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Comparative morphology of the pyloric armature of adult mosquitoes (Diptera: Culicidae)

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ABSTRACT

The structure of the pyloric armature, hypothesized to aid in blood-meal digestion or parasite resistance, was compared quantitatively among the following 8 species in 5 genera of adult mosquitoes from the southeastern United States: *Aedes albopictus*, *Aedes japonicus*, *Aedes triseriatus*, *Anopheles punctipennis*, *Culex pipiens s.l.*, *Culex restuans*, *Orthopodomyia signifera*, and *Toxorhynchites rutilus*. Females differed significantly among species in the structure of spines composing the armature, with *Aedes* spp. forming one general group, *Culex* spp. another, and *An. punctipennis* and *Or. signifera* a third. Relationships of species based on structural characters of the armature were consistent with recent culicid phylogenies. Although pyloric armature has been noted in mosquitoes and other insects, this is the first quantitative investigation of the mosquito pyloric armature.

1. Introduction

Chitinous spines, also called spicules or microspines, are borne on the cuticular gut lining of the cibariuim, pharynx, and pylorus of arthropods, such as the larvae of Simuliidae and Lepidoptera and the adults of Ephemeroptera, Diplopoda, and phlebotomine Psychodidae (Trembley, 1951; Byers and Bond, 1971; Christensen et al., 1971; McGreevy et al., 1978; Elzinga, 1998; Kim and Adler, 2009).

The pyloric armature of adult mosquitoes is a collection of posteriorly directed spines posterior to the pyloric valve (Richins, 1938; Trembley, 1951). It has received scant attention, relative to the cibarial and pharyngeal armature. Although species differences have been reported anecdotally (Trembley, 1951; Vaughan et al., 1991), quantitative analyses are lacking. Eysell (1905) called the spines of the pyloric armature "Chitin-Nadeln" and noted they "projected downward" and were arranged in "regular" formation, possibly referring to rows. Thompson (1905) described the "ileocolon" (i.e., pylorus) as a pumping apparatus "roughened by bristle-like chitinous papillae which point caudad," and described the armature as a "hirsute belt." De Boissezon (1930) referred to the armature as "poils chitineux hérissés." Richins (1938) described the pylorus as having "rough spines projecting caudad into the lumen," while Snodgrass (1959) stated that "the inner wall of the pyloric funnel is armed in some species with numerous small spines directed posteriorly," and Christophers (1960) reported "a fine cuticular lining which carries backwardly projecting spinous processes." Christophers (1960) also noted "the spines are not unlike those seen on the larval cuticle in some situations, namely a thorn-like apex which is continued into from four to six fine spines projecting in a horizontal plane." Trembley (1951), who presented the only light-microscope photographs, found "pyloric spines" of 6–16 μm in "irregular rows" that changed from "fine and comblike" to "heavier," anterior to posterior, in *Aedes aegypti*, and reported pyloric spines in both sexes of nine additional species. Two scanning electron micrographs of the spines of *Ae. aegypti* were published as part of a larger study of the gut (Dapples and Lea, 1974).

The pyloric armature might aid mechanical filtering and concentrating of host erythrocytes from serum, and its structure might vary with size and shape of erythrocytes (Vaughan et al., 1991; Lyimo and Ferguson, 2009). The armature, in combination with peristalsis of the pylorus, also might aid in hemolysis of host blood cells (Vaughan et al., 1991), a function attributed to the cibarial armature (Coluzzi et al., 1982; Chadee et al., 1996). The foregut armature shreds filarial nematodes (e.g., *Wuchereria bancrofti*) ingested in mosquito blood meals (McGreevy et al., 1978). Accordingly, the pyloric armature might aid in killing L1 larvae of *Dirofilaria* spp. These larvae move into the Malpighian tubules

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through openings in the pyloric valve (J. McCall, pers. comm.) – a strategy different from that of other filarioid nematodes that cross the midgut into the hemocoel (Macdonald and Ramachandran, 1965).

A quantitative understanding of pyloric armature potentially can elucidate mechanisms responsible for vector competence and host choice, and aid taxonomy and phylogenetic inference. Our objective was to compare the pyloric armature of adult mosquitoes representing five genera and eight species to test the hypothesis that species differ significantly in spine structure.

2. Materials and methods

2.1. Collection and preparation

Mosquitoes were obtained June–September 2009 with gravid and light traps at the Greenville (34°50.58′N 82°23.24′W, Greenville Co.) and Riverbanks (34°00.58′N 81°04.56′W, Richland Co.) zoos and April–May 2011 in Clemson (34°39.18′N 82°50.03′W, Pickens Co.), South Carolina. After identification, zoo samples were stored at −20 °C before dissection, while Clemson samples were dissected fresh. Frozen mosquitoes were rehydrated for 1–3 days in a 10% Alconox® solution in a refrigerator before dissection.

Photographs and measurements were taken of the pyloric armature of four males and females each of *Aedes albopictus* (Skuse), five females of *Aedes japonicus* Yamada, two males and females each of *Aedes triseriatus* (Say), three females of *Anopheles punctipennis* (Say), four females of *Culex pipiens s.l.* Linnaeus, five females of *Culex restuans* Theobald, one female of *Orthopodomyia signifera* (Coquillett), and one female of *Toxorhynchites rutilus* (Coquillett). All images are deposited on a CD, with voucher specimens, in the Clemson University Arthropod Collection, Clemson, South Carolina.

2.2. Dissections

Each mosquito was oriented laterally in a drop of phosphate-buffered saline and held with a pin through the thorax. The eighth abdominal segment was pinched with forceps, and the gut was removed by pulling the forceps posteriorly. The gut and attached eighth abdominal segment were dragged into a drop of 10% KOH and cleared for 3–4 h at room temperature, with solution added to compensate for evaporation. The gut then was dragged by the eighth abdominal segment into a drop of 50% acetic acid on the slide. The posterior abdomen was severed from the gut and a coverslip applied to the drop with the gut, resulting in either an anterior to posterior view of the pylorus interior or a lateral view of the pylorus exterior (Fig. 1).

2.3. Terminology

Because “spines” is the term most commonly used in the literature (e.g., Trembley, 1951; Vaughan et al., 1991), we use this term to describe the individual spiculate projections of the cuticular intima in the mosquito pylorus. Each spine consists of a “pedicel” and one or more “teeth.” The “base” is where the spine originates anteriorly in the pyloric intima, and the “apex” is the distal tip of each tooth. The pedicel meets the teeth at the “junction.”

2.4. Images and measurements

Pyloric armature was viewed and photographed under phase-contrast at 50×, 125×, 250×, 500×, and 1250× with a Jenoptik camera (ProgRes Speed XT core 5) on an Olympus BH-2 compound microscope. The following measurements were made on photographs of the armature in the ImageJ software program (Abràmoff et al., 2004): length of the pylorus, junction width, pedicel width, pedicel length, tooth length, and number of teeth (Fig. 2). Measurements were taken for up to five spines per specimen in each of the first (anterior) and second (middle) third of the pylorus. Distances between adjacent spine bases were measured throughout the pylorus (Fig. 3).

Anterior and middle spines were scored, when clearly visible, for whether 1) the line, or junction, where the teeth met the spine pedicel was straight (teeth flush) or irregular (variation in teeth attachment line); 2) the teeth were barbed (i.e., flared at the apex like a spearhead) or unbarbed; and 3) the spines were pointed and closed (i.e., base of spine coming to a complete point), pointed and open (i.e., base approaching a point but not complete), or truncate (i.e., no noticeable point) (Fig. 3). Pylori were scored for whether 1) spines in the pylorus were sparse (distance between spines $>$ one spine width), regular (distance between spines $\leq$ one spine width, but not overlapping), or dense (overlapping spines); and 2) spines were or were not in horizontal rows.

Posterior spines generally were less elaborate than those in the anterior and middle regions, often having only two teeth or being toothless spicules. Posterior spines, therefore, were not compared among species.

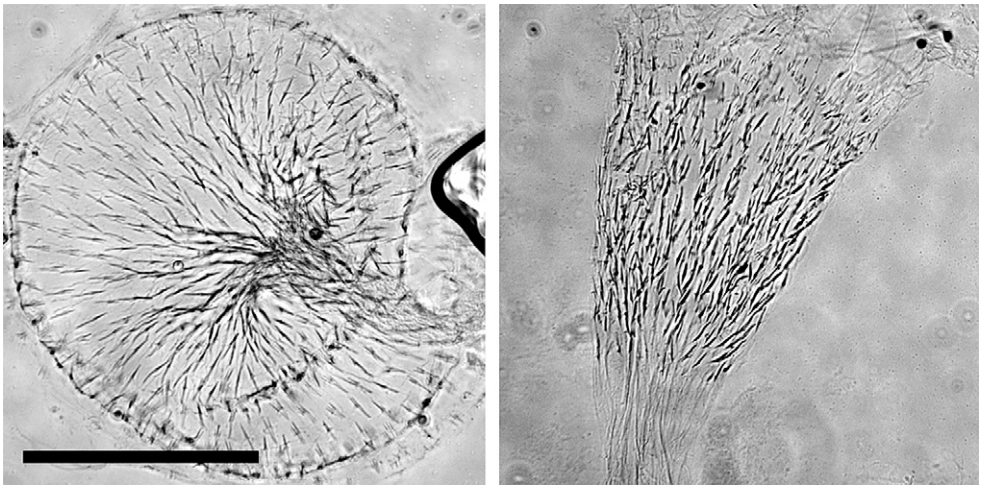

Fig. 1. Two views (phase-contrast) of slide-mounted pylori from females of *Cx. pipiens s.l.* Left: anterior to posterior view of the pylorus interior; right: lateral view of the pylorus exterior. Scale bar = 100 um.