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Original article (Orijinal araştırma)

Impact of maternal age on performance of the progeny in *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae)

Ana yaşının *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) oğul döllerinin performansına etkisi

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Abstract

Maternal age is the age of an insect at the time of depositing an egg and is an important factor impacting on the properties of the progeny. This study determined the influence of maternal age on the preadult total development time, larval development time, pupal development time, pupal weight and adult longevity of *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae). This study was conducted in 2018 in the Biology Laboratory of Science Teaching, Sinop University. The insects were grouped into four groups (1, 5, 10 and 15 d-old) and they were kept under laboratory conditions (28 ± 2°C and 65 ± 5% RH and continuous darkness). Larval development time, total preadult development time and longevity decreased with maternal age whereas pupal development time increased. Although the development time of the progeny of younger females lasted longer, total longevity increased. Although, the progeny of younger females had increased longevity, progeny of older females was advantaged in terms of development time.

Keywords: *Galleria mellonella*, insect development, longevity, maternal age

Öz

Ana yaşı, bir böceğin yumurtayı bıraktığı andaki yaşıdır ve oğul dölün özelliklerini etkileyen önemli bir faktördür. Bu çalışma ana yaşının *Galleria mellonella*'nın (L., 1758) (Lepidoptera: Pyralidae) ergin öncesi gelişim süresi, larva gelişim süresi, pupa gelişim süresi, pupa ağırlık ve ergin ömür uzunluğu üzerindeki etkisini belirledi. Bu çalışma 2018 yılında Sinop Üniversitesi, Fen Bilgisi Eğitimi, Biyoloji Laboratuvarı'nda yapıldı. Böcekler dört gruba ayrıldı (1, 5, 10, 15 gün yaşlı ve 28 ± 2 °C, %65 ± 5 bağıl nem ve devamlı karanlık) ve laboratuvar şartları altında tutuldu. Larva gelişim süresi, toplam ergin öncesi gelişim süresi ve ömür uzunluğu yaşla azalırken pupa gelişim süresi arttı. Genç anaların oğul döllerinin gelişim süresi daha uzun sürmesine rağmen, ergin ömür uzunluğu arttı. Genç analardan elde edilen oğul döller ömür uzunluğu bakımından avantajlıyken, yaşlı analardan elde edilen oğul döller gelişim süresi bakımından avantajlı olarak saptanmıştır.

Anahtar sözcükler: *Galleria mellonella*, böcek gelişimi, ömür uzunluğu, ana yaşı

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Introduction

Maternal age is the age of an insect at the time of depositing an egg (Karsavuran & Anaç, 2014). It is known that maternal age has an impact on parameters such as oviposition, egg mass, number of emerged insects, fecundity, survival ratio of the adult insects and longevity (Fiore, 1960; Ambrose et al., 1988; Rossiter, 1991; Gavrilov et al., 1997; Mohaghegh et al., 1998a, 1998b; Fox et al., 2003; Mishra & Omkar, 2004; Tucic et al., 2004). Legaspi & O'Neil (1994) found that the maternal age delayed the nymphal development in *Podisus maculiventris* (Say, 1832) (Hemiptera: Pentatomidae). In some studies, it was found that progeny of young females had higher fecundity than the progeny of older females (Mishra & Omkar, 2004; Zehnder & Hunter, 2007; Montoya & Farfan, 2009).

The greater wax moth, *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae*)* can easily be bred under laboratory conditions. For this reason, it is frequently used as a model insect in different fields of biology, such as physiology, molecular biology, microbiology and biochemistry. It is also an important insect used under laboratory conditions as a natural host in rearing parasitoid insects in biocontrol studies, in studies on substances harmful for the environment, e.g., insecticides, and in determining the pathogenicity of microorganisms (Hood et al., 2003; Büyükgüzel & Kalender, 2009; Ciesielczuk et al., 2015; Hosamani et al., 2017; Kecko et al., 2017).

The impact of maternal age on longevity, development time and morphological and physiological traits of progeny is a factor to be taken into consideration in maintaining laboratory cultures of insects, and in studies on such cultures. Mohaghegh et al. (1998a) found that the eggs deposited by old females (young individuals 2-4 weeks-old and old individuals 7 weeks-old) were smaller in *P. maculiventris*, the individuals were lighter and the development was delayed in the generations arising from young females. Development time of insects obtained from the older females was 29.0 ± 0.33 d for females and 28.7 ± 0.26 d for males, whereas these values were 26.7 ± 0.17 and, 26.7 ± 0.21 d, respectively for younger individuals (Mohaghegh et al., 1998a). In the study conducted by Şimşek et al. (2015) on *Rhyzobius lophanthae* (Blaisdell, 1892) (Coleoptera: Coccinellidae), it was found that total development time and survival successes decreased as the maternal age increased and the oviposition time increased as the age increased. In some studies, the progeny of younger females is more fecund as than the progeny of older females (Mishra & Omkar, 2004; Zehnder & Hunter, 2007; Montoya & Farfan, 2009). Therefore, reproduction of young individuals has been suggested as important for continuity in the cultures. Also, the quality of the insects used as hosts is very important in biological control studies.

Accordingly, in this study, the impact of maternal age on the longevity and development time of *G. mellonella* were assessed, comparing the performance of the progeny of 1, 5, 10 and 15 d-old *G. mellonella* adults.

Materials and Methods

Setting up the cultures

The study used cultures of successive laboratory stocks of *G. mellonella.* Initial cultures were formed from adults obtained from hives infested with *G. mellonella* obtained from beekeepers in Sinop (Sinop Beekeepers Association, Sinop, Turkey). The cultures were started by placing the adults in 500-ml glass jars (500 × 140 × 84 mm, DTO070, ISOLAB, Istanbul, Turkey) covered with a cloth which did not prevent air flow. Honeycomb without honey was used to feed the insects. The honeycomb was kept in a deep freezer to eliminate parasites and then were sterilized. They were given to insects every day as needed. In order to assist pupation, folded pieces of paper (100 × 100 mm) were put into the jars. Adults reared under laboratory conditions for at least three generations were used in the experiments.

Forming the age groups

In order to find out the influence of maternal age on performance of progeny, four groups were formed based on adult age (time since eclosion): 1, 5, 10 and 15 d-old. The adults taken out of stock culture were placed in a separate experimental jar to form the 1 d-old group. For the other age groups, adults were kept for 5, 10 and 15 d in separate jars, and they were put into their own experimental jar, when they reached the required age. The insects in each experiment were kept in separate jars so that they were not allowed to mate until they reached the required age. A total of 20 insects were put in each experimental jar. At this stage, they were allowed to mate and lay eggs. The insects were kept in these jars for 5 d. They were removed from the jars after 5 d. The individuals which came out of these jars were taken in experiments. For the parameters assessed, measurements were made on 15 individuals, i.e., 45 in total. Honeycomb was used as food in all cases.

All experimental jars were monitored daily. For larval development time, the date each larva pupated was recorded. In each experiment, 15 pupae were taken randomly selected for subsequent assessment. Total larval development time was calculated. The 15 pupae were placed in different Petri dishes (90 × 15 mm, ISOLAB). The date the insects emerged as adult was recorded and the pupal development time calculated. They were weighed the first day they reached pupal stage. The time adult insects emerged was used to determine longevity, with each placed in separate jars (250 ml) without allowing them to mate, but allowing them to continue to feed. They were checked every day. Results were recorded for three repeat experiments for each age group with 45 insects in total.

The experiments were conducted under laboratory conditions of 28 ± 2°C, 65 ± 5% RH and continuous darkness. This study investigated the influence of maternal age on *G. mellonella* larval development time, pupal development time, total preadult development time, pupal mass and adult longevity.

Data analysis

SPSS 21.0 software (IBM, Armonk, NY, USA) program was used for the statistical assessment of the data. Averages were obtained for the 15 insects in each group of the three experiments (45 insects in total) and then statistical analyses (n = 3 in each age group). Firstly, a normality test was conducted for the groups. According to Kolmogorov-Smirnova and Shapiro-Wilk tests, it was found that the data were not normally distributed. Since the data were not normally distributed and in order to understand whether there were differences between groups, we performed Kruskal-Wallis test for each age group. Differences were found between groups according to Kruskal-Wallis test ($p < 0.05$). Mann-Whitney U test was used to determine if age group differences existed ($p < 0.001$).

Results

Figure 1 shows the influence of maternal age on *G. mellonella*'s total preadult development time ($H = 109$, $p < 0.001$, $df = 3$), larval development time ($H = 157$, $p < 0.001$, $df = 3$) and pupal development time ($H = 135$, $p < 0.001$, $df = 3$; Kruskal-Wallis test $p < 0.001$). Total development time and larval development time decreased with increase in maternal age whereas pupal development time increased (Man-Whitney U, $p < 0.001$).

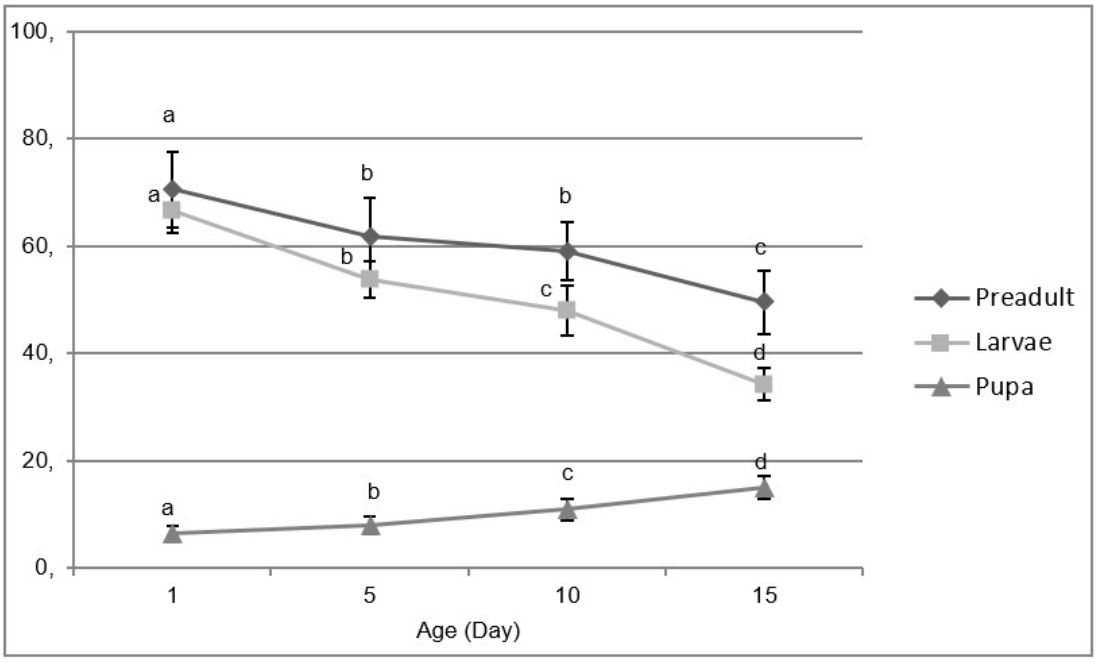

Figure 1. Effect of maternal age on total preadult, larval and pupal development time of the progeny of *Galleria mellonella*. The values with the same letters the same line is not significantly different (Man-Whitney U, $p < 0.001$); n = 3 for each experiment with each an average of 15 individuals.

Figure 2 and Figure 3 show the influence of maternal age on *G. mellonella*'s longevity and pupal mass. Longevity ($H = 40.9$, $p < 0.001$, $df = 3$; Kruskal-Wallis test $p < 0.001$) decreased with the increase in age (Man-Whitney U, $p < 0.001$). While there were significant differences in pupal mass among the age groups, the difference was not consistent ($H = 12.1$, $p < 0.001$, $df = 3$; Kruskal-Wallis test $p < 0.05$; Man-Whitney U, $p < 0.001$, Figure 3).

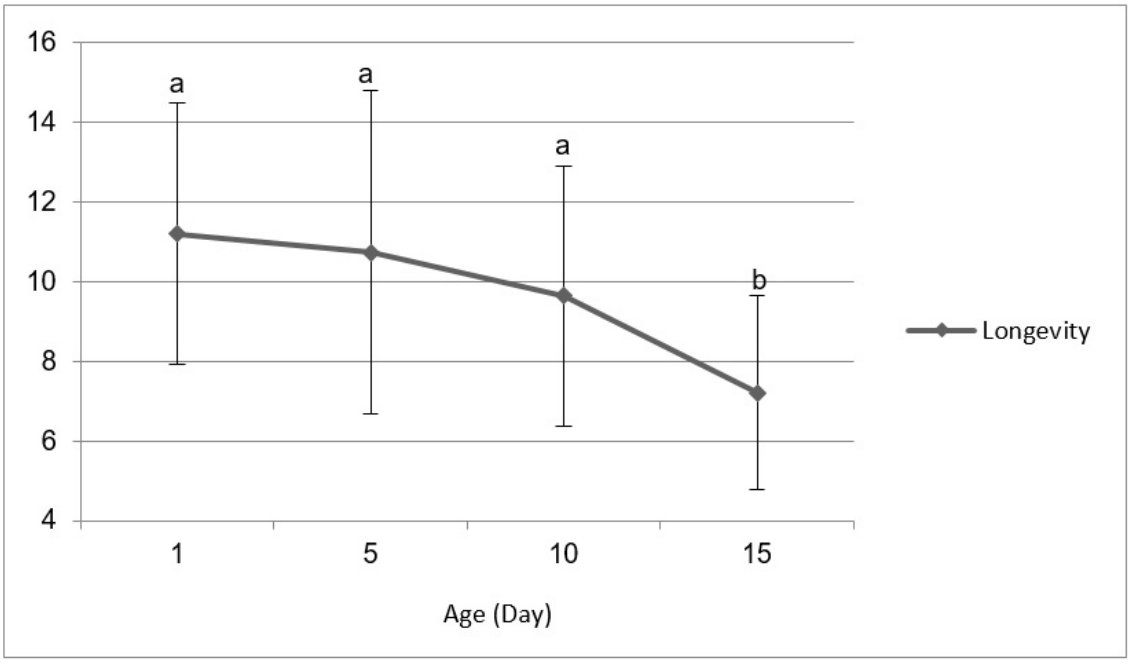

Figure 2. Effect of maternal age on longevity of the progeny in *Galleria mellonella*. The values with the same letters are not significantly different (Man-Whitney U, $p < 0.001$); n = 3 for each experiment with each an average of 15 individuals.

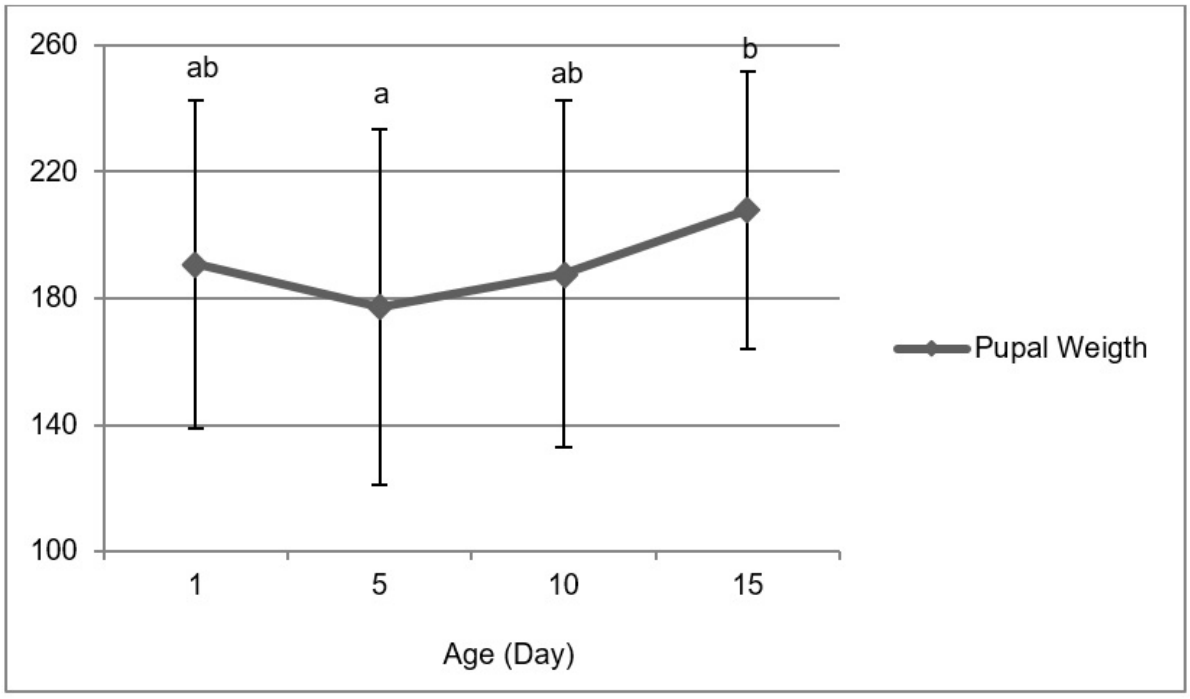

Figure 3. Effect of maternal age on pupal mass of the progeny in *Galleria mellonella*. The values with the same letters are not significantly different (Man-Whitney U, $p<0.001$); n = 3 for each trial with each an average of 15 individuals.

Discussion

In this study, it was found that larval development time in *G. mellonella* decreased with the increase in maternal age whereas pupal development time increased (Figure 1). The total preadult development time decreased. Adult longevity was found to be shorter in the progeny of older females than younger females (Figure 2). This could result from weaker nutrient composition older egg-laying females passed to its eggs for the next generation. In a study on *Eupelmus vuilleti* (Crawford, 1913) (Hymenoptera: Eupelmidae), Muller et al. (2017) found that eggs and progeny of older females had less glycogen and protein. Consistent with the results of that study, there are some studies which have found that older maternal age reduces total development time, larval development time and longevity (Fiore, 1960; Şimşek et al., 2015). In a study on *Aphis nerii* (Fonscolombe, 1879) (Hemiptera: Aphididae), Parris et al. (2007) observed that the progeny of older females matured more quickly, consistent with the Lansing effect and had shorter longevity. Lansing (1947) stated that longevity could be influenced through a factor that could be transmitted through eggs from the parent female.

Priest et al. (2002), Lind et al. (2015) and Bock et al. (2019) attributed the effects of maternal age on development and longevity to some mutations that occur with aging and stated that genetic factors could influence the following generations. However, they suggested that the maternal effect on longevity mechanism was not fully clarified and that more detailed studies were required. The decreased longevity found in the current study of the progeny of older females could be due to both egg quality and the genetic factors suggested by those authors.

While Ludwig & Fiore (1960), Lints (1978), Phelan & Frumhoff (1991) and Şimşek et al. (2015) found that the development time of progeny of older females was shorter. Muller et al. (2017), Wasserman & Asami (1985), Legaspi & O'Neil (1994) and Mohaghegh et al. (1998a) suggested the opposite. Harvey (1977) could not find any changes on development rates in terms of age. This shows that the development time of species from older females can differ between species.

Correspondingly, probably at the pupal stage, pupae originating from younger females became adults earlier. This could be due to the fact that they complete the required changes and organ formations quicker. The progeny of the oldest egg-laying females of the present study had the shortest lifespan, which implies that individuals from younger females are healthier and can thus have a shortened pupal stage. In the present study, pupal mass was affected less and inconsistently by maternal age (Figure 3). Eggs of old females have reduced hatching, higher death rates and shorter development time than young females (Fox, 1993; Mishra & Omkar, 2004; Karsavuran & Anaç, 2014). According to the "consumption of reproduction sources hypothesis" in the female, these maternal influences can influence egg size, egg survival rate, development time, hatching, pupal development time and adult longevity (Yanagi & Miyatake, 2002). Although development time is often shorter in insects from old females (Ludwig & Fiore, 1960; Phelan & Frumhoff, 1991; Zehnder & Hunter, 2007), in this study pupal time was significantly shorter in insects from young females. As far as we could observe, population density in insects from young females was higher.

In the present study, shorter longevity and longer pupal time of the progeny of older females can be explained by lower quality of their eggs (Mousseau & Dingle, 1991; Phelan & Frumhoff, 1991). This can occur as a result of older egg-laying females rapidly depleting the required resources for egg development. Decreased provisioning of eggs influences nutrient composition of the hatch larvae (Fox & Dingle, 1994). From a study on *Oncopeltus fasciatus* (Dallas, 1852) (Hemiptera: Lygaeidae), Phelan & Frumhoff (1991) suggested that females pass a special biological signal to eggs for a faster embryonic and nymphal development. This in turn can influence the life cycle and performance of hatched insects. In our results, although the development time of the progeny of younger females lasted longer, longevity increased. While the progeny of younger females is advantaged in terms of longevity, progeny of older females are advantaged in terms of development time.

According to these results, more detailed study is needed to determine which age group will be more advantaged the most. A focus on determination of fecundity and larval death rates in future studies would be important.

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