Original Article

Selection for age at reproduction changes pre-mating period and mating frequency in *Zeugodacus cucurbitae*: impacts on insect quality control

Takahisa Miyatake

Laboratory of Evolutionary Ecology, Graduate School of Environmental and Life Science, Okayama University, Tsushima-naka 111, Okayama 7008530, Japan

***Correspondence:** E-mail: miyatake@okayama-u.ac.jp

Running title: *Age at reproduction in melon fly*

Key words: *Zeugodacus cucurbitae*, mass-rearing, mating, melon fly, quality control, sterile insect technique, SIT, Diptera, Tephritidae, correlated responses

Accepted: 30 March 2021

Abstract

In the mass-rearing of insects for sterile insect technique (SIT), it is important to maintain the quality of mass-reared males so that they can compete with wild males in mating with wild females. Mass-reared insects sometimes have shorter pre-mating periods and higher mating frequencies than their wild conspecifics. Indeed, this was the case for mass-reared individuals of the melon fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), used in the SIT program to eradicate the melon fly on the Southwest Islands of Japan. Therefore, I hypothesized that divergent artificial selection for age at reproduction altered these traits in the melon fly as well. To examine the effects of eggs produced by younger parents on each generation, six artificially selected lines were established, for which eggs were collected from young (Y lines) and old (O lines) females. Then, the pre-mating periods and mating frequencies in males and females of the selected lines were compared. The results show a decreased pre-mating period and an increased mating frequency in younger lines compared to older lines, which was driven by males rather than females. These traits ensure efficient offspring production in mass-rearing, and are likely advantageous to the success of SIT programs. The impact of artificially selecting for these traits, especially in males, on insect quality and the efficiency of SIT is discussed.

Abbreviated abstract (max. 80 words)

Insect quality is important when mass-rearing for sterile insect technique (SIT). Mass-reared melon fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae), have shorter pre-mating periods (i.e., younger parents) and more mating events (i.e., more offspring) than their wild conspecifics. I established artificially selected lines in which eggs were collected from younger and older females, and showed that younger lines had shorter pre-mating periods and more mating events than older lines. The impact of these traits on insect quality and SIT efficiency is discussed. [80 words]

Graphic for Table of Contents

Graphical abstract

Introduction

The sterile insect technique (SIT) requires the release of large numbers of sterile males of the target pest into the environment; the males are allowed to mate with wild females to reduce the number of target pests over time (as this results in no offspring), and eradicate wild pest populations (Knipling, 1955, 1959). In recent years, attempts to control pests – such as invasive *Drosophila* spp. (Sassù et al., 2019), tephritid fruit flies (Dyck et al., 2006), and mosquitoes (Zheng et al., 2019) – with SIT have increased worldwide (Koyama et al., 2004; Damiens et al., 2019). An important issue for the success of SIT is quality management of mass-reared insects (e.g., FAO/IAEA/USDA, 2003; Calkins & Parker, 2005; Simmons et al., 2010; Gilchrist et al., 2012; Fanson et al., 2014; Hoffmann & Ross, 2018; Leppla & De Clercq, 2019). Variation exists in an organism's heritable traits, which are evolutionarily altered by selection pressures. Because SIT requires continuous insect rearing for generations, quality control requires attention to be paid to genetic changes across generations by selection or inbreeding (Mackauer, 1976; Miyatake & Yamagishi, 1993; Haymer, 1995; Miyatake, 1998a; Sánchez-Rosario et al., 2017).

SIT has eradicated the melon fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), from Japan's Southwest Islands (Koyama et al., 2004). During the mass production of *Z. cucurbitae*, attention was paid to insect quality management (Miyatake, 1998a). In the process of mass-rearing, individuals who lay many eggs at a younger age have a selective advantage, because it speeds up the generation process (Miyatake & Yamagishi, 1999). In *Drosophila melanogaster* Meigen, populations artificially selected for younger (Y lines) or older (O lines) age at reproduction varied in several characteristics. Females of the O line had lower insemination rates when younger, and higher insemination rates when older, than females of the Y line (Service, 1993). In young flies, sexual differences were visible; males of the O line had a higher mating frequency than those of the Y line, whereas females of the Y line had a higher mating frequency than those of the O line (Pletcher et al., 1997). Overall, in young flies, individuals of the Y lines had higher mating frequencies than individuals of the O lines, but in older flies, this was the other way around (Sgrò et al., 2000). New insights on relationships between aging and life-history traits in *D. melanogaster* and other invertebrate

models have since been provided (see Ackermann et al., 2001; Kapahi et al., 2017); therefore, it is worth evaluating how mass-rearing specifically affects life-history traits in *Z. cucurbitae*. Furthermore, it is important to evaluate whether the methods by which mass-rearing intentionally selects for parents who lay many eggs at a young age have a positive or negative effect on the quality of the insects that are released (see Miyatake, 1998a).

In *Z. cucurbitae*, selecting for females that lay a large number of eggs at a young age improves efficiency in the production of flies, resulting in two correlated responses: a younger age at peak fecundity (Soemori & Nakamori, 1981), and a shorter pre-oviposition period (Nakamori et al., 1976; Sugimoto, 1978; Soemori & Nakamori, 1981; Nakamori, 1987) in mass-reared females compared to wild flies.

The pre-mating period (age until mating) and mating frequency (with wild females) are important traits of sterile males that determine a successful SIT program. Predators may feed on the flies, and sterile males have a short lifespan (Miyatake, 2002). Compared to wild males, mass-reared males have a shorter pre-mating period and a shorter time to remating (Kuba and Soemori 1998). However, whether these differences are the result of selection for age at reproduction during mass-rearing has not been examined. Heritability in the length of the pre-mating period has been observed in *Z. cucurbitae* (Miyatake, 1998b), suggesting genetic linkage in this reproductive trait. Therefore, in the present study, I compared the lengths of the pre-mating period and mating frequencies in *Z. cucurbitae* populations artificially selected for younger or older age at reproduction.

Materials and methods

Insects and selection protocols

The *Z. cucurbitae* base population that underwent selection was a mass-reared strain that was maintained at the Okinawa Prefectural Pest Control Technology Center, Okinawa, Japan, according to the methods described by Nakamori & Kakinohana (1980) and Nakamori et al. (1992). The strain originated in 1985, with 19 281 larvae gathered from bitter gourd fruits, *Momodrica charantia* L. (Cucurbitaceae), collected in the southern part of Okinawa Island, Japan (Kakinohana, 1996).

The method of artificial selection for age at reproduction has been

described in Miyatake (1997). In brief, three replicate 'young' and 'old' lines (total of six lines) were produced from the base population. Selection started in June 1992, and was initiated in pupae, which were divided into three populations. Each population was placed in a rearing cage (30 by 30 by 4 cmsize), and the adults (ca. 1000 flies per cage) were allowed to eclose in each cage. When the adults were 10–15 or 55–60 days old, eggs were collected from the flies in each cage for 13 h using artificial oviposition cylinders. The lines originating from the eggs collected at the two age groups were classified as young lines (Y lines) and old lines (O lines).

All rearing activities and experiments were conducted in a laboratory at 25 ± 2 °C and L14:D10 photoperiod (light phase: 05:30–19:30 hours). Approximately 2400 eggs (0.3 ml) from each line were placed on 300 g of artificial larval medium (Nakamori et al., 1992) in a sample container (7.7 cm high, 13 cm diameter). Each cup was kept separately in a larger sample container (9.2 cm high, 15 cm diameter) filled with water (80 ml). The water was exchanged for pupation substrate, which consisted of a 7:1 mixture of sawdust and water, at 4 days after egg seeding. Mature larvae exited the larval medium and pupated in the pupation substrate. The pupae were transferred to a plastic cup (200 ml), and placed in separate rearing cages. This selection regime was maintained for 45 generations for Y lines and 16 generations for O lines.

Bioassays

Two bioassays were performed to assess the differences in the pre-mating period and mating frequency between the Y and O lines, in earlier and later generations. In the first assay, the traits were measured in generation 24 of the Y line and generation 9 of the O line, at the same time. The large differences in number of generations is due to the selection regimes for Y and O lines; i.e., eggs were collected when adult age was 10–15 days for Y lines and 55–60 days for O lines (see Miyatake, 1997). For two replicated lines (Y-1, Y-2, O-1, O-2), males and females were assayed separately. I did not include the Y-3 and O-3 lines due to the workload at the time. Fifty newly emerged males and females of each line were selected, and each individual was put into an adult rearing cup (80 mm diameter, 40 mm height) (Miyatake, 1996) with a virgin of the opposite sex aged over 20 days derived from the base population. This assay was conducted to measure the two

traits in males and females of each line separately. Mating was observed every night (at approximately 20:00 hours) for 30 days, because the melon fly mates once a day in the evening, and copulation continues until morning. This observation period accounted for all the mating events in the adults.

In the second assay, the traits were measured in generation 45 of the Y line and generation 16 of the O line, at the same time. Males and females that had emerged from the same line on the same day were randomly selected and paired, and consequently, males and females were not distinguished in the results. For three replicated lines (Y-1, Y-2, Y-3, O-1, O-2, O-3), individuals were assayed. Thirty males and 30 females of each line were paired. Mating was observed according to the methods described above.

Statistical analysis

All statistical tests were conducted using JMP v.12.2.0 (SAS Institute, Cary, NC, USA). Mixed-design ANOVA followed by Tukey's honestly significant difference (HSD) tests were performed to analyze the length of the pre-mating period and the number of mating events, using the two separate selection regimes as fixed factors (i.e., Y/O), and replicate lines (i.e., 1 and 2 in assay 1, and 1, 2, and 3 in assay 2) as random factors ($\alpha = 0.05$).

Results

In the first assay, which compared generation 24 of the Y line and generation 9 of the O line, males and females were measured separately. Y-line males had a shorter pre-mating period (Figure 1A) and higher mating frequency (Figure 1C) than O-line males when they were allowed to copulate with females derived from the base population (Table 1). However, for females, there was no difference in the length of the pre-mating period between Y and O lines (Figure 1B), but O-line females had a lower mating frequency than Y-line females (Figure 1D) when they were allowed to mate with males from the base population (Table 1).

In the second assay, which compared generation 45 of the Y line and generation 16 of the O line, there was no distinction between males and females, because each couple was derived from the same line. Y-line flies had a shorter pre-mating period (Figure 2A) and a higher mating frequency (Figure 2B) than O-line

flies (Table 2).

Discussion

Zeugodacus cucurbitae Y-line flies had a shorter pre-mating period and higher mating frequency than O-line flies when males and females were allowed to mate with partners from the same line. Because this pattern was also visible for males that were allowed to mate with partners from the base population (mass-reared but not selected insects), this suggests that these traits depend on males, not females. In other words, males control the length of the pre-mating period and the number of mating events. Also in *Anastrepha obliqua* (Macquart), males control the reproductive behavior of females (Hernández et al., 2017). Interestingly, in the current experiments with *Z. cucurbitae*, the females modified their reproductive behavior, decreasing the age at first oviposition and decreasing the period of oviposition when mated with mass-reared males.

In *D. melanogaster*, a few studies are concerned with mating frequency in diverged lines selected for younger (Y lines) and older (O lines) ages at reproduction. O-line females had lower insemination rates at younger ages (0 weeks) and higher insemination rates at older ages (6 weeks) than Y-line females (Service, 1993). Sexual differences were observed when using young (3–7 days old) adult flies; that is, O-line males had a higher mating frequency than Y-line males, whereas Y-line females had a higher mating frequency than O-line females (Pletcher et al., 1997). The response to selection for age at reproduction is at least in part a consequence of changes in the development of female reproductive behavior; Y-line individuals had higher mating frequencies than O-line individuals at a younger age, but lower mating frequencies at an older age (Sgrò et al., 2000).

Why these traits differ in populations selected for age at reproduction has yet to be resolved (Service, 1993; Pletcher et al., 1997; Sgrò et al., 2000). It may be due to varying environmental conditions (Chippindale et al., 2001), population origin (Charlesworth & Hughes, 1996), and/or molecular pathways involved in the linkage between targeted traits (Pletcher et al., 2002). Because studies in *D. melanogaster* were conducted using paired males and females from the same line, they are most comparable to the results of the second assay in the current study. The results presented here are consistent with those of Sgrò et al. (2000), where Y-

line flies mated more often than O-line flies as relatively young adults. The current study was also conducted using relatively young adults (approximately 20 days after eclosion). In *Z. cucurbitae*, when controlling for mates (using individuals from the base population as sexual partners), the mating frequency was higher in both males and females of the Y line. Conversely, in *D. melanogaster*, O-line males had a higher mating frequency than Y-males, and Y-females had higher mating frequency than O-females (Pletcher et al., 1997). However, in the current study, the effects of fly age on the measured traits were not assessed – this would be interesting to include in future comparisons between Y and O lines in *Z. cucurbitae*.

In the mass-rearing of *Z. cucurbitae*, eggs are collected from young parents to ensure efficient offspring production (Miyatake & Yamagishi, 1999). Therefore, artificial selection for younger age at reproduction during the mass-rearing process caused an indirect response: the pre-mating period was shortened and the mating frequency was increased. As mentioned above, comparisons of these traits between mass-reared and wild strains showed that the mass-reared strains had a shorter pre-mating period and higher mating frequency than wild flies (Nakamori et al., 1976; Sugimoto, 1978; Soemori & Nakamori, 1981; Nakamori, 1987). Heritability of the pre-mating period was also found in males and females of *Z. cucurbitae* (Miyatake, 1998b).

The present results suggest that the shortened pre-mating period and increased mating frequency in mass-reared flies are dependent on artificial selection for younger age at reproduction. These responses in mass-reared flies, e.g., shorter pre-mating period and higher mating frequency, may be advantageous to the success of the SIT eradication program. This is because males begin courting wild females soon after being released into the field, and may be able to mate with wild females multiple times. It has been suggested that artificial selection pressure during mass-rearing may actively produce males with traits (benefits) that favor successful SIT program (Miyatake & Yamagishi, 1993; Meats et al., 2004; Hoffmann & Ross, 2018), and the present results support this hypothesis.

Several aspects of artificial selection are detrimental to a successful SIT program. Y-line females that mate at a younger age are known to have a shorter life span than O-line females that mate at an older age (Miyatake, 1997). Also, although melon flies mate once a day in the evening (males mate every day, and females

mate several times during their life), Y-line flies mate earlier in the day than O-line flies (Miyatake, 2002). Artificial selection for life-history traits can alter mating time, affecting the *cryptochrome* clock gene, probably because of effects of the selection for developmental period (Matsumoto et al., 2008; Fuchikawa et al., 2009). The shortened lifespan and earlier mating time observed in the young lines might have negative consequences for a successful SIT program. This might not only apply for species that mate in the afternoon, such as *Z. cucurbitae*, but also for species that mate in the morning, such as *Anastrepha* spp. (Hernández et al., 2017). Thus, non-matching mating times between mass-reared males and wild females may decrease the efficacy of SIT programs, because they depend on mating of released males with wild females (e.g., Miyatake, 1998a; Matsuyama & Kuba, 2009; Hernández et al., 2017; Pérez et al., 2021). Furthermore, sterile males released in the field die earlier than wild males; thus, the opportunities to mate with wild females decrease. Note that the potentially detrimental effects of sterilization by irradiation have not been addressed in the current study.

The method to collect eggs from young females to improve reproductive efficiency when propagating insects is not limited to *Z. cucurbitae*. It is likely a common practice in the mass-rearing of other organisms used in SIT programs or biological control agents for other species (Boller, 1979; Calkins & Parker, 2005; Gilchrist et al., 2012; Hoffmann & Ross, 2018; Rhode et al., 2020; Ammaghami et al., 2021). In the future, it is necessary to consider the costs and benefits of producing mass-reared strains for SIT programs with regard to sustainability in terms of mating behavior and reproductive schedules.

Acknowledgements

This work was mainly supported by the Japan Society for the Promotion of Science Grant-in-Aid for Scientific Grants, KAKENHI 18H02510.

References

Ackermann M, Bijlsma R, James AC, Partridge L, Zwaan BJ & Sterns SC (2001) Effects of assay conditions in life history experiments with *Drosophila melanogaster*. Journal of Evolutionary Biology 14: 199–209.

Calkins CO & Parker AG (2005) Sterile insect quality. Sterile Insect Technique:

Principles and Practice in Area-Wide Integrated Pest Management (ed. by VA Dyck, J Hendrichs & AS Robinson), pp. 269–296. Springer, Dordrecht, The Netherlands.

Charlesworth B & Hughes KA (1996) Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence. Proceedings of the National Academy of Sciences of the USA 93: 6140–6145.

Chippindale AK, Gibson JR & Rice WR (2001) Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. Proceedings of the National Academy of Sciences of the USA 98: 1671–1675.

Damiens D, Marquereau L, Lebon C, Goff GL, Gaudillat B et al. (2019) *Aedes albopictus* adult medium mass rearing for SIT program development. Insects 10: 246.

Dyck VA, Hendrichs J & Robinson AS (2006) Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Fanson BG, Sundaralingam S, Jiang L, Dominiak BC & D'Arcy G (2014) A review of 16 years of quality control parameters at a mass-rearing facility producing Queensland fruit fly, *Bactrocera tryoni*. Entomologia Experimentalis et Applicata 151: 152–159.

FAO/IAEA/USDA (2003) Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies, v.5.0. International Atomic Energy Agency, Vienna, Austria.

Fuchikawa T, Sanada S, Nishio R, Matsumoto A, Matsuyama T et al. (2009) The clock gene *cryptochrome* of *Bactrocera cucurbitae* (Diptera: Tephritidae) in strains with different mating times. Heredity 104: 387–392.

Ghaemmaghami E, Fathipour Y, Bagheri A, Talebi AA & Reddy GVP (2021) Quality control of the parasitoid wasp *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) over 45 generations of rearing on *Sitotroga cerealella*. Insect Science 28: 180–190.

Gilchrist AS, Cameron EC, Sved JA & Meats AW (2012) Genetic consequences of domestication and mass rearing of pest fruit fly *Bactrocera tryoni* (Diptera:

Tephritidae). Journal of Economic Entomology 105: 1051–1056.
Haymer DS (1995) Genetic analysis of laboratory and wild strains of the melon fly (Diptera: Tephritidae) using random amplified polymorphic DNA-polymerase chain reaction. Annals of the Entomological Society of America 88: 705–710.
Hernández E, Liedo P, Toledo J, Montoya P, Perales H & Ruiz-Montoya L (2017) Fitness of mass-reared males of *Anastrepha obliqua* (Diptera: Tephritidae) resulting from mating competition tests in field cages. Journal of Economic Entomology 110: 2325–2333.
Hoffmann AA & Ross PA (2018) Rates and patterns of laboratory adaptation in (mostly) insects. Journal of Economic Entomology 111: 501–509.
Kakinohana H (1996) Studies on the mass production of the melon flies, *Bactrocera cucurbitae* (Coquillett). Bulletin of the Okinawa Agricultural Experiment Station 16: 1–102 (in Japanese with English summary).
Kapahi P, Kaberlein M & Hansen M (2017) Dietary restriction and lifespan: lessons from invertebrate models. Ageing Research Reviews 39: 3–14.
Knipling EF (1955) Possibilities of insect control or eradication through the use of sexually sterile males. Journal of Economic Entomology 48: 459–462.
Knipling EF (1959) Sterile-male method of population control. Science 130: 902–904.
Koyama J, Kakinohana H & Miyatake T (2004) Eradication of the melon fly, *Bactrocera cucurbitae*, in Japan: importance of behavior, ecology, genetics, and evolution. Annual Review of Entomology 49: 331–349.
Kuba H, Soemori H (1988) Characteristics of copulation duration, hatchability of eggs and remating intervals in the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Japanese Journal of Applied Entomology and Zoology 32: 321–324.
Leppla NC & De Clercq P (2019) History of the International Organization for Biological Control Global Working Group on Mass Rearing and Quality Assurance. Journal of Insect Science 19(2): 7.
Mackauer M (1976) Genetic problems in the production of biological control agents. Annual Review of Entomology 21: 369–385.

Matsumoto A, Ohta Y, Itoh TQ, Sanada-Morimura S, Matsuyama T et al. (2008) Period gene of *Bactrocera cucurbitae* (Diptera: Tephritidae) among strains with different mating times and sterile insect technique. Annals of the Entomological Society of America 101: 1121–1130.

Matsuyama T & Kuba H (2009) Mating time and call frequency of males between mass-reared and wild strains of melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Applied Entomology and Zoology 44: 309–314.

Meats A, Holmes HM & Kelly GL (2004) Laboratory adaptation of *Bactrocera tryoni* (Diptera: Tephritidae) decreases mating age and increases protein consumption and number of eggs produced per milligram of protein. Bulletin of Entomological Research 94: 517–524.

Miyatake T (1997) Genetic trade-off between early fecundity and longevity in *Bactrocera cucurbitae* (Diptera: Tephritidae). Heredity 78: 93–100.

Miyatake T (1998a) Genetic changes of life history and behavioral traits during mass-rearing in the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). Researches on Population Ecology 40: 301–310.

Miyatake T (1998b) Genetic variation in pre-mating period of the mass-reared melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). Applied Entomology and Zoology 33: 29–33.

Miyatake T (2002) Pleiotropic effect, clock genes, and reproductive isolation. Population Ecology 44: 201–207.

Miyatake T & Yamagishi (1993) Active quality control in mass reared melon flies: quantitative genetic aspects. Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques, pp. 201–213. IAEA, Vienna, Austria.

Miyatake T & Yamagishi (1999) Rapid evolution of larval development time during mass-rearing in the melon fly, *Bactrocera cucurbitae*. Research on Population Ecology 41: 291–297.

Nakamori H (1987) Variation of reproductive characters in wild and mass-reared melon flies, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Japanese Journal of Applied Entomology and Zoology 31: 309–314.

Nakamori H & Kakinohana H (1980) Mass-production of the melon fly, *Dacus*

cucurbitae Coquillett, in Okinawa, Japan. Review of Plant Protection Research 13: 37–53.

Nakamori H, Kakinohana H & Soemori H (1976) Method of mass rearing melon flies, *Dacus cucurbitae* Coquilett (Diptera: Tephritidae). 3. Mass collection of eggs. Journal of Okinawa Agriculture 14: 1–5 (in Japanese with English summary).

Nakamori H, Kakinohana H & Yamagishi M (1992) Automated mass production system for fruit flies based on the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Advances in Insect Rearing for Research and Pest Management (ed. by TE Anderson & NC Leppla), pp. 441–454. Westview Press, Boulder, CO, USA.

Pérez J, Mendez V, Yuval B & Taylor PW (2021) Domestication-related changes in sexual performance of Queensland fruit fly. Insect Science (doi.org/10.1111/1744-7917.12873).

Pletcher SD, Fukui HH & Curtsinger JW (1997) Mating behavior in *Drosophila melanogaster* selected for altered longevity. Evolution 51: 303–307.

Pletcher SD, Macdonald SJ, Margueries R, Certa U, Sterns SC et al. (2002) Genome-wide transcript profiles in aging and calorically restricted *Drosophila melanogaster*. Current Biology 12: 712–723.

Rhode C, Badenhorst R, Hull KL, Greenwood MP, Bester-van der Merwe AE et al. (2020) Genetic and phenotypic consequences of early domestication in black soldier flies (*Hermetia illucens*). Animal Genetics 51: 752–762.

Sánchez-Rosario M, Pérez-Staples D, Toledo J, Valle-Mora J & Liedo P (2017) Artificial selection on mating competitiveness of *Anastrepha ludens* for sterile insect technique application. Entomologia Experimentalis et Applicata 162: 133–147.

Sassù F, Nikolouli K, Caravantes S, Taret G, Pereira R et al. (2019) Mass-rearing of *Drosophila suzukii* for sterile insect technique application: evaluation of two oviposition systems. Insects 10: 448.

Service PM (1993) Laboratory evolution of longevity and reproductive fitness components in male fruit flies: mating ability. Evolution 47: 387–399.

Sgrò CM, Geddes G, Fowler K & Partridge L (2000) Selection on age at reproduction in *Drosophila melanogas*ter: female mating frequency as a

correlated response. Evolution 54: 2152–2155.
Simmons GS, Suckling DM, Carpenter JE, Addison MF, Dyck VA & Vreysen MJB (2010) Improved quality management to enhance the efficacy of the sterile insect technique for lepidopteran pests. Journal of Applied Entomology 134: 261–273.
Soemori H & Nakamori H (1981) Production of successive generations of a new strain of the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae) and reproductive characteristics in mass rearing. Japanese Journal of Applied Entomology and Zoology 25: 229–235 (in Japanese with English summary).
Sugimoto A (1978) Egg collection method in mass rearing of the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Japanese Journal of Applied Entomology and Zoology 22: 60–67 (in Japanese with English summary).
Zheng X, Zhang D, Li Y, Yang C, Wu Y et al. (2019) Incompatible and sterile insect techniques combined eliminate mosquitoes. Nature 572: 56–61.

Figure captions

Figure 1 Mean (± SE) (A, B) pre-mating period (days) and (C, D) mating frequency (no. matings per day) in (A, C) male and (B, D) female melon fly, *Zeugodacus cucurbitae*, in two lines selected for a younger (Y) age at reproduction (generation 24) and two lines selected for an older (O) age at reproduction (generation 9) (n = 50). Flies were allowed to copulate with conspecifics from a non-selected population. Means within a panel capped with different letters are significantly different (Tukey HSD test: $P<0.05$). Note the difference in scale on the vertical axes in panels C and D.

Figure 2 Mean (± SE) (A) pre-mating period (days) and (B) mating frequency (no. matings per day) in male and female melon fly, *Zeugodacus cucurbitae*, in three lines selected for a younger (Y) age at reproduction (generation 45) and three lines selected for an older (O) age at reproduction (generation 16) (n = 30). Flies were allowed to copulate with conspecifics from the same line, so males and females were not distinguished. Means capped with different letters are significantly different (Tukey HSD test: $P<0.05$).

1

Table 1 ANOVA for mating traits of melon fly, *Zeugodacus cucurbitae*, in two lines (replications) selected for a younger age at reproduction (generation 24) and two lines selected for an older age at reproduction (generation 9)

Sex	Trait	Factor	d.f.	F	P
Male	Pre-mating period	Line	1,1	98.181	<0.0001
		Line*replication	1,1	0.301	0.58
	Mating frequency	Line	1,1	134.958	<0.0001
		Line*replication	1,1	0.294	0.59
Female	Pre-mating period	Line	1,1	0.457	0.50
		Line*replication	1,1	1.724	0.19
	Mating frequency	Line	1,1	12.386	0.0005
		Line*replication	1,1	0.985	0.32

Table 2 ANOVA for mating traits of melon fly, *Zeugodacus cucurbitae*, in three lines (replications) selected for a younger age at reproduction (generation 45) and three lines selected for an older age at reproduction (generation 16)

Traits	Factor	d.f.	F	P
Pre-mating period	Line	1,1	32.826	<0.0001
	Line*replication	2,2	0.100	0.10
Mating frequency	Line	1,1	16.693	<0.0001
	Line*replication	2,2	0.197	0.82

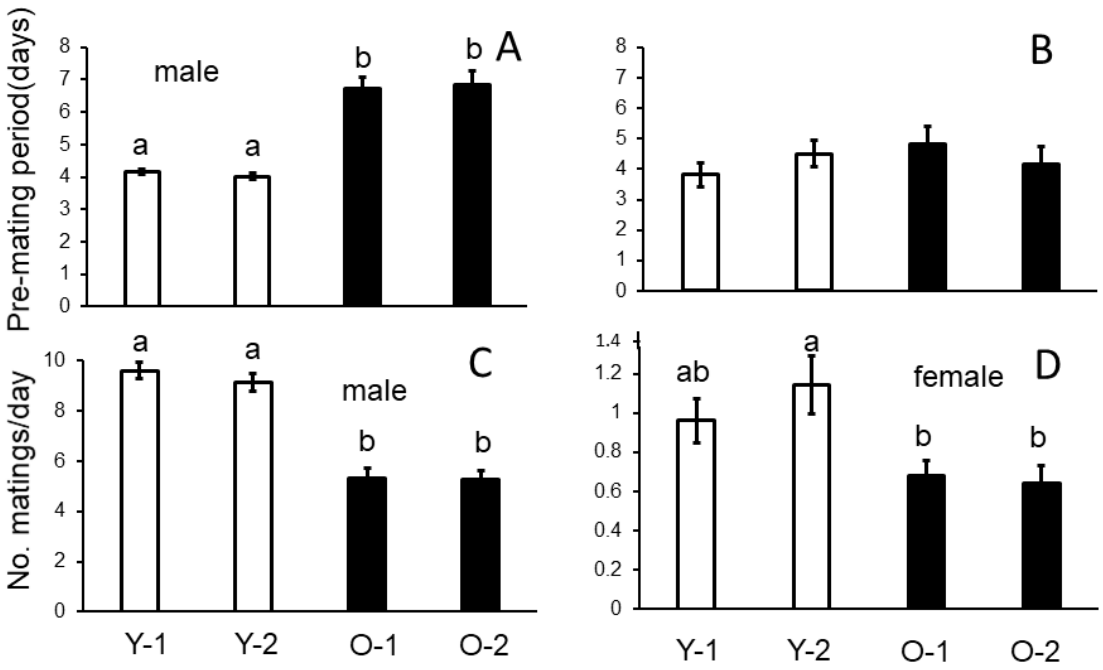

Fig. 1

-Please, label the panels A B C and D

-In panel D, place the statistics letters directly above the error bars, centered

-In Panel D, prolong the y-axis to 1.4 (so that all data fall within the scale indicated on the axis)

-Remove ‘male’ and ‘female’ <both 2x>. Instead, add ‘Male’ and ‘Female’ as a title above the resp. top panel <centered; with capital initial letter>

-Top panels, add unit to text label on y-axis: Pre-mating period (days)

-Lower panels, replace text label on y-axis: No. matings/day

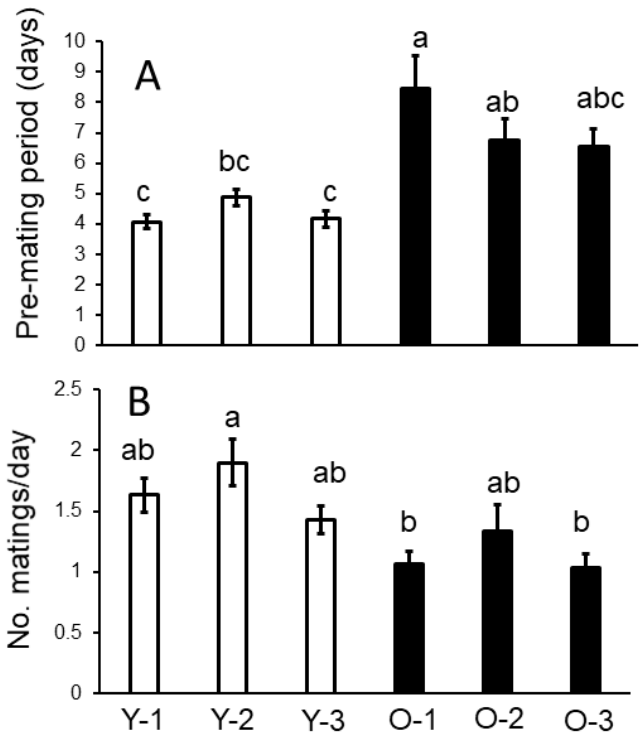

Fig. 2

-Please, label the panels A and B

-Significance letters: centered relative to the columns

-Top panel, add unit to text label on y-axis: Pre-mating period (days)

-Lower panel, replace text label on y-axis: No. matings/day

1 Graphical abstract

Mass rearing of *Bactrocera cucubitae*

2

3 Please check spelling of species name in the graphical abstract. In the main text,
4 the genus is *Zeugodacus*, and the species name is spelled *cucuRbitae*