CHRISTOPHERS' STAGE DURATIONS AND EFFECT OF INTERRUPTED BLOOD MEAL IN THE MOSQUITO *AEDES CASPIUS* (DIPTERA: CULICIDAE)

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Summary:
Christophers' stages durations and effect of interrupted blood meal were investigated in laboratory to study the gonotrophic cycle of *Aedes caspius* (Pallas, 1771). A first experiment was done with replete females (full blood meal) and females with an interrupted blood meal. Females were then regularly dissected, the durations of Christophers' stages I, II, III, IV, V were up to 8, 8, 32, 8, 48 h, respectively. A second experiment was done with replete females, females with an interrupted blood meal and females with an interrupted blood meal completed 24 h later. Interrupted females matured 21 ± 5 follicles, interrupted-completed females 92 ± 11, and replete females 120 ± 8 follicles.

KEY WORDS : *Aedes caspius*, Christophers' stages, blood meal, gonotrophic cycle.

Résumé : DURÉES DES STADES DE CHRISTOPHER ET EFFET DE L'INTERRUPTION DU REPAS SANGUIN CHEZ LE MOUSTIQUE *AEDES CASPIUS* (DIPTERA : CULICIDAE)

*Les durées des stades de Christopher et l'effet de l'interruption du repas sanguin sont estimés en laboratoire pour étudier le cycle trophogonique d'*Aedes caspius *(Pallas, 1771). Une première expérience est faite avec des femelles gorgées à réplétion et des femelles dont le repas de sang a été interrompu. Les femelles sont ensuite régulièrement disséquées. Les durées des stades de Christopher I, II, III, IV et V vont jusqu'à 8, 8, 32, 8 et 48 h, respectivement. Une seconde expérience est réalisée avec des femelles à réplétion, des femelles interrompues et des femelles interrompues dont le repas est complété 24 h plus tard. Les femelles interrompues ont développées 21 ± 5 follicules, les femelles interrompues-complétées 92 ± 11 et les femelles à réplétion 120 ± 8 follicules.*

MOTS CLÉS : Aedes caspius, *stades de Christopher, repas sanguin, cycle trophogonique.*

INTRODUCTION

For more than 50 years, entomologists have estimated the physiological age and the gonotrophic cycle duration of vector mosquitoes (Clements, 1992, 1999). For vectorial studies, entomologists were interested in estimating the physiological age and the hematophagous activity (Detinova, 1963). These data gave information on survival and on egg numbers laid by a mosquito during its life span (Service, 1993). More recently, it allowed developing demographic models (Southwood & Henderson, 2000).

Female fecundity depends on the number of gonotrophic cycles and their durations. Christophers' stages are a part of gonotrophic cycle, they correspond to the different stages of follicles maturation (Christophers, 1911). An abdominal distension above a minimum blood ingestion induces an inhibition of the host seeking behaviour of *Aedes aegypti* (Klowden & Lea, 1978) and there is a correlation between fecundity and blood intake in quality as well as in quantity for *Aedes* and *Culex* species (Woke *et al.*, 1956; Shelton, 1972). Under this threshold, vitellogenesis is not induced (Woke *et al.*, 1956; Clements, 1992).

Aedes caspius (Pallas, 1771) is widely distributed in Europe (Gabinaud, 1975) and breeds in a variety of places (Rioux, 1958). This species is remarkable for its economical importance as it induces a huge nuisance (Porretta *et al.*, 2005). *Ae. caspius* can harbour filaria such as *Dirofilaria immitis* (Roubaud & Colas-Belcour, 1937) or can transmit arboviruses such as Tahyna virus (Hannoun *et al.*, 1966). It is one of the vectors of Rift Valley Fever in Egypt (Beier *et al.*, 1987). Akhter *et al.* (1982) successfully infected Pakistanese *Ae. caspius* with West Nile virus in the laboratory and this virus was isolated from this species in Ukraine (Vinograd & Obukhova, 1975).

This study was aimed to deepen our knowledge of *Ae. caspius* gonotrophic cycle. For this species, there was a relationship between Christophers' stage durations and temperature (Sinègre, 1974), however the effect of an interrupted blood meal was not known. First of all, the durations of Christophers' stages and the effect of an interrupted blood meal on these durations were investigated. Then, the number of matured follicles was

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compared between replete females (full blood meal), females with an interrupted blood meal and females with an interrupted blood meal completed 24 h later. For this, a method without quantification of blood meal size, except as replete or partial meals, was used.

MATERIALS AND METHODS

Adult sampling, rearing methods and blood meals

Ae. caspius females used in the experiments were collected in the Camargue (southern France; 4° 24' 03'' E; 43° 30' 11'' N) with a Mosquito-Magnet® trap (American Biophysics Corporation, Greenwich, USA). The trap was positioned in a field near a representative *Ae. caspius* breeding site and was left there until a sufficient number of females was collected (from one to four nights).

After each catch night, adults were brought back to the laboratory. Species were identified and all the *Ae. caspius* were put in a 40 × 40 × 40 cm cage. 100 females were killed and dissected to determine the parity rate using the Detinova method (Detinova, 1963). The other adults were reared on controlled environmental conditions at 28 ± 1.5° C (mean ± confidence interval), 50 ± 10 % relative humidity, 16:8 light:dark, and were fed with water diluted honey.

The water diluted honey was removed 24 h before the blood meal. The blood meals were taken on a guinea pig between 8:00 and 10:00 AM. One group of females was fed to repletion ("replete" females) and another had interrupted blood meals by taking off the guinea pig ("interrupted" females). The replete and interrupted females were taken the way they came to hand and isolated in different cages with a laying-box (a piece of cotton wool soaked in water) and were fed with water diluted honey.

Estimation of Christophers' stages durations

In a first experiment, females were sampled from 9 to 11 June 2004. A blood meal was given on 14 June 2004 until 200 replete females and 200 interrupted females were isolated. From 14 to 19 June 2004, 10 females were dissected every 8 h (1:30 am, 9:30 am, and 5:30 pm), the last dissection was done 120 h after the blood meal. The presence of blood in the stomach and the Christophers' stage were noted. Time of first oviposition after a blood meal was noted.

Egg development

In the first experiment, the number of matured follicles at stages IV and V was counted from stage V on, a paired-t-test (Zar, 1999) was used to compare replete females to interrupted females. In a second experiment, other females were caught on 2 and 3 September 2004. A blood meal was given on 6 September 2004. Three cages, each containing 30 females, were used: the first with replete females, the second and the third with interrupted females. In the third cage, blood meal was completed until repletion on 7 September 2004; these females were named "interrupted-completed". On 9 September 2004, all females were freeze killed. Presence of blood in the stomach and Christophers' stage were noted. From stage V on, the number of matured follicles (stages IV and V) was counted. The normality of the number of matured follicles of the first and second experiments was tested using a Shapiro-Wilk test (Zar, 1999). The comparison of matured follicles number between the three groups of females was done with an ANOVA and the pairwise comparison if significant ANOVA was done with the Fisher's least significant differences (LSD; Zar, 1999). All statistical tests were done with SYSTAT® 9 (SPSS Inc., 1999).

RESULTS

Christophers' stage durations

Blood was found in all the dissected females for both experiments. Blood digestion lasted up to 48 h. For Christophers' stage I, II, III, IV, V the durations were up to 8, 8, 32, 8, 48 h, respectively (Table I). The duration from Christophers' stage I to the beginning of stage V was up to 56 h. The first oviposition occurred 104 h after the blood meal. There were no differences in the durations of Christophers' stages between replete females and interrupted females with matured follicles (Table I).

	Interrupted		Replete	
Time (h)	**CS**	**N**	**CS**	**N**
Blood meal	I	10	I	10
8	II	4	II	5
16	III	4	III	10
24	III	5	III	10
32	III	7	III	10
40	III	6	III	10
48	IV	5	IV	9
56	V	4	V	9
64	V	8	V	9
72	V	6	V	10
80	V	6	V	10
88	V	6	V	10
96	V	7	V	10
104	V	9	V	10
112	V	5	V	10
120	V	5	V	10

Table I. – First experiment: Christophers' stages according to time for replete females (full blood meal) and females with an interrupted blood meal. N is the female number that reached the Christophers' stage (CS) on the 10 dissected females.

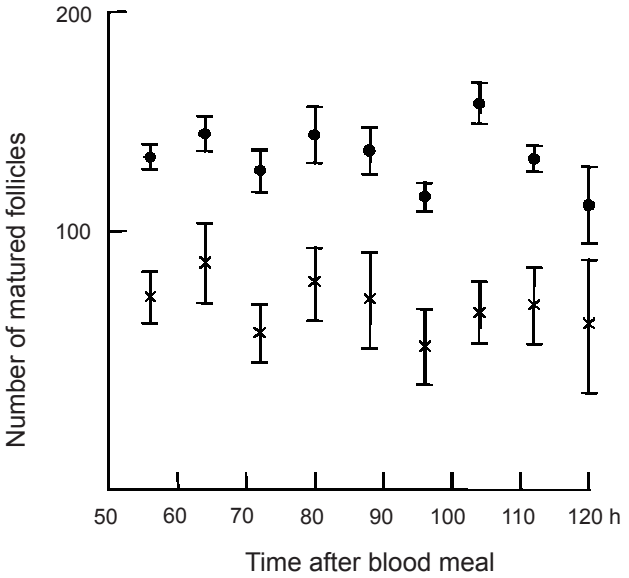

Fig. 1. – First experiment: means and standard errors of the matured follicle numbers according to time after the blood meal for replete (●) and interrupted (×) females. The number of matured follicles was counted only from stage V on (the first stage V was observed 56 h after the blood meal).

EGG DEVELOPMENT

The parity rate was of 7.5 % for the first experiment and 63.3 % for the second. The number of matured follicles was normally distributed for the first and the second experiments (Shapiro-Wilk test, $p = 0.098$ and $p = 0.058$). During the first experiment, 33 ± 8 % of interrupted females had not developed mature follicles and remained at stage I. The matured follicle numbers were significantly higher in replete than in interrupted females with matured follicles (paired-t-test; $n = 9$, $t = -19.64$, $df = 8$, $p < 0.001$; Fig. 1). In the second experiment, differences in Christophers' stages were noted between interrupted, interrupted-completed and replete females (Table II). There were significant differences in the matured follicle numbers between interrupted with matured follicles, interrupted-completed with matured follicles and replete females (ANOVA; $F = 15.45$; $df = 2, 64$; $p < 0.001$) and the three pairwise comparison test were significant (Fisher's LSD test, $p < 0.05$). It was higher in replete females (mean ± standard error = 120 ± 8 follicles), then came interrupted-completed females (92 ± 11 follicles) and then interrupted females (21 ± 5 follicles).

Christophers' stage	Interrupted	Interrupted-completed	Replete
I	46	0	0
II	4	0	0
III	0	0	0
IV	0	54	3
V	50	46	97

Table II. – Second experiment: percentage of females in each Christophers' stage 77 h after a blood meal.

DISCUSSION

The aim of this study was, firstly, to estimate the durations of Christophers' stages and the effect of an interrupted blood meal on these durations. Secondly, the effect of completed a blood meal after 24 h on the number of matured follicles was investigated.

The dissections were done every 8 h, thus the true duration of Christophers' stage I, for example, was not 8 h but between 0 h (the blood meal) and 8 h (the first dissection after the blood meal). For Sinègre (1974) the duration from blood meal to oviposition was 96 h at 25° C and 60 h at 32° C. Our estimation of the duration from Christophers' stage I to the beginning of stage V is 56 h at 28° C and there was a delay of 48 h between the first Christophers' stages V and the first oviposition which occurred after 104 h. Dégallier (1979) noted that 84 h old eggs of *Culex portesi* reached their final structure but were not fully developed until the fifth day. This delay could explain our results, however the delay to the first oviposition in our study was greater than that of Sinègre (1974) and the observed relative stability in the number of matured follicles from 50 to 120 h (Fig. 1) may indicate that the delay of 48 h could also be due to egg retention.

The quantity of blood ingested was not controlled but all the dissected females had taken some blood and, in both experiments, some interrupted females did not mature follicles. Thus under a threshold (unknown in these experiments) the volume of blood may be not sufficient to induce vitellogenesis, as was noted for other species (Klowden & Lea, 1978; Clements, 1992). Clements (1992) suggested that above this threshold, there is a correlation between the quantity of blood intake and the number of eggs produced. The number of matured follicles for replete females was significantly higher than that in the interrupted females (Fig. 1). So a correlation may exist between blood intake and the number of eggs produced by *Ae. caspius*.

Mating status (Klowden & Chambers, 1991), parity (Guilvard, 1983) and adult body weight (Shelton, 1972; Briegel, 1990) affect fecundity. *Ae. caspius* spermathecae were not examined. The observed differences of number of matured follicles between replete and interrupted females in the first experiment and between replete, interrupted-completed and interrupted females in the second experiment could be explained by the mating, the parity status and/or the body weight. As for each experiment, all the females collected in the field were put in the same cage and as during blood feeding, replete or interrupted females were collected

randomly, therefore for each experiment, the percentage of mated females, the parity rate and the mean body weight should not have been significantly different between the female groups. Thus the biases of mating, parity status and body weight could be considered unimportant compared to blood meal size.

Lea *et al.* (1978) concluded that after an interrupted blood meal some follicles of *Ae. aegypti* are programmed to maturation and others to resorption. It is possible to dissect ovaries to observe dilatations which are the remnants of aborted follicles (Detinova, 1963). This method differentiates if the follicles are programmed to resorption or if vitellogenesis did not occur. In our second experiment, interrupted-completed females matured significantly less follicles than replete females. However, dilatations were not looked for when we have dissected ovaries and in parous females a resorbed follicle can not be differentiated from a previous oviposition, therefore it was not possible in this second experiment to know if follicles were resorbed or if vitellogenesis did occur.

The population growth rate is one of the fundamentals of demographic models and is calculated with the net reproductive rate per generation, itself estimated from the survival and the fecundity of individuals (Southwood & Henderson, 2000). The fecundity of a female depends on the number of gonotrophic cycle during her life span, and this number of gonotrophic cycle depends on the durations of Christophers' stages. Therefore, results obtained in this study could be considered to realize a demographic model of *Ae. caspius*. This would allow to deepen the demography of *Ae. caspius* populations on the Mediterranean littoral, and thus to better control their harmful effect.

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