View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by RIMI

LIFE TABLES AND REPRODUCTIVE PARAMETERS OF *PHLEBOTOMUS NEGLECTUS* TONNOIR, 1921 (DIPTERA, PSYCHODIDAE) UNDER LABORATORY CONDITIONS

V. IVOVIĆ[1], V. CHANIOTIS[2], MARIJA VUJANIĆ[1], B. BOBIĆ[1], ALEKSANDRA NIKOLIĆ[1], IVANA KLUN[1], TIJANA ŽIVKOVIĆ[1] and OLGICA DJURKOVIĆ-DJAKOVIĆ[1]

[1]*Department of Parasitology, Institute for Medical Research, University of Belgrade,* 11000 Belgrade, Serbia
[2]*Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, Faculty of Medicine, University of Crete,* 71110 Heraklion, Greece

Abstract: Laboratory investigations of the biology of the sand fly, *Phlebotomus neglectus*, including bionomic factors collected on the life history, behavior and feeding preferences of this species and the characteristics of its developmental biology are presented. In addition, we quantified the parameters of the population dynamics and life history of this species under laboratory conditions which are crucial for a better understanding of its role as a vector of *Leishmania* parasites in the eastern Mediterranean area.

Key words: Life tables, Phlebotominae, *P. neglectus*, laboratory colony, reproductive parameters.

UDC 595.771:591.1

INTRODUCTION

Phlebotomus neglectus is a Palaearctic species spreading over central and eastern Mediterranean, from northern Italy up to the southeast of Turkey and the eastern banks of the Mediterranean Sea (Leger & Depaquit, 2002). This species has never been recorded in either Africa or Cyprus (Leger et al., 2001).

We present the successful laboratory rearing of *P. neglectus* collected from the southernmost part of its range, namely Crete. While comparatively rare in other parts of its range, in Crete *P. neglectus* is the most abundant species caught over a three-year period of systematic trapping. We quantified numerically the parameters of the population dynamics of a laboratory colony using life table statistics as a template. An experimental cohort of 100 females was selected as a representative sample of a laboratory colony, and ten successive generations were reared, allowing us to clarify in detail all aspects of its dynamics. Valuable data were also collected on the life history, behavior and feeding preferences of reared *P. neglectus*, and the characteristics of its developmental biology are described.

Both human visceral leishmaniasis and canine leishmaniasis, caused by *Leishmania infantum* MON-1, are endemic along the Aegean and Mediterranean coasts and occur sporadically in other regions (Ertabaklar et al., 2005). This study provides initial data for further research on *P. neglectus* which is necessary to elucidate its relationship with the distribution of leishmaniasis and in particular its apparent role as a vector of *L. infantum* and most likely of sand fly-borne viruses in the Mediterranean.

MATERIALS AND METHODS

The laboratory colony of *P. neglectus* originated from adults caught in Fodele (35, 38º N, 24, 95º E), an inland village 25 km west of Heraklion, Crete. All the sand flies were collected in June 2003 over a period of four successive nights, either by hand with battery-powered aspirators or with CDC light traps. Aspirators were used to collect the sand flies from

Table 1. Life table and stage-specific mortality.

	eggs	1st instar	2nd and 3rd instar	4th instar	pupae	adults	females	eggs
l_x (live individuals)	827	722	580	519	470	441	420	219
d_x (mortality)	105	142	61	49	29	21	210	219
qx (finite mortality rate)	0.127	0.197	0.105	0.094	0.062	0.048	0.500	1.000
Sx (finite survival rate)	0.873	0.803	0.895	0.906	0.938	0.952	0.500	0
Apparent mortality (%)	12.70	19.67	10.52	9.44	6.17	4.76	50.00	100.00
Real mortality (%)	12.70	17.17	7.38	5.93	3.51	2.54	25.39	26.48
Irreplaceable mortality (%)	7.38	12.45	6.05	5.32	3.39	2.54		
A.n.ad*	61	90	35	28	16	11		

walls on an illuminated porch of a house, while the CDC traps were placed inside a chicken pen adjacent to the house. The stone walls surrounding the house offered suitable hiding places for the sand flies during the day. In total, 170 sand flies were transported alive to the laboratory for colonization; those caught by aspirators were transferred to pots with humid plaster of Paris for transportation on the night of capture, while the ones caught by CDC traps were transported the same way the following morning. An experimental cohort of 100 females, the parental generation, was selected from the wild-caught sand flies, and after the first blood feeding, transferred into rearing vessels.

We followed the procedure and techniques used for establishing and maintaining a colony described by Chaniotis et al. (2000) with some modifications. The sand flies were kept in an incubator, in the dark, at a temperature ranging between 27 and 28°C and with a relative humidity of approx. 80%. During the winter, the temperature was raised to 30°C. Stage-specific mortality was recorded after the hatching of the eggs and the molting of larvae from ten successive generations, and life table statistics were calculated according to Southwood (1978) and Krebs (1999).

The larvae of *P. neglectus* are surface feeders, mobile and very active through all immature stages. The average duration of larval stages was 8.5 days (eggs-1st instar), 6 days (1st-2nd instar), 5.5 days (2nd-3rd instar), 7 days (3rd-4th instar), 7.5 days (4th-pupae) and 10 days (pupae-adults). Therefore, the total generation time was 44.5 days (Table 2). We did not observe any significant difference in the duration of the developmental periods related to the season of the year.

Pupae

The pupation of *P. neglectus* occurred mainly on the walls of the incubation vessels, with only a few pupae remaining on the surface. The length of pupation was not affected by the season of the year. The mortality of pupae was very low, with 95% developing into adults.

Adults

The average female-to-male sex ratio was 1.087. Copulation occurred regardless of nutritional state, but was much more frequent after the females engorged. We did not observe any copulation during blood feeding.

Table 2. Developmental time of each instar

	egg-1st instar	1st -2nd instar	2nd -3rd instar	3rd -4th instar	4th st-pupae	pupae-adult	Total
In days	8.5 ±0.50	6 ±0.58	5.5 ±0.65	7 ±0.71	7.5 ±0.65	10 ±0.58	**44.5**
SD	0.707	1.000	1.291	1.581	1.291	1.000	
SE	0.50	0.58	0.65	0.71	0.65	0.58	

P. neglectus fed readily on rabbits, humans and chickens. Females hesitated to land immediately on the skin of the host and pierced it up to four times until reaching full engorgement.

The gonotrophic cycle is concordant, but some females also accepted a second blood meal. No relation between the fecundity and the number of blood meals was recorded. Although some females survived oviposition, most died within the following 24 h. Only some females accepted a second blood meal after laying eggs. The duration of blood feeding decreased between the first and second preoviposition feeding after the eggs were laid by almost two-fold. The average time of the first feeding was 5 min 26 s, while the average postoviposition feeding lasted 3 min 46 s. The adult longevity differed significantly between males and females, with most of the males dying within the first two weeks. The average female life span was 19 days, and the maximum age recorded was 42 days.

Analysis of the stage-specific mortality

A vertical life table was constructed based on the l_x (live individuals) and d_x (mortality) at each instar (average based on ten successive generations) (Table 1). Stage-specific mortality was based on data in the vertical life table according to Southwood (1978).

DISCUSSION

The distribution of *P. neglectus*, shown by Chaniotis (1994) and Maroli et al. (2002) suggests that its developmental biology is related to a more humid climate. For this reason, the sand flies were kept in an incubator with a relative humidity of approx. 85% and temperature ranging between 27°C and 28°C: the colony was established without infertility problems.

Similar to *P. ariasi,* another sand fly species belonging to the same subgenus (*Larroussius*) (Killick-Kendrick and Killick-Kendrick, 1987), the larvae of all instars are remarkably motile and do not burrow. But unlike the latter species or *P. perniciosus* (Ready and Croset, 1980), *P. neglectus* from Crete, kept under the laboratory conditions does not go to diapause. We assume that this is because of the fixed physiological adaptations of this species to the very mild Mediterranean climate.

A comparison of the net reproductive rate (Ro=2.105 daughter females per cohort female) obtained in this study and data on other species such as *L. shannnoni* (Ro=23.5), or *L. spinicrassa* (Ro=8.4) (Escovar et al., 2004), suggests that the population increase of *P. neglectus* under laboratory conditions is low, and consequently the maintenance of a laboratory colony of this species is difficult. However, the observed low reproductive rate of *P. neglectus* may, at least in part be due to the keeping of the colony in total 24 h darkness.

The mortality rates (apparent, real and irreplaceable) were highest in the 1st, and lowest in the 4th instar stage. This may be attributed to a number of factors including the increased sensitivity of the 1st instar to the quality of food provided and its reduced mobility, as well as fungal

and bacterial infections, and oscillations of the microclimatic conditions during handling.

P. neglectus females were found to be anautogenous, as are many other species of phlebotomine sand flies from both the Old World (Hanafi et al., 1999) and the New World (Chaniotis, 1975).

Successful engorgement from human, rabbit and chicken skin suggests that *P. neglectus* is probably an opportunistic feeder in nature, feeding on various vertebrate hosts. Further field and laboratory studies are needed to determine the host preferences of this species in nature. Compared to *P. neglectus,* females of *P. similis* are much more aggressive in feeding, landing to feed very quickly, and engorging fully with a single bite (Ivović et al., 2007).

In conclusion, the presented results show that despite the difficulties during the laboratory colonization of *P. neglectus*, this species can nonetheless be successfully colonized to facilitate studies on new phlebotomine repellent efficiency and its vectorial capacity for other strains of *Leishmania*. The observed blood-feeding behavior as well as the latest isolation of *Leishmania* promastigotes from *P. neglectus* (Leger et al., 1988; Garifallou et al., 1989; Ivovic et al., 2004) verify the vectorial status of this sand fly species as the most important vector of human and canine leishmaniasis in Crete and the eastern Mediterranean.

Acknowledgements – The authors are grateful to the I.K.Y scholarship foundation from Greece for financial support to V. Ivović. The study has been partially supported by a grant (No. 145002) from the Ministry of Science and Technologies of Serbia.

REFERENCES

Chaniotis, B. (1975). A new method for rearing *Lutzomyia trapidoi* (Diptera: Psychodidae), with observations on its development and behavior in the laboratory. *J. Med. Entomol.* **12**, 183-188.

Chaniotis, B., Garcia, G. and *I. Tselentis* (1994). Water wells as a habitat of sand fly (Diptera, Psychodidae) vectors of visceral leishmaniasis in Greece. *J. Med. Entomol.* **33**, 269-270.

Chaniotis, B., Spyridaki, I., Sculica, E. and *M. Antoniou* (2000). Colonization of *Phlebotomus neglectus* (Diptera: Psychodidae), the major vector of visceral leishmaniasis in Greece. *J. Med. Entomol.* **37** (3), 346-348.

Ertabaklar, H., Ozensoy Toz, S., Taylan Ozkan, A., Rastgeldi, I.S., Balcioglu, C. and *Ozbel, Y.* (2005). Serological and entomological survey in a zoonotic visceral leishmaniasis focus of North Central Anatolia, Turkey: Corum province. *Acta Trop* **93**, 239-246.

Escovar, J., Bello, F.J., Morales, A., Moncada, L. and *E. Cardenas* (2004). Life tables and reproductive parameters of *Lutzomyia spinicrassa* (Diptera: Psychodidae) under laboratory conditions. *Memorias do Instituto Oswaldo Cruz* **99,** 603-607.

Garifallou, A., Hadjiantoniou, M., Schnur, L.F., Yuval, B., Warburg, A., Jacobson, R.L., Pateraki, E., Patrikoussis, M., Schlein, Y. and *C. Sérié* (1989). Epidemiology of human and canine leishmaniasis of the island of Zakinthos. In: DT Hart (Editor), Leishmaniasis. Plenum Publishing Corporation, 1011-1015.

Hanafi, A.H., Kanour, JR W.W., Beavers, M.G. and *E.G. Tetreault* (1999). Colonization and bionomics of the sandfly *Phlebotomus kazeruni* from Sinai, Egypt. *Med. Vet. Entomol.* **13**, 295-298.

Ivović, V., Depaquit, J., Léger, N., Urano, A. and *B. Papadopoulos* (2004). Sandflies (Diptera: Psychodidae) in the Bar area of Montenegro (Yugoslavia). 2. Presence of promastigotes in *Phlebotomus neglectus* and first record of *P. kandelakii*. *Ann. Trop. Med. Parasitol.* **98**, 425-427.

Ivović, V., Ivović M., Chaniotis, B. and *Y. Tselentis* (2007). The establishment, maintenance and productivity of a laboratory colony of *Phlebotomus similis* Perfiliew, 1963 (Diptera: Psychodidae). *Parasitol. Res.* **101**(1), 229-231.

Killick-Kendrick, R. and *M. Killick-Kendrick* (1987). The laboratory colonization of *Phlebotomus ariasi* (Diptera: Psychodidae). *Annales de Parasitologie Humaine et Comparée* **62**, 354-356.

Krebs, J.C. (1999). Ecological Methodology. 2nd Edn. Benjamin/Cummings, imprint of Addison Wesley Longman, Inc. (ISBN 0-321-02173-8), pp. 620.

Léger, N., Gramiccia, M., Gradoni, L., Madulo-Leblond, G., Pesson, B., Ferte, H., Boulanger, N., Killick-Kendrick, R. and *M. Killick-Kendrick* (1988). Isolation and typing of Leishmania infantum from *Phlebotomus neglectus* on the island of Corfu, Greece. *Trans. Roy. Soc. Trop. Med. Hyg.* **82**, 419-420.

Leger, N., Depaquit, J., Ferte, H., Rioux, J.A., Gantier, J.C., Michaelides, A. and *P. Economides* (2001). Les phlebo-

tomes de l'Ile de Chypre III – Inventaire faunistique. *Parasite* **8**, 11-20.

Leger, N. and *J. Depaquit* (2002). Systematique et biogeographie des phlebotomes (Diptera: Psychodidae). *Annales de la Societe Entomologique de France* (n.s.) **38**, 163-175.

Maroli, M., Khoury, C., Bianchi, R., Ferroglio, E. and *A. Natale* (2002). Recent findings of *Phlebotomus neglectus* Tonnoir, 1921 in Italy and its western limit of distribution. *Parassitologia* **44**, 103-109.

Ready, P.D. and *H. Croset* (1980). Diapause and laboratory breeding of Phlebotomus perniciosus Newstead and Phlebotomus ariasi Tonnoir (Diptera: Psychodidae) from southern France. *Bull. Entomol. Res.* **70**, 511 – 523.

Southwood, T.R.E. (1978). Ecological methods, with particular reference to the study of insect population. Chapman and Hall, London, pp. 524.

ЖИВОТНИ ЦИКЛУС И ОСНОВНИ ПАРАМЕТРИ РЕПРОДУКЦИЈЕ ВРСТЕ *PHLEBOTOMUS NEGLECTUS* TONNOIR, 1921 (DIPTERA, PSYCHODIDAE) У ЛАБОРАТОРИЈСКИМ УСЛОВИМА

В. ИВОВИЋ[1*], В. ХАНИОТИС[2], МАРИЈА ВУЈАНИЋ[1], Б. БОБИЋ[1], АЛЕКСАНДРА НИКОЛИЋ[1], ИВАНА КЛУН[1], ТИЈАНА ЖИВКОВИЋ[1] и ОЛГИЦА ЂУРКОВИЋ-ЂАКОВИЋ[1]

[1]*Одељење за паразитологију, Институт за медицинска истраживања, Универзитет у Београду,* 11000 Београд, Србија
[2]*Лабораторија за клиничку бактериологију, паразитологију, зоонозе и географску медицину, Медицински факултет, Универзитет на Криту,* 71110 Ираклион, Грчка

У раду су приказани резултати истраживања основних карактеристика животног циклуса и понашања врсте флеботомина *Phlebotomus neglectus* у лабораторијским условима. Такође, нумерички су приказани основни параметри динамике популације и помоћу таблица живота одређени су кључни фактори животног циклуса у циљу бољег разумевања ове врсте флеботомина као најважнијег преносиоца паразита *Leishmania infantum* у источном Медитерану.