

Prediuresis in female *Phlebotomus* sandflies (Diptera: Psychodidae)JOVANA SÁDLOVÁ¹, JOSEF REISHIG² and PETR VOLF¹¹Department of Parasitology, Faculty of Science, Charles University, Viničná 7,
128 44 Prague 2, Czech Republic; e-mail: volf@beba.cesnet.cz²Department of Biology, Faculty of Medicine, Charles University, Karlovarská 48,
301 00 Pilsen, Czech Republic**Psychodidae, *Phlebotomus papatasi*, *Phlebotomus duboscqi*, sandflies, urine, diuresis, *Leishmania*, transmission**

Abstract. Sandfly females, while feeding on the host, excrete urine to concentrate proteins of the blood-meal and restore weight and water balance. This process, analogous to prediuresis in mosquitoes, was observed in 100% of *Phlebotomus papatasi* (Scopoli) and 85% of *P. duboscqi* Neveu-Lemaire females studied. Individual females, however, differed in duration of prediuresis and in the number of ejected urine droplets. In both species the prediuresis generally started 1–2 min after the commencement of feeding and the variation in urine production was positively correlated with the length of feeding. The first one or two droplets were opaque whitish while the remaining ones were clear. Erythrocytes were found sporadically in first droplets of some females. Representative prediuresis in *P. duboscqi* included 27 droplets, i.e., about 325 nl urine in total, ejected during 8 min of feeding. The study revealed prediuresis in *P. papatasi* and *P. duboscqi* as a regular physiological process which may have consequences in transmission of infective diseases.

Introduction

Phlebotomine sandflies are vectors of *Leishmania* parasites, causative agents of diseases, representing an important human health problem in many countries. *Leishmania* promastigotes develop in the sandfly gut and are transmitted into the vertebrate host principally by the bite of infected female (for review see Killick-Kendrick, 1990; Schlein, 1993). Our group, however, recently found viable promastigotes of *Leishmania major* in urine droplets which infected sandfly females discharge during feeding (Sádlová and Volf, unpubl.). This finding suggests that contaminative transmission may additionally occur in the sandfly-*Leishmania* system and led us to the study of sandfly diuresis.

Diuresis, the rapid production of urine containing principally water and salts, was described in detail in bloodfed mosquitoes (reviewed by Clements, 1992). Similarly to sandflies, mosquito females periodically ingest a large volume of bloodmeal. Such a meal makes flight difficult and produces water- and salt-loads which threaten the homeostasis of the haemolymph. The blood-feeding habit, therefore, necessitates the rapid excretion of water and sodium ions. Soon after the mosquito female begins feeding, fluid is rapidly absorbed from the bloodmeal and excreted via Malpighian tubules. An initial secretion of the Malpighian tubules (tubular fluid), which is iso-osmotic with the haemolymph, is modified in the rectum before being expelled as urine (Spring, 1990). In addition, the anal discharge of urine apparently serves to rapidly concentrate the nutritive elements of the bloodmeal (Briegel & Rezzonico, 1985).

In sandflies, the digestive processes are thought to resemble those in mosquitoes on the basis of similar functional morphology (Rudin & Hecker, 1982). Diuresis and prediuresis of sandflies, however, has yet not been studied.

Throughout the study, the term “urine” is used according to Clements (1992) for the complete excretory fluid discharged to the exterior. Excretion of urine during bloodfeeding is called “prediuresis”, while “diuresis” (sensu stricto) denotes excretion after feeding has stopped (Briegel & Rezzonico, 1985).

Material and methods

Phlebotomus papatasi (Cyprus strain) and *P. duboscqi* (Senegal strain) were maintained on 50% sucrose at 25–26°C and a 14L : 10D photoperiod. Flies used to establish colonies were received from the Imperial College in Ascot.

Twenty females of each species, 5–6 days post-emergence, were allowed to feed on a human finger. The feeding process was observed with a stereomicroscope, recorded and analyzed afterwards. Urine droplets were caught on a piece of coverslip held with forceps behind the anus. Air dried drops were fixed by methanol, stained with Giemsa and subsequently examined for the presence of erythrocytes. Total feeding time was measured from insertion of the fascicle into the skin (if multiple probing occurred, the last penetration was measured) to female take off. Excretion time designates the length of prediuresis; i.e., the period from appearance of the first urine droplet to female take off (they flew away immediately after withdrawing their fascicles from the skin).

Additionally, the feeding of sandfly females on anaesthetized animals (hamsters, mice) or a human arm was repeatedly videorecorded in order to evaluate exact time course of excretion and the volume of droplets. Volume of urine droplets was determined by measuring the diameter of an apparently spherical droplet. This method gave data on the actual volume flow rates expressed in nl/min.

Statistical evaluations were made using the PC program Statgraphics, version 5.0. Non-parametric tests were used for the analysis of droplets numbers, excretion time and feeding time where data distribution were significantly different from the normal distribution.

Results

The basic characteristics of prediuresis for both species are shown in Table 1. *P. papatasi* had a significantly longer excretion time and produced significantly higher number of droplets than *P. duboscqi* (Fig. 1). Prediuresis was observed in all *P. papatasi* flies. However, in one female, droplets were not ejected away and urine accumulated on the anus. In *P. duboscqi*, three females did not expel urine and flew away after a very rapid engorgement.

TABLE 1. The comparison of prediuresis characteristics in *P. papatasi* (PAP) and *P. duboscqi* (DUB) females. The difference between species was tested by non-parametric (Mann-Whitney) test.

	Droplets number		Excretion time (s)		Feeding time (s)	
	PAP	DUB	PAP	DUB	PAP	DUB
Median	20.5	6	193	96	254	206
Lower quartile	13	1.5	138	69	214	137
Upper quartile	33	12.5	224	159	338	255
No.	20	20	20	17	20	20
Difference between species	P = 0.0007 Z = -3.3725		P = 0.0044 Z = -2.8498		P = 0.0498 Z = -1.9617	

Besides interspecific differences, considerable intraspecific variations in urine production were observed, especially in *P. papatasi* (Fig. 1). In both species, the variation in droplet numbers coincided with feeding times. Generally, the longer the total feeding time, the higher the number of droplets (Spearman test; coefficient 0.5912, $P = 0.0100$ for *P. papatasi* and coefficient 0.6055, $P = 0.0083$ for *P. duboscqi*).

The first droplet appeared most often within the range 50–92 s and 70–125 s (lower and upper quartiles) with median 69 and 92 s in *P. papatasi* and *P. duboscqi*, respectively. The later start of prediuresis in *P. papatasi*, the longer was the feeding time (Spearman test, coefficient 0.7245, $P = 0.0016$). In *P. duboscqi*, however, a correlation between the start of prediuresis and the length of feeding was not found (coefficient 0.3899, $P = 0.1188$).

A time course of prediuresis based on videorecorded *P. duboscqi* included twenty seven droplets ejected during 8 min of feeding (Fig. 2). The first droplet was released after 91 s and then the time

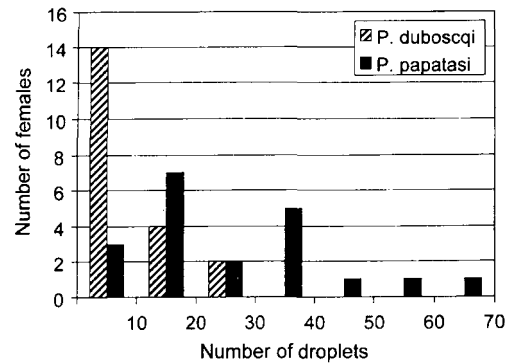


Fig. 1. Frequency histogram depicting urine production of *P. papatasi* and *P. duboscqi* females feeding on human finger.

intervals shortened to the rate of about one droplet per 10 s. The highest urine flow rate (8 droplets/min) was reached in the 7th min of feeding. The volumes of urine droplets in this female ranged from 2.7 nl (the first one) to 21.9 nl with the average $12.1 (\pm 2)$ nl. In total, the female excreted about 325 nl of urine during bloodfeeding.

Extrusion of a droplet of urine takes about a second and involves a slow tilting upwards of the terminal abdominal segments. The droplet is then very quickly and forcefully ejected, often over 5 mm (Fig. 3). After this, the terminalium quickly returns to its original position. The first 1–2 droplets were often opaque whitish, the following ones were clear. Sporadically, the first droplets were reddish with blood; erythrocytes were found in the first droplets of five (25%) *P. papatasi* and two (10%) *P. duboscqi* females.

Discussion

Prediuresis in sandfly females starts when the midgut is partially filled by blood. We suppose that, similarly to mosquitoes, abdominal distension could be the initial stimulus that leads to the induction of prediuresis. Correlation between total feeding time and the start of diuresis was found in *P. papatasi* but not in *P. duboscqi*. The latter resembles the situation reported for *Aedes aegypti* (L.) by Mellink et al. (1982).

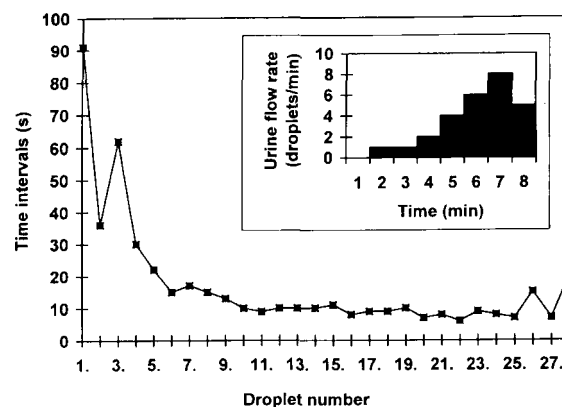


Fig. 2. Time course of urine excretion of *P. duboscqi* female feeding on human arm.

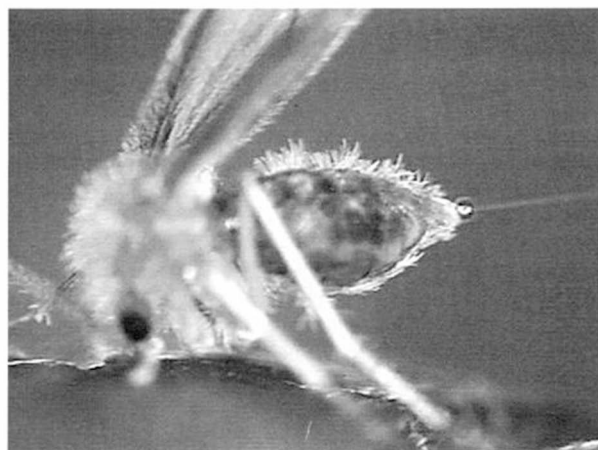


Fig. 3. Ejection of the urine by *P. duboscqi* female during blood-feeding. A picture taken from a video recording.

In mosquitoes, the urine excretion usually begins in less than 2 min after the commencement of feeding (i.e., while the insect is still engorging) (Williams et al., 1983; Spring, 1990) and passes through three phases which differ in the rate of flow and urine composition. The first phase with a high rate of urine flow takes the first few minutes (peak phase) and is followed by a steady decline (post peak phase) to a relatively stable low flow rate (late phase) (Williams et al., 1983; Clements, 1992). In our experiments, the high urine production within the first minutes of feeding coincides probably with the peak phase of urine production described for mosquitoes.

Certain species/strains of mosquitoes discharge clear fluid while others dispose a reddish fluid containing erythrocytes. High variability was described also for excretion rates, number of droplets, and their size (Nijhout & Carrow, 1978; Jones & Brandt, 1981; Briegel & Rezzonico, 1985; Vaughan et al., 1991). In our experiments, *P. papatasi* and *P. duboscqi* sandflies produced a predominately clear fluid, only the first urine drops contained blood in some cases. This indicates efficient filtration and resembles the situation in *Anopheles albimanus* Wiedemann and *A. quadrimaculatus* Say (Briegel & Rezzonico, 1985) and in *Anopheles freeborni* Aitken (Nijhout & Carrow, 1987).

Anopheline mosquitoes, which are unable to distend the midgut with bloodmeal efficiently, use prediuresis to accumulate nutrients and significantly increase protein intake. This, in consequence, leads to enhanced fecundity (Briegel & Rezzonico, 1985). A similar role of prediuresis can be predicted for sandflies. Because of the correlation between feeding time and the number of urine droplets (i.e., the volume of urine excreted) sandfly females which feed slowly may withdraw relatively larger amounts of blood from the host. On the other hand, a longer feeding time increases the probability to evoke host defensive behaviour. As indicated by great intra-species variations in the feeding habits of *P. papatasi* and *P. duboscqi*, this trade-off might be solved individually by each of the females within a species.

ACKNOWLEDGEMENTS. We thank R. Killick-Kendrick (Imperial College, Ascot) for providing *Phlebotomus* flies used to establish our colonies. The study was supported by the Ministry of Education of the Czech Republic (project VS96/142).

References

- BRIEGEL H. & REZZONICO L. 1985: Concentration of host blood protein during feeding by anopheline mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* **22**: 612–618.
- CLEMENTS A.N. 1992: *The Biology of Mosquitoes. Vol. 1. Development, Nutrition and Reproduction.* Chapman and Hall, London, 509 pp.
- JONES J.C. & BRANDT E. 1981: Fluid excretion by adult *Aedes aegypti* mosquitoes. *J. Insect Physiol.* **27**: 545–549.
- KILLICK-KENDRICK R. 1990: The life-cycle of *Leishmania* in the sandfly with special reference to the form infective to the vertebrate host. *Ann. Parasitol. Hum. Comp.* **65**: 37–42.
- MELLINK J.J., POPPE D.M.C. & VAN DUIN G.J.T. 1982: Factors affecting the blood-feeding process of laboratory strain of *Aedes aegypti* on rodents. *Entomol. Exp. Appl.* **31**: 229–238.
- NIJHOUT H.F. & CARROW G.M. 1978: Diuresis after a bloodmeal in female *Anopheles freeborni*. *J. Insect Physiol.* **24**: 293–298.
- RUDIN W. & HECKER H. 1982: Functional morphology of the midgut of a sandfly as compared to other hematophagous Nematocera. *Tissue Cell* **14**: 751–758.
- SCHLEIN Y. 1993: *Leishmania* and sandflies: Interactions in the life cycle and transmission. *Parasitol. Today* **9**: 255–257.
- SPRING J.H. 1990: Endocrine regulation of diuresis in insects. *J. Insect Physiol.* **36**: 13–22.
- VAUGHAN J.A., NODEN B.H. & BEIER J.C. 1991: Concentration of human erythrocytes by anopheline mosquitoes (Diptera: Culicidae) during feeding. *J. Med. Entomol.* **28**: 780–786.
- WILLIAMS J.C., HAGEDORN H.H. & BEYENBACH K.W. 1983: Dynamic changes in flow rate and composition of urine during the post-bloodmeal diuresis in *Aedes aegypti* (L.). *J. Comp. Physiol.* **153**: 257–265.

Received July 21, 1998; accepted September 25, 1998