.) and white sapote or matasano (*Casimiroa edulis* Llave & Lex) collected in the Soconusco region and in the Comiteca Tojolabal plateau, Chiapas, México. Infested fruits were brought to the laboratory and placed in plastic trays (60x50x10 cm) with vermiculite. Once third instar larvae were mature, they were extracted from the fruits and placed in 27x16x4 cm containers with vermiculite at 60% humidity, to promote pupation.

131 At eclosion, adult flies were sorted by sex and placed in glass cages (30x30x30 cm) with water
132 and standard laboratory diet (3:1 sugar: yeast hydrolyzate enzymatic, [ICN Biomedicals, Costa
133 Mesa, California, USA]). Mass-reared and wild flies were maintained under these conditions until
134 they reached sexual maturity (~14 days for mass-reared flies and ~19 days for wild flies). Wild
135 flies were collected for each test in each generation.
136
137 *Rearing process*
138 Standard rearing processes were followed for each treatment and generation. Adults were
139 maintained in 30x30x40 cm cages. At the top of the cage, a 16 cm in diameter egging chamber
140 was placed. This was made of black tergal cloth coated with silicon, containing water to avoid egg
141 desiccation. The eggs laid in the chamber were placed over wet black satin cloth in a 19 cm in
142 diameter Petri dish. After ~4-5 days the larvae hatched and were transferred to the artificial larval
143 diet in plastic trays at a density of 5 larvae per gram of diet (Domínguez et al., 2010).
144 Approximately 10 day later, when the larvae were mature and ready to pupate, they were sorted
145 out from the diet and placed in 27x16x4 cm containers with vermiculite at 60% humidity to
146 promote pupation. After 10 days, pupal size was standardized by means of a pupa sorter machine
147 with 10 size categories (FAO/IAEA/USDA, 2003). Based on size frequency distribution, the most
148 frequent categories, 7 (2.30-2.45 mm in diameter), 8 (2.45-2.60 mm in diameter) and 9 (2.60-2.75
149 mm in diameter), were used. Fourteen days after pupation, pupae were sorted out from the
150 vermiculite and placed in 30x30x30 cm glass cages for adult emergence. At emergence, adults
151 were sorted by sex and maintained in independent cages, at similar densities (200 – 250 flies per
152 cage), provided with water and food as described above, until they reached sexual maturity.

153 *Treatments*

154 Three colonies were maintained under the previously described standard rearing process:

155 1) Laboratory (L). This was a control colony with no selection applied. Parental flies were taken
156 as a random sample from the standard rearing at the Moscafrut facility.

157 2) Alpha Selection (AS). This was the colony based on only a single selection event on the parental
158 flies. The colony was started with laboratory males that successfully mated with wild females, in
159 competition with wild males. For the selection process, 100 laboratory males, 100 wild males, and
160 100 females were released in each of 8 field cages. Laboratory males were 14 days-old and wild
161 flies were 19 – 23 days old. Laboratory males that successfully mated (intromission longer than
162 one minute) were selected. The process was repeated the following day, using the selected males
163 and maintaining the 1:1 laboratory: wild male ratio and the 2:1 male: female ratio. The process
164 was repeated until selected males accounted for ~1% of the initial cohort (800 males). This
165 normally occurred after 3 consecutive days. Selected males were placed in reproduction cages with
166 water and standard food under laboratory conditions. Then, laboratory sexually mature females
167 were introduced in these cages at a 3:1 female: male ratio. The same number of females was added
168 every 3 days for 12 days to assure enough offspring of the F1 generation from the selected males.

169 3) Beta Selection (BS). In this colony, the selection process was repeated in each generation during
170 3 more generations (until F4). The field cage tests used to assess mating performance was the
171 initial step for the selection process. AS males that successfully mated with wild females were
172 selected and tested again the next day. The process was repeated until ~1% of the initial cohort
173 was selected. In the next generations, BS males that successfully mated were selected following
174 the same procedure and their offspring were obtained as described above. Figure 1 graphically

describes the process and indicates the precise number of insects used in the field cage tests and the number and percent of selected males in each generation.

Mating competitiveness

Virgin males, age 14-17 days old of each of the three colonies (L, AS, BS) and 19-23 days old wild males and females were used in the field cage tests. Five days before the test, males were marked with a small dot of acrylic paint (Baco® Mexico City) on the thorax, using different colors to identify each treatment. Marked males were maintained in the laboratory in 15x15x25cm cages, provided with water and standard food.

Tests started 2 hours before sunset and finished just after sunset, when it was completely dark. In each field cage, 75 to 100 males of each colony (L, AS, BS) were released together with 75 to 100 wild males. Twenty minutes later, 150 wild females were released into the cage. Mating pairs were collected in vials and the time at mating and mating duration were recorded. Mating latency was defined as the time from the release of the females in the field cage to the time of the first mating. Mating duration was defined as the time each pair remained in copula.

Each cage was considered as a replicate. The number of replicates were 4, 6, 3 and 3 for F1, F2, F3 and F4 generations, respectively. The density of flies per cage varied according to the number of treatments evaluated. In the F1 generation only L and AS males were tested, BS males were added in the next 3 generations. The 1:1 male: male ratio and the 2:1 male: female ratio were constant in the four generations tested (Figure 1).

198 *Mating inhibition*

199 Wild females that mated with males from the different treatments were placed individually in 7
200 cm diameter by 10 cm long labeled containers, provided with water and standard diet, under
201 laboratory conditions. Three days later a 15-22 days old laboratory virgin male was introduced in
202 each container. Individuals were observed during 2 hours and the number of rematings were
203 recorded according to the type of male (L, AS, BS, W) females had initially mated with. The
204 proportion of females remating after 3 days from the first mating was used as an indicator of the
205 initial male´s ability to inhibit female remating.

206

207 *Demographic parameters*

208 Immature and adult survival, fecundity and fertility of the offspring of each treatment (L, AS, BS)
209 and each generation were estimated to see if selection based on sexual competitiveness was related
210 to these biological attributes.

211 Immature survival was estimated from an initial sample of 100 eggs for each colony, obtained
212 from the egging chambers described above. These eggs were placed on black satin fabric placed
213 over a moistened sponge in plastic Petri dishes (9 cm diameter). These dishes were observed daily,
214 for 8 days, under the microscope to determine egg hatch. For larval survival, eggs ready to hatch
215 were placed on artificial diet (two eggs per gram of diet) formulated according to the Moscafrut
216 facility mass-rearing process (Domínguez et al., 2010). The diet was placed in plastic Petri dishes
217 (9 cm diameter) with 50 g of diet. Once the larvae reached the third instar and were ready to pupate,
218 survival was recorded, and the larvae were placed in 6 cm in diameter by 5 cm high plastic
219 containers with vermiculite at 60% humidity. Pupal survival was estimated from the proportion of
220 adults that emerged. Ten replicates were done for each treatment and generation.

221 Adult survival, fecundity and fertility were estimated from samples of 50 flies, 25 males and 25
222 females for each treatment and each generation. At eclosion, individual pairs (male and female)
223 were placed in 7 cm in diameter by 10 cm high containers provided with water and standard diet.
224 The number and sex of dead individuals was recorded daily, until the last individual died.
225 Each container had an egging device on top. The egging device consisted of a plastic cup that fit
226 into the mouth of the bottle (6 cm in diameter by 3 cm high). The bottom of the cup was replaced
227 with black satin fabric coated with silicon and the cup was filled with water. Females laid their
228 eggs through the silicon-coated fabric and the eggs were collected daily from the water with a
229 pipette. The eggs of each individual female were placed on black satin fabric placed over a
230 moistened sponge in a plastic Petri dish. Fecundity was estimated from the number of eggs laid
231 per female per day. Fertility was considered as the number of eggs that hatched (fertile eggs).
232 Collected eggs were observed under the microscope six days later to record the number of hatched
233 eggs.
234
235 *Statistical analysis*
236 For the mating competitiveness test, the number of matings by each treatment in each field cage
237 were transformed to proportions. These data were subject to one-way analysis of variance
238 (ANOVA). In generations where the ANOVA assumptions of normality and homoscedasticity did
239 not fit, Box-Cox transformations were used. When differences among treatments were significant
240 ($P<0.05$) in each generation, a Tukey HSD (Honestly Significant Different) test was done to
241 compare means.
242 Mating latency and copula duration data were adjusted to a linear mixed-effects model. This was
243 because of the interactions among flies in the field cage and the one level hierarchy, where the

244 cage was considered the random effect and the treatments were considered the fixed effects. When
245 differences were significant ($P<0.05$), a Tukey multiple means comparison was made.
246 Mating proportions of each treatment through the 3 generations were analyzed as a split-plotthe
247 design to determine if there were interactions between treatments (L, W, AS, BS) and generations
248 (F2, F3, F4). Tukey multiple means comparisons were made where differences were significant.
249 This analysis was made only for the last 3 generations, because in the F1 generation the BS
250 treatment was absent. To balance the number of replicates, in F2 generation 3 replicates were
251 selected at random.
252 Mating inhibition was measured as the proportion of wild females that remated. Contingency
253 tables were used for the analysis on the condition of the female (remated / did not remate), and the
254 male treatment of the initial mating (W, L, AS or BS). In each of the generations where significant
255 difference was found, associations between factors were verified by calculating the proportion of
256 contribution of each cell in the table in relation to the value of χ^2 (Zar, 2010).
257 Immature survival was considered as the number of individuals that survived to the next stage.
258 Data were analyzed by a lineal regression model adjusted to generalized least squares, to allow for
259 variance heterogeneity and residuals dependency. In generations where significant differences
260 were found, Tukey multiple means comparisons were made.
261 Adult demographic parameters were estimated following methods described by Carey (1993).
262 Survival curves were analyzed by Log-rank test for interval censured data considering that
263 mortality was recorded every 24 hours (Therneau & Grambsch, 2000; Fay & Shaw, 2010). Gross
264 fecundity ($\sum m_x$) was analyzed by ANOVA, followed by HSD or Fisher LSD test. Fertility rates
265 were analyzed by binomial logistic regression and Tukey multiple mean comparison test.

Analyses were carried out using R software version 3.2.4 (R Core Team, 2016) and the packages Icens (Gentleman & Vandal, 2016) MLEcens (Maathuis, 2013) and nlme (Pinhero et al., 2016).

Results

Mating competitiveness

Wild males (W) were the most successful in terms of the proportion of matings obtained in each of the four generations evaluated (Figure 2). However, differences were significant only for the first 3 generations (F1: $F_{2,9}$ = 39.449, P = 3.517e-05; F2: $F_{3,20}$ = 4.3329, P = 0.0165; F3: $F_{3,8}$ = 19.52, P < 0.0004; and F4: $F_{3,8}$ = 2.969, P = 0.0971). No significant differences were observed among AS, BS and L males, although in the F4, the selected colonies performed better and were closer to the wild males than the L males. There were not significant differences among generations ($F_{2,6}$ = 0.456, P = 0.654), but differences among treatments were significant ($F_{3,18}$ = 13.316, P = 8.05e-05). The *post hoc* test confirmed that wild males were more successful in mating than males of the three rearing colonies (L, AS, BS). There was no significant interaction between generations and treatments ($F_{6,18}$ = 2.095, P = 0.105).

Mating latency and copula duration

Mating latency tended to be longer for wild males than for mass-reared males, and selected males tended to be closer to wild males, but significant differences were only observed in the F2 generation ($F_{3,209}$ = 5.7793, P = 8e-04) (Table 1). There were no significant differences in copula duration, except in the F2 generation, where wild males had the longest duration ($F_{3,209}$ = 8.0470, P < 0.0001).

289 *Mating inhibition*

290 The percentage of wild females that remated ranged from 6.3 to 25.5% (Figure 3). In the F1
291 generation, females that mated with wild males showed the lowest remating percentage, but
292 differences were not significant ($\chi^2_2 = 0.1192$, P = 0.9421). In the F2 generation, again females that
293 first mated with wild males showed the lowest percentage of remating and in this case differences
294 were significant ($\chi^2_3 = 10.779$, P = 0.0129). Tukey *post hoc* tests revealed significant differences
295 between females that mated with W males and females that mated with AS males. Differences in
296 remating frequency were not significant in F3 ($\chi^2_3 = 1.8517$, P = 0.6038) and F4 generations (χ^2_3
297 = 2.4911, P = 0.4769).

298

299 *Demographic parameters*

300 Immature survival decreased in each generation. There were significant interactions between
301 treatments and stages of development in F2 and F3 ($F_{2,83} = 9.2672$, P = 0.0002 and $F_{2,83} = 15.2699$,
302 P < 0.0001, respectively), but not in F1 ($F_{1,55} = 0.0388$, P = 0.8445). Survival in the L control was
303 greater than in the treatments with selection (AS, BS), and was lower in BS for all developmental
304 stages (Figure 4). According the multiple mean comparisons, in F2, survival in the L control was
305 greater than in the selection treatments in the 3 developmental stages (larva, pupa and adult). In
306 F3, larval survival was different among the 3 treatments and L individuals showed the greatest
307 survival. No differences were found in survival to pupae and adults.

308 Adult demographic parameters are shown in table 2. Flies from the BS colony were evaluated only
309 in F2 and F3 generations, while the parameters of W flies were from only one cohort. Wild flies
310 showed greater female life expectancy and lower reproductive rates compared with the three mass-
311 reared colonies. In mass-reared flies, female life expectancy deceased throughout the rearing

312 generations, with the only exception in the case of the BS colony. There was not a clear trend in
313 male life expectancy, but the F3 generation showed the lowest values in the three mass-reared
314 colonies.
315 There were significant differences in male and female survival (Figure 5). In females, differences
316 were found in the 3 generations (F1: $\chi^2_2 = 7.9493$, P = 0.0187; F2: $\chi^2_3 = 18.7137$, P = 0.0003 y
317 F3: $\chi^2_3 = 26.8686$, P = 6.273e-06). According to the post hoc test W females showed greater
318 survival than females from the mass-reared treatments (L, AS, BS) in the 3 generations, except in
319 the F2 generation where the difference between W and L was no significant. There were not
320 significant differences among mass-reared treatments. In males, no significant differences were
321 found in F1 and F2 generations (F1: $\chi^2_2 = 3.0935$, P = 0.2129; F2: $\chi^2_3 = 2.6082$, P = 0.4560). In
322 F3 there were significant differences (F3: $\chi^2_3 = 15.9752$, P = 0.0011). According to multiple mean
323 comparisons, W and L males had greater survival than AS and BS males, with no differences
324 between L and W or between AS and BS males.
325 Gross and net fecundity rates were greater in the mass-reared colonies than in the wild strain.
326 However, these rates decreased with rearing generations. The only exception was the BS colony,
327 where fecundity rates increased from the F2 to the F3 generations (Table 2). In the F1 generation,
328 gross fecundity of the L colony was significantly greater than the fecundity of the AS colony ($F_{1,45} = 10.055$, P = 0.0027). In F2, no significant differences were found ($F_{2,53} = 1.1822$, P = 0.3146)
330 and in the F3 generation differences were significant ($F_{2,52} = 3.4648$, P = 0.0386). According to
331 Fisher LSD test, BS flies showed significantly greater fecundity than L and AS flies. Figure 6
332 shows the gross fecundity schedules for the four treatments in the 3 rearing generations. Wild flies
333 showed a longer oviposition period, but daily egg production was lower than in the mass-reared
334 colonies during peak production. The reproductive period for the three mass-reared colonies was

335 reduced in each generation. Regarding fertility, significant differences were observed in the three
336 generations (F1: $\chi^2_1 = 215.43$, $P < 2.2e\text{-}16$; F2: $\chi^2_2 = 1834.3$, $P < 2.2e\text{-}16$ y F3: $\chi^2_2 = 406.18$, $P <$
337 2.2e-16) (Figure 7). Multiple mean comparisons indicated differences among the 3 treatments and
338 the 3 generations. In F1, L flies showed greater fertility than AS flies. However, in F2 and F3
339 generations, AS and BS showed the greatest fertility rates, and L the lowest (Figure 7).

340 **Discussion**

341 Mating field cage tests confirmed that wild males were more competitive than mass-reared males
342 throughout the four generations evaluated. This negative effect of mass-rearing conditions on
343 mating competitiveness has been documented for a number of tephritid species, including *A.*
344 *ludens* (Briceño et al., 1998; Miyatake, 1998; Lance et al., 2000; Ávila et al., 2011; Meza et al.,
345 2014).

346 The effect of selection (AS and BS) was not as expected despite the high level of selection pressure
347 (F1 = 98.5%, F2 = 98.7%, F3 = 95.1%). In the F4 generation more matings were achieved by
348 males from the AS and BS colonies than males from the L colony and these were closer to the W
349 males, but differences were not significant. The positive effect of selection on mating
350 competitiveness was shown by McInnis et al. (2002) in *C. capitata,* and by Bosa et al. (2016) in
351 *A. ludens* and Quintero et al. (2016) for a genetic sexing strains of *A. ludens*. A possible explanation
352 for this unexpected result could be attributed to the use of large numbers of L females for
353 reproduction. These females were used because they were already adapted to mass-rearing
354 conditions and facilitate the production of large numbers of offspring. However, their genetic
355 contribution could result in greater variation and no significant effect on their male offspring
356 mating competitiveness. Indeed, mass-rearing seems to affect more female parameters than male
357 parameters (Meza et al., 2014).

Another possible explanation might be that the initial numbers used in our selection experiments (800 in the parental generation and 450 to 225 in the subsequent generations) were not large enough, or that the number of initial males due to the strength of selection (6 to 11) was too small. Population size is a fundamental aspect in selection experiments, since size is related to genetic variation, recombination probabilities and genetic drift (Falconer & Mackay, 1996; Conner, 2003; Jalvingh et al., 2014). Inadvertent selection on other traits, inbreeding and genetic drift could counteract the effect of selection. Based on previous results (Harris et al., 1986; McInnis et al., 2002; Rull et al (2015); Bosa et al., 2016; Quintero et al., 2016), the potential of selection in colony management to improve field performance of sterile males could not be discarded, but questions regarding the optimal population size and selection strength should be addressed.

Mating latency tended to be longer in W males than in mass-reared males, although differences were only significant in the F2 generation. According to Schutze et al. (2015) in *Bactrocera dorsalis* (Hendel) the differences in mating latency between laboratory and wild flies are closely associated to light incidence during sunset. Here, although no significant differences were observed, it is interesting that selected males increased this time to mate, more closely resembling W male behavior. Shorter mating latency of mass-reared *A. ludens* flies compared with W males has been previously reported (Meza et al., 2014; Quintero et al., 2016). A longer mating latency means that males start calling and courting later (Bosa et al., 2016). This can be an adaptation of W males to reduce the time they are vulnerable to predators. But under our field cage conditions, the advantage of delaying the time to start calling and courting was not observed because flies were protected from predators. A longer mating latency could also be due to males performing more elaborate or longer pre-copulatory courtships, which would coincide with the higher mating success of W males. If mass-reared strains start calling before W males this could be advantageous

381 and is reflected in a shorter mating latency. However, across generations this did not result in
382 higher mating success. Only in the second generation, the BS colony tended to increase mating
383 latency. Mating latency is probably determined by a combination of female and male factors. In
384 *C. capitata* wild females reject males with short premount courtship (Briceño & Eberhard, 2000),
385 and in *A. ludens* wild females are also considered choosier than laboratory females (Meza et al.,
386 2014). The shorter mating latency of mass-reared males could thus be attributed to males being
387 more aggressive or having shorter courtship sequences than W males. In *C. capitata* pre-mount
388 courtships of mass-reared males is significantly shorter than those of wild males (Briceño &
389 Eberhard, 2000). The fact that there was no consistent increase in mating latency across
390 generations suggests again that the female component in determining latency to mate is strong.
391 No pattern was identified in copula duration, despite the significant differences found in the F2
392 generation. Apparently, these values could be completely random and not be a good measure of
393 sexual competitiveness. Pérez-Staples et al. (2010) proposed that copula duration is controlled by
394 the female and it is not a good indicator of male performance.
395 Also, no consistent differences or patterns were found in mating inhibition that could establish a
396 relationship between the type of male in the first copula and remating propensity. Our results
397 suggest that mass-reared males have the same potential as wild males to inhibit remating (Abraham
398 et al., 2012, 2014; Meza et al., 2014). This could be interpreted as a beneficial trait for SIT, but the
399 effect of irradiation for sterilization should be taken into account.
400 Immatures survival suggests that L flies are well adapted to the larval diet and laboratory
401 conditions and selection on mating competitiveness had a negative effect on survival. This could
402 be a trade-off between these traits, as was reported for *B. cucurbitae* developmental time and
403 sexual performance (Miyatake 1995). The low survival from egg to first instar larva in the BS

404 treatment could be attributed to the large female: male ratio that resulted in non-fertilized eggs.
405 Further research, trying to understand the relationship between survival of immatures and sexual
406 performance could be worthwhile.
407 Greater life expectancy of wild females, compared with mass-reared strains, has been reported for
408 several tephritid species (Liedo & Carey, 1996). This difference could be explained by the cost of
409 reproduction. Mass-reared females produce more eggs in a shorter time requiring the depletion of
410 reserves with consequent shorter life expectancy (Carey et al., 2008). Male life expectancy was
411 greater than female´s and no difference or pattern was detected among treatments. The gradual
412 decrease of survival with generations was a common feature in all rearing colonies. This could be
413 attributed to the rearing conditions. To obtain enough eggs and to avoid selection for fast
414 development, eggs from females of different ages were mixed. This probably resulted in
415 inadvertent negative selection on adult survival.
416 Reduction in reproductive rates through the rearing generations was unexpected. Almost all studies
417 comparing wild and mass-reared flies indicate that in mass-reared adapted strains, developmental
418 time is shorter and fecundity rates are greater (Liedo et al., 1992, 2007; Vargas et al., 2000; Meats
419 et al., 2004; Ekesy et al., 2007; Hernández et al., 2009). This could also be explained by the
420 inadvertent selection resulting from our rearing conditions. The reduction in reproductive rates
421 was a combination of a narrower reproductive window, a decrease in the number of eggs per day
422 during the peak oviposition period and the decrease in female survival. The BS colony was an
423 exception. In this case, female life expectancy and reproductive rates significantly increased from
424 one generation (F2) to the next (F3). This suggests that if this selection process continues for more
425 generations, fecundity rates could reach the level of the L control.

426 Fertility rates increased in the colonies where selection was applied in the F2 and F3 generations.
427 This was an unexpected result, but in this case, it is favorable from the perspective of mass-rearing
428 to SIT application.
429 Our results suggest that selection on mating competitiveness might have a trade-off with other
430 biological attributes, such as survival and reproduction. Perhaps these traits are polygenic and the
431 selection could have resulted in epistatic and/or pleiotropic process (Carey et al., 2005; Jalvingh
432 et al., 2014; Miyatake, 1998; Rull et al., 2015). Despite the unexpected changes in survival and
433 reproduction through rearing generations, the differences between wild and mass-reared flies were
434 sustained. This suggests that adaptation to rearing conditions is well fixed. If mating
435 competitiveness and other traits related to field performance (i.e. predator avoidance, tolerance to
436 extreme environmental conditions) could be improved by selection, this will represent a favorable
437 contribution to more efficient SIT. Understanding the genetic basis of complex polygenetic traits
438 and their trade-offs, as proposed by Benelli et al. (2014), could contribute to the selection of
439 genotypes adapted to both, mass-rearing and field conditions.
440

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448

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627 **Figure legends**

628 Figure 1. Flow diagram of the process showing selection events starting from the laboratory
629 parental males through 3 generations (F1, F2 and F3). The initial number of males tested and the
630 number and percentage of males selected is shown.

631

632 Figure 2. Mating competitiveness. Proportion of matings in field cage tests by AS, BS, L and W
633 males in four consecutive generations (A = F1, B = F2, C = F3 and D = F4). Different letters
634 indicate significant differences among treatments within each generation according to Tukey HSD
635 test with $P < 0.05$.

636

637 Figure 3. Wild female mating percentage (light gray) with males from different treatments (W, L,
638 AS and BS), and rematings (dark gray) with L virgin males during four consecutive generations
639 (A = F1, B = F2, C = F3, and D = F4). Different letters within each generation indicate significant
640 differences among treatments according to Tukey HSD test with $P < 0.05$

641

642 Figure 4. Number of surviving individuals in each stage (larva, pupa, adult) in each treatment (L,
643 AS, BS) per generations (A = F1, B = F2, C = F3). Each point represents a replicate and the solid
644 line represents the mean.

645

646 Figure 5. Survival of females (left) and males (right) from three mass-rearing colonies (L, AS
647 and BS) and the W strain during three consecutive generations (Females: A = F1, B = F2 and C
648 = F3; Males: D = F1, E = F2 and F = F3). The same data from the wild strain was used as a
649 control for the three generations.

650

651 Figure 6. Gross fecundity. Mean number of eggs laid per female per day in each of the three
652 mass-reared colonies (L, AS and BS) and the W strain during three consecutive generations (A =
653 F1, B = F2 and C = F3). The same data from the wild strain was used as a control for the three
654 generations.

655

656 Figure 7. Average of the proportion of hatched eggs (fertility) in each treatment (L, AS, BS)
657 during 3 consecutive generations (A = F1, B = F2, C = F3).

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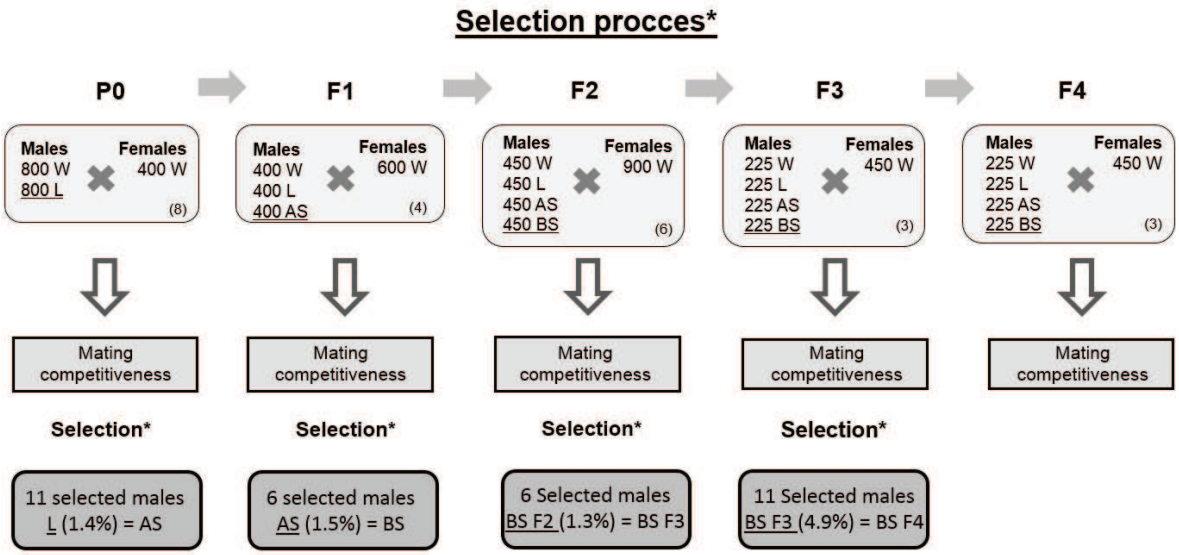
660 Figure 1



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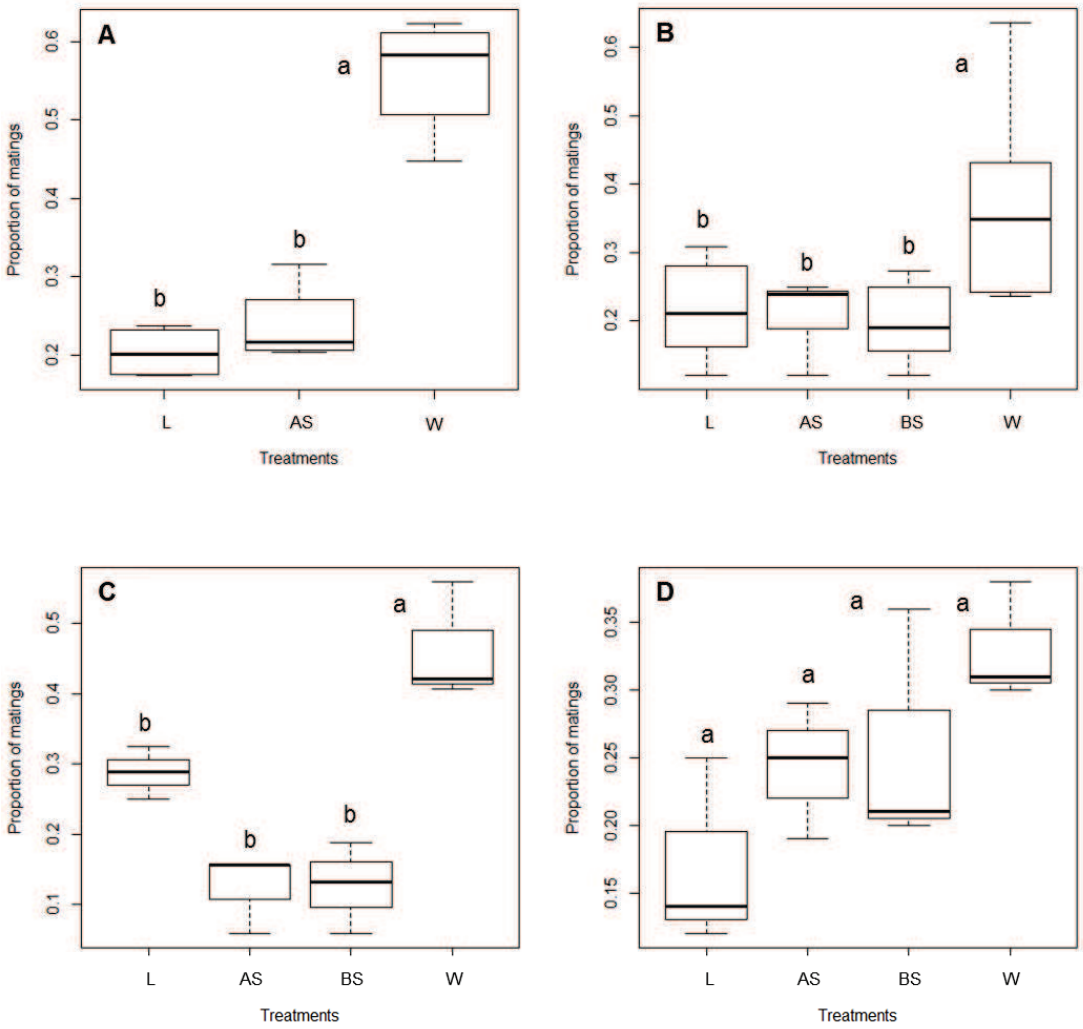
664 Figure 2



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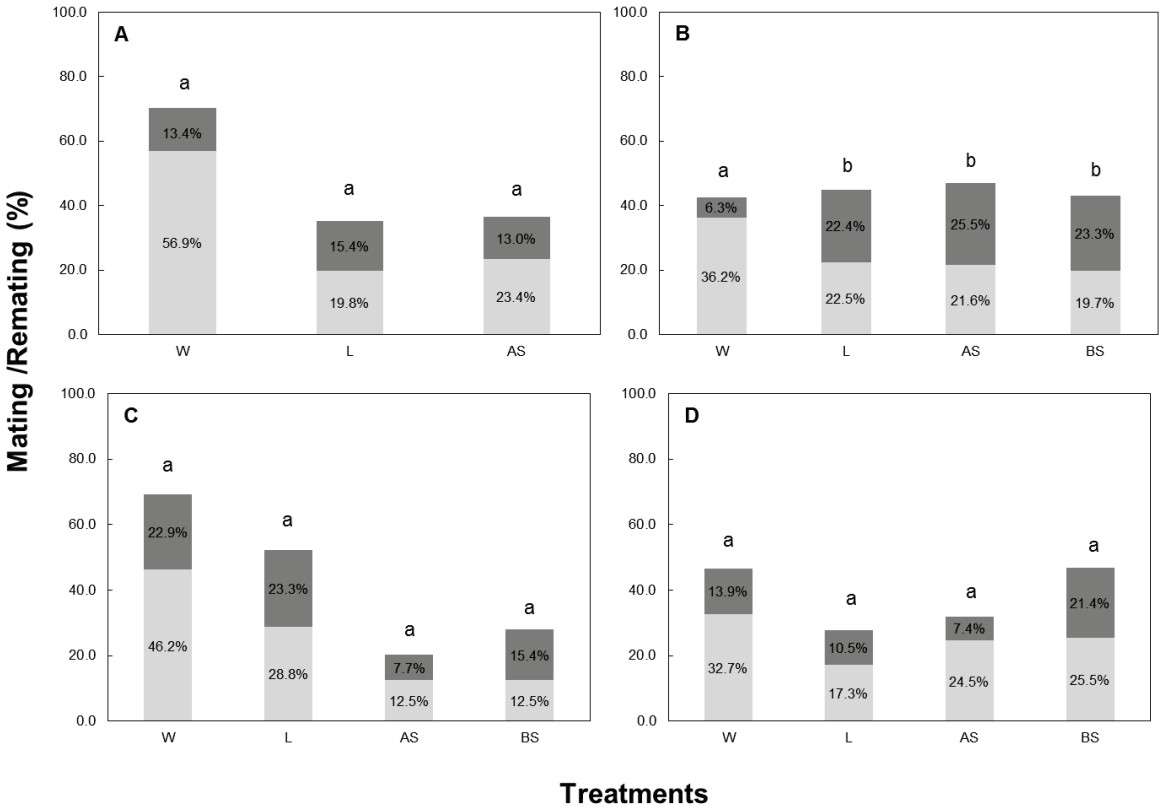
669 Figure 3



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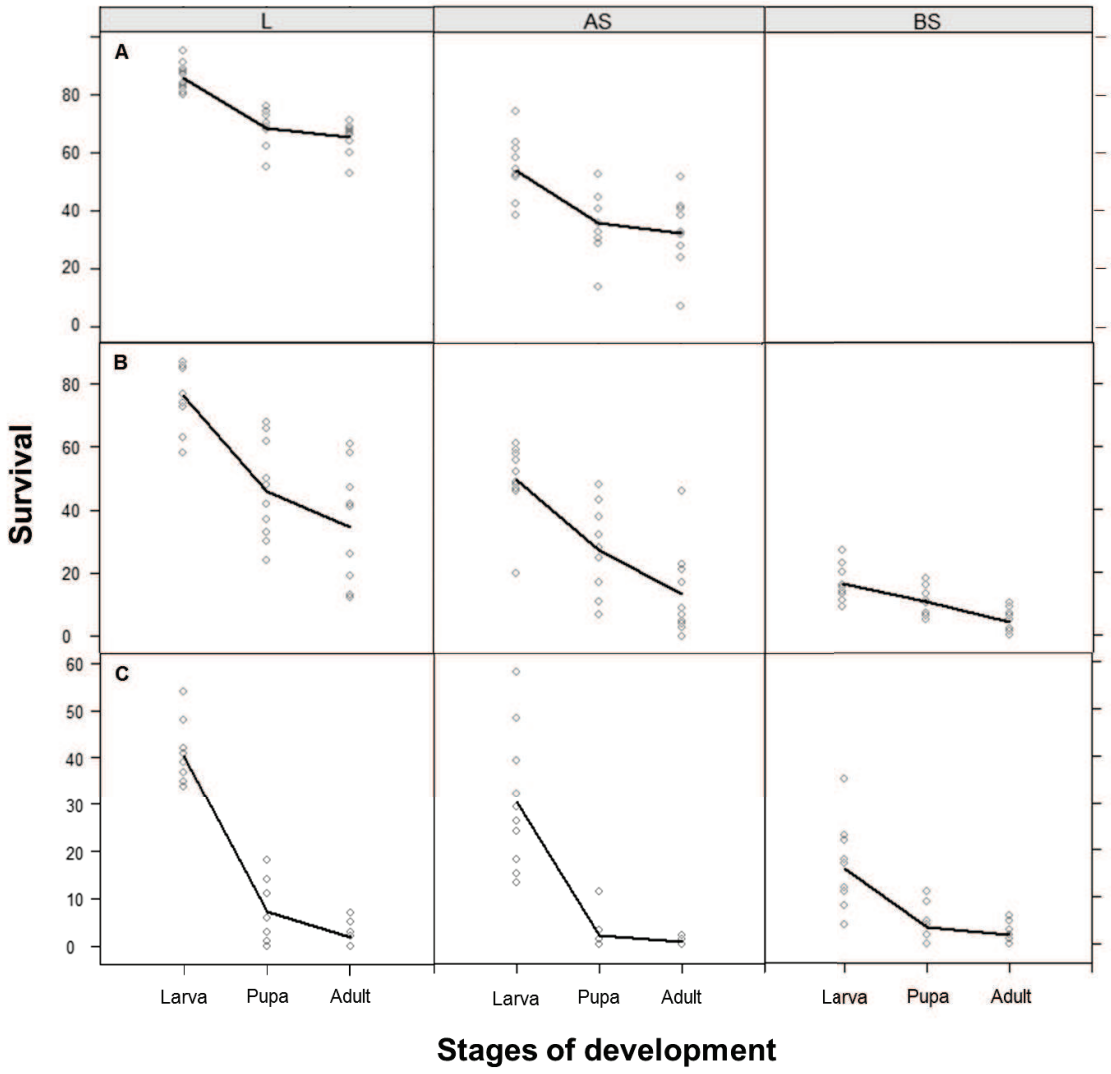

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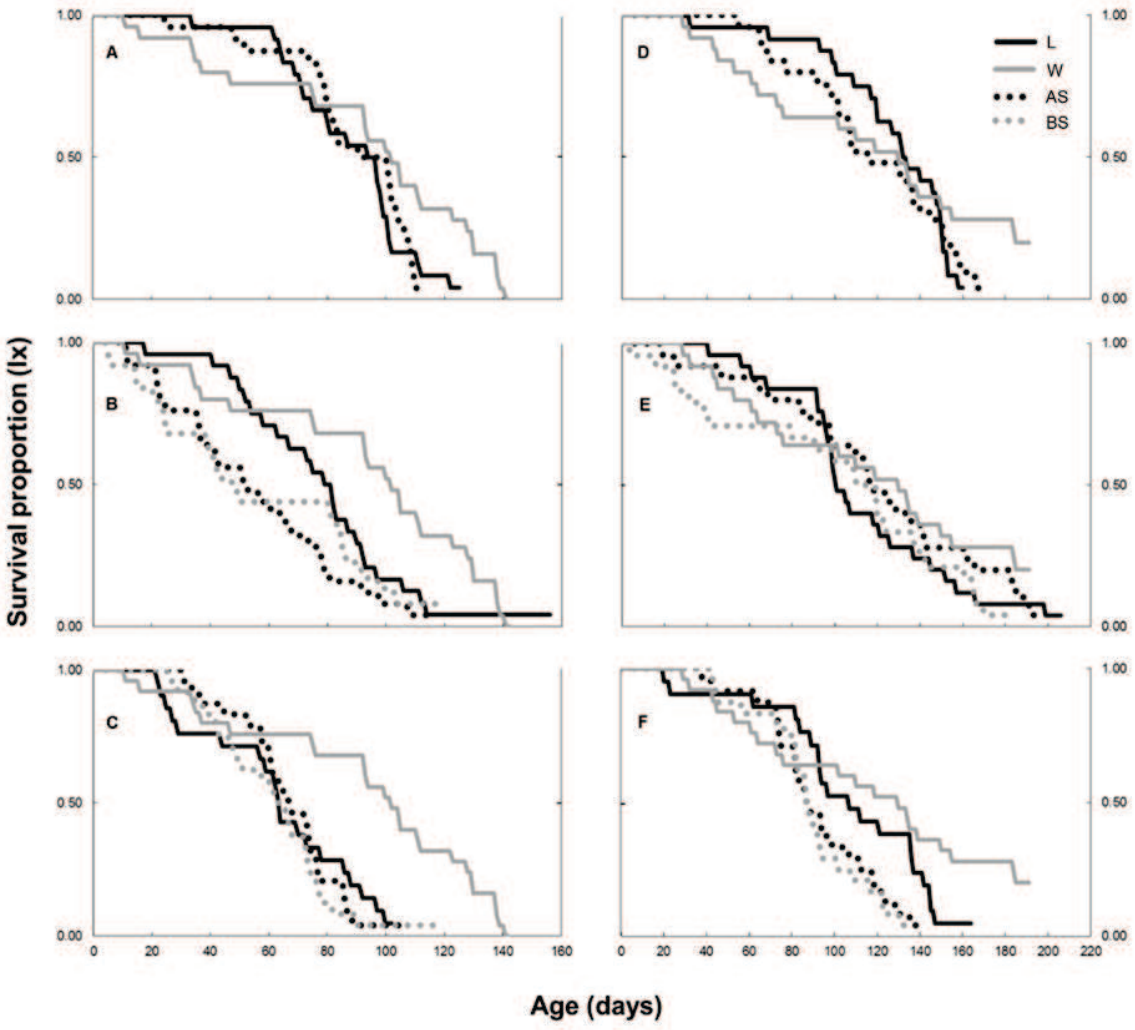

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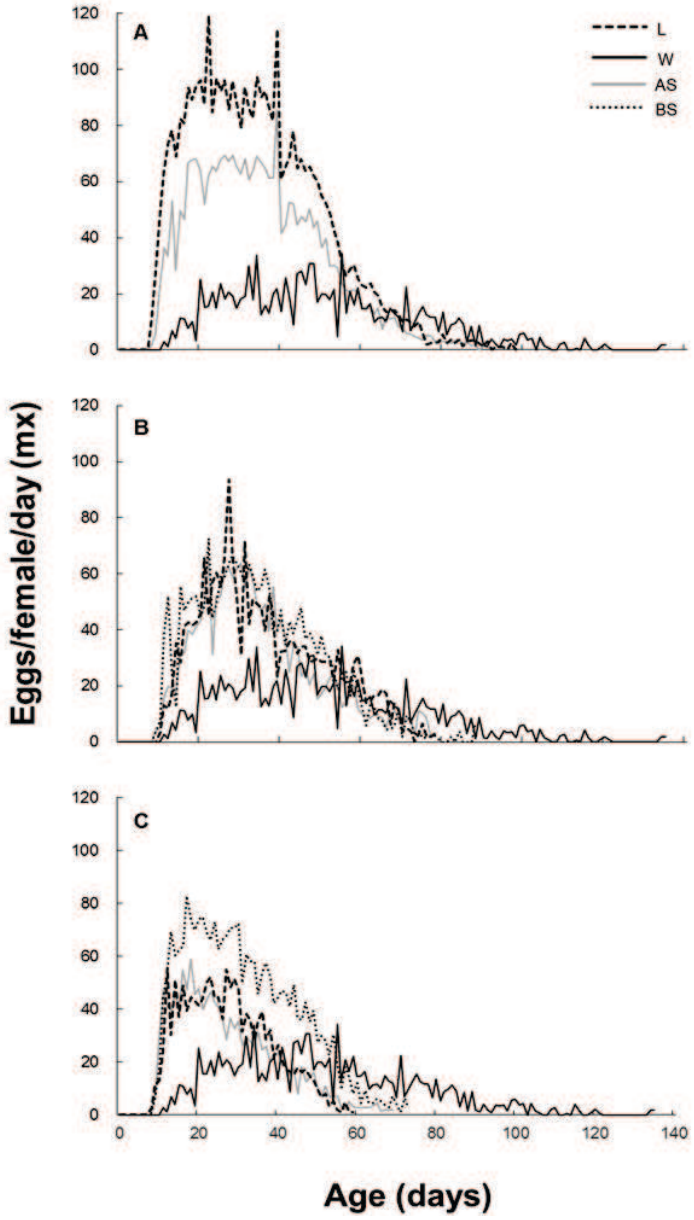
686 Figure 6



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690 Figure 7

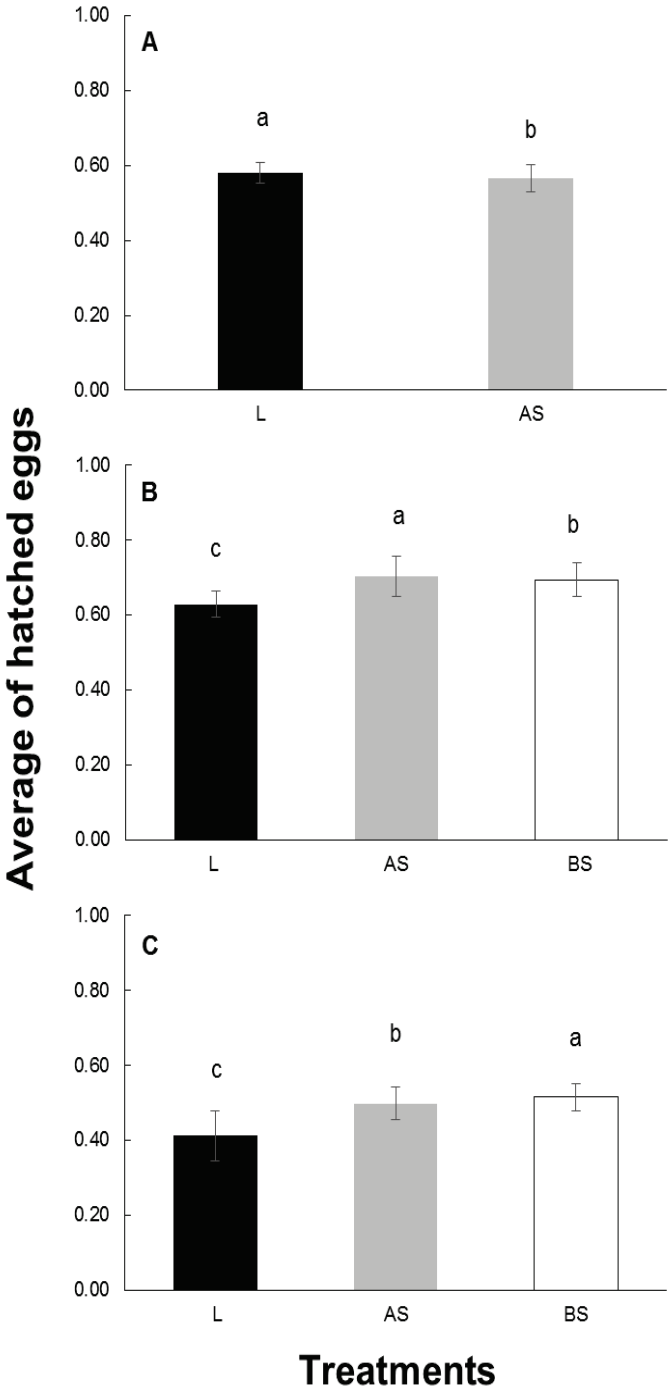

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Table 1. Mean (±SE) mating latency and copula duration (minutes) of wild flies mated with males from different treatments in field cage tests. Different letters in each column indicate significant differences according to Tukey HSD test with $P < 0.05$.

Treatments males	Mating latency				Copulation duration			
	F1	F2	F3	F4	F1	F2	F3	F4
Wild	84 ± 2 a	91 ± 3 b	83 ± 3 a	52 ± 2 a	37 ± 3 a	60 ± 4 b	36 ± 5 a	39 ± 5 a
Laboratory	78 ± 5 a	72 ± 4 a	76 ± 5 a	46 ± 4 a	33 ± 4 a	42 ± 4 a	56 ± 10 a	40 ± 8 a
Alpha Selection	84 ± 4 a	72 ± 4 a	70 ± 6 a	49 ± 3 a	42 ± 5 a	33 ± 4 a	36 ± 9 a	37 ± 6 a
Beta Selection	—	81 ± 5 ab	89 ± 5 a	47 ± 3 a	—	42 ± 5 a	57 ± 17 a	44 ± 6 a

719 Table 2. Demographic parameters of male and female *Anastrepha ludens* flies under different
720 selection methods for mass-rearing.

721

722

Parameter	Wild	Laboratory			Alpha Selection			Beta Selection	
		F1	F2	F3	F1	F2	F3	F2	F3
e_0 male (days)	118.66	126.38	112.98	105.69	117.90	119.34	92.17	100.38	88.75
e_0 female (days)	92.02	87.79	77.50	62.64	88.25	55.26	66.46	57.26	61.29
Gross fecundity (mx)	1256.67	3984.53	2110.73	1426.01	2736.22	1938.37	1373.43	2295.36	2550.70
Net fecundity (lxmx)	994.72	3860.54	1909.17	1231.43	2605.46	1360.80	1307.67	1510.28	2257.63
Daily eggs (net)	10.80	43.97	24.63	19.66	29.52	24.63	19.68	26.38	36.83
Daily eggs (gross)	13.65	45.39	27.24	22.76	31.01	35.08	20.67	40.09	41.62

III. Discusión General y Conclusiones

Los resultados de nuestro trabajo proporcionan nuevas evidencias que muestran la complejidad del proceso de selección fundamentado en el éxito de apareamiento, destacando que el éxito reproductivo se encuentra asociado a inherentes costos o compromisos biológicos (“trade-off”).

De acuerdo con nuestro objetivo de comparar la competitividad sexual de machos de *A. ludens* provenientes de un solo evento de selección (SA) con machos provenientes de selección continúa a lo largo de cuatro generaciones (SB), los resultados de las pruebas de competitividad sexual indicaron que no existió diferencia entre las dos variantes del proceso de selección (SA y SB). Al comparar el desempeño de los machos provenientes de colonias seleccionadas con el testigo de laboratorio (L), en la cuarta generación, se pudo apreciar que aunque no hubo diferencia significativa, sí hubo una tendencia a una mayor competitividad.

Por otro lado, los rasgos de historia de vida, en particular la fecundidad y fertilidad, mostraron que el efecto de la selección continua (SB) resultó en aumentos que son favorables para la cría masiva.

Estudios previos de selección artificial fundamentados en el desempeño sexual de los machos (Bosa et al., 2016; Quintero et al., 2016), aunado a nuestros resultados permiten especular acerca de las principales causas que pudiesen haber enmascarado el potencial del proceso de selección. Destaca los elevados porcentajes de presión de selección, los cuales fueron >95%, en adición a la variabilidad y al decremento del tamaño poblacional (800 en parentales hasta 225 en las últimas generaciones) lo que pudiese haber resultado en recombinación y

deriva genética, contrarrestando el efecto de la selección (Falconer y Mackay 1996, Conner 2003, Jalvingh et al. 2014).

Otra razón pudiese ser las altas relaciones de hembras de laboratorio: machos exitosos, que fueron utilizadas para garantizar la descendencia de los machos seleccionados, lo que resultó en una mayor variación genética que pudo disminuir la frecuencia de los genes asociados a la competitividad sexual de los machos seleccionados.

Los resultados obtenidos en la sobrevivencia de los estados inmaduros, particularmente la sobrevivencia o eclosión de huevos, constituyen un reflejo de las altas proporciones de hembras de laboratorio: machos exitosos, debido a que la gran cantidad de hembras utilizadas y el número reducido de machos, sobretodo en el tratamiento de selección continua (SB), pudieron provocar que muchas hembras no se hayan apareado y por lo tanto muchos huevos no fueran fertilizados.

La latencia a obtener una cópula tendió a ser más larga en las moscas silvestres (W) y más corta en los machos de laboratorio (L). Los machos de las líneas seleccionadas (SA y SB) tuvieron una latencia a la cópula intermedia, aunque las diferencias no fueron significativas. Esto puede deberse a que la latencia a la cópula es determinada por las hembras, que en nuestro caso siempre fueron silvestres (Briceño y Eberhard 2002). Los machos de laboratorio generalmente iniciaban el llamado y comportamiento de cortejo más temprano que los silvestres, pero esto no resultó en mayores copula (Bosa et al. 2016, Meza et al. 2014). Respecto a la duración de la cópula, no se encontró una asociación entre la duración de la cópula y el tratamiento, y esto pudo deberse a que este parámetro está regulado

principalmente por las hembras y todas las hembras utilizadas fueron de origen silvestre (Pérez-Staples et al. 2010).

No se encontraron diferencias significativas en la habilidad de los machos seleccionados en inhibir la receptividad sexual de las hembras con las cuales copularon, ya que no existió una relación entre el tipo de macho de la primera cópula con la propensión de la hembra a copular nuevamente. Esto sugiere que los machos de cría masiva tienen el mismo potencial que los silvestres para inhibir el reapareamiento, lo cual resulta favorable para la técnica del insecto estéril, aunque habría que considerar el efecto de la irradiación (Gavriel et al. 2009, Pérez-Staples et al. 2012).

Las diferencias en la sobrevivencia de adultos silvestres y laboratorio fueron las típicas reportadas en otros estudios (Foote y Carey 1987, Vargas & Carey 1989, Sivinsky 1993, Liedo y Carey 1996, Liedo et al. 2007). En los primeros 20 a 30 días de edad las moscas de laboratorio registraron una mayor sobrevivencia que las silvestres, posteriormente hubo una acelerada mortalidad en las de laboratorio, reflejándose en una menor longevidad que las moscas silvestres. Entre las líneas seleccionadas y la colonia de laboratorio no seleccionada (testigo) las diferencias fueron menores y en algunos casos no significativas.

Aunque los machos silvestres fueron más competitivos respecto a los de las colonias de laboratorio (L, SA, SB), los rasgos de historia de vida sugieren que la competitividad sexual puede estar comprometida con otros atributos biológicos, como la sobrevivencia o la reproducción (Carey et al. 2005). La comprensión de estos rasgos poligénicos y sus compromisos (trade-offs) representan una ventana de opciones para mejorar el desempeño del macho estéril.

Conclusiones:

- Se observaron diferencias consistentes entre moscas silvestres y de laboratorio en la competitividad sexual, sobrevivencia de adultos y fecundidad. No se observaron diferencias o fueron menos consistentes en latencia a la cópula, duración de la cópula e inhibición al apareamiento.
- Las diferencias entre moscas de laboratorio no seleccionadas y seleccionados fueron menores o no significativas.
- Los machos de laboratorio mostraron mayor sobrevivencia de inmaduros, mientras que los tratamientos de selección beta y selección alfa mostraron una mayor fecundidad y fertilidad.
- Los procesos de selección no indujeron una mayor competitividad sexual en los machos.
- La mayor fecundidad y fertilidad de los tratamientos con selección puede contribuir a mejorar la cría masiva.
- La menor sobrevivencia, particularmente de hembras de cría masiva, puede explicarse como el costo de la reproducción ya que tuvieron mayores tasas de fecundidad.

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