DEGRADATION OF RED IMPORTED FIRE ANT (HYMENOPTERA: FORMICIDAE) YOLK SPHERES

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ABSTRACT

Transmission electron microscopy reveals that the eggs of the red imported fire ant, *Solenopsis invicta* Buren, contain yolk spheres that are evenly distributed throughout the center of the egg. Yolk degradation occurs sequentially as evidenced by the simultaneous observation of three stages of yolk platelet degradation within the eggs.

Key Words: Insecta, *Solenopsis invicta*, egg, oogenesis.

RESUMEN

La microscopía electrónica de transmisión revela que los huevos de la hormiga de fuego *Solenopsis invicta* Buren, contienen esferas de yema que se distribuyen parejamente a lo largo del centro del huevo. La observación simultánea de tres etapas de degradación en las esferas de yema dentro de los huevos evidencia que la degradación de la yema ocurre secuencialmente.

The most abundant subcellular organelles of mature oocytes in arthropods are yolk granules, constituting approximately 80% of the total protein present (Medina et al. 1988). Arthropod yolk spheres (granules or platelets) are phosphoglycolipoproteins with varying amounts of phosphorus, lipids, and carbohydrates (Yamashito & Indra-

sith 1988). Much of the yolk material consists of vitellin, a protein that is derived from vitellogenin (Kunkel & Nordin 1985; Tata & Smith 1979; Wallace 1985). Vitellin, which comprises 60-90% of the total mass of yolk proteins (Kunkel & Nordin 1985), is transported through insect haemolymph and accumulates as yolk granules in the developing oocyte by pinocytosis (Postlethwait & Giorgi 1985). The yolk protein composition of numerous arthropods, including many orders of the class Insecta, have been determined (Bownes & Hames 1977; Yamashita & Indrasith 1988). For example, yolk spheres of the fruit fly (*Drosophila*) constitute about 80% of the total protein of the mature oocyte (Vallejo et al. 1981). However, the degradation of yolk in arthropods is not a process that is well understood.

The red imported fire ant, *Solenopsis invicta* Buren, was introduced into the United States from South America around 1940 and has rapidly become a major pest in the southern U.S.A. Although much is known of its basic biology and behavior, relatively little attention has been given to oogenesis, vitellogenesis, and embryogenesis. However, one study indirectly addressing these subjects was initiated to understand the mode of action of the insect growth regulator fenoxycarb on the red imported fire ant (Glancey & Banks 1988). Fenoxycarb caused a lack of cell differentiation involving oocytes, trophocytes, and follicular epithelial cells. In addition, because nurse cells did not develop, oocytes did not contain the necessary yolk spheres. Therefore, eggs did not develop, and the colony died due to lack of worker replacement (Glancey & Banks 1988). In that study, the sequestration of yolk in untreated eggs and the lack of yolk spheres in treated eggs with concomitant egg reabsorption were clearly displayed. A review of the literature reveals that study as the only one portraying yolk sphere formation in oocytes of the red imported fire ant. Our paper, herein, is the first to describe the appearance of naturally occurring yolk degradation in fertilized eggs of ants, and in particular, of the red imported fire ant.

Materials and Methods

Red imported fire ant colonies were collected from Abilene and Victoria, Texas, in the spring of 1991 and maintained at approximately 28°C. Oviposited eggs were removed from colonies with a camel hair brush after the ants were immobilized by carbon dioxide. Samples were fixed for three hours at room temperature in 2% glutaraldehyde, 0.1 M phosphate buffer (pH 7.0) and several drops of the wetting agent Triton-X. Eggs were subsequently washed in 0.1 M phosphate buffer (pH 7.0) and post-fixed with osmium tetroxide. Eggs were next washed in 0.1 M phosphate buffer (pH 7.0) and dehydrated through a graded ethanol series. To remove the ethanol, specimens were rinsed twice with 100% acetone and stored over a dehydrating material ($CuSO_4$). Eggs were embedded in Spurr's low viscosity medium (Spurr 1969). Sorvall MT-2B and glass knives were used for ultramicrotomy. Sections were stained with methanolic uranyl acetate and lead citrate, and micrographs were taken using a Hitachi HS-9 transmission electron microscope.

Results and Discussion

Electron micrographs showed the eggs to be filled with large and dense storage organelles (yolk spheres). Yolk platelets were distributed evenly throughout the center of eggs, whereas the periphery of eggs were relatively free of yolk granules (Fig. 1B). However, early embryonic cells were located at the periphery of the egg near the vitelline membrane (Fig. 1A and B). The yolk is thus somewhat initially separated from tissue of the developing embryo. We observed three categories of yolk granules: 1)

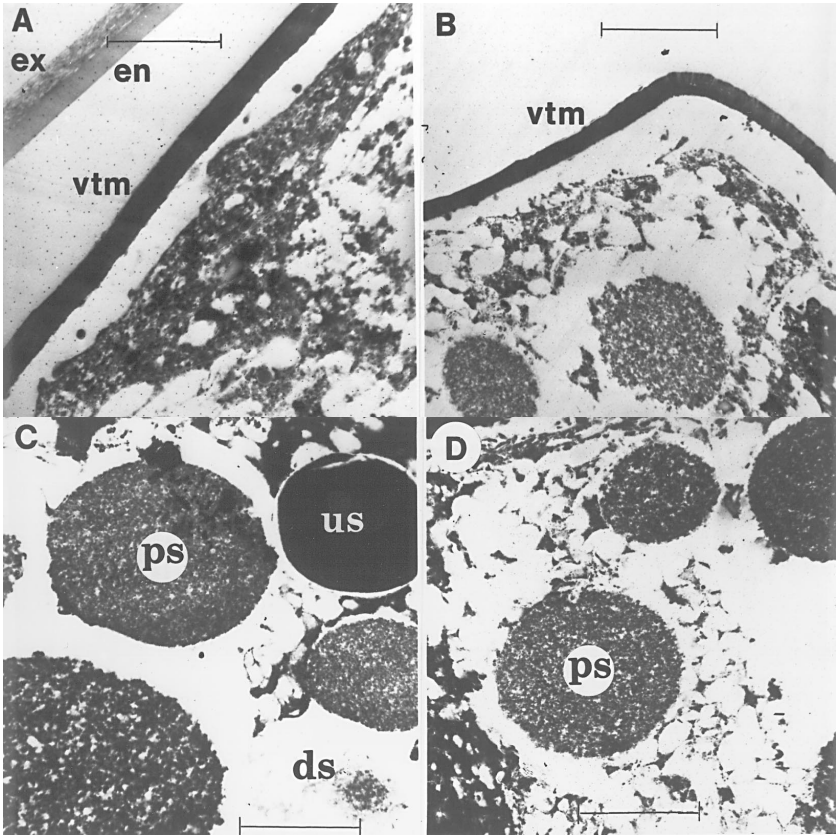

Figure 1. Transmission electron micrographs of cross sections of red imported fire ant eggs showing sequential degradation of yolk spheres: us = undegraded yolk spheres; ps = partially degraded yolk spheres; ds = degraded yolk spheres; ex = exochorion; en = endochorion; vtm = vitellin membrane (Fig. 1A, bar = 36 µm; Fig. 1B, bar = 50 µm; Fig 1C, bar = 52 µm; Fig. 1D, bar = 67 µm).

large, membrane bound, undegraded dense yolk spheres; and 2) partially degraded and 3) degraded yolk spheres (Fig. 1C). Not all yolk platelets in red imported fire ant eggs are degraded at the same time, but rather, yolk sphere utilization proceeds sequentially.

Our cytological observations of yolk degradation (Fig. 1C) in red imported fire ant eggs support earlier studies showing sequential yolk degradation in other arthropods. For example, proteinase activity is involved in yolk granule organelle degradation during embryogenesis in *Drosophila*, and this activity is developmentally regulated (Medina et al. 1988). Fagotto (1991) states that yolk spheres of the eggs of an African soft tick (*Ornithodoros moubata*) are dense and neutral and, that during later stages, these spheres are degraded through acidification. Furthermore, acidic protease activity is responsible for yolk degradation in the migratory locust, *Locusta migratoria* (McGregor & Loughton 1974). Although Glancey & Banks (1988) observed less yolk during reabsorption of developing oocytes in red imported fire ant queens after treat-

ment with an insect growth regulator, proteinase is inactive during those stages of egg formation. Therefore, normal yolk degradation would not be observable. Proteinase is only activated in the egg subsequent to fertilization (Medina et al. 1988).

Although common mechanisms of yolk degradation may exist among evolutionarily distant species (Fagotto 1991), the assumption should not be made that these degradation mechanisms are applicable to other systems (Yamashita & Indrasith 1988). However, the general mechanism for yolk degradation in many insects has been shown to be sequentially controlled (Yamamoto & Takahashi 1993), and proteins within these yolk spheres are differentially hydrolyzed.

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