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SELİM SÜALP ÇAĞLAR

İSMAİL KUDRET SAĞLAM

MUHAMMET MUSTAFA AKINER

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Research Article

The influence of photoperiod on size and development of ovarioles in insecticide resistant and susceptible strains of the house fly *Musca domestica* L. (Diptera: Muscidae)

Selim Süalp ÇAĞLAR[1,*], İsmail Kudret SAĞLAM[1], Muhammet Mustafa AKINER[2]

[1]Hacettepe University, Faculty of Science, Department of Biology,
Ecological Sciences Research Laboratories (ESRL), 06800, Beytepe, Ankara - TURKEY

[2]Rize University, Faculty of Arts and Science, Biology Department, Zoology Branch,
Milli Piyango Campus, Rize - TURKEY

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Abstract: The ovariole development of laboratory-reared insecticide resistant and sensitive strains of the house fly, *Musca domestica* L. (Diptera: Muscidae) under different photoperiod regimes was examined. Flies were reared under laboratory conditions of 75 ± 5% RH, 25 ± 1 °C and 0:24, 6:18, 12:12, and 18:6 h L:D photoperiods. Each day, 20 female flies were collected from a cohort of females that had emerged on the same date. Ovarioles were dissected from the collected adults and microscopically examined to determine overall length and developmental stage. For the resistant strain, development was most rapid under the photoperiod regimes with more hours of darkness and steadily decreased with increasing hours of light. However, development of the susceptible strain was most rapid at both photoperiod extremes of 0:24 and 18:6 h L:D. Results also showed that the effect of both photoperiod and resistance status and the interaction between these factors had a significant effect on ovariole development.

Key words: House fly, *Musca domestica*, ovariole development, photoperiod, insecticide resistance

Fotoperiyodun dirençli ve duyarlı karasinek *Musca domestica* L. (Diptera: Muscidae) soylarının ovaryol gelişimi üzerindeki etkisi

Özet: Bu çalışmada laboratuarda yetiştirilmiş olan karasinek *Musca domestica* L. (Diptera: Muscidae)'nın insektisit dirençli ve duyarlı soylarının, farklı fotoperiyot rejimleri altındaki ovaryol gelişimleri incelenmiştir. Sinekler laboratuar ortamında %75 ± 5 RH, 25 ± 1 °C ve 0:24, 6:18, 12:12 ve 18:6 s A:K fotoperiyot rejimleri altında yetiştirilmiştir. Her gün, dişilerden oluşan bir kohort içerisinden, aynı gün pupadan çıkmış olan 20 dişi sinek örneklenmiştir. Örneklenen bireylerin ovaryolleri dissekte edilerek mikroskop altında incelenmiş ve ovaryollerin uzunlukları ve gelişim evreleri belirlenmiştir. Dirençli soylar için en hızlı gelişimin, karanlık periyodu daha uzun olan fotoperiyot rejimleri altında olduğu gözlemlenirken, gelişim hızının aydınlık oranının artması ile düzenli bir şekilde azaldığı belirlenmiştir. Buna karşılık duyarlı soylarda en hızlı gelişimin, 0:24 ve 18:6 saat A:K fotoperiyot rejimleri altında olduğu belirlenmiştir. Sonuçlarımız aynı zamanda direnç durumunun, fotoperiyodun ve bunlar arasında olan etkileşimin ovaryol gelişimini önemli derecede etkilediğini göstermiştir.

Anahtar sözcükler: Karasinek, *Musca domestica*, ovaryol, gelişim, fotoperiyot, insektisit direnci

* E-mail: sualp@hacettepe.edu.tr

Introduction

Ovarioles are the functional units of the insect ovary. The ovariole development stage was correlated with mating in the house fly, *Musca domestica* L., by Adams and Hintz (1969). Similarly, Hodin and Riddiford (2000) correlated ovariole number and development with reproductive potential and fitness.

Photoperiod commonly affects development time and adult size of insects (Musolin and Saulich, 1997). Photoperiod is a stable harbinger of seasonal changes, and can induce the insect to prepare physiologically and behaviorally for such changes. The influence of photoperiod as an anticipatory clue in the life history of an insect is dependent on the life stage(s) capable of perceiving photoperiod changes and the extent to which these stages overlap with the critical photoperiodic shifts (Ruberson et al., 2000). One of these physiological changes is the reduction in juvenile hormone (JH) titer in adult females (De Kort, 1990). In conjunction with ecdysone, this hormone mediates ovariole development in Diptera (Kelly et al., 1987).

Since the research by Crow (1957), it has been noted that resistant and susceptible strains differ in fitness characteristics, such as development time, fecundity, and fertility. Because resistant individuals are not common before selection with pesticides, it is generally assumed that resistant genotypes must have pleiotropic effects that result in reproductive and developmental disadvantages for the resistant types in the absence of pesticides (i.e. selection agent). Çağlar (1987, 1991) showed that the maturation time of embryos, larvae and pupae and the overall mean development time of insecticide resistant *M. domestica* was longer than that of insecticide susceptible populations under the same rearing conditions. The reproductive capacity of the resistant strains was also lower. In a later study, Çağlar and Sağlam (2005) showed that there were consistent differences in testis development time between resistant and susceptible strains of *M. domestica*, bred under different photoperiod regimes. For all of the photoperiod conditions examined, resistant strains always showed a higher capacity for growth than the susceptible strains.

Therefore, there is ample evidence that changes in photoperiod could have important effects on the development rate of ovarioles in house flies and that this response could be different between susceptible and resistant individuals, which have important differences in developmental times. Understanding the mechanisms behind differential reproductive capacities of resistant and susceptible strains of *Musca domestica*, and an increased knowledge of the environmental cues that drive this differentiation, could have important consequences for pest and vector management.

In light of this, the objective of the present study was to examine the influences of different photoperiod regimes on selected ovariole development parameters in relation to insecticide resistant and susceptible strains of the house fly under laboratory conditions.

Materials and methods

House flies resistant to organophosphate and pyrethroid insecticides (Çağlar et al., 2000) were collected by sweep net from a municipal refuse site near Ankara, Turkey, in 2000 and were subsequently bred for 44 generations in the laboratory. The WHO insecticide susceptible strain, which was obtained from The Danish Pest Infestation Laboratory in 1996, was used as a reference strain for comparison and was subsequently bred for 83 generations in our laboratory without exposure to insecticides. Prior to experimentation, the resistant status of the resistant stock was checked via bioassays and the stock was found to be highly resistant against all tested insecticides (LC_{50}: 478.8 mg/L, Malathion: 186 mg/L, Fenitrthion: 15.9 mg/L, and Propoxur) with reference to the susceptible WHO stock where LC_{50} values varied between 1.1 and 7.1 mg/L.

The *M. domestica* colonies were reared under laboratory conditions of 75 ± 5% RH and 25 ± 1 °C. The insect colonies were normally maintained at 12:12 h L:D photoperiod. For the present study, separate colonies were maintained at 0:24, 6:18 and, 18:6 h L:D photoperiods for each strain of *M. domestica*. Each photoperiod cabinet contained 2 florescent lights (40 W, 1640 lumen light intensity). The total light regime of 24:0 could not be included in the current study since rearing of colonies under this regime was unsuccessful.

House fly eggs were collected on dental rolls soaked with milk, which were then placed on the larval medium (500 g wheat bran, 120 g powdered milk, and 500 mL water). The larval medium was arranged to contain 1 gram per larva as modified from Çağlar (1991). The adults were fed a sugar cube and milk solution (water + milk powder).

A new cohort was set up, consisting of females that emerged as adults on the same day. A subset of 20 females was collected from this cohort on consecutive days after post-emergence for ovariole dissections. Collected females were dissected in Ringer's Solution (Kennedy, 1949) under a Leica binocular microscope at 10× (Leica Zoom 2000 Model, Germany). The dissected ovaries were fixed in a 1:3 glacial acetic acid and 96% ethanol solution (5 min) followed by a 3:2:1 methanol, chloroform, and propionic acid solution (5 min) (Pienaar, 1955), stained with aceto-carmine (3 min) (Conn et al., 1960), and mounted (Kennedy, 1949).

Total length (distal end of the terminal filament to apical end of the first egg chamber) of excised ovarioles was determined. The developmental stage of each ovariole was determined by microscopic observation using the criteria of Schwartz (1965) as follows: Stage 1, the spherical primary follicle was the larger of the 2 follicles and contained granular cytoplasm with no oocyte differentiation, and nurse cells became clearly defined; Stage 2, oocyte formation had begun and yolk granules were deposited. The oocyte occupied up to half the length of the follicle, and the nurse cells had about doubled in size by the end of this stage; Stage 3, the primary follicle had become more ovate and the oocyte occupied about half to three-fourths of the follicle, and the nurse cells were quite large; Stage 4, in the primary follicle the nurse cells and the follicular epithelium surrounding the oocyte had begun degeneration, the third follicle was completed, the beginning of the fourth follicle appeared as a slight enlargement of the germarium, and both of the second and third follicles were still in stage one; Stage 5, the oocyte occupied the whole primary follicle, the chorion pattern was easily discernible, and the micropyle was apparent. Stage determination of ovarioles was repeated each day until the first oviposition by a female within the treatment was noted.

Ovariole development was measured, as change in ovariole length and statistical tests were conducted using total ovariole length obtained at the end of the maturation phase. All variables were screened for normality (one-sample Kolmogorov-Smirnov) and tested for equal variances (Levene's test). No statistical deviation from normality or inequality of variances was detected in any of the samples. The effects of photoperiod, and insecticide resistance status (resistant/susceptible) on ovariole development were examined by univariate analysis of variance (ANOVA) using a 2-way factorial design. Photoperiod and resistant status were included as fixed factors. A 2-way factorial design enabled us to test the following hypotheses: (1) no effect of photoperiod on ovariole development; (2) no effect of population on ovariole development, and (3) no interaction between photoperiod and resistant status. Post-hoc tests between groups were conducted by using the Tukey HSD test, which is based on equal sample sizes. In addition, to test the effects of resistance status (resistant/susceptible) on total ovariole length in different photoperiod regimes, pairwise ANOVAs were performed separately for strains in each photoperiod regime.

Results

Daily mean length of ovarioles for resistant and susceptible *M. domestica* reared under the 4 photoperiod regimes are summarized in Figure 1. General trends in ovariole development stage and time taken until first oviposition for each treatment are summarized in Table 1. For the resistant strain, development was most rapid under the photoperiod regimes with more hours of darkness, 4 days to first oviposition for 0:24 and 6:18 h L:D treatments. Development time increased to 5 days for first oviposition at 12:12, and 8 days at the 18:6 h L:D photoperiod.

Development of the susceptible strain was most rapid at the photoperiod extremes of 0:24 and 18:6 h L:D, with first oviposition in both treatments at 5 days followed by 6 days at 18:6 and 7 days at 12:12 h L:D photoperiods. Development to individual stages using the criteria of Schwartz (1965) followed the same general trend of oviposition across treatments.

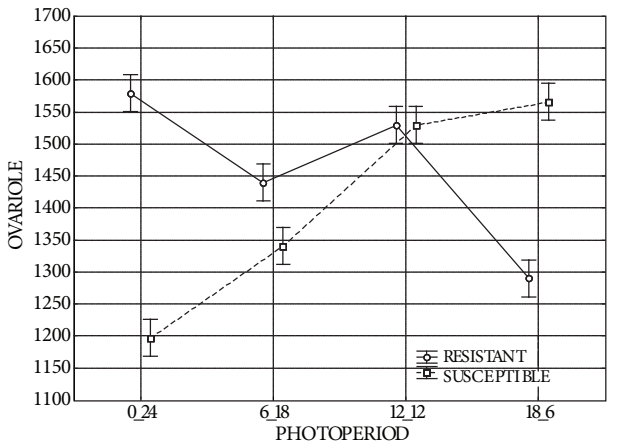

Figure 1. Mean length ± sd of ovarioles dissected from insecticide resistant (A) and susceptible (B) *Musca domestica* females collected daily from separate laboratory colonies under different photoperiod regimes.

Least square means comparing the effects of resistance status and photoperiod on total ovariole length as deduced by a 2-way factorial ANOVA are given in Figure 2 and raw values are given in Table 2. Univariate analysis revealed a highly significant effect of photoperiod and resistance status on total ovariole length ($F_{3, 72} = 42.14$, $P < 0.001$; $F_{1, 72} = 25.22$, $P < 0.001$). In addition, the interaction of the 2 parameters (photoperiod and resistant status) also had a significant effect on ovariole development ($F_{3, 72} = 176.48$, $P < 0.001$), indicating that resistant and susceptible population gave differential responses to photoperiod (Figure 1).

Ovariole length in the resistant strain had significantly higher values than the susceptible strain in the 0:24 and 6:18 photoperiod regimes (Tukey HSD, $P < 0.001$, Figure 2), whereas in the 18:6 photoperiod regime, ovariole length in the susceptible strain was significantly higher (Tukey HSD, $P < 0.001$, Figure 2). In the 12:12 photoperiod regime, ovariole length showed no significant differences between resistance status (Tukey HSD, $P = 1$, Figure 2). Overall, there was a significant increase in ovariole length in the susceptible strain when photoperiod changed from 0:24 to 18:16 and all pairwise differences were significant (Tukey HSD $P < 0.001$, Figure 2). In contrast, ovariole length in the resistant strain generally decreased from 0:24 to 18:6 with all pairwise differences being significant (Tukey HSD $P < 0.001$, Figure 2).

Table 1. Maturation stage (Schwartz, 1965) and date of first oviposition of insecticide resistant and susceptible *Musca domestica* ovarioles dissected from 20 females collected daily from separate laboratory cultures with different photoperiod regimes.

Photoperiod (h L:D)	Fly strain	Female age (days)						
		1	2	3	4	5	**6**	**7**
0:24	Resistant	2	3	3-4	5*	-	-	-
	Susceptible	2	2-3	3-4	4	5*		
6:18	Resistant	1	2-3	4	5*	-	-	-
	Susceptible	1	2-3	3	4	4	5*	
12:12	Resistant	2	3-4	4	4-5	5*	-	-
	Susceptible	2	2-3	3-4	4	4	4-5	5*
18:6	Resistant	1	2	3	3	4	4	5*
	Susceptible	1	2	3	4-5	5*	-	-

*First oviposition

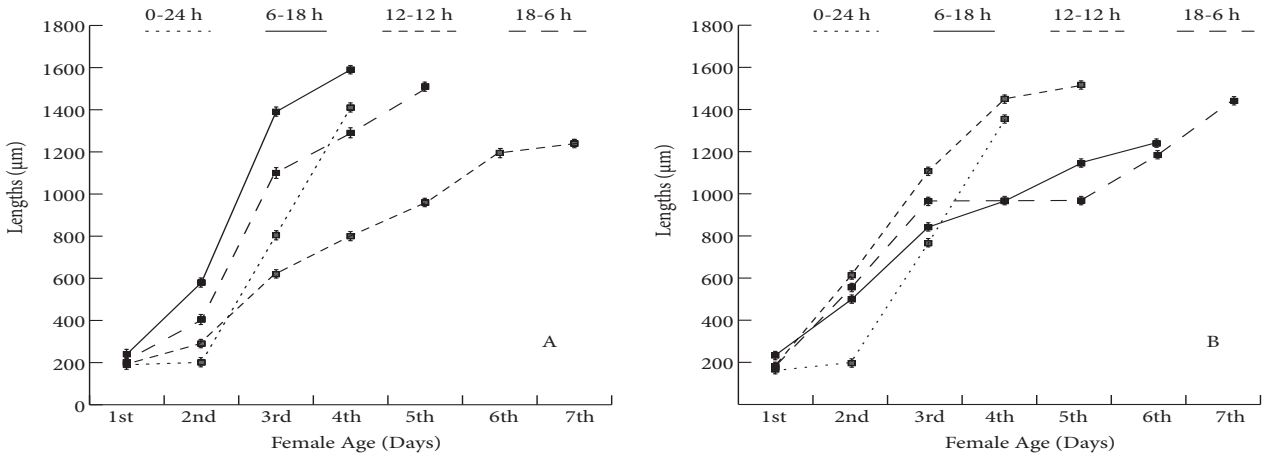

Figure 2. Least square means for resistant and susceptible strains of *Musca domestica* reared under different photoperiod regimes. Vertical bars denote 95% confidence intervals.

Table 2. Mean total ovariole lengths of resistant and susceptible strains at different photoperiod regimes.

Strain	Photoperiod (h L:D)	N	Mean (µ) ± sd
Resistant	0:24	20	1580 ± 39.420
	6:18	20	1440 ± 40.564
	12:12	20	1530 ± 46.027
	18:6	20	1290 ± 48.922
Susceptible	0:24	20	1197 ± 44.538
	6:18	20	1341 ± 47.056
	12:12	20	1530 ± 50.879
	18:6	20	1566 ± 47.487

Discussion

It is well known that selection for resistance has important effects on development times of pre-adult stages of *M. domestica* (Şisli et al., 1984; Çağlar, 1991, 1993). A recent selection experiment, wherein we selected for the development of resistance in *M. domestica* for 5 generations, showed that although selection did not result in any significant difference in reproductive traits or capacity, there was a significant increase in pre-adult development times (Kuyucu, 2007). The present study indicates that in contrast to pre-adult developmental phases, maturation (i.e. developmental times) of ovarioles did not differ between resistant and susceptible strains in the standard 12:12 L:D photoperiod regime and differences in ovariole maturation between resistant and susceptible strains were only evident once photoperiod regimes were off balance in total light and darkness hours.

Photoperiod is often used by insects as a measure of seasonal change and as cues for activities including reproduction or diapause. Diapause is induced mainly by short photoperiods (De Wilde et al., 1959), which also act in its maintenance (Tauber et al., 1986). The finding of a peak of ecdysteroids in pre-diapause adults of both sexes by Briers and De Loof (1981) has led to the suggestion that diapause induction results from a change in hormonal balance between juvenile hormone and ecdysteroids, rather than from a depletion of juvenile hormone alone. Şişli (1964, 1965) showed that absolute darkness and an 18:6 h L:D photoperiod promoted ovariole

maturation in *Aelia rostrata*, which was included in ovariole diapause. Similarly, Tauber and Tauber (1969) showed in *Chrysopa carnea* a sharp decrease in fecundity in a 18:6 h L:D photoperiod. Beetles (*Leptinotarsa decemlineata*) reared under a long-day photoregime (18 h photophase) from egg entered the reproductive phase 5 days after adult emergence. Under short-day conditions (10 h photophase) the adults did not show reproductive activity and entered diapause 10-12 days after adult emergence (De Kort et al., 1982). In the present study we observed similar results with development of ovarioles slowing down with increasing daylight hours. However, the retardation of development was not enough to force females into diapause as all females laid eggs. The most rapid development time and the largest ovariole length were recorded at the short photoperiod (0:24 h L:D) and the slowest development time and shortest ovariole length were recorded at the long photoperiod (18:6 h L:D) for the resistant population. These results came not only from the restriction in growth due to hormonal effects caused by increased daylight hours, but also the effects of resistance on the development of the housefly. When the same analyses were conducted for the susceptible strain, the most rapid development time and the largest ovariole length were recorded at the long photoperiod while, the slowest development time at the medium photoperiod (12:12 h L:D) and smallest ovariole length were recorded at the short photoperiod (Tables 1 and 2). Therefore, it is clear that resistant and susceptible populations respond differently to photoperiod.

The present study indicates that resistant strains have increased growth capacity in photoperiod regimes with longer hours of darkness, as evidenced by faster development times, and larger total ovariole length when compared to the susceptible strain and to photoperiod regimes with longer daylight hours. Therefore, it would seem that changes in hormonal activity brought on by increased exposure to light have more severe effects on resistant strains when compared to susceptible strains and that these effects are masked once daylight hours decrease, enabling resistant populations to attain higher growth capacities. However, is unclear whether this difference is a result of plasticity in hormonal control mechanisms or inherent genetic differences, and future research should heavily concentrate on the inherent physiological mechanism controlling development pathways in resistant and susceptible individuals.

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