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Physical features and chitin content of eggs from the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*: Connection with distinct levels of resistance to desiccation

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ABSTRACT

Mosquito eggs are laid in water but freshly laid eggs are susceptible to dehydration, if their surroundings dry out at the first hours of development. During embryogenesis of different mosquito vectors the serosal cuticle, an extracellular matrix, is produced; it wraps the whole embryo and becomes part of the eggshell. This cuticle is an essential component of the egg resistance to desiccation (ERD). However, ERD is variable among species, sustaining egg viability for different periods of time. While *Aedes aegypti* eggs can survive for months in a dry environment (high ERD), those of *Anopheles aquasalis* and *Culex quinquefasciatus* in the same condition last, respectively, for one day (medium ERD) or a few hours (low ERD). Resistance to desiccation is determined by the rate of water loss, dehydration tolerance and total amount of water of a given organism. The ERD variability observed among mosquitoes probably derives from diverse traits. We quantified several attributes of whole eggs, potentially correlated with the rate of water loss: length, width, area, volume, area/volume ratio and weight. In addition, some eggshell aspects were also evaluated, such as absolute and relative weight, weight/area relationship (herein called surface density) and chitin content. Presence of chitin specifically in the serosal cuticle as well as aspects of endochorion external surface were also investigated. Three features could be related to differences on ERD levels: chitin content, directly related to ERD, the increase in the egg volume during embryogenesis and the eggshell surface density, which were both inversely related to ERD. Although data suggest that the amount of chitin in the eggshell is relevant for egg impermeability, the participation of other yet unidentified eggshell attributes must be considered in order to account for the differences in the ERD levels observed among *Ae. aegypti*, *An. aquasalis* and *Cx. quinquefasciatus*.

1. Introduction

Mosquitoes transmit pathogens that cause diverse human diseases and hence vector control has an important role to block their propagation (WHO, 2013). This is particularly relevant when no vaccines or specific drugs are available, as is the case for dengue and chikungunya viruses (Teixeira et al., 2015).

Although eggs and embryos have the potential to be a suitable control target (Beament, 1989), the egg is the least known life stage in mosquitoes. According to Hinton (1981), insect eggs can be

Abbreviations: ERD, egg resistance to desiccation.

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classified in three groups, depending on external requirements: (i) those that only necessitate oxygen, (ii) eggs that need oxygen and water and (iii) eggs requiring oxygen, water and nutrients. Mosquito eggs belong to the second group and are laid on or near the water surface. Freshly laid eggs increase in size and weight due to water uptake and are susceptible to water loss under dry conditions (Gander, 1958; Kliewer, 1961; Clements, 1992; Rezende et al., 2008).

The eggshell protects the developing embryo from biotic and abiotic stresses, and helps to maintain its water balance. Mosquito eggshell is comprised of three layers: exochorion, endochorion and serosal cuticle (Clements, 1992). Both the exochorion and endochorion are present when mosquito eggs are laid (Monnerat et al., 1999), since they are produced by female follicle cells in the ovaries during choriogenesis (Clements, 1992; Chapman, 1998) (Fig. 1A). The innermost serosal cuticle in turn is an extracellular matrix produced during the first third of mosquito embryogenesis by the extraembryonic serosa, after it completely wraps the embryo (Rezende et al., 2008; Vargas et al., 2014) (Fig. 1B and C).

Mosquito eggs desiccate and die in arid conditions before serosal cuticle formation. This cuticle therefore increases eggshell impermeability (Fig. 1C), allowing eggs to remain viable for many hours, if exposed to a dry environment during embryogenesis (Rezende et al., 2008; Goltsev et al., 2009). Apart from these commonalities, differences in the levels of resistance to desiccation among eggs from distinct mosquitoes species have been described. At the end of embryogenesis *Culex quinquefasciatus*, *Anopheles aquasalis* and *Aedes aegypti* eggs present, respectively, low, medium and high levels of resistance to desiccation since they can survive outside water for, respectively, 5, 24 and at least 72 h (Vargas et al., 2014) (Fig. 1D).

Desiccation resistance is defined as the capacity of any organism to withstand in an arid environment without loss of viability. Organisms that survive drought for longer periods are considered to bear a higher desiccation resistance. Three factors are related to desiccation resistance: rate of water loss, dehydration tolerance (the minimum body water content prior to death) and the whole water content of an organism (Hadley, 1994, Gibbs et al., 1997, Gray and Bradley, 2005).

The egg resistance to desiccation (ERD) phenomenon was previously named 'embryonic desiccation resistance', or 'EDR' (Rezende et al., 2008; Goltsev et al., 2009; Vargas et al., 2014). We revised this term due to two conceptual inaccuracies: (i) the ability to withstand desiccation is not a feature of the embryo *per se*, but rather of the egg as a whole; (ii) resistance to desiccation is present in all life stages of any organism; it is not exclusive of embryos, as 'EDR' might suggest.

The ERD differences observed in the three mosquitoes at the end of embryogenesis (Vargas et al., 2014 and Fig. 1D) can be related to particular factors of each species, like egg size, structure of the three eggshell layers and of the larval cuticle, as well as to variations in presence or amount of metabolites such as glycerol, trehalose, glycogen or triacylglycerols inside the egg (Sota and Mogi, 1992; Hadley, 1994; Sawabe and Mogi, 1999; Gray and Bradley, 2005).

As an attempt to unravel the nature of the distinct ERD levels, we compared several physical aspects of eggs and eggshells of *Ae. aegypti*, *An. aquasalis* and *Cx. quinquefasciatus*. In addition, chitin presence in the serosal cuticle and eggshell chitin content were also evaluated. Interesting and significant differences have been observed in most of the studied features, and some of them could be related to the different degrees of ERD presented by the three mosquito species.

2. Material and methods

2.1. Mosquitoes rearing, synchronous egg laying and exochorion removal

Ae. aegypti, *An. aquasalis*, and *Cx. quinquefasciatus* mosquitoes from colonies of the Laboratório de Fisiologia e Controle de Artrópodes Vetores, IOC, Fiocruz, Rio de Janeiro, RJ, Brazil were reared as previously described (Vargas et al., 2014). Briefly, immature mosquitoes developed at 26 ± 1 °C and were fed with fish or cat food. Adult mosquitoes were kept in cages at 26 ± 1 °C and fed *ad libitum* with a 10% sucrose solution. Blood meals required for egg produc-

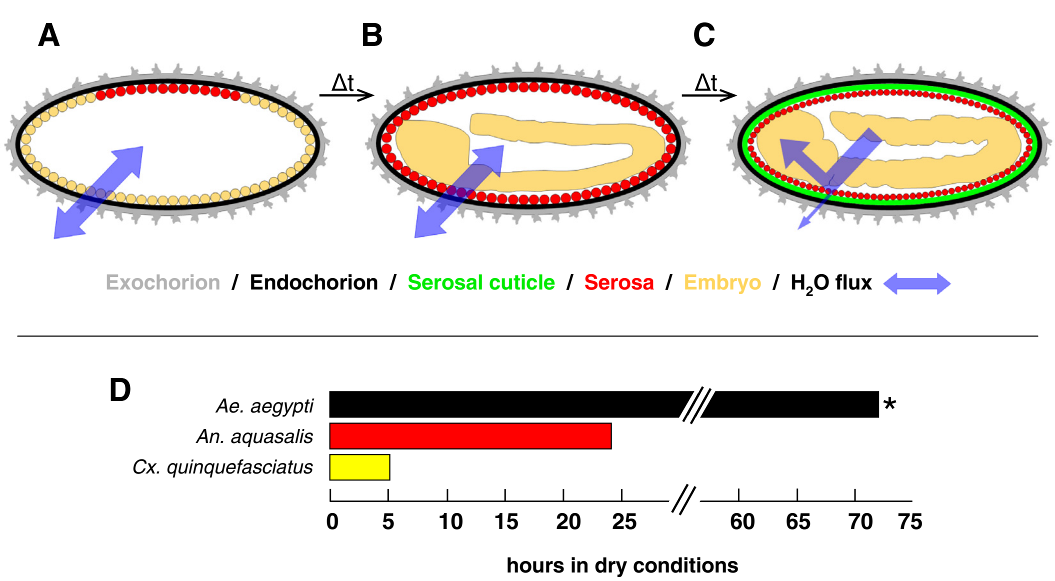

Fig. 1. Mosquito eggshell layers and egg resistance to desiccation. (A) Immediately after oviposition, eggshells are comprised of maternally produced exochorion and endochorion. (B) During embryogenesis, serosal cells surround the embryo and, subsequently, (C) secrete the serosal cuticle that considerably decreases water flow. (D) *Aedes aegypti*, *An. aquasalis* and *Cx. quinquefasciatus* eggs were transferred from water to dry conditions (20–55% relative air humidity) from 80% of complete embryogenesis on. Bars indicate the longest periods of time that eggs keep high viability. Adapted from Rezende et al. (2008), Vargas et al. (2014). *'At least', in this case: the ability of *Ae. aegypti* eggs to preserve high viability under dry conditions for periods much longer than 72 h is well-known (Kliewer, 1961; Christophers, 1960).