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Antennal Hair Erection in Male Mosquitoes: A New Mechanical Effector in Insects

Abstract. *Male* Anopheles *mosquitoes erect their antennal hairs prior to mating. The erectile mechanism resides in a unique annulus at the base of each hair whorl. It appears that the insect regulates the degree of hydration of this annulus. When the annulus is made to swell the attached hairs are pushed to their erect position.*

Male mosquitoes are sexually attracted to the flight sound of the female. The long fibrillae (hairs) on a male's antennae are set in resonant vibration by the female's hum. This vibration, transmitted via the shaft of the antenna, stimulates receptor neurones (scolopidia) in the bulbous antennal base (*1*). Sound can only be perceived when the antennal hairs stand nearly perpendicular to the antennal shaft. In many species of mosquitoes the antennal hairs of the male are recumbent against the antennal shaft during most of the day and night and are erected only for a few hours, usually about dusk. Mating is limited to the period of antennal hair erection since this is the only time that males can perceive the females (*1-3*). Erection of each whorl of hairs is under direct neural control (*3*). We report here the structure and properties of the effector mechanism that erects the antennal hairs of *Anopheles stephensi*. This mechanism is of a type heretofore unknown in animals but similar to that used by *Mimosa*, Venus's-flytrap, and certain grasses to fold their leaves (*4*).

Antennal hairs are arranged in 12 evenly spaced whorls along the length of the antenna. Each whorl is attached to the periphery of a doughnut-shaped annulus that is, in turn, suspended from the antennal shaft by a stiff chitinous flange (Fig. 1). The annulus stains intensely blue with methylene blue at pH 7. In addition, it stains a very pale blue with Mallory's trichrome. These staining reactions differ radically from those of chitin and from those of the antennal cuticle; they indicate that the annulus is rich in basophilic protein (*5*).

A deep slit runs along the distal face of the annulus. In antennae with recumbent hairs this slit is tightly closed (Fig. 1A), whereas in antennae with erect hairs the slit is widely agape (Fig. 1B). Measurements on sections of 35 antennae with hairs erected to various degrees revealed a direct proportionality between the angle of gape of the annulus and the angle between the hair and the antennal shaft.

Three types of cells are present in the space immediately below each hair whorl. A tormogen cell and the cell body of a sensory neuron occur at the base of each hair (*6*) (Fig. 1D). In addition, large irregular cells are attached to the entire inner surface of the annulus. The appear-

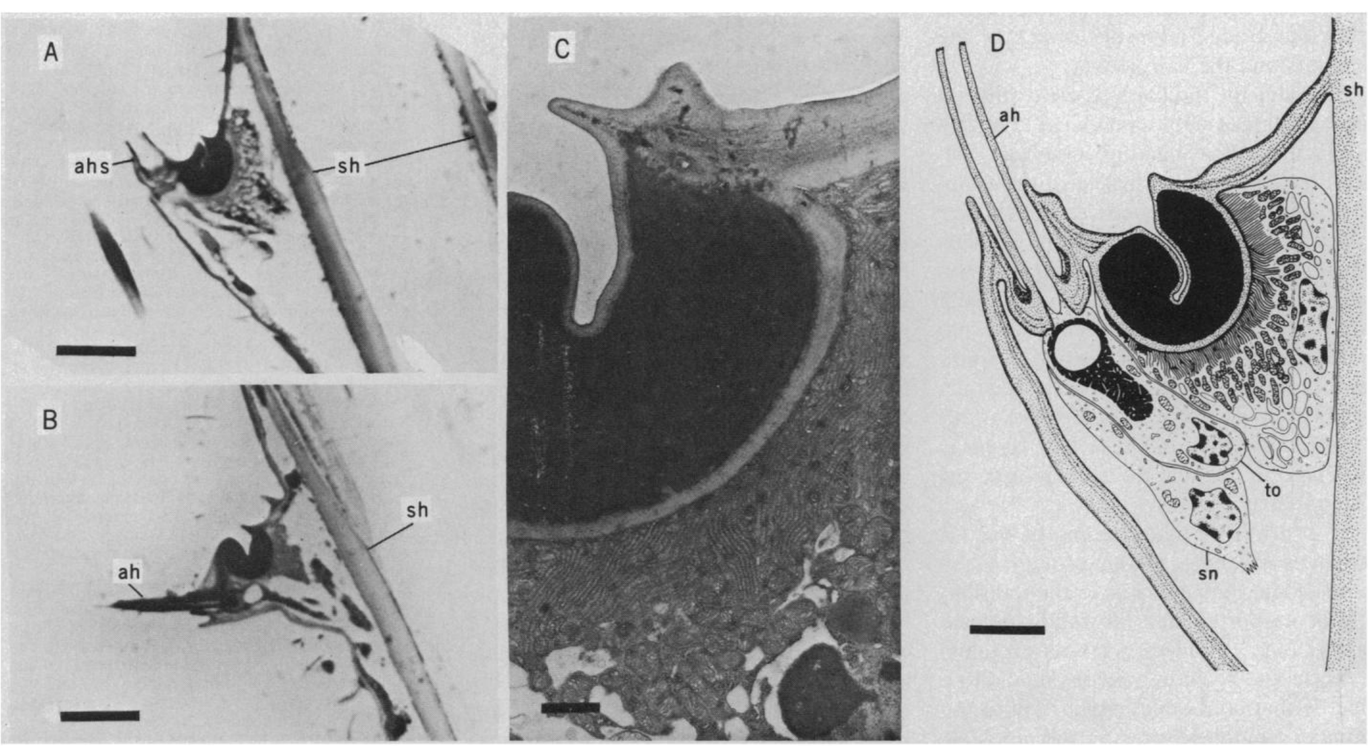

Fig. 1. (A) Thin section through one hair whorl of an antenna with recumbent hairs. (B) Section through antenna with erect hairs. Note that slit in dark-staining object (annulus) at base of hair is now open. Scale bar, 10 μm. (C). Electron micrograph of a portion of annulus and its subtending cell. Scale bar, 1 μm. (D) Diagrammatic reconstruction, based on electron micrographs, of the erectile mechanism of an antennal hair. Scale bar, approximately 5 μm. Abbreviations: *ah*, antennal hair; *ahs*, hair socket; *sh* skeleton of antennal shaft; *sn*, cell body of sensory neuron of hair; and *to*, tormogen cell of hair.

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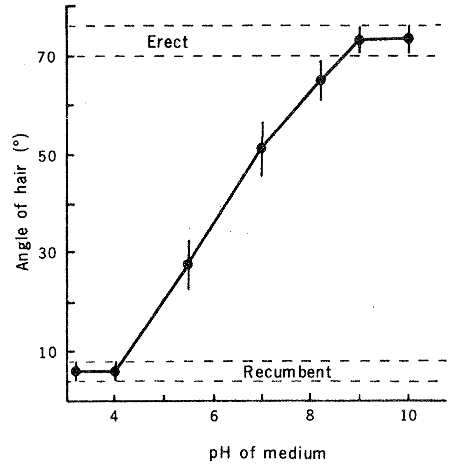

Fig. 2. Effect of pH on erection of hairs on dead antennae. Ordinate indicates angle between hair and antennal shaft. Regions between dashed lines are ranges of angles found in antennae with fully erect or fully recumbent hairs. The bars indicate the entire range of angles measured after incubation for 1 hour at each pH.

ance of these subtending cells changes markedly in the course of hair erection. In antennae with recumbent hairs their cytoplasm appears heavily vacuolated, whereas vacuoles are virtually absent when hairs are erect (Fig. 1, A and B). We believe that the annulus in conjunction with its subtending cells constitute the effector mechanism for hair erection.

Electron microscopy reveals several functionally significant features of this system. The apical surfaces of the cells that subtend the annulus bear numerous closely packed microvilli. Immediately beneath the microvilli occurs a layer of densely packed mitochondria (Fig. 1, C and D). The annulus itself is not homogeneous in structure but is composed of a thin (0.1 to 0.4 μm) electron-lucent cortex around a massive electron-dense medulla (Fig. 1, C and D). No substructure was discernible in either cortex or medulla at magnifications up to 40,000 diameters. The cortex is continuous with and has the same electron-density as the cuticle of the antenna. A short, thick, cuticular bridge joins the cortex of the annulus and the hair socket.

In order for the hairs to erect, the slit in the annulus must open wide. For this to occur without kinking or severely deforming the convex surface of the annulus, one of two processes must take place. Either the volume of the annulus must increase while its perimeter remains constant, or the perimeter must shrink while the volume remains constant. Differentiation of the annulus into a thin cortex and a massive medulla lends support to the hypothesis that differential swelling of these two layers is involved in opening the slit and erecting the antennal hairs.

It is probable that opening of the slit involves swelling of the proteinaceous core of the annulus, rather than shrinking of its cortex, for the following reasons. Ionic polymers (such as proteins) hydrate and swell in aqueous media to a degree that is in direct proportion to the number of ionized sites on the molecule (*7*). Swelling is minimal at the isoelectric point and increases as the pH rises above or falls below the isoelectric point. The basophilic (cationic) character of the annulus has already been noted and suggests an isoelectric point below pH 7. We measured the effect of pH on hair erection in isolated antennae that had been air-dried and kept either frozen or at room temperature for several weeks (*8*). We found a continuous, smooth relationship between pH and the angle to which hairs were erected (Fig. 2). At pH 4.5 hairs were fully recumbent, whereas at pH 8.5 they were fully erect with intermediate degrees of erection at intermediate values of pH. The constant slope of the curve in Fig. 2 indicates that the isoelectric point of the material of the annulus lies at or below pH 4.5 (*9*).

We conclude, therefore, that the annulus swells as pH increases (*10*). Whether or not the pH of the annulus changes in the course of natural hair erection we cannot ascertain, but the large cells that subtend the annulus seem ideally suited to effect such a change. Ultrastructural evidence indicates that these cells are specialized for secretion or active transport, and the range of pH over which hair erection occurs in isolated antennae certainly seems biologically reasonable (*11*). Since swelling of the medulla would require a source of water for hydration (*12*), it is noteworthy that the volume of the subtending cells decreases dramatically when the slit in the annulus opens (Fig. 1, A and B). These subtending cells embrace the entire internal surface of the annulus and, thus, must participate in any transport of material into and out of the acellular annulus.

We propose, therefore, that antennal hairs of male *Anopheles* are erected when the pH of the annulus is raised by some activity of the subtending cells. Increased pH leads to hydration and swelling of the medulla of the annulus, forcing the slit in the annulus to open. Since the proximal portion of the annulus is anchored to the antennal shaft, opening of the slit will cause the distal portion of the annulus to rotate outward. This rotation, through a direct mechanical link, causes the hair to swing to its erect position.

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5. Antennal cuticle, including the flanges that support the annulus, stained a pale blue with methylene blue and exhibited the typical red color of chitinous cuticle with Mallory's stain. We examined the possibility that the substance of the annulus might be resilin. We found that the annulus did not exhibit the characteristic fluorescence at 420 nm of resilin. Also, resilin characteristically stains red with Mallory's trichrome, while the annulus does not.
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8. Isolated antennae were submerged in 15 m*M* buffer with 0.02 percent Triton-X for 1 hour, after which the angles between the antennal shaft and hairs of the third and fourth whorls were measured. Antennae used in these experiments were dead. Their response to pH, therefore, reflects the chemical and mechanical behavior of the acellular parts alone. By changing the pH of the bathing medium, dead antennae can be made to move their hairs alternately between fully erect and recumbent positions indefinitely. Only changes in pH evoke this response in dead antennae. Alteration of ionic strength (with NaCl) or osmolarity (with sucrose) of the medium at pH 7 had no effect on the position of the antennal hairs.
9. Typical isoelectric points of insect cuticular proteins are pH 3.4 to 5.7 [A. C. Neville, *Biology of the Arthropod Cuticle* (Springer-Verlag, New York, 1975), p. 351; R. H. Hackman, *Comp. Biochem. Physiol. B* **49**, 457 (1974).
10. The state of hydration of *Rhodnius prolixus* cuticle changes little between pH 6 and 10 (*11*). The cortex of the annulus is continuous with and structurally indistinguishable from the cuticle. If we assumed that the physical properties of the cortex too are like those of cuticle then we would indeed have a nonswelling perimeter for the annulus.
11. S. E. Reynolds [*J. Exp. Biol.* **62**, 81 (1975)] has shown that the structurally less specialized epidermal cell of *Rhodnius prolixus* can control and alter the pH and plasticity of the overlying cuticle. It is, of course, possible that factors in addition to pH come into play in vivo [but see (*8*)]. Such factors might restrict the pH range over which the erectile response occurs.
12. Using cross-sectional models of the annulus we calculate that the volume of the medulla must increase by about 25 to 30 percent to achieve the maximum observed degree of gape of the slit.
13. We thank Drs. S. Vogel and S. A. Wainwright for critical comments on the manuscript.

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