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Artificial selection, pre-release diet, and gut symbiont inoculation effects on sterile male longevity for area-wide fruit-fly management

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Abstract

Longevity is an important life-history trait for successful and cost-effective application of the sterile insect technique. Furthermore, it has been shown that females of some species – e.g., *Anastrepha ludens* (Loew) (Diptera: Tephritidae) – preferentially copulate with 'old', sexually experienced males, rather than younger and inexperienced males. Long-lived sterile males may therefore have greater opportunity to find and mate with wild females than short-lived males, and be more effective in inducing sterility into wild populations. We explored the feasibility of increasing sterile male lifespan through selection of long-lived strains and provision of pre-release diets with added protein, and inoculated with bacterial symbionts recovered from cultures of the gut of wild *Anastrepha obliqua* (Macquart). Artificial selection for long-lived *A. ludens* resulted in a sharp drop of fecundity levels for F1 females. Nevertheless, the cross of long-lived males with laboratory females produced a female F1 progeny with fecundity levels comparable to those of females in the established colony. However, the male progeny of long-lived males*laboratory females did not survive in higher proportions than laboratory males. Provision of sugar to *A. obliqua* adults resulted in increased survival in comparison to adults provided only with water, whereas the addition of protein to sugar-only diets had no additional effect on longevity. Non-irradiated males lived longer than irradiated males, and supplying a generic probiotic diet produced no noticeable effect in restoring irradiated male longevity of *A. obliqua*. We discuss the need to evaluate the time to reach sexual maturity and survival under stress for long-lived strains, and the inclusion of low amounts of protein and specific beneficial bacteria in pre-release diets to increase sterile male performance and longevity in the field.

Introduction

The sterile insect technique (SIT) is an environment friendly, area-wide, species-specific pest control method consisting of mass production, sterilization, and release of high densities of insects into target pest populations (Vreysen & Robinson, 2011). Released sterile males induce sterility by transfering non-viable sperm to wild females, thereby hampering their reproductive capacity (Hendrichs et al., 2002). SIT has been successfully applied to eradicate or suppress several species of insects of agricultural, veterinary, and medical importance (Vreysen et al., 2007). However, SIT application has historically faced a conflict between the need to produce and deliver large quantities of insects and the need for sterile males to behaviorally perform and achieve the maximum number of matings with choosy wild females (Hendrichs et al., 2002; Rull et al., 2012).

Cost-effective mass rearing for SIT requires artificial selection of early-developing highly fecund individuals that concentrate their reproductive output over a short period of time. Such selection can have negative effects on correlated traits that might be of importance for successful SIT application (Sørensen et al., 2012). The effects of

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artificial selection during mass rearing on performance of SIT males have been thoroughly investigated, with particular emphasis on male courtship and mating success (Cayol et al., 1999, 2002; Lux et al., 2002; Rull et al., 2005). However, much less effort has been put at documenting the effects of artificial selection on other life-history traits (Miyatake, 1997a,b, 1998), some of which can be particularly important for SIT.

Whereas early maturation and high fecundity are desirable traits among females in mass-rearing facilities, a long lifespan or increased longevity is a desirable trait among sterile males in the field. Pérez-Staples et al. (2010) documented that in the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), females preferred to copulate with males that were old and sexually experienced rather than with younger ones without sexual experience. The ability to gain sexual experience can be a critical trait for sterile males that managers of mass-production facilities need to be considered in their production and quality control schemes.

Early female reproduction and high fecundity result in cost reductions during mass rearing, and long-lived sterile males have greater opportunity to transfer sperm to wild females with the added benefit of increasing the duration of time periods between succesive releases in a particular area. Unfortunately, a negative correlation between early reproduction and adult longevity has been documented for mass-reared melon flies, *Bactrocera cucurbitae* (Coquillett) (Miyatake, 1998), and several other insect species under artificial selection (Flatt, 2011). Therefore, mass-reared males may frequently exhibit shorter lifespans than their wild counterparts (Miyatake, 1997a).

Another important aspect affecting performance under SIT is an adult diet. In general, carbohydrates affect survival, whereas protein is important for maturation, fecundity, and sexual performance (Jacome et al., 1995; Blay & Yuval, 1997; Papadopoulos et al., 1998; Drew & Yuval, 2000; Aluja et al., 2001; Yuval et al., 2002; Teal et al., 2004; Pérez-Staples et al., 2007). Protein inclusion in pre-release diets often results in boosting male sexual performance (Shelly et al., 2002; Pérez-Staples et al., 2008), yet high doses can have negative effects on male longevity (Kaspi & Yuval, 2000; Yuval et al., 2007; Utgés et al., 2013). Irradiation has been found to affect the ability of sterile males to absorb protein, because it damages the gut lining, where symbiotic bacteria normally contribute to nutrient assimilation (Lauzon & Potter, 2012). Administration of probiotic diets has resulted in restoring the capacity for protein assimilation in medflies [*Ceratitis capitata* (Wiedemann)], with a concomitant boost in sexual performance (Ben-Ami et al., 2010; Hamden et al., 2013). However, less is known on the effects of probiotic diets on longevity and on the effects of irradiation on the ability of flies to absorb carbohydrates. Although Sacchetti et al. (2014) found no effect of probiotic diet administration on olive fly longevity, Behar et al. (2008) observed an increase in medfly longevity when sugar-fed flies were also fed with an *Enterobacter*. Yuval et al. (2013) later speculated that beneficial bacteria in the medfly gut may increase individual fitness by acting as a barrier against deleterious bacteria. Restoring the state of the gut with probiotic diets could also aid in increasing irradiated male longevity.

The Mexican fruit fly, *A. ludens*, and the West Indian fruit fly, *Anastrepha obliqua* (Maquart), are currently being managed in Mexico through programs including SIT (Gutiérrez-Ruelas & Santiago, 2008). The Mexican fruit fly is considered the most important tephritid pest of citrus in Mexico, whereas the West Indian fruit fly is the most important pest of mangos (Aluja et al., 1996; Birke et al., 2013). Long-term studies of pest population dynamics in commercial orchards suggest that the most sustainable strategy for tephritid control is area-wide management (Aluja et al., 2012), a strategy where SIT fits very well (Vreysen et al., 2007). Although the Mexican Fruit Fly Campaign has been successful in eradicating and reducing some populations of these pests (Reyes et al., 2000; Gutiérrez-Ruelas & Santiago, 2008), there is still room for improvement. Among other aspects, mass-reared male life expenctancy for *A. ludens* and *A. obliqua* has been estimated at only 4–5 days in the field (Hernández et al., 2007; Utgés et al., 2013), although these estimates are based on the probability of recapture by highly inefficient traps (McPhail traps recover only from 0.5 to 6% of released adults). Therefore, increasing sterile-male longevity could significantly increase overall effectiveness and results in cost reductions or expansion of areas under SIT.

Here, we investigate factors affecting male longevity of *A. ludens* and *A. obliqua* to increase the efficacy of SIT. We attempted to address the problem by focusing on three angles: artificial selection for male longevity, adult pre-release diet, and restoration of bacterial flora of irradiated adults.

Materials and methods

Selection for male longevity

Selection of long-lived adults. *Anastrepha ludens* pupae were obtained from a colony held for over 20 years for research purposes in the laboratories of the Red de Manejo Biorracional de Plagas y Vectores at the Instituto de Ecología in Xalapa, Veracruz, Mexico. At emergence, 50 males and 50 females were placed in 30 × 30 × 30-cm Plexiglas cages with free access to water and food (3:1 ratio of sugar and hydrolyzed protein). The number and sex of

dead adults was recorded every 2 days until 30% of males in any cage were dead. Surviving males and females in each cage were considered as 'long lived' and used to establish crosses to obtain an F1 generation.

F1 pupal recovery. A new cohort of mature pupae (close to adult emergence) was obtained from the laboratory colony (hereafter named as 'normal'). At emergence, adults were sexed and assigned to different crossing treatments in Plexiglas cages as described above. One cage received 50 long-lived males and 50 normal females, one cage received 50 normal males and 50 long-lived females, and one cage received 50 long-lived males and 50 long-lived females. When normal females reached sexual maturity (8 days), an artificial egg-laying device consisting of a bottomless Petri dish with silicone-covered tergal cloth filled with water was placed on the top of cages to receive eggs. Eggs were collected from devices on a daily basis for seven consecutive days. Eggs recovered were incubated for 4 days on a piece of black organdy cloth placed over a moist piece of cotton in a 10-cm-diameter Petri dish. Hatching eggs were then seeded in 250 g of artificial diet in 500-ml cylindrical plastic containers to recover F1 pupae. After 8 days, pupae were washed from diet with tap water, counted, and placed according to treatment in 500-ml plastic containers with vermiculite as pupation medium. Plastic containers were sealed with a perforated lid and placed at 26 °C, 65% r.h. until adult emergence (ca. 10 days).

F1 longevity. In a second set of experiments, the longevity of F1 adults obtained from the cross of long-lived males and normal females was compared with laboratory flies (normal males*normal females). Five Plexiglas cages containing 50 males and 50 females of each of the two treaments were set up at emergence of adults obtained from the first experiment, and a similar age cohort of the laboratory colony. The number and sex of dead adults was recorded every 2 days for every cage until 30% of males were dead. The time in days necessary to reach 30% male mortality was recorded for every treatment and control cage. Egg-laying devices as described above were placed on top cages. Egg clutches were counted for 11 consecutive days and eggs were incubated and seeded in diet (as described before). F2 pupae from the cross of F1 males*F1 females were recovered for a third set of experiments.

F2 longevity. F2 adults were placed at emergence in Plexiglas cages (only four cages with 32 males and 32 females each could be set up). Four cages with 32 normal males and 32 normal females were also set up as controls. Male and female mortality was recorded every 2 days as described before. Egg-laying devices were also placed on cages and the clutches and the total number of eggs were counted.

Effect of diet and irradiation regime on longevity of *Anastrepha obliqua*

Mass-reared *A. obliqua* pupae were obtained from the Moscafrut mass-rearing facility at Metapa, Chiapas, Mexico. Pupae were placed in plastic bags under hypoxia, 48 h before adult emergence. One lot was irradiated at 80 Gy, and a second lot was not irradiated. Pupae were shipped to Xalapa, Veracruz, and hypoxia was broken within 12 h upon arrival. Pupae were placed in cages according to irradiation treatment (non-irradiated and irradiated) awaiting adult emergence. At emergence, 10 males and 10 females were transferred from large cages to 3-l plastic cages. For each irradiation treatment, seven cages were left without water or food, seven cages received only water, seven cages received water and a sucrose only diet, and seven cages received water and a diet consisting of sucrose and protein (3:1). The number of dead adults was recorded daily for each cage until all adults were dead.

Effect of probiotic diet on longevity of irradiated and non-irradiated *Anastrepha obliqua*

Wild *A. obliqua* were obtained from infested hogplum, *Spondias mombin* L. (Anacardiaceae), in Apazapan, Veracruz. Pupae were recovered using methods described in Lopez et al. (1999) and kept in Plexiglas cages until emergence of adults. Adults were maintained with water and a mixture of sugar and hydrolyzed protein (3:1) as a source of food until gut extraction. Five days after emergence, flies were dipped in a soap solution, surface disinfected with 70% ethanol, and washed with a phosphate buffered saline (PSB) solution. Guts were sterilely extracted under a dissecting microscope and individualy kept in 750 µl of PSB. Extracted guts were then homogenized using a Vortex mixer. All homogenates of 10 fly guts were put together and used to inoculate growth medium. Gut homogenate was plated in 10 Petri dishes (100 µl per dish) with tryptic soy agar. Five plates were incubated at 28 °C and five plates at 24 °C during 3 days. All plates were then washed with sterile water and bacteria re-suspended together in sterile water at an optical density of 0.3 at 550 nm estimated by using a GENESYS 10S Vis spectrophotometer (Thermo Fisher Scientific, Grand Island, NY, USA). Bacterial suspension (750 µl) was added to 40 mg of a protein and sugar diet (3:1) and mixed to prepare the probiotic diet. A similar diet additioned with the same volume of water was used for control as a non-probiotic diet. We did not attempt to identify the bacterial community in

A. obliqua's gut and assumed, as it has been reported for *A. ludens*, that most bacterial species are similar to those normally found in the guts of other tephritid species (Kuzina et al., 2001).

Irradiated and non-irradiated *A. obliqua* pupae were obtained from the Moscafrut mass-rearing facility, and kept in cages with water only until adult emergence. Ten adult fly couples (20 adult flies), irradiated and non-irradiated, were transferred to 3-l plastic cages and provided with ad libitum access to either a probiotic or non-probiotic diet during 12 days (four treatments). In all cages, diet was replaced after 12 days with fresh 'non-treated' diet. Thereafter, diet was replaced in cages every 12 days until the end of the experiment. In total, eight replicates per treatment were set up (32 cages). Mortality of flies in all cages was monitored daily for 100 consecutive days.

Statistical analysis

The number of pupae recovered from seeding eggs produced by females in cages, and female and male mortality after 47 days were compared among treatments by means of Kruskal–Wallis tests followed by two-tailed multiple comparisons. Male and female longevity was compared by means of a Wilcoxon matched pairs test. Fecundity of F1 and F2 females was compared to fecundity of laboratory females by means of Mann–Whitney U-tests. The effect of diet and irradiation regime on longevity (expressed as the number of days needed to reach 100% mortality) of *A. obliqua* was compared among treatments with a two-way ANOVA. Finally, the effect of irradiation regime and diet (non-inoculated and probiotic) on the number of adults surviving after 100 days was compared between sexes and treatments with a generalized linear model. All statistical analyses were performed with STATISTICA v. 7 (StatSoft, Tulsa, OK, USA).

Results

Selection for male longevity

F1 pupal recovery. The cross of long-lived males with normal females produced more pupae than the cross of normal males*long-lived females and the cross of long-lived males*long-lived females (Kruskal–Wallis test followed by two-tailed multiple comparisons: H = 13.78, d.f. = 2, P = 0.001; n = 21) (Figure 1).

F1 longevity. After 47 days, more of the females of *A. ludens* had died (mean ± SD = 42.8 ± 19.0%) than of the males (24.2 ± 8.8%) (Wilcoxon matched pairs test: T = 1.5, Z = 3.07, P = 0.002; n = 14). Mortality after 47 days of males or females did not differ among generations (Kruskal–Wallis test, males: H = 3.98, P = 0.41; females: H = 0.72, P = 0.69; both d.f. = 2, n = 14) (Figure 2).

F1 fecundity. The number of egg clutches laid in cages was not different between normal (mean ± SD = 193.2 ± 19.03 clutches per cage) and F1 females (114.2 ± 93.71) (Mann–Whitney U-test: Z = −0.31, P = 0.75) or between normal (201.8 ± 31.7) and F2 females (217 ± 36.6) (Z = 0.76, P = 0.46). The total number of eggs laid by normal and F2 females were 6 804 (1 360.3 ± 2 560 eggs per cage) and 7 692 (1 538.4 ± 252 eggs per cage).

Effect of diet and irradiation regime on longevity of irradiated and non-irradiated *Anastrepha obliqua*

Non-irradiated flies fed with sugar or with sugar and protein lived longer than irradiated flies fed with similar diets, and flies fed with sugar and sugar + protein lived longer than flies provided with only water or nothing (Figure 3).

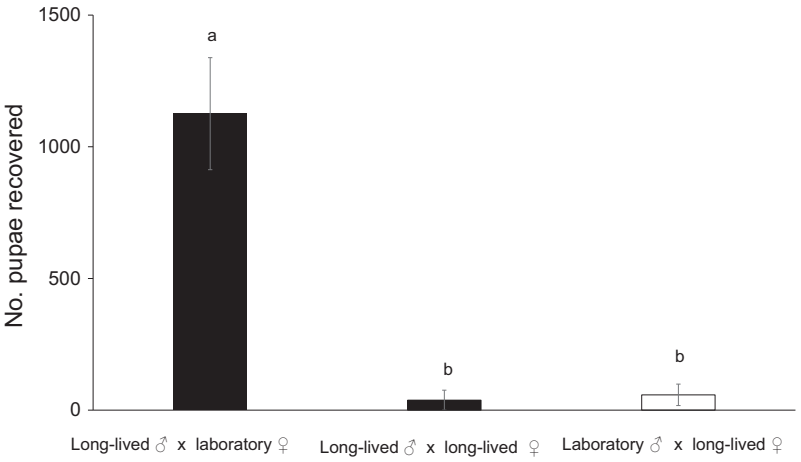

Figure 1 Mean (± SE) number of pupae recovered from eggs laid by *Anastrepha obliqua* laboratory females crossed with long-lived males, long-lived females* long-lived males, and long-lived females*laboratory males. Bars capped with different letters are significantly different (Kruskal–Wallis test: P<0.05).

The number of days needed to reach 100% mortality was influenced by fertility status (two-way ANOVA: $F_{1,48} = 6.18$, $P = 0.016$) and diet ($F_{3,48} = 108.26$, $P<0.001$). The interaction between the factors was not significant ($F_{3,48} = 2.15$, $P = 0.11$).

Effect of probiotic diet on longevity of irradiated and non-irradiated *Anastrepha obliqua*

Male *A. obliqua* survived in greater numbers than females (mean ± SD = 5.15 ± 2.09 vs. 2.27 ± 1.92) after 100 days, and although non-irradiated males survived in greater numbers than irradiated males (5.85 ± 1.75 vs. 4.45 ± 2.21), numbers of surviving *A. obliqua* females did not differ according to fertility status (non-irradiated: 1.75 ± 1.25, irradiated: 2.80 ± 2.33) (Figure 4). A generalized linear model revealed a significant effect of sex ($F_{1,72} = 42.22$, $P<0.001$) and the interaction between sex and fertility status (irradiated vs. non-irradiated) ($F_{1,72} = 7.66$, $P = 0.007$) on number of surviving *A. obliqua* adults after 100 days. Fertility status ($F_{1,72} = 0.16$, $P = 0.69$) and diet (normal vs. probiotic) had no effect on *A. obliqua* survial ($F_{1,72} = 0.08$, $P = 0.78$). The interactions were not significant: sex*diet ($F_{1,72} = 0.03$, $P = 0.86$), fertility status*diet ($F_{1,72} = 0.16$, $P = 0.69$), and sex*diet*fertilty status ($F_{1,72} = 0.38$, $P = 0.54$).

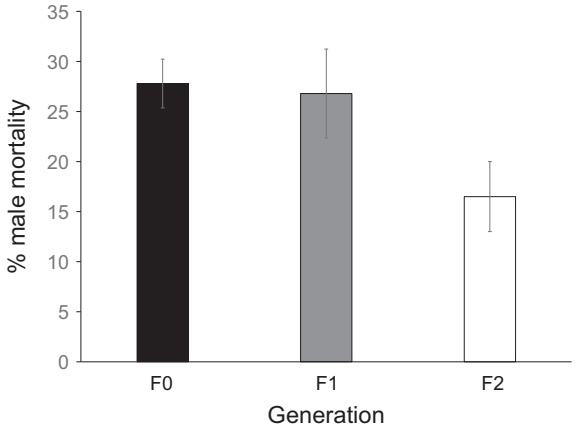

Figure 2 Mean (± SE) *Anastrepha obliqua* male mortality (%) after 47 days for laboratory males (F0), F1 males produced by crossing laboratory females with long-lived males, and F2 males from the cross of F1 females and F1 males. Means did not differ significantly (Kruskal–Wallis test: $P>0.05$).

Discussion

Artificial selection for long-lived *A. ludens* resulted in a sharp drop of fecundity levels for F1 females. Nevertheless, the cross of long-lived males with laboratory females produced a female F1 progeny with fecundity levels comparable to those of females in the established colony. However, the male progeny of long-lived males*laboratory females did not survive in greater proportions than laboratory males. Provision of sugar to *A. obliqua* adults resulted in increased survival in comparison to adults provided only with water, whereas the addition of protein to sugar-only diets had no additional effect on longevity. Non-irradiated males lived longer than irradiated males, yet supplying a probiotic diet produced no noticeable effect in restoring irradiated male longevity of *A. obliqua*.

Miyatake (1997a) found a genetic trade-off between early fecundity and longevity of mass-reared *B. cucurbitae*,

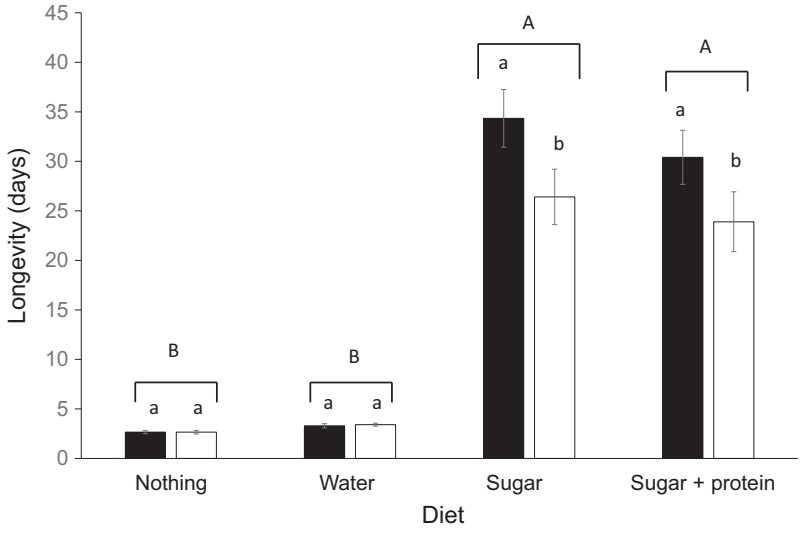

Figure 3 Mean (± SE) longevity (days) of *Anastrepha obliqua* adults developing from non-irradiated (black bars) or irradiated (white bars) pupae, fed nothing, water, sugar + water, or sugar + protein + water. Different letters capping bars indicate significant differences among diets (capital letters) and fertility status (lower case letters) (two-way ANOVA, followed by Tukey HSD: $P<0.05$).

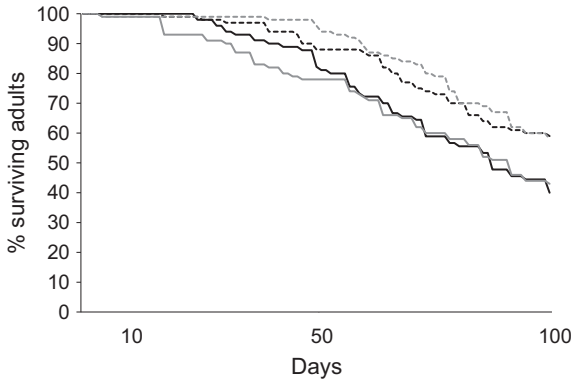

Figure 4 Survival (%) of *Anastrepha obliqua* adults over 100 days according to fertility status – developed from non-irradiated (dotted lines) or irradiated (solid lines) pupae – and diet – probiotic (black lines) or non-inoculated (gray lines).

where the mass-reared population exhibited a negative genetic correlation between these two demographic traits suggesting antagonistic pleiotropy. A similar trade-off has been reported for *Drosophila melanogaster* Meigen (Flatt, 2011; but see Khazaeli & Curtsinger, 2013) and appears to be occurring with *A. ludens*. Direct selection for adult longevity during mass rearing will therefore possibly result in a shift toward late reproduction and a drop in fecundity during the early stages after sexual maturation. This scheme is therefore not likely to be adopted by mass-rearing facilities because it would entail significant increases in costs and complicated logistics during mass production. Additionally, it has to be considered that long-lived lines may perform well in the laboratory, but not in the field, as it has been found for artificially selected strains of *D. melanogaster* where individuals stemming from long-lived lines are less likely to locate food sources in the field than individuals originating from control strains (Wit et al., 2013), or exhibit reduced fitness in comparison to normal strains due to physiological and behavioral factors (Buck et al., 2000). Furthermore, little is known about relative sexual performance of long-lived lines of tephritids used in SIT programs (Miyatake, 1998) and it is possible that long-lived individuals attain sexual maturity much later than adults from mass-reared strains (Miyatake, 1997a, 1998), a fact that may affect SIT efficiency.

Our results revealed that the addition of a pre-release diet including sugar to *A. obliqua* had a positive effect on the survival of sterile males compared to males provided only with water (which lived less than 5 days). Although the addition of protein to a sugar diet had no additional positive effect on longevity and did not cause a significant reduction of lifespan either, this protein-supplemented diet has been reported to increase sexual performance and reduce lifespan for medflies (Kaspi & Yuval, 2000; Yuval et al., 2007) and *A. ludens* and *A. obliqua* (Utgés et al., 2013). The reduction of lifespan of protein-fed males in other studies was observed under field conditions or stress (subsequent starvation), whereas our observations were performed on flies held under artificially constant environmental conditions and permanent access to pre-assigned diets. In fact, San Andrés et al. (2009) found greater longevity for protein-fed than for protein-deprived medflies with continous access to food and water, and an inverse effect for medflies fed with protein + sugar or sugar only for 5 days and subsequently deprived of food and water. In any case, protein is added to adult pre-release diets because it has been found to improve sexual performance of sterile males (Yuval et al., 2007; Pérez-Staples et al., 2008). In species exhibiting a male lekking mating system (where two or more males call and display in mating territories devoid of resources to attract females for mating), males fed both sugar and protein are more attractive and acceptable to wild females than unfed males (Yuval et al., 2007). Although we did not test sexual performance of *A. obliqua* males in this study, evidence for other species of *Anastrepha* (Aluja et al., 2001; Liedo et al., 2013) suggest that including protein in pre-release diets will boost sexual performance. Additionally, reduced proportions of protein in the diet for *A. obliqua* and *A. ludens* could result in a reduction of costs for area-wide programs without detriment to male mating competitivness, as reported by Liedo et al. (2013). Reducing the amount of protein in the adult diet of male *A. fraterculus* to one-eighth, everything else kept equal, did not affect sexual performance or sperm transfer to wild females either (Abraham et al., 2013; Liendo et al., 2013). Comparing longevity under stress for adults of these species fed high and low proportions of protein in the diet could indicate the most effective and least expensive adult pre-relase diet for SIT.

Irradiated *A. obliqua* males and females lived significantly shorter than non-irradiated adults, a finding previously reported for this and other tephritid species under SIT (Barry et al., 2003; Toledo et al., 2004; Collins et al., 2009). In general, radiation primarily affects cells undergoing division; insects therefore are exposed to radiation during late development to maximize genetic damage to mature germ cells and minimize somatic damage (Bakri et al., 2005; Robinson, 2005). However, during late devolpment, somatic cell division is at a minimum; yet some somatic cells, especially in the gut, continue to divide and will be affected by radiation (Robinson, 2005). This may be related to lifespan reduction of irradiated flies. Specifically, radiation has been found to destroy the lining of the gut in sterile medflies. Irradiated flies exhibit signs of damage to midgut tissue, cellular organelles, and gut

microbiota not observed in non-irradiated flies (Lauzon & Potter, 2012).

Symbiotic bacteria have been identified in the gut of several species of Tephritidae (Rubio & McFadden, 1966; Fitt & O'Brien, 1985; Lauzon et al., 2000; Kuzina et al., 2001; Marchini et al., 2002) and play a key role in protein absorption (Behar et al., 2005; San Andres et al., 2007). By adding symbionts to probiotic pre-release diets, damage to the gut lining of irradiated medflies may have reverted and nutrient absorption capacity restored. This addition has resulted in a boost in sexual performance of irradiated males in comparison to males fed a non-inoculated diet (Niyazi et al., 2004; Ben-Ami et al., 2010). Here, irradiated *A. obliqua* feeding on a protein-rich probiotic diet did not experience significant increases in overall longevity; perhaps, because under our experimental conditions protein did not enhance survival of irradiated flies. Several species of bacteria in different families have been isolated from the gut of flies in the genus *Anastrepha* (Kuzina et al., 2001), including *Pseudomonas aeruginosa* (Schroeter) *Migula*, a species known to be pathogenic to arthropods. Behar et al. (2008) suggested that beneficial bacteria in the family Enterobacteriaceae may prevent the proliferation of colonies of harmful bacteria normally found in the medfly gut at low population levels. Yuval et al. (2013) further reported that irradiated medflies fed with diets innoculated with Enterobacteriaceae lived significantly longer than sugar-fed flies, suggesting that the dominant presence of the enterobacterial community in the gut contributes to longevity. They further reported that the sole addition of *Klebsiella oxytoca* (Flügge) Lautrop to the diet improved mating performance of irradiated medflies. The specific composition of the bacterial comunity used in pre-release probiotic diets appears therefore decisive in the result of gut restoration. We may have failed in providing the proper symbionts to generate such a beneficial gut environment in irradiated *A. obliqua* and further experiments involving inoculation of dominant beneficial species could yield more encouraging results.

In summary, increasing male longevity in the field may be achieved through selection of a strain stemming from the cross of long-lived males and laboratory females. However, males from such a strain may take longer to mature and could also be exposed to predation and other mortality factors for prolonged periods before being able to induce sterility into wild populations. Periodic refreshment of inbred bisexual laboratory strains with wild males may be a better option because it could not only improve sexual performance, but also result in an increase in longevity of released males. Pre-release diets including sugar and low amounts of protein may also result in a boost in sexual performance without affecting longevity, and inoculation of these diets with specific beneficial bacteria may aid in reverting radiation damage to the gut lining of sterile males, potentially affecting their longevity.

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