ARTÍCULO ORIGINAL

Effects of blood ingestion on patterns on the chorion of eggs of Lutzomyia ovallesi (Diptera: Psychodidae)

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ABSTRACT

Chorion patterns on eggs of Lutzomyia ovallesi fed on seven types of blood were studied using scanning microscopy. The results showed that the different blood sources produced no differences in the patterning. The eggs had a chorion with sinuous longitudinal bridges and a few transverse bridges that formed rectangular shapes. These results confirm that chorion patterns on the eggs of sand flies are a useful taxonomic parameter.

Key words: *Lutzomyia ovallesi*, blood ingestion, egg chorion, scanning microscopy.

INTRODUCTION

Chorion patterns on sand flies eggs are significantly different between species and have been proposed as a taxonomic tool for species identification and classification, since this feature makes it possible to distinguish between species that are morphologically very similar.¹⁻⁸ On the other hand, have been found intraspecies differences in the exochorion of *L. gomezi* which were explained as the result of geographical differences.⁹ Also exists intraspecies variations in the exochorion of *Phlebotomus pedifer*, *P. aculeatus* and *P. martin* which made it difficult to use this feature for species identification.¹⁰ The same phenomenon was recorded in females of *L. gomezi* in Colombia and Brazil.¹¹ These differences were produced by microenvironmental factors and require further study to determine precisely what factors produced them.¹⁰ The morphological differences in the

chorion between species are due to specific adaptation to the environmental conditions of sites where eggs were laid and the small intraspecies differences in *L. verrucarum* are associated with the geographical isolation of colonies in different Andean valleys.⁸ The intraspecies differences between colonies of *L. gomezi* in Panama-Colombia and Venezuela-Brazil and colonies of *L. verrucarum* result from isolation due to geographical barriers and separation.⁵

Since the factors producing variations in chorion patterns in the same species of sand flies are still unknown, and since blood is a determining factor in egg production and maturation, it seemed possible that these variations might be due to different types of blood. Thus the present work is a scanning microscopy study of the chorion patterns on eggs of *Lutzomyia ovallesi* fed on seven different types of blood.

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MATERIALS AND METHODS

Females of *L. ovallesi* were used. These came from a laboratory colony established with specimens collected in El Arenal, Ejido, in the state of Merida, Venezuela at 1 360 mts. a.m.s.l. Following the techniques described previously,¹² the colony is kept in an incubator at $25^{\circ}\text{C} \pm 1^{\circ}$ with a relative humidity of $80\% \pm 10\%$ in the Parasitology Laboratory, "José F. Torrealba", in the University of Los Andes, Mérida, Venezuela.

Blood from healthy animals was collected in citrate tubes, with 0.2 ml of citrate per ml of blood. Blood sources were horse (*Equus caballus*), chicken (*Gallus domesticus*), pig (*Sus scrofa domestica*), cow (*Bos taurus*) and goat (*Capra hircus*). Human (*Homo sapiens sapiens*) and dog (*Canis familiaris*) blood was also used.

Two-day-old females were used, artificially fed to repletion using chicken membrane. They were kept in an incubator at $25^{\circ}\text{C} \pm 1^{\circ}$ with a relative humidity of $80\% \pm 10\%$, with 12 hours of light and 12 hours of darkness. As a dietary supplement they were given a 50% fresh saccharose solution, renewed daily.

Chorion patterns on the eggs

Eggs

Three-day-old *L. ovallesi* eggs were used. These came from different females of each group fed on the blood of 7 different hosts. The eggs were washed for 2 minutes in 7.2 pH phosphate buffer at 4°C . They were then fixed in glutaraldehyde following the method described previously.⁵

Scanning microscopy

After washing, the eggs were fixed for 2 hours at 4°C in glutaraldehyde in 0.1M 7.2 pH phosphate buffer. They were then washed in 7.2 pH phosphate buffer at 4°C after fixing, for 1 hour in a 1% osmium tetroxide solution in a 7.2 pH phosphate buffer at 4°C . They were washed in a phosphate buffer and dehydrated in an alcoholic series (30%, 50%, 70%, 80%, 90%, 95% and 100%), and then exposed to the air to dry overnight. They were coated with gold (Au) in an SPI ion coater for 90 seconds at 18 mAmp and at a pressure of 0.3 mbar.

Observation and photography

The chorion of the eggs was viewed and

photographed using a Hitachi scanning electron microscope (S-2500). Observations were made to determine the values for the sides of the polygonal cells from the exochorion of eggs of *L. ovallesi* fed on the blood of 7 vertebrate hosts and one-way variation analysis (ANOVA) was used.

RESULTS

No differences were observed with regard to the patterns of the exochorion of eggs of *L. ovallesi* fed on the blood of 7 vertebrate hosts (Figures 1 - 7).

The chorion had sinuous longitudinal bridges and a few transverse bridges that formed rectangular shapes. This pattern was the same for each one of the eggs of *L. ovallesi* fed on the different types of blood in the study.

Table 1 shows the average values for the sides of the polygonal cells from the exochorion of eggs of *L. ovallesi* fed on the blood of 7 vertebrate hosts. Variation analysis (g.1 6 and 37) no shows significant differences ($p = 0.316$).

DISCUSSION

The chorion of insect eggs functions as protection for the embryo from the environment, preventing desiccation and regulating gaseous interchanges. These are some of the functions associated with the structure of the chorion, which may also have a taxonomic value.⁴ Chorion patterns in sand flies have only been studied in a limited number of species due to the difficulty of

Table 1. Average values for the sides of the polygonal cells from the exochorion of eggs of *Lutzomyia ovallesi* fed on the blood of 7 vertebrate hosts

Blood source	Average (mm)
Dog	10.333
Human	12.100
Cow	11.000
Chicken	10.583
Pig	8.500
Goat	11.083
Horse	11.733

Variation analysis (g.1 6 and 37) no shows significant differences ($p = 0.316$).

Figure 1. Chorion pattern of an egg of *L. ovallesi* fed on horse blood, bar = 17.5 μ m (a); fed on dog blood, bar = 15 μ m (b) and fed on pig blood, bar = 15 μ m (c).

finding eggs in the natural state and to the difficulty of establishing laboratory colonies.^{5,7,10}

Chorion patterns on eggs of *L. ovallesi* fed on blood from 7 different hosts were within the parameters described for this species, with sinuous longitudinal bridges and a few transverse

bridges that formed rectangular shapes.^{5,7}

There were no significant differences in the average values of the polygonal cell sides of the exochorion of *L. ovallesi*.

These results would seem to indicate that blood source does not modify chorion patterns in *L. ovallesi*; the experiment should be repeated for those species where differences have been observed between different colonies.

The chorion is secreted by cells in the follicular epithelium when eggs are laid; it is viscous, allowing the egg to adhere to the substrate. When dry, the chorion assumes its characteristic patterns.^{4,8} The genital atrium of phlebotomines change shape as eggs passed through.¹³ On the other hand, the shape and size of the genital atrium in 17 species of sand flies found in Venezuela is a constant feature of individuals from the same species and differentiates them from individuals of other species.¹⁴ In the same way, spines on the genital armature can be used as a classification feature. This suggests that the parameters of chorion patterns

Figure 2. Chorion pattern of an egg of *L. ovallesi* fed on human blood, bar = 15 μm (a); fed on goat blood, bar = 15 μm (b); fed on cow blood, bar = 15 μm (c) and fed on chicken blood (d), bar = 15 μm .

are mainly the result of the shape and size of the genital atrium and spines, features dependent on genetic information in sand flies. For *Lutzomyia* spp. chorion patterns are a useful taxonomic tool.

RESUMEN

Se estudio la escultura del corion de los huevos de *Lutzomyia ovallesi* alimentadas con siete tipos de sangre utilizando microscopia de

barrido, los resultados no muestran diferencias estructuras del corion de los huevos de *L. ovallesi* alimentadas con las distintas fuentes sanguíneas estudiadas, presentando los huevos un corion con puentes longitudinales sinuosos con pocos puentes que se cruzan formando áreas rectangulares, estos resultados confirman el uso de la escultura del corion de los huevos de los flebotominos como un buen parámetro taxonómico.

REFERENCES

- 1.- WARD RD, READY P A. Chorionic sculpturing in some sandfly eggs (Diptera: Psychodidae). J Entomol 1975; 50: 127-34.
- 2.- GEBRE-MICHAEL T, LANE R P. Scanning electron microscopy of eggs of *Phlebotomus* (synphlebotomus) *martini* and *P. (Syn.) celiae* (Diptera: Phlebotominae). Parassitologia 1991; 33: 261-6.
- 3.- FAUSTO A M, MAROLI M, MAZZINI M. Ootaxonomy investigation of three sandfly species (Diptera: Psychodidae) from Italy. Parassitologia 1991; 33: 225-8.
- 4.- FAUSTO A M, MAROLI M, MAZZINI M. Ootaxonomy and eggshell ultrastructure of *Phlebotomus* sandflies. Med Vet Entomol 1992; 6: 201-8.
- 5.- FAUSTO A M, FELICIANGELI M D, MAROLI M, MAZZINI M. Ootaxonomic investigation of five *Lutzomyia* species (Diptera: Psychodidae) from Venezuela. Mem Inst Oswaldo Cruz 2001; 96: 197-204.
- 6.- ENDRIS R G, YOUNG D G, PERKINS P V. Ultrastructural comparison of eggs surface morphology of five *Lutzomyia* species (Diptera: Psychodidae). J Med Entomol 1987; 24: 412-5.
- 7.- FELICIANGELI M D, CASTEJON O C, LIMONGI J. Egg surface ultrastructure of eight New World phlebotominae sand flies species (Diptera: Psychodidae). J Med Entomol 1993; 30: 651-6.
- 8.- PÉREZ E, OGUSUKU E. Chorion patterns on eggs of *Lutzomyia* sandflies from the Peruvian Andes. Med Vet Entomol 1997; 11: 127-33.
- 9.- ZIMMERMAN J H, NEWSON H D, HOOPER G R, CHRISTENSEN H A. A comparison of the egg surface structure of six anthropophilic phlebotomine sandflies (*Lutzomyia*) with the scanning electron microscope (Diptera: Psychodidae). J Med Entomol 1977; 13: 574-9.
- 10.- ROGO L R, KOKWARO E D, MUTINGA M J, KHAMALA C P M. Differentiation of vector species of phlebotominae (Diptera: Psychodidae) in Kenya by chorionic sculpturing of their eggs. J Med Entomol 1992; 29: 1042-4.
- 11.- SIERRA D, VELEZ I D, URIBE S I. Electronic microscopy of eggs as a taxonomic parameter. Biol Dir Mal San Amb 1995; 30: 327-36.
- 12.- KILLICK-KENDRICK R, LEANEY A J, READY P D. The establishment, maintenance and productivity of laboratory colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). J Med Entomol 1977; 13: 429-40.
- 13.- TANG Y, AÑEZ N. The genital atrium as new charter for distinguishing parous and nulliparous Neotropical sandflies. Ann Trop Med Parasitol 1996; 90: 203-6.
- 14.- VALENTA D T, AÑEZ N, TANG Y, KILLICK-KENDRICK R. The genital atrium as good taxonomic character to distinguish between species of phlebotomine sandflies (Diptera: Psychodidae) from Venezuela. Ann Trop Med Parasitol 1999; 93: 389-99.

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