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SHORT COMMUNICATIONS

A SCANNING ELECTRON MICROSCOPE STUDY OF THE BLUE REFLECTING PARTICLES IN *ENALLAGMA CIVILE* (HAGEN) (ZYGOPTERA: COENAGRIONIDAE)[1]

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The size distribution and physical arrangement in hypodermal cells of the reflecting particles responsible for the Tyndall-blue coloration of *E. civile* was compared for males and females. They are shown to be at least temporarily continuous with the endoplasmic reticulum. Micrographs are provided that suggest that these particles may indeed be composed of subparticles.

INTRODUCTION

MASON (1923, 1926) postulated that the blue coloration found in *Enallagma* was caused by very small particles, less than 0.5μ, reflecting only a select subset of those wavelengths comprising visible light. This phenomenon is referred to as the Tyndall effect. The wavelengths reflected are a function of reflecting particle size and the refractive index of both the particles and the medium which surrounds them. He suggested that these particles resided in the cuticle and that a pigment layer, which absorbed all visible light except that within the blue spectrum, underlaid these particles. BECKER (1941a, 1941b, 1942) noted that many blue and green odonates have light-scattering particles in their hypodermal cells overlying a layer of ommochrome pigment-containing bodies. This description corresponds most closely to the color producing system we have observed in *Enallagma*.

One of the most detailed studies of the ultra-structure of color producing

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cells in odonates was concerned primarily with documenting the presence, and operation, of chromatophores, i.e. hypodermal cells capable of producing physiological color change (VERON, O'FARRELL & DIXON; 1974). This study used transmission electron microscopic techniques and concentrated on Australian Odonata. We here present a scanning electron microscope study of a member of the most speciose genus, *Enallagma*, of zygopterans in North America.

MATERIAL AND METHODS

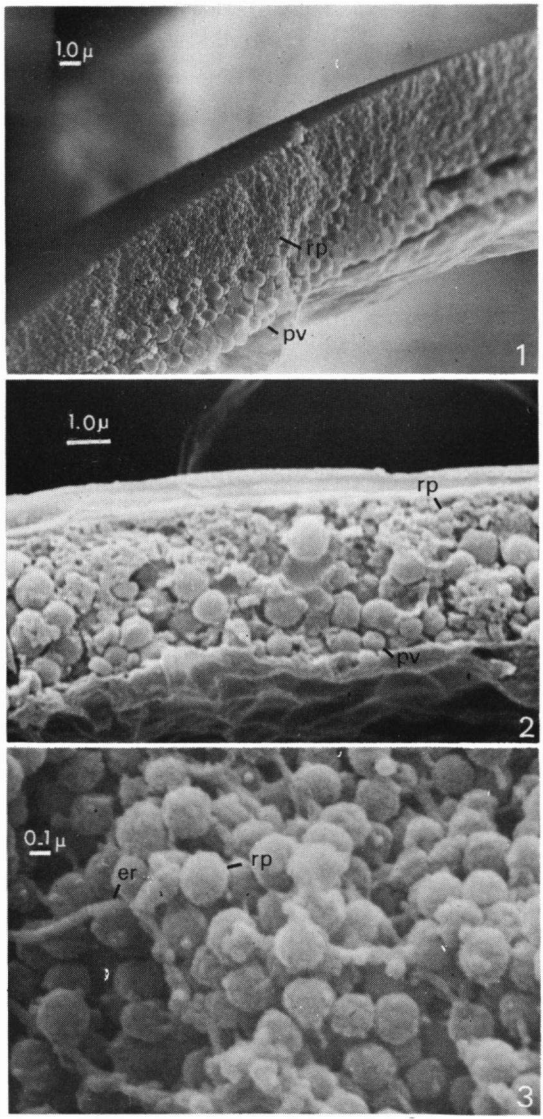
Specimens of *E. civile* were collected during September and October 1980 from a slow moving creek, Trading House Creek, on The University of Texas at Arlington campus, in Tarrant County, Texas. Mature individuals were sacrificed by immersing them in plastic vials containing a two percent solution of glutaraldehyde that was phosphate buffered (pH - 7.4). Following trial fixations of 1-8 hours, a glutaraldehyde fixation period of two hours was chosen as sufficiently effective for whole specimen fixations. After glutaraldehyde fixation, the organisms were rinsed with the phosphate buffer and allowed to fix in one percent osmium tetroxide. A stock laboratory solution of four percent OsO_4 was diluted to proper concentration by a phosphate buffer. Following one hour of fixation in this OsO_4 solution the specimens were again rinsed. Specimens were then dehydrated through stages of sequentially higher concentrations of ethanol, which ended at 100% ethanol. The individuals were freeze-fractured in small cylinders containing liquid N_2. The collected pieces of each individual were then critically-point dried.

The pieces were coated with gold-palladium using a Hummer Jr. sputter coater. Then each specimen was examined at 15-30 KV using a JEOL-35 scanning electron microscope.

RESULTS

Male *E. civile* have very tightly packed arrangements of reflecting particles and pigment vesicles underlying the blue regions of their abdomen (Fig. 1). The cells have a cross section of approximately 7μ and contain reflective particles in their distal 4μ, the remaining 3μ being packed with pigment vesicles. A random sample of the reflecting particles was measured and yielded a mean diameter of 0.225μ with a standard deviation of 0.015μ. The pigment vesicles are less spherical but are approximately five times as large. The interface between pigment vesicles and reflecting spheres is quite regular and approximately straight in blue regions. This interface is more undulatory under black abdominal regions.

Females have hypodermal cells having cross sectional diameters of approximately 3.5μ. Rust brown females have quite irregular groupings of pigment vesicles and reflecting particles in hypodermal cells underlying their brown regions (Fig. 2). These cells appear to contain a higher proportion of pigment vesicles than corresponding male cells. Blue females, in contrast, have a higher proportion of reflective particles and the pigment vesicles are primarily confined to proximal portions of the cell. While the pigment vesicles in females were comparably sized to those in males, their reflective particles had



Figs. 1-3. *Enallagma civile* (Hag.): (1) Cross section of a hypodermal cell obtained from an abdominal blue region of a male; — (2) Cross section of a hypodermal cell (overlaid by cuticle) obtained from an abdominal brown region of a female; — (3) A close up view of the reflecting particle region obtained from a male. — (*rp* = reflecting particles; — *pv* = pigment vesicles; — *er* = endoplasmic reticulum).

a mean diameter of 0.347μ with a standard deviation of 0.039μ.

The reflecting particle region of the hypodermal cell is extensively traversed by endoplasmic reticulum which is apparently connected with these particles (Fig. 3). Close inspection indicates that many small particles (perhaps nascent reflecting particles) are attached to this endoplasmic reticulum. The reflecting particles themselves have evaginations, suggesting that they have a subparticulate composition.

DISCUSSION

Adult species of *Enallagma* may be separated into two major color categories: those that are bright blue and those that have yellow or orange markings amongst black (NEEDHAM & HEYWARD, 1929). *E. civile* is included within the blue group. In addition, *E. civile* is sexually dimorphic in coloration and collections generally yield mature females that are either blue or rust brown. This dichromatism may be similar to that found in *Ischnura* (WAL-

KER, 1953; JOHNSON, 1972), or may be the result of physiological color change. MAY (1976) found that male *E. civile* coloration did not respond to temperature changes, but did not test females. Our results also do not address this question for females, but do suggest that the brown color can be attributed to the sporadic arrangement of pigment vesicles and reflecting spheres in their hypodermal cells. This arrangement corresponds well visually to micrographs of *Austrolestes annulosus* in its temperature influenced dark phase (VERON, O'FARRELL & DIXON, 1974).

Our results (Fig. 3) also indicate that the physical composition of the reflecting bodies consists of a complex of subparticles. Previous research by VERON, O'FARRELL & DIXON (1974), suggested that the reflecting bodies consisted of a walled central core surround by an, "....electron-lucent space which is often transversed by varying numbers of radiating electron-dense lines, perhaps indicating fracture planes in solid crystalline material".

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