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When mothers anticipate: Effects of the prediapause stage on embryo development time and of maternal photoperiod on eggs of a temperate and a tropical strains of *Aedes albopictus* (Diptera: Culicidae)

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ABSTRACT

Background: The diapause of *Aedes albopictus* is maternally induced by photoperiod and initiates at the pharate larvae stage in eggs. This pre-diapause results in enhanced survival eggs during the winter. This study aims to disentangle the effects of photoperiod and diapause on embryonic developmental time and egg size in *A. albopictus*. A temperate strain capable to perform diapause and a tropical strain unable of diapause were reared at 21 °C with long-(LD) and short-day (SD) lengths. Four distinct traits were studied on embryos and eggs were measured at the end of embryogenesis.
Results: The chronologies of embryo development for both strains were influenced by maternal photoperiod, especially in the temperate strain in which the development of SD eggs took longer than LD eggs. The delay increased gradually in the temperate strain, and reached up to 38 h at the end of embryogenesis. The kinetics of embryogenesis differed among the temperate and the tropical strains, each one of the 4 studied traits showing differences. For example the serosal cuticle was secreted precociously in the tropical strain. Egg width and volume are influenced by the maternal photoperiod and the strain × photoperiod interaction. For both strains, larger eggs were laid by female reared under SD when compared to LD.
Conclusions: The influence of several maternal effects was demonstrated in this study. The diapause process modifies greatly the length of embryogenesis in the temperate strain, whereas the maternal photoperiod has a direct influence on egg size and embryogenesis regardless of the strain considered. These findings provide useful data on chronology of embryonic development for integrative biology studies of egg pre-diapause stages.

1. Background

The Asian tiger mosquito *Aedes albopictus* is an emerging model for the study of the eco-physiology of egg diapause. It is a successful invasive species now present in all continents excepted Antarctica (Bonizzoni et al., 2013). It was first introduced in tropical countries around the 18th century, in water-stock of migrants' ships, and a second wave of invasion is still ongoing in tropical areas (Delatte et al., 2011). The colonization of temperate areas began in the middle of the 20th century, through used tire traffic by ships from Asian subtropical areas (Kuno, 2012; Urbanelli et al., 2000). As it is an effective vector for many arboviruses (Gratz, 2004), this species represents a new public health threat for the US and Europe. Its role as the exclusive vector in epidemics in temperate areas has been proven for chikungunya and dengue fevers (Rezza et al., 2007; Vega-Rua et al., 2013). There is a remote risk that diapausing eggs will be infected by arboviruses (Guo et al., 2007); this could lead to the persistence of arboviruses in mosquito populations in temperate countries.

Abbreviations: HAE, hour after egg laying; LD, long daylight; SD, short daylight.

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Diapause is an adaptation and a complex physiological process defined as "a form of dormancy that is hormonally programmed in advance of its onset and is not immediately terminated in response of favorable conditions" (Denlinger and Armbruster, 2014). Diapausing individuals are indeed usually more resistant to harsh environmental conditions than non-diapausing ones. Desiccation (Sota and Mogi, 1992a; Urbanski et al., 2010a) and cold resistance are generally enhanced during diapause (Hanson and Craig, 1995) and post-diapause (Thomas et al., 2012) as for *A. albopictus* eggs. Diapause also enhances nutritive resources (Hahn and Denlinger, 2011), and even irradiation resistance (Brower, 1980) in insects. Numerous examples show that the diapause status of the progeny is determined by the exposure of ovipositing females to short photoperiod (Mousseau and Fox, 1998). The annual variation in day length is the most reliable indicator of seasonality in temperate areas. The photoperiodism, defined as the ability to determine day length with precision and to react to changes, is in most organisms a major adaptation to avoid harsh periodical environmental conditions (Danilevskii, 1965; Tauber et al., 1986). As a result it is used to initiate biological events essential to survival, such as reproduction, migration and dormancy (Danilevskii, 1965).

Egg diapause is a useful phenotype to study photo-induced maternal effects. Maternal effect is environmentally modulated transgenerational phenotypic plasticity (Mousseau and Fox, 1998). Investigating the pre-diapause process in the egg is of particular interest to elucidate the molecular process of the photo-induced maternal effects, from maternal induction to phenotypic initiation. Egg diapause is currently associated with any condition of suspended hatchability in temperate species that overwinter as cold hardy eggs. As described in Lepidoptera, egg diapause can be initiated early in the embryogenesis phase in the late gastrula stage as in the silkworm *Bombyx mori*, or at the end of the embryogenesis in the pharate larva stage, with a fully-developed larva still contained and compacted in the egg, as in *Lymantria dispar* and *Antheraea yamamai* (Denlinger and Armbruster, 2014). The Asian tiger mosquito has only one clearly defined stage of diapause, the pharate larva (Vinogradova, 2007). The changes in the eggs occurs during diapause preparation, before the initiation *sensu stricto* (Koštál, 2006), resulting in phenotypes with differences in morphology, development time and physiology. The developmental period preceding the stage of diapause initiation is frequently prolonged in insects (Denlinger, 2002; Harrat and Petit, 2009). This increased duration is linked to changes in metabolism, including protein synthesis and additional lipid storage (Denlinger, 2002).

In addition to diapause effects, photoperiod generates direct impacts on mosquito development and life history traits. For example, some larvae of *Aedes* and *Culiseta* species cannot reach maturity in the absence of light exposure (Clements, 1963), and the development time of *A. albopictus* larvae from the US is affected by the rearing photoperiod (Yee et al., 2012). Nevertheless it can be difficult to discriminate between effects of the mechanisms of a photoperiod-induced diapause and direct effects of photoperiod on organisms. It is the case for *Aedes mariae*, where diapause-programmed females preferentially seek sheltered holes in rock pools, providing them an appropriate hibernaculum against winter events like storms (Coluzzi et al., 1975). We can use tropical and temperate populations of *A. albopictus* to study this type of phenomenon in mosquitoes. Tropical strains are unable to perform diapause, contrary to temperate and subtropical strains which perform photo-induced diapause (Pumpuni, 1989). This fundamental difference between strains occurs naturally, contrary to other biological models of insects where strains must be artificially selected (Lee et al., 1997). It allows for the set-up of a discriminative comparison test of the consequences of change in daylength on the embryonic development of eggs with or without diapause.

The objective of this paper is to disentangle the effects of photoperiod and diapause on egg size and embryonic developmental time in *A. albopictus*. We predict that diapause induction in *A. albopictus* eggs will generate a prolonged embryo development sometime before the diapausing initiation. To test this prediction, we will investigate the effects of photoperiod and of the diapause syndrome by recording the size of eggs as related to an indicator of mother size (maternal wingspan), and by hourly monitoring the appearance of four features representing successive steps in the embryo development. The simultaneous study of a diapausing temperate strain and a non-diapausing tropical strain under long and short daylengths will allow us to disentangle the effects on development of the daylength experienced by the mother.

2. Methods

2.1. Ethic statement

The animal facility of the "Entente Interdépartementale pour la Démoustication du littoral méditerranéen" has received accreditation from the French Ministry of Agriculture to perform experiments on live guinea pig (permit number B34-172-29) in appliance of the French and European regulations on care and protection of Laboratory Animals.

2.2. Mosquitoes

Two strains of *A. albopictus* were used in this study. The European temperate strain named SPAM was collected in 2007 in the coastal area of Nice, France (43° 41′ 45″ N, 7° 16′ 17″ E). The tropical strain is native of La Reunion Island, located south-east of Africa near the Madagascar island, and was collected in 2011 in the coastal area of Saint-Denis Providence city (20° 52′ 44″ S, 55° 26′ 53″ E). The F16-F17 and F2-F3 maternal generations were used respectively for the temperate and tropical strains.

2.3. General rearing protocol

Mosquitoes of both strains were maintained in a laboratory room under a constant environment of 21.5 ± 0.3 °C, 80.1 ± 2.4% relative humidity, a photoperiod of 16 h of light and 8 h of darkness. Larvae were reared in batches of 500 larvae per pan (30.5 × 20 × 6 cm) in 2 l tap water and fed with 3.5 g of milled dog food during larvae development. This standardized protocol was chosen to produce an optimal expression of photoperiodic response, as it has been shown that this response is sensitive to temperature and larval diet (Pumpuni et al., 1992). After pupation, 500 pupae were placed per pan and transferred in cages in photoperiodic chambers. They were either submitted to non-diapausing long-days conditions (LD) with a light:dark cycle of 16 h:8 h, or short-days conditions (SD) inducing diapause in temperate strain with a light:dark cycle of 9 h:15 h. Photoperiodic chambers consisted of windowless plastic boxes (65 × 65 × 40 cm) with a zipper opening in black-cloth placed in the rearing room. Individual chambers were maintained at a constant temperature of 21.5 ± 0.4 °C and 79.1 ± 2.3% relative humidity, using a fan-produced air flow and a periodic air dampening system made of a water pot stirred using an aquarium air-pump. Light cycle was computer controlled and generated with a 3 watt bar of 42 white LED per photoperiodic chamber. There was no light transition between photophase and scotophase. Adult mosquitoes were left in the cages in photoperiodic chambers and were supplied with a 10% sucrose solution. The first blood meal was provided on anesthetized guinea pig 10 days after emergence, to make sure enough