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Differential response to larval crowding of a long and a short lived medfly biotype

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Abstract

Response of endophytic fruit fly species (Tephritidae) to larval crowding is a form of scramble competition that may affect important life history traits of adults, such as survival and reproduction. Recent empirical evidence demonstrates large differences in adult life history traits, especially longevity, among Mediterranean fruit fly (*Ceratitis capitata*; "medfly") biotypes obtained from different regions of the world. However, whether the evolution of long lifespan is associated with response to stress induced by larval crowding has not been fully elucidated. We investigated, under constant laboratory conditions, the response of a short and a long-lived medfly biotype to stress induced by larval crowding. Survival and development of larvae and pupae and the size of resulting pupae were recorded. The lifespan and age-specific egg production patterns of the obtained adults were recorded. Our findings reveal that increased larval density reduced immature survival (larvae and pupae) in the short-lived biotype but had rather neutral effects on the longed-lived one. Only larvae of the long-lived biotype were capable of prolonging their developmental duration under the highest crowding regime to successfully pupate and emerge as adults. Response of emerging adults to larvae crowding conditions were similar in the two medfly biotypes. Those individuals emerging from high larval density regimes had reduced longevity and fecundity. The importance of our findings to understand the evolution of long lifespan is discussed.

Keywords

Plasticity; plastic response; stressful conditions; life history traits; longevity

INTRODUCTION

Insect population dynamics are affected by a series of factors influencing both immature development and the adult stage (Wilbur, 1980). Intraspecific competition on food availability caused by increased larval density affects both immature survival and

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developmental duration (Applebaum & Heifetz, 1999), (Gibbs, Lace, Jones, & Moore, 2004). In scramble competition, resources that represent the limiting factor are allocated equally among individuals of a given population. Therefore, all community members suffer the same adverse consequences in cases where available resources are exhausted (Speight, 2008). In contest competition, some individuals exploit greater amounts of the available resources by preventing access of others. Contest competition results in more stable population dynamics relative to scramble, since under continuous depletion of available resources, a proportion of individuals always acquires essential resources for survival, development and breeding. In contrast, scramble competition may lead to habitat overexploitation and population collapse because very few or none of the individuals acquire enough resources for survival and reproduction (Speight, 2008).

Various forms of stress, such as larval crowding during development induce a metabolic cost for insect immature stages, expressed as increased energetic expenditure in activities that in most cases function against development, i.e somatic maintenance (Buchanan, 2000). However, the magnitude of larval crowding on shaping insect developmental parameters is determined by the type of interactions among larvae and the quality of available food (Fielding, 2004). Adult crowding may affect both survival and reproduction, while larval crowding can affect size, survival and developmental rates. Crowding during the larval stage may shape important fitness elements of the emerging adults, such as survival and reproduction conferring considerable impacts to future generations (Peters & Barbosa, 1977).

In frugivorous, endophytic, insect selection of oviposition sites is considered a form of energy-demanding maternal investment to select locations where offspring are more likely to survive (Díaz-Fleischer, 2000). For example, females of many insect species avoid laying eggs into already infested fruit, a behavior that may has evolved to minimize levels of intraspecific competition among larvae due to the limited food sources inside fruits (Godfray & Parker, 1992). In many tephritid (Diptera: Tephritidae) species, including the Mediterranean fruit fly (medfly) *Ceratitis capitata*, rejection of already infested fruit is mediated by the use of host-marking pheromone that deters oviposition by subsequently arriving females (Papaj, Averill, Prokopy, & Wong, 1992; Papaj, Roitberg, & Opp, 1989; Prokopy, Ziegler, & Wong, 1978). Since medfly larvae must complete their development exclusively within the fruit in which they are deposited as eggs, host marking possibly functions to reduce intraspecific competition among progeny of multiple females (Papaj & Messing, 1996). Indeed, Debouzie (1989) referring to the biotic factors causing mortality in populations of tephritid species argues that in species where females lay their eggs in large fruits, such as *C. capitata*, the average number of larvae in a fruit is always lower than the threshold value above which the deleterious effects of competition appear. For example, the total number of medfly larvae in a peach or in a sour orange never exceeded 100, while their average number was generally lower than 20 per fruit resulting usually in excess of food availability rather than complete depletion (Cirio et al. 1972, from Debouzie, 1989). A series of studies suggest that despite the functional importance of oviposition-deterring pheromone, medfly females actively deposit eggs into pre-existing oviposition punctures formed by conspecifics, leading to increased larval density inside infested fruits (Papaj, Averill, Prokopy, & Wong, 1992; Papaj, Katsoyannos, & Hendrichs, 1989). A possible

explanation of this behavior is that females gain important benefits associated with increased proportions of egg-laying attempts that result in successful deposition of a clutch, reduction in the time required to deposit a clutch and avoidance of ovipositor wear allowing females to establish their own sites in future efforts (Papaj & Messing, 1996). However, theoretically, it is expected that female medflies would oviposit into already infested fruit when gains resulting from such a behavior exceed costs from increased larvae competition inside fruit (Papaj & Messing, 1996). Therefore, studies dealing with the effects of larval density on the biology of *C. capitata* is of high ecological importance. Today only few studies, have addressed effects of larvae density on medfly fitness traits (Dukas, Prokopy, & Duan, 2001; Papaj, Roitberg, & Opp, 1989; Prokopy & Duan, 1998). They demonstrate a decline in larval survival when density of *C. capitata* eggs per kumquat fruit *Fortunella japonica* (Rutaceae) increased from 1 to 32 (Papaj, Roitberg, & Opp, 1989), and kumquats with more than one egg clutch (Dukas, Prokopy, & Duan, 2001). In addition, the latest study revealed that the increment of egg density inside the fruit leads to a decrease in larval growth rate and pupal mass. Interestingly, Dukas and colleagues (2001) point out that these differences, although not significant, may have a great effect on the fitness of both immature stages and adults. Nonetheless, there are no such studies exploring at the same time the effects of larval competition on the biology of both immature stages and emerging adults.

Individuals in natural populations usually experience variable and perhaps also stressful conditions such as food scarcity and extremes in temperature and water availability. Longevity under natural conditions is highly dependent on both the frequency and intensity of stressful conditions that are encountered as well as the ability of living organisms to cope with these difficulties (Vermeulen & Loeschcke, 2007). The positive relationship between longevity and the ability of living organisms to deal with stressful conditions has been the subject of extensive research in recent years. Additionally, a great number of studies with model organisms such as *Drosophila* species have dealt with the detection of stress resistance genes that ultimately mediate lifespan (Vermeulen & Loeschcke, 2007 and references therein). In medfly, a considerable number of studies have focused on the effects of stressful conditions during the adult stage such as high density, radiation exposure, and reduction of food nutrition value on both longevity and female reproduction (Carey, Liedo, Harshman, Zhang, Muller, Partridge, & Wang, 2002; Carey, Liedo, Muller, Wang, Love, Harshman, & Partridge, 2001; Carey, Liedo, & Vaupel, 1995). Moreover, the effects of cold stress on adult survival, behavior, gene expression and lipid profiles have been recently elucidated (Pujol-Lereis, Fagali, Rabossi, Catala, & Quesada-Allue, 2016; Pujol-Lereis, Rabossi, & Quesada-Allue, 2014). Thermal conditions during pupal development on adult flight performance have been also explored recently (Esterhuizen, Clusella-Trullas, van Daalen, Schoombie, Boardman, & Terblanche, 2014). However, to our knowledge, none to date have explicitly studied the effects of larval competition on adult demographic traits.

Recent studies suggest that medfly biotypes, from different parts of the globe, sharing different invasion histories have evolved remarkably different life histories (Diamantidis, Carey, Nakas, & Papadopoulos, 2011a; Diamantidis, Carey, & Papadopoulos, 2008; Diamantidis, Papadopoulos, Nakas, Wu, Muller, & Carey, 2009). Adult lifespan was several weeks shorter in flies obtained from Kenya, Hawaii, and Guatemala compared to those from Greece, Portugal and Brazil. It seems that seasonal/temperate environments select for longer

adult lifespan to bridge gaps in availability of oviposition resources. Moreover, medfly have evolved divergent stress responses that likely enhance survival under different climates (Weldon, Nyamukondiwa, Karsten, Chown, & Terblanche, 2018). Whether such adaptive responses are accompanied with response to other stressful conditions, such as larval crowding has yet to be explored. Such data would only broaden our understanding regarding response of different biotypes to stressful conditions but may also provide additional information regarding the relationship between early stress and late performance at the individual level. Here we tested the hypothesis that two different medfly biotypes with an inherent shorter and longer adult lifespan express similar responses to crowding during the larval stage and subsequent performance of adults. We tested under identical laboratory conditions the effect of larval density on: (1) the survival and developmental times of larvae and pupae, (2) pupal size (length and weight), and (3) adult longevity and female reproductive potential of a long-lived and short-lived biotype of the medfly. Based on the abovementioned literature we predicted that larval crowding will similarly reduce immature survival rates and size (pupae) in both biotypes, increase their developmental times and confer similar fitness cost on the adults in terms of reduced longevity and fecundity.

Materials and methods

Experimental conditions and flies used

The experiments were conducted in the laboratory of Entomology and Agricultural Zoology at the University of Thessaly at 25 ± 1 °C, 65 ± 5% relative humidity and a photoperiod of L14 : D10 with photophase starting at 07:00 h. Light was provided by daylight fluorescent tubes and by natural light from four windows with the intensity inside the test arena ranging from 1500 to 2000 Lux.

Experiments involved two medfly biotypes, one with a long (long-lived) and one with a short (short-lived) adult lifespan respectively. The classification of these two biotypes as long-lived and short-lived was based on the results of a previous study suggesting an approximately 1.5 fold variation in longevity within each sex (Diamantidis, Papadopoulos, Nakas, Wu, Muller, & Carey, 2009). Moreover, this variation remained pretty much at the same levels following laboratory adaptation of both biotypes, suggesting a genetically controlled and maintained trait under common garden conditions (Diamantidis, Carey, Nakas, & Papadopoulos, 2011b). Specifically, the flies of the long-lived biotype were of the F_1 laboratory generation originating from infested mandarin oranges (*Citrus reticulata*) collected from the broader area of Volos, Greece. In order to remove any effects in life history traits resulting from the host fruit, we reared this population for one generation on an artificial diet before subjecting them to experimental trials. The demographic parameters of this population had been determined earlier and they were found to be very similar to the population of Chios, Greece, a well-documented long-lived biotype (Diamantidis, Papadopoulos, Nakas, Wu, Muller, & Carey, 2009). The flies of the short-lived population were of the F_{25} laboratory generation originated from coffee (*Coffea arabica*) berries collected from Antigua, Guatemala. Details regarding colony maintenance and rearing procedures of the two biotypes are given by Diamantidis and colleagues (2011b).

Effects of larval density on the biological parameters of immature stages

The eggs of the two populations were transferred with a fine brush from artificial oviposition substrates to, black colored filter paper fitted in a 9 cm Petri dish and moistened with distilled water. In each Petri dish, 450-500 eggs were placed and kept at 25 °C for 48 hours until larvae eclosed.

Immediately after hatch, larvae from each medfly biotype were transferred with a soft brush, under a binocular stereoscope (Leica MZ 12, Wetzlar, Germany), onto a nutrient substrate comprising cotton pads (3 cm in diameter and 0.4 cm in high) impregnated with 3 ml of an artificial liquid diet (Boller, 1985). The cotton pads were placed inside small, 30 ml plastic cups, sealed with a plastic lid bearing 50-60 evenly distributed holes 1 mm in diameter to allow adequate ventilation of the cup. The newly hatched larvae from each population were randomly assigned to six different densities (treatments). The amount of larval food (3 ml) was constant across all treatments. The treatments used, the number of replicates of each treatment and the total number of larvae used for each population are given in Table 1. The number of replicates in each larval density treatment was adjusted to provide a balanced number for statistical analysis that were conducted at individual level (see results).

All treatments were maintained in a walk-in chamber at 25±1 °C until completion of the larval stage. Three days after placing larvae onto the nutrient substrates, the small cups were placed in larger plastic cups (diameter: 6 cm) containing a thin layer of sand for pupation. Daily, during the last two hours of photophase (19:00 - 21:00 h), commencement of the pupal stage was checked and recorded in all density regimes for both biotypes. Soon after pupation, all pupae were individually placed into 1.5 ml Eppendorf tubes. All pupae were weighed one day after pupation using a precision (±0.1mg) electronic scale (Precisa 40SM-200A, Switzerland), while their length was measured under a stereomicroscope equipped with a graduated scale. Pupae were kept at 25 °C and adult emergence was recorded daily.

Effects of larval density on adult demographic traits

Immediately after adult emergence from each treatment (larval density regime), pairs consisting of a male and a female from the two medfly biotypes were placed into small cages. Each cage comprised a 0.4 L capacity transparent plastic cup (height 12.5 cm high, upper diameter 6.5 cm, base diameter 9.2 cm) provided with ample adult food (a mixture of yeast hydrolysate, sugar and water at a ratio of 4 : 1 : 5, respectively). In the side of each cup, an opening of 25 cm^2 covered with nylon mesh was formed for ventilation. The base of each cage included a 9.2 cm in diameter Petri dish lid with an oviposition substrate fitted into a 5 cm diameter hole formed into the center. Each oviposition substrate consisted of a red, plastic, hollow hemisphere (diameter 5 cm) with 40-50 evenly distributed 1 mm in diameter holes (hereafter called a ‘dome’) through which females laid their eggs. Small cages were placed upon 9 cm in diameter Petri dishes provided with tap water to maintain adequate humidity in the interior of the domes, whereas adults had access to drink via a small piece of wick. To stimulate oviposition, plastic cups containing 5 ml of freshly-squeezed orange juice were placed beneath the domes. The orange juice was replaced every two days. Adult mortality and female fecundity were recorded as a daily egg count until

death. The adults used in the experiments involved both biotypes from four different treatments: 1, 15, 60 and 120 larvae / 3 ml of artificial diet (Table 1). We performed 40 replicates (pairs of adults) for each of the four treatments and each one of the two biotypes. In total, 320 adults were used for each medfly biotype.

Statistical analysis—The effects of medfly biotype (categorical variable), larval density (treated as continous variable) and their interaction on a) larval and pupal survival rates, b) larval and pupal developmental durations, c) pupal length and weight and d) female fecundity, were studied using General Linear Modeling techniques. The effects of medfly biotype, larval density and sex on adult lifespan were assessed using the Cox proportional hazards model with censoring. Pairwise comparisons were conducted using the log rank (Mantel–Cox) test. The same analysis was also performed to assess the effects of medfly biotype and larval density on female reproductive periods (pre-oviposition, oviposition and post-oviposition period) since they represent time “to event” as in the case of lifespan. A two-way analysis of variance (ANOVA) was performed to assess the effects of medfly biotype and larval density on female fecundity rates followed by Tukey’s HSD post hoc test to separate means. Data analysis was performed using IBM SPSS 25 (IBM Corp., Armonk, NY).

Results

Effects of larval density on the biological parameters of immature stages

Immature survival—Larval survival rate was significantly affected by both medfly biotype and crowding (Wald test x^2_1=9.022, p=0.003 and x^2_1=7.907, p=0.005 respectively). The interaction between medfly biotype and crowding was also significant (Wald test x^2_1=10.689, p=0.001) suggesting that larval crowding influenced in a differential way larval survival rate in each of the two medfly biotypes tested. Indeed, larval survival rate in the long-lived medfly biotype was very high (above 95%) regardless of larval density, while the opposite was evident for the short-lived biotype (Fig.1A). Likewise, pupal survival rate was significantly affected by both medfly biotype and crowding (Wald test x^2_1=19.301, p<0.001 and x^2_1=5.792, p=0.016 respectively). The interaction between medfly biotype and crowding was not significant (Wald test x^2_1=3.585, p=0.058). Pupal survival rates among different larval density regimes ranged from 93 to 98 % in the long-lived medfly biotype and from 68 to 86 % in the short-lived biotype (Fig. 1B).

Pupal length and weight—Larval crowding negatively affected male pupae length ($F_{3, 1002}$=348.77, p<0.001; t=−30.92, p<0.001). Overall medfly biotype was a significant predictor of pupal length (t=−9.64, p<0.001). The interaction between medfly biotype and crowding was also significant (t=13.10, p<0.001) indicating a stepper rate of reduction on male pupae length of the long-lived biotype compared to the short-lived one the in response to increased larval density (Fig. 2A). Likewise, larval crowding ($F_{3, 863}$ =330.08, p<0.001; t= −30.46, p<0.001) and biotype (t=−10.77, p<0.001) were significant predictors of pupal length in females. As in the case of males, the interaction between medfly biotype and crowding was also significant (t=13.45, p<0.001) indicating that larval crowding affected female pupae length in a differential way in each of the two medfly biotypes (Fig. 2B).

Larval crowding negatively affected pupal weight in males ($F_{3, 1001}$=632.34, p<0.001; t=−36.56, p<0.001). Overall pupae of the long lived biotype were heavier that that of the short lived biotype (t=−6.06, p<0.001). The interaction between medfly biotype and crowding was also significant (t=5.91, p<0.001) indicating a differential rate of reduction on male pupae weight between the short-lived and the long-lived biotype in response to increased larval density (Fig. 3A). Likewise, larval crowding ($F_{3, 863}$=602.43, p<0.001; t=−36.64, p<0.001) and biotype (t=−5.83, p<0.001) were significant predictors of female pupal weight. The interaction between medfly biotype and crowding was also significant (t=6.93, p<0.001) indicating that increased larval crowding reduced female pupae weight at higher rates in the long lived biotype compared to the short lived one (Fig. 3B).

Immature stages developmental duration—Larval developmental duration was significantly affected by crowding but not medfly biotype (Wald test x^2_1=104.772, p<0.001 and x^2_1=1.224, p=0.269 respectively). The interaction between medfly biotype and crowding was significant (Wald test x^2_1=28.787, p<0.001) suggesting that larval crowding influenced in a differential way larval developmental duration in each of the two medfly biotypes tested. Indeed, larval crowding increased the developmental duration only in larvae of the long-lived medfly biotype (Figure 4). This increase was most evident in the density of 120 larvae/3 ml of food for both males and females.

Pupal developmental duration in males was significantly affected by crowding but not medfly biotype (Wald test x^2_1=69.738, p<0.001 and x^2_1=3.447, p=0.063 respectively). The interaction between medfly biotype and crowding was not significant (Wald test x^2_1=0.038, p=0.846). Intermediate larval densities appeared to increase pupal developmental duration of males in both medfly biotypes (Fig. 5A). Likewise, pupal developmental duration in females was significantly affected by crowding but not medfly biotype (Wald test x^2_1=39.876, p<0.001 and x^2_1=2.275, p=0.131 respectively). The interaction between medfly biotype and crowding was not significant (Wald test x^2_1=0.081, p=0.776). Crowding affected females' pupal developmental duration in the same way as in males (Fig. 5B). A quadratic model for crowding provided better fit to the data in this case.

Effects of larval density on adult demographic traits

Survival—Larval crowding during development resulted in shorter adult female lifespan of both biotypes (Table 2). Likewise, male lifespan followed similar trends in response to increased larval density with one exception regarding the 60 larvae/3 ml of food density. Cox regression revealed medfly biotype (x^2_1=28.651, p<0.001), larval density (x^2_1=10.727, p=0.001) and sex (x^2_1=201.753, p<0.001) as significant predictors of adult lifespan (Table 3). The interaction between medfly biotype and sex was also significant (x^2_1=27.870, p<0.001). However, the interactions between medfly biotype and larval density and between sex and larval density were not significant (x^2_1=0.105, p=0.746 and x^2_1=0.762, p=0.383 respectively).

Survival rates for males and females of the long-lived and the short-lived medfly biotype reared in the four different larval densities regimes are given in Figure 6. Female survival of the long-lived biotype was high until day 40. After this point, female survival derived from

the density of 120 larvae / 3 ml of food decreased at a faster rate than that of the females of the other three treatments (Fig. 6). Female survival of the short-lived biotype showed similar trends among different treatments of larval density, i.e high survival up to day 20 and gradual decline of survival after that point (Fig. 6). Male survival of the long-lived biotype was high until day 60 in all larval crowding regimes (Fig. 6). After this age there was a gradual decline in survival for males of all treatments of the long-lived biotype (Fig. 6). Similar trends in survival were observed for males of the short-lived biotype derived from the four larval densities tested, i.e high survival up to day 40 and gradual decline in survival after this age (Fig. 6).

Fecundity—Table 4 provides female fecundity rates of both medfly biotypes at different larval density regimes. Analysis of variance revealed that increased larval density reduced female fecundity ($F_{3, 312}$=7.2, p<0.001) while medfly biotype had no significant effects ($F_{1, 312}$=0.08, p=0.77). The interaction between larval density and medfly biotype was not significant ($F_{3, 312}$=1.3, p=0.24).

Cox regression model revealed medfly biotype (x^2_1=82.585, p<0.001) as significant predictor of female pre-oviposition period but not larval density (x^2_1=2.181, p=0.140). Pre-oviposition period displayed a slight increase among treatments in the case of the short-lived biotype, while it decreased in the long-lived biotype (Table 5). Similarly, medfly biotype (x^2_1=111.339, p<0.001) but not larval density (x^2_1=1.998, p=0.157) was a significant predictor of female oviposition period. Female oviposition period in both long-lived and short-lived biotype slightly decreased in response to increased larval density (Table 5). On the other hand, both medfly biotype (x^2_1=11.164, p=0.001) and larval density (x^2_1=11.381, p=0.001) were significant predictors of female post-oviposition period. The interaction between medfly biotype and larval density however was not significant (x^2_1=1.920, p=0.166). Female post-oviposition period in both biotypes decreased as the larval density during development increased (Table 5).

An event-history diagram (Carey, Liedo, Muller, Wang, & Vaupel, 1998) showing age-specific reproductive patterns of females derived from the four different larval densities for the long-lived and the short-lived medfly biotype is given in Figure 7. It appears that the high egg-laying (> 20 eggs) period for females of the short-lived biotype was observed between days 10 and 30 in all four larval densities. In the case of the long-lived biotype, the high egg-laying period in all four treatments was more evenly distributed throughout the female oviposition period.

Age-specific reproduction schedule of females is given in Figure 8. Egg-laying (20-30 eggs/female / day) peaked for the long-lived females in all treatments around day 30 of age. Peak of egg laying (35-50 eggs/female/day) for the short-lived females was observed in all treatments at approximately day 20 of age (Fig. 8). Therefore, it appears that the age-specific reproduction schedule of females is not influenced by the larval crowding (Fig. 7 & 8).

Discussion

This study reveals that two Mediterranean fruit fly biotypes respond differently to the stressful conditions resulting from high density of larvae during development. In particular, contrary to our predictions, an increase in larval density: reduces the survival of immature stages (larvae and pupae) at a higher rate in the short-lived biotype, while increases larval developmental duration only in the long-lived one. Additionally, different patterns on the reduction of immature size (pupal length and weight) were observed. On the other hand, adults of both biotypes suffer reduced longevity and fecundity in response to increased larvae crowding. It seems that adults of the long lived biotype suffered a higher cost, at least as far as longevity is regarded, compared to short live biotype.

An increase in larval density reduces both larval and pupal survival in the short-lived biotype, but increases larval developmental duration of the long-lived biotype. Density-dependent natural selection theory may explain the differential response of the two medfly biotypes, according to which the evolution of several of the biological characteristics of the organisms is the result of the population density experienced by their ancestors (Mueller, 1991). Several of the predictions of this theory are confirmed by laboratory studies, mainly in *Drosophila* species, where the selection based on different density conditions among individuals of a population can lead to genetic differentiation among resulting biotypes for diverse biological characteristics (Borash & Ho, 2001; Mueller, 1991; Mueller, Gonzalezcandelas, & Sweet, 1991; Mueller, Guo, & Ayala, 1991; Roper, Pignatelli, & Partridge, 1996; Sokolowski, Pereira, & Hughes, 1997). For example, populations of *D. melanogaster*, that were reared for several generations in the laboratory under conditions of increased larval density, exhibit higher survival and prolonged larval growth rates compared to others that were maintained at low larval densities (Mueller, 1991). In this study, the higher larval survival in populations that were reared under conditions of increased larval density is attributed to the avoidance of pupation on the surface of the nutrient substrate, where the mortality rate is high. Larval densities of the two medfly biotypes tested under their natural environments from which they originated is unknown. Temperate medfly populations, such as the long-lived in our study, are faced with seasonal and dispersed in time reproductive opportunities (hosts) compared with the tropical ones (short-lived) (Diamantidis, Papadopoulos, Nakas, Wu, Muller, & Carey, 2009). This suggests that temperate populations may have evolved more opportunistic strategies that allow them to exploit ephemeral sources. Under this context, high larval densities inside fruits may occur under host scarcity periods. Therefore, differences in the larval survival rates under high crowding conditions are likely to reflect different larval competition levels of the two biotypes ancestor populations in the wild.

Contrary to the expected outcome, laboratory adaptation for 25 generations of the short-lived biotype did not result in high survival rates under increased larval crowding conditions. Although its rearing conditions can be regarded as relaxed, 50-100 larvae per 10 ml of artificial diet, this density could have promoted resistance against crowding since it is far higher than the typical densities found in nature (Debouzie, 1989). In this case for example, larvae could have developed mechanisms to compensate the deleterious effects of their feeding activity waste products as explained below. On the other hand, adaptation to the

artificial diet *per se* could have shaped important larval biological traits such as developmental time and survival rates (Leftwich, Nash, Friend, & Chapman, 2017).

In order to complete their development, larvae of holometabolous insects must reach a physiologically designated set point known as “critical size/weight” at which metamorphosis occurs. Although larval feeding continues for some time after the critical size is attained, the interval between reaching critical size and pupation is not affected by nutrition as larvae will attempt to pupate even in the absence of food (De Moed, Kruitwagen, De Jong, & Scharloo, 1999; Edgar, 2006). Therefore, the plastic response of pupal/adult size to larval nutrition reflects the growth occurring after the critical size is reached, whereas variation in larval developmental duration mainly reflects the time needed to reach critical size (Vijendravarma, Narasimha, & Kawecki, 2012). In our study, medfly biotype is a significant predictor of pupal size with the long-lived one producing larger pupae in all crowding regimes except the last one relative to the short-lived. This finding suggests that the critical size in the long-lived biotype is reached faster than in the short-lived providing the opportunity for the formation of larger pupae. Moreover, the fact that larval survival (i.e pupation) in the long-lived biotype is not affected by crowding regimes (contrary to the short-lived) indicates a smaller critical size for metamorphosis initiation relative to the short-lived. Interestingly, under the highest crowding regime, only the larvae of the long-lived biotype were capable of prolonging their developmental duration to reach critical size. The prolongation of larval developmental periods is the major mechanism that permit temperate medfly populations to survive during winter periods inside host-fruits (Papadopoulos, Carey, Katsoyannos, & Kouloussis, 1996). On the other hand, tropical populations develop under favorable conditions all year round which might have prevent larvae to evolve such developmental plasticity. Consequently, the observed differences regarding the prolongation of larval developmental duration between the long-lived and the short-lived biotypes in our study is probably the outcome of divergent selection pressures.

Our findings show that the increase in the density of the larvae during their development results in a reduction of pupal length and weight of both *C. capitata* biotypes tested. The completion of medfly larvae development requires the accumulation of a minimum amount of nutrients that should takes place within species-specific time limits (Nestel, Nemny-Lavy, & Chang, 2004). The growth of larval species of the Tephritidae family in high density conditions is accompanied, in several cases, by qualitative degradation of the artificial diet used for their rearing (Debouzie, 1989). Medfly larvae are particularly sensitive to changes in the nutritional quality of their food, and they can choose the "best" nutritional area within the substrate they grow (Zucoloto, 1987). Therefore, larvae that grow at elevated densities probably receive smaller amounts of nutrients per unit of time than those grow at low density as they spent more time searching for those parts of the developmental substrate with high nutrient content and quality. This results in larvae that develop under crowded conditions to need more time in order to accumulate the minimum amount of nutrients necessary for pupation. In addition, larvae that grow at elevated density increase in size and weight at a reduced rate as compared to those that grow at low density as they consume smaller amounts of nutrients per unit of time. Thus, when the timeframe within which larval development is to be completed tends to be exhausted, those that grow under high crowding

conditions may have received the minimum amount of nutrients needed for pupation, yet they are smaller in size and weight than those that develop under low density conditions.

One parameter which is likely to play an important role in reducing larval survival as well as in prolonging their developmental time under conditions of increased crowding is the presence of urea and uric acid in the larval nutrient substrate (Botella, Moya, Gonzalez, & Mensua, 1985). The effect of these substances, byproducts of nitrogen metabolism, to larval survival and growth rates under increased density conditions, has been studied in *D. melanogaster* (Botella, Moya, Gonzalez, & Mensua, 1985). *Drosophila melanogaster* larvae, in conditions of high density during their growth, inevitably ingest large amounts of urea and uric acid. Indeed, in cases of particularly high larval density, the consumption of these substances exceeds their rate of excretion through metabolism. This can lead either to a reduction of larvae survival due to toxic effects of urea and uric acid or to the expense of significant amounts of energy by larvae for the chemical degradation of the excess of these substances. The increased rate of chemical degradation of these substances is probably responsible for the increment of larval developmental duration under high crowding conditions (Botella, Moya, Gonzalez, & Mensua, 1985). In addition, populations of *D. melanogaster* that were reared for several generations in the laboratory under high larval density conditions seem to tolerate high levels of urea better than others that were maintained under low larval densities (Mueller, 1991). Therefore, the increased survival and larval developmental duration observed in the long-lived biotype of our study under the high crowding conditions may be due to a more efficient management of nitrogen metabolic products relative to the larvae of the short-lived biotype.

Adults originated from larvae grown under high density conditions exhibited lower average lifespan and reduced female fecundity rates compared to those derived from larvae grown under low density conditions in both biotypes used. This is most likely associated with the smaller size and weight of pupae observed under high larval crowding conditions. In a demographic study of *C. capitata*, it was found that female fecundity increased with size (Krainacker, Carey, & Vargas, 1989). In the same study, pupal size had no effect on adult survival. However, it should be noted that Krainacker and colleagues recorded adults' survival up to the mass rearing discard age (day 14 at which the lifetime production of pupae per female is maximum). Adults originated from larger pupae exhibited a higher average expected adult lifespan and female fecundity rates compared to others from smaller pupae in three species of the genus *Anastrepha* of the Tephritidae family, namely *A. obliqua*, *A. ludens* and *A. serpentine* (Liedo, Carey, Celedonio, & Guillen, 1992). The fact that larger adults in our study showed greater lifespan and higher female reproduction rates is probably due to their ability to store larger quantities of reserve substances than adults of smaller size. Such substances are likely to play an important role both in adult survival and female fecundity, presumably by maintaining body tissues in a good condition. The greater reduction in adult lifespan and female fecundity of the long-lived biotype compared to that observed for adults of the short-lived biotype under conditions of increased larval density (Tables 2 and 4), is probably due to a different biotype-specific interaction between their genetic background and adult size.

Overall the results of the current study have broad implications for understanding the response of different medfly biotypes to stressful condition that may reflect thriving and persistence in hostile conditions. The wide geographic distribution and extremely polyphagy of this species suggest a

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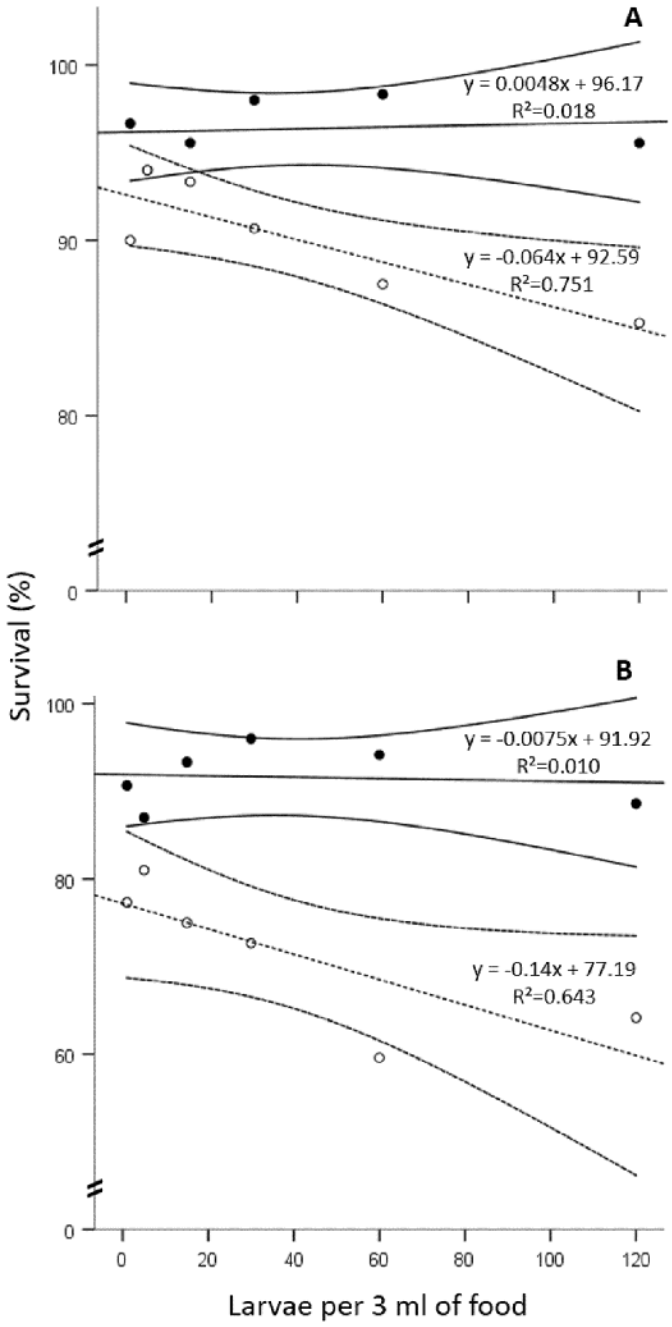

Figure 1.

Linear relationship between larval density and the survival of larvae (A) and pupae (B) for the long-lived (black dots) and the short-lived (white dots) medfly biotype.

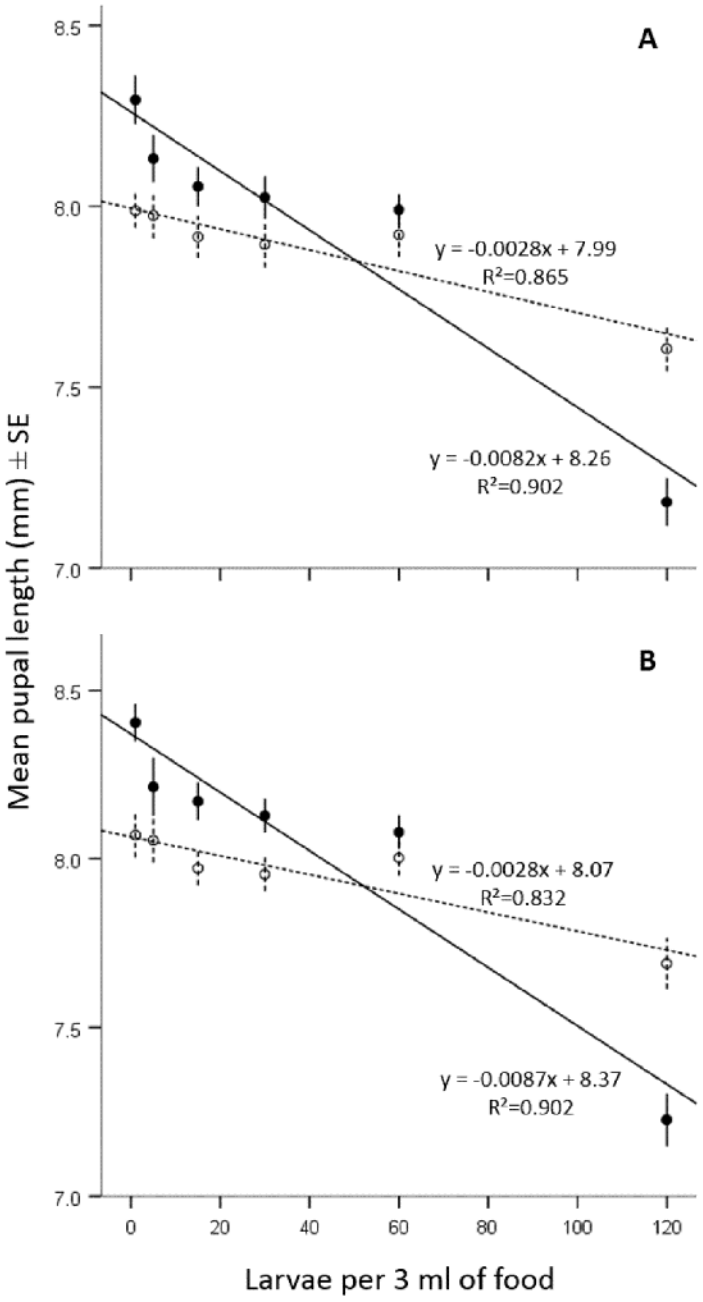

Figure 2.

Linear relationship between pupal length and larval density for male (A) and female (B) pupae for the long-lived (black dots) and the short-lived (white dots) medfly biotype.

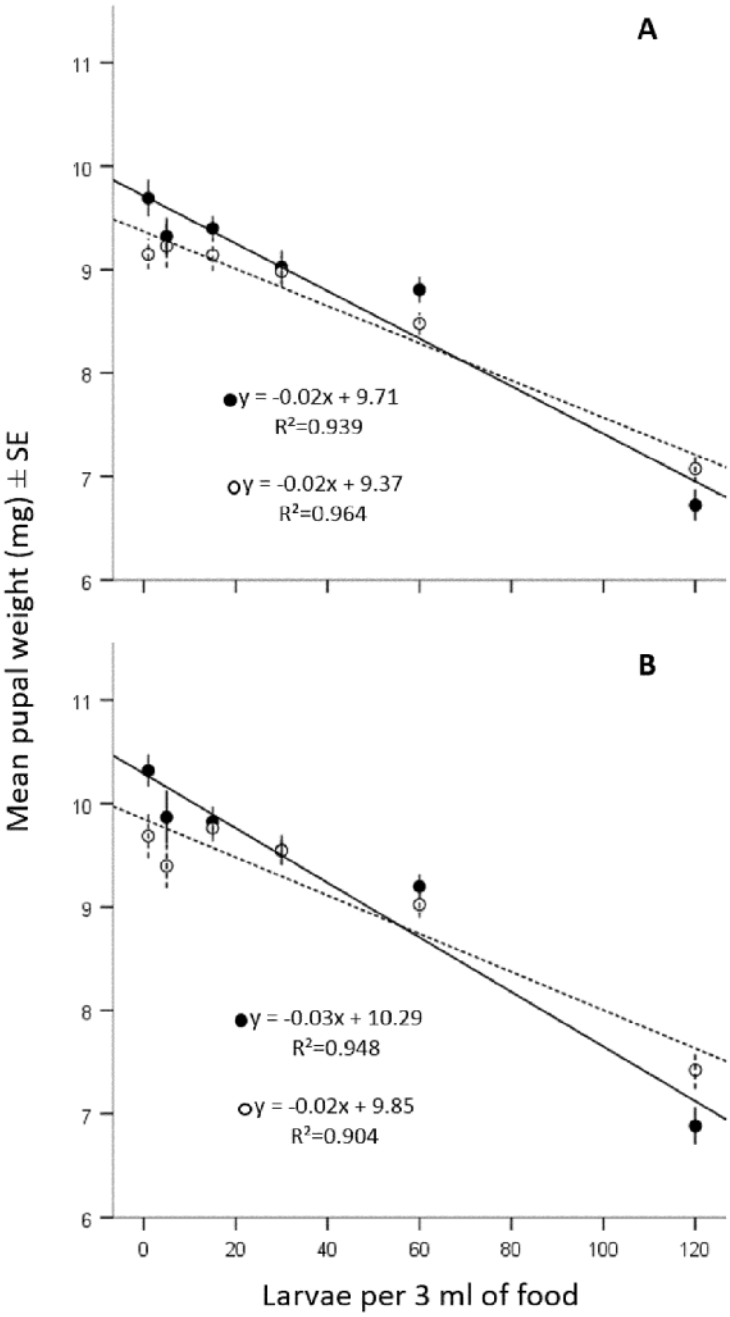

Figure 3.

Linear relationship between pupal weight and larval density for male (A) and female (B) pupae for the long-lived (black dots) and the short-lived (white dots) medfly biotype.

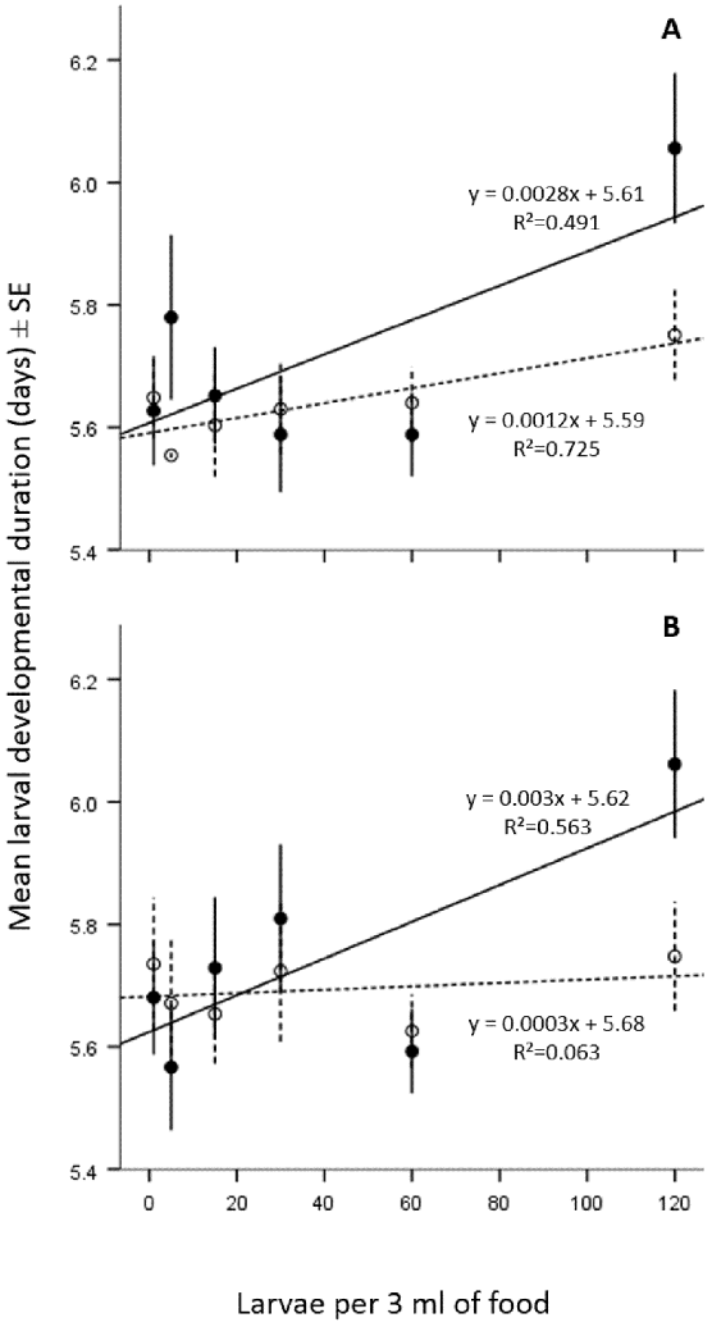

Figure 4.

Linear relationship between larval developmental duration for male (A) and female (B) larvae for the long-lived (black dots) and the short-lived (white dots) medfly biotype.

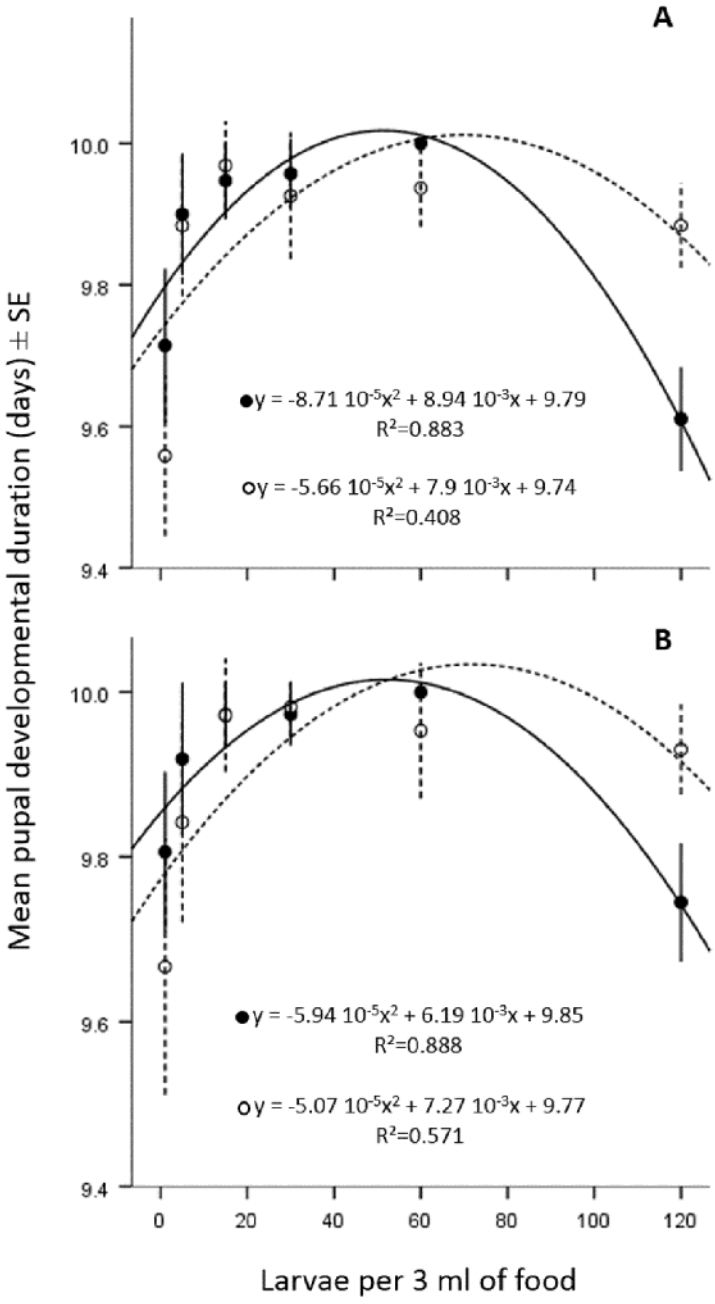

Figure 5.
Quadratic relationship between pupae developmental duration for male (A) and female (B) pupae for the long-lived (black dots) and the short-lived (white dots) medfly biotype.

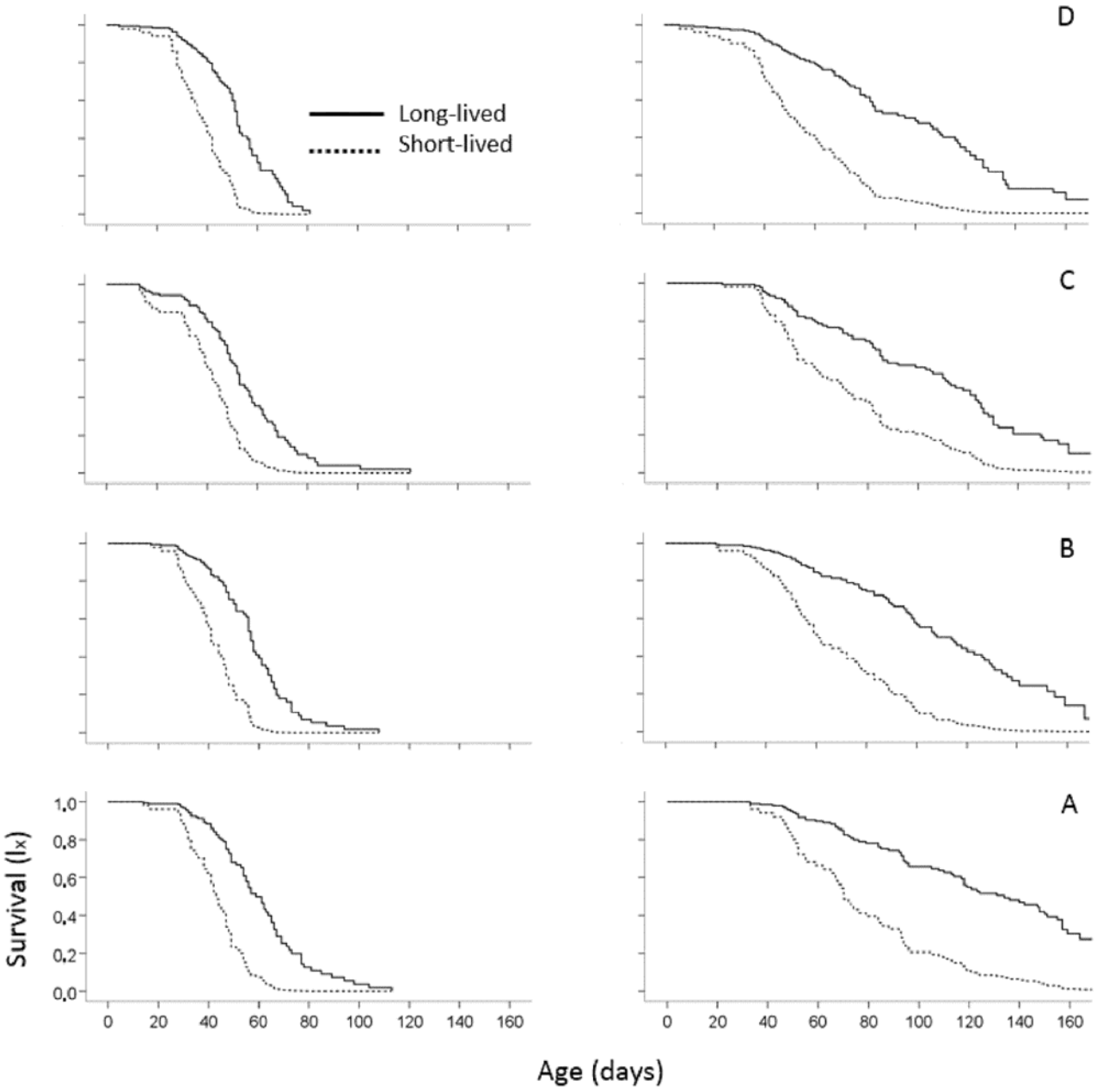

Figure 6.

Female (left column) and male (right column) age-specific survival curves of the two medfly biotypes obtained from larvae reared in four crowding densities [(A) 1, (B) 15, (C) 60 and (D) 120 larvae / 3 ml of food].

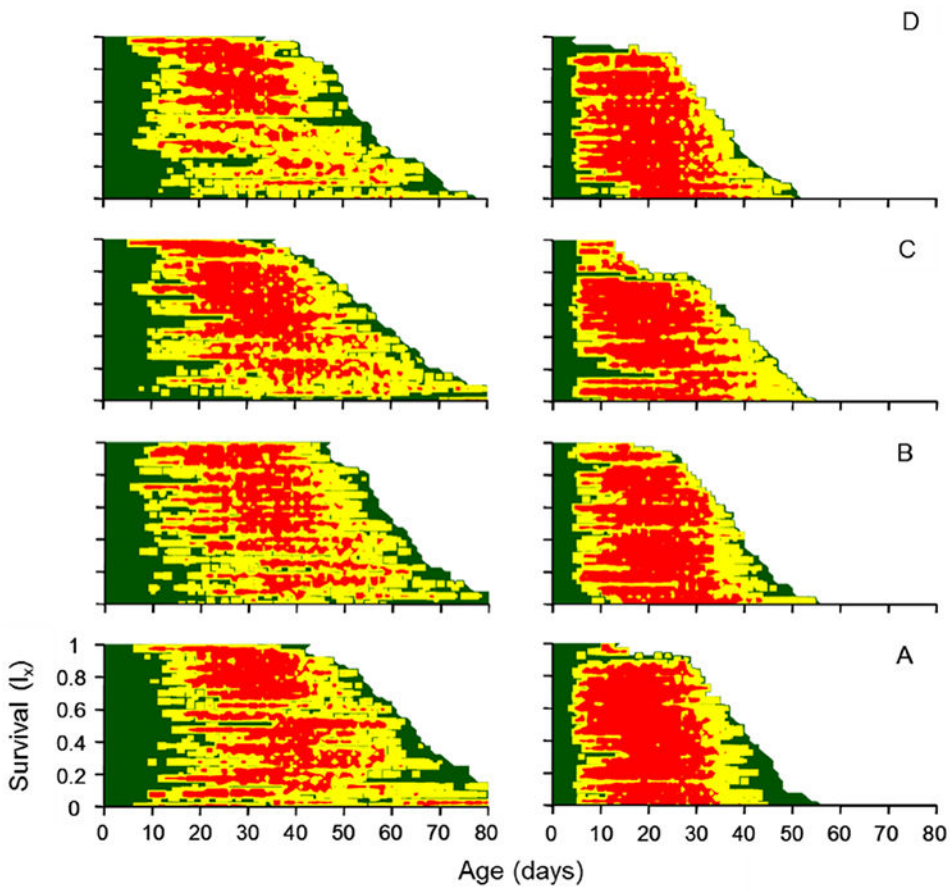

Figure 7.

Event history diagram of females derived from four different larval densities [(A) 1, (B) 15, (C) 60 and (D) 120 larvae / 3 ml of food], for the long-lived (left column) and the short-lived (right column) medfly biotype. Each horizontal line represents the longevity of a single female and different colours designate the level of reproduction for each age. Green: 0 eggs, yellow: 1-20 eggs, red:> 20 eggs. Forty females were used from each larval density for both medfly biotypes.

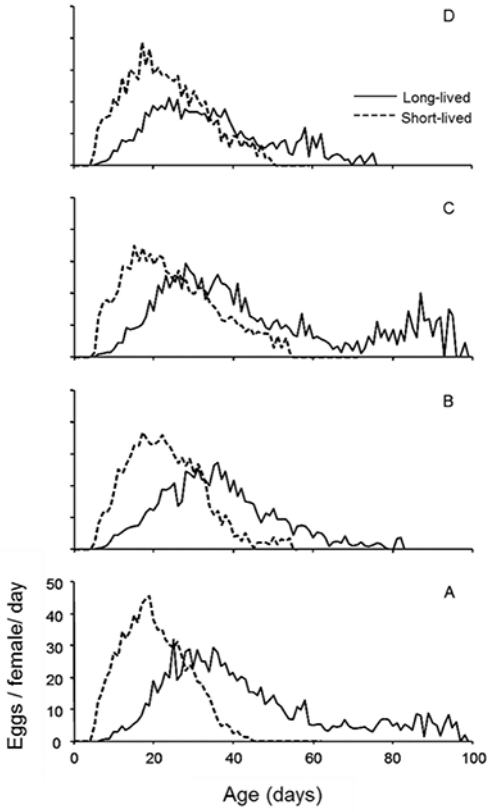

Figure 8.

Female age-specific reproduction schedule for the long-lived and the short-lived medfly biotype derived from four different larval densities [(A) 1, (B) 15, (C) 60 and D120 larvae / 3 ml of food]. Forty females were used from each larval density for both medfly biotypes.

Table 1.

Larval density treatments applied to both medfly biotypes.

	Larvae per 3 ml of artificial diet (treatments)					
	1	**5**	**15**	**30**	**60**	**120**
Number of replicates	150	20	12	5	4	3
Number of larvae	150	100	180	150	240	360

Table 2.

Average and maximum lifespan of females and males of the long-lived and the short-lived medfly biotype in relation to larval crowding during development.

Larvae per 3 ml of food	Average lifespan (days ± SE)				Maximum lifespan (days)			
	Long-lived		Short-lived		Long-lived		Short-lived	
	Females	Males	Females	Males	Females	Males	Females	Males
1	67.8 ± 2.5 a	119.0 ± 5.3 a	39.8 ± 1.6 a	82.1 ± 6.8 a	113	195	63	170
15	64.2 ± 2.0 a	102.1 ± 5.2 b	38.7 ± 1.5 a	70.6 ± 5.3 ab	108	172	57	159
60	61.3 ± 2.7 ab	105.0 ± 4.2 b	37.5 ± 2.2 a	71.3 ± 6.4 ab	121	156	72	171
120	57.0 ± 1.7 b	95.0 ± 5.6 b	35.3 ± 1.7 a	57.3 ± 4.9 b	81	174	60	160

Means in the same column followed by the same letter do not differ significantly (log-rank test)

Table 3.

Variables of the Cox proportional hazards model on the effect of medfly biotype, sex and larval crowding on the adult lifespan.

Source of variation	β	SE	Exp(β)	*P*
Medfly biotype	−0.768	0.143	0.464	< 0.001
Sex	2.356	0,166	10.544	< 0.001
Larval density	0.005	0.002	1.005	0.001
Medfly biotype x Sex	−0.888	0.168	0.412	< 0.001
Medfly biotype x Larval density	−0.001	0.002	0.999	0.746
Sex x Larval density	−0.002	0.002	0.998	0.383

Table 4.

Female reproductive parameters of the long-lived and the short-lived medfly biotype obtained from larvae reared in four crowding regimes.

Larvae per 3 ml of food	Fecundity (eggs per female)			
	Maximum ($\sum_{x=\alpha}^{\beta} Mx$)		Average (± SE)	
	Long-lived	Short-lived	Long-lived	Short-lived
1	990.6	877.6	821.2 ± 43.0 a	810.3 ± 55.0 a
15	765.1	856.3	710.8 ± 42.8 a	745.3 ± 45.8 a
60	1041.6	892.6	760.0 ± 39.6 a	677.5 ± 56.9 a
120	644.2	822.4	558.5 ± 33.0 b	654.5 ± 45.3 a

Means in the same column followed by the same letter do not differ significantly (Tukey's HSD test). Mx = total numbers of eggs laid by the average female at age x (Carey, 1993).

Table 5.

Female reproductive periods for the long-lived and the short-lived medfly biotype obtained from larvae reared in four crowding regimes.

Larvae per 3 ml of food	Average duration (days ± SE)					
	Pre-oviposition		Oviposition		Post-oviposition	
	Long-lived	Short-lived	Long-lived	Short-lived	Long-lived	Short-lived
1	17.1 ± 1.1 a	7.6 ± 0.7 a	42.6 ± 2.1 a	27.7 ± 1.3 a	8.1 ± 1.3 a	4.5 ± 0.7 a
15	14.3 ± 0.9 ab	8.4 ± 0.7 a	43.2 ± 1.4 a	27.3 ± 1.3 a	6.7 ± 1.2 a	3.0 ± 0.5 ab
60	15.9 ± 1.2 a	8.6 ± 0.8 a	39.6 ± 1.9 a	27.0 ± 2.0 a	5.8 ± 1.2 a	2.0 ± 0.4 b
120	12.5 ± 0.6 b	8.4 ± 0.6 a	39.2 ± 1.4 a	26.6 ± 1.4 a	5.3 ± 0.7 a	1.8 ± 0.4 b

Means in the same column followed by the same letter do not differ significantly (log-rank test)