

THE PRESUMED ROLE OF WING SENSORY STRUCTURES IN THE UNIQUE MATING BEHAVIOR OF THE ENDANGERED EUROPEAN MAYFLIES *PALINGENIA LONGICAUDA* (OLIVIER) AND *PALINGENIA FULIGINOSA* (GEORGI) (INSECTA, EPHEMEROPTERA)

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Large sensilla coelaconica (sensory peg in a pit) occur on the principal concave dorsal and ventral anterior wing veins of the fore and hind wings of male imagos of *Palingenia longicauda* and *P. fuliginosa*. Since female *Palingenia* do not possess these structures this suggests that these structures are chemoreceptive and may aid males in locating females or they may aid males in their peculiar flight pattern near and in contact with the water surface (hygroreceptive?).

In the summer of 1991 on the River Tisza we video recorded the flight pattern of male imagos (often at a shutter speed of $1/4000$ second). The flight pattern of the male differed depending on the size of the swarms. At all times males fly close to the water. When females emerge the males are often on the water and the large wings have a sculling like motion that seems to indicate maximum exposure of the wing sensory structures to the area on the water just in front and to the sides of the male. It appears like the males are looking for females with their wings.

INTRODUCTION

While the diverse adult visual system of mayflies has captured the attention of many, we really have little or no knowledge of the role other senses may play in the behavior of these insects. The role of chemoreception in mate location in mayflies has received little direct study. In our study we report on the structure and ultrastructure of unique wing receptors in Palingeniidae. We also report on detailed observations of apparent male mate location behavior in *Palingenia longicauda*, which appear to support a chemosensory role of the wing receptors in mate location.

The biology of *Palingenia longicauda* has received intensive study recently and the reader is referred to the following treatises for further information on this fascinating species (RUSSEV, 1987; ANDRIKOVICS *et al.*, 1992; SARTORI *et al.*, 1995; LANDOLT *et al.*, 1995; LANDOLT *et al.*, 1997).

METHODS

Light, scanning and transmission electron microscopy were generally by standard methods. Specimens for light and scanning electron microscopy were from ethanol preserved specimens from the collections of G. Edmunds, Jr. and W.L. Peters and collected by B.K. Russev (*P. longicauda*) and T. Soldán (*P. fuliginosa*). Specimens of *P. longicauda* for transmission electron microscopy were collected into glutaraldehyde by P. Landolt and then shipped from Hungary to the US where they were further processed. We also collected specimens into ethanol and formaldehyde. We sought to determine the presence of pores in coeloc-

ic pegs in part by placing formaldehyde preserved wings in a solution of crystal violet. The penetration of the stain through the pegs would indicate the presence of a pore in the peg cuticle (SLIFER, 1960; 1977).

We observed the emergence mating behavior of *Palingenia longicauda* from June 16 to July 16, 1991 on the Tisza River at Doba, Hungary. The largest mass emergence occurred on June 27, the second largest mass emergence occurred on June 29, and fair to medium emergences occurred around these dates from June 25 to July 5. Trace to small (all or mostly males) emergences occurred around the above dates from June 16 until July 12. All emergences were video recorded with a standard consumer-grade video camcorder. Most video shooting was at $1/4000$ per second when the light was sufficient.

RESULTS

Structure of sensory receptors

The large receptors of the male imago can be seen even with the dissecting microscope at 45x total magnification. They are composed of pits approximately 10-13 μm in depth, about 13-15 μm in diameter with a central peg in the middle of the pit approximately equal in length to the depth of the pit (Figs 1A-F). These receptors are known in the literature as sensilla coelaconica (e.g. KAPOOR, 1985) and will often be referred to in the rest of this paper as the large receptors. When observing these receptors with the compound light microscope and thereby visualizing the entire surface and under surface (beneath the wing cuticle), these structures have a wine-glass shape where the pit is the «bowl of the glass» with an apparent under cuticle stem (Fig. 2). The length of the stem is

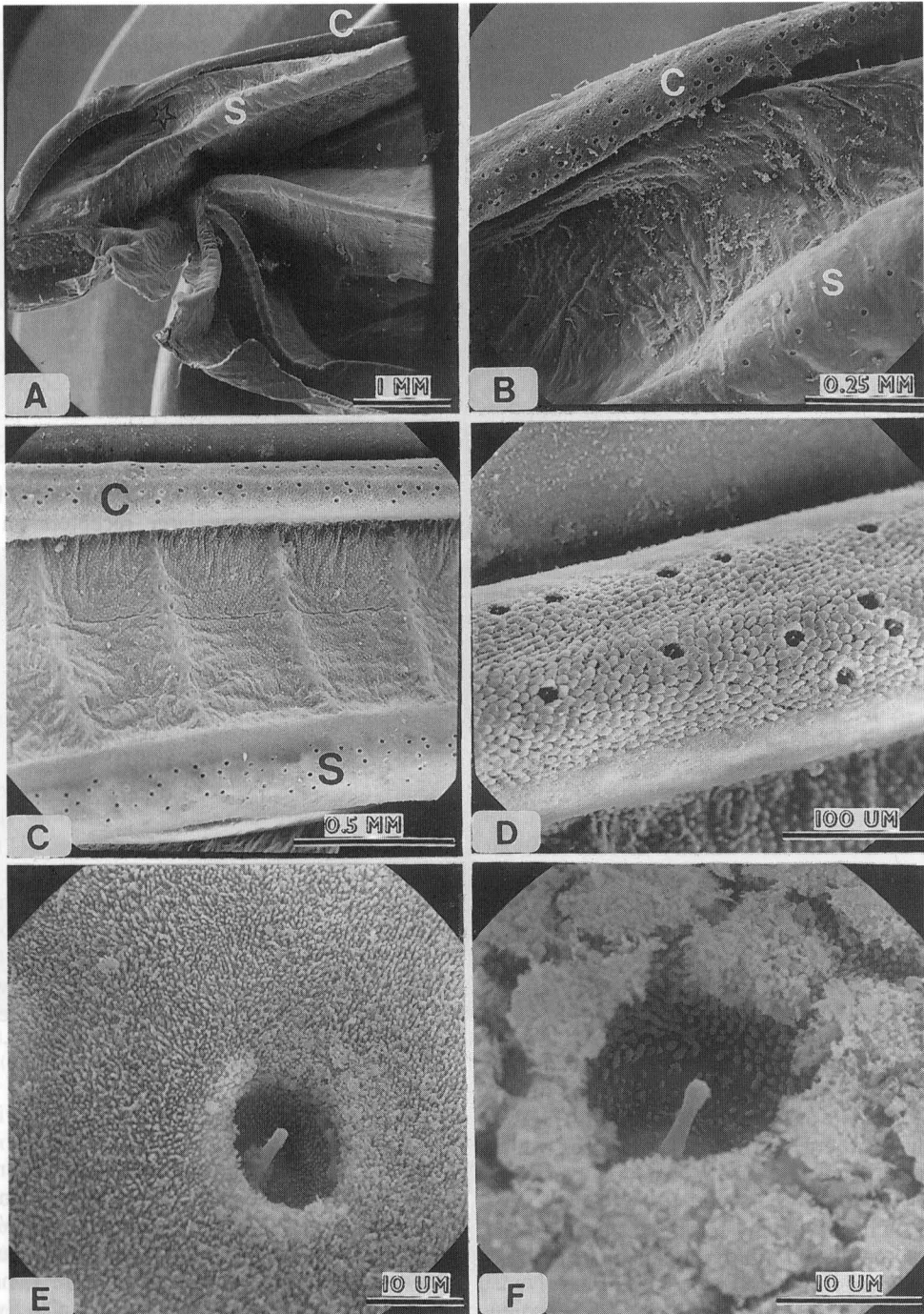


Fig. 1. Scanning electron micrographs of structures of *Palingenia* wings (abbreviations: C = costa, S = subcosta). A: Ventral view of base of left forewing from a male imago. Starred area is enlarged in B; B: Magnified view of costa and subcosta seen in A. The large sensory receptors (peg in a pit) are abundant on both veins; C: Ventral view of forewing costa and subcosta showing the large wing receptors; D: Ventral view of costa showing the large wing receptors; E: Large sensory receptor on subcosta; F: Large sensory receptor on costa.

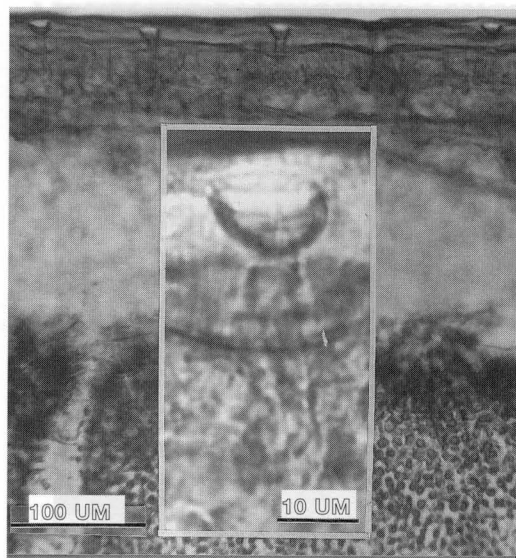


Fig. 2. Large subcostal sensory receptors from a male imago (light microscopy micrographs).

about equal to the depth of the pit. The stem is where the sensory neurons and dendrites occur. Smaller but similar receptors occur in lesser numbers on the concave surfaces of some wing veins (see distribution below). These are about half the size or less than the large receptors. Most are coeloconic pegs (peg in a pit), although the pit in some appears to be absent.

Ultrastructure of sensory receptors and innervation

The large receptors appear to be innervated, as dendrites (fine filaments in Fig. 3) appear to contact the coeloconic pegs and sensory neurons occur at the base of the stem (Fig. 3). We have not yet observed any pores which might indicate a chemosensory function. However, we have stained formaldehyde preserved wings with crystal violet and the pegs appear to take up the dye indicating the presence of pores.

Location of sensory receptors

Male: No receptors are found on the wings of the subimago male, however receptors of the imaginal wings can be seen below the sub-

imaginal wing cuticle. All concave veins of the dorsal surface of the forewing have receptors (small or large). Only the costa and radius R_1 veins have the large noticeable receptors, which occur from the base of the wing to the apex, but are most abundant in the basal region of the wing (Figs 1A-D). The other concave veins have a small number of smaller (difficult to see by compound light microscopy) receptors (as described in the structure section).

The ventral surface of the imaginal male wing is similar to the dorsal surface. Most of the large receptors on the costae are near anterior edge. The subcosta has a large number of large receptors from the base to apex of the vein. The subcosta also possesses some smaller receptors too. Other ventral concave veins possess few receptors of any kind. The male imaginal hind wing possesses many large receptors on the ventral concave surface of the subcostal vein.

Female: Females only possess small receptors and their distribution is similar to that described for the male imago.

Flight behavior of male imagos during small emergences when generally only males appear to be present

Male imago flight during small emergences is generally characterized by a patrolling flight just above the water surface. The male flies about equally up and downstream (approximately 20-40 m plus range) and at a fairly rapid speed. During these small emergences we often saw small birds which observed and then caught these patrolling males. Some fish were also seen to jump partially out of the water to catch these males.

Flight behavior of male imagos during mass emergences when many females are emerging

Male flight behavior during large emergences when many females were present, was much different than during small emergences. When females were present males greatly reduced their search range to the area where females were actively emerging. Males were also flying on the water where their caudal filaments and posterior abdomen were on the water surface, and where their posterior edge of their fore wings was barely above the water surface (fig. 4). Males would often hover in place or over

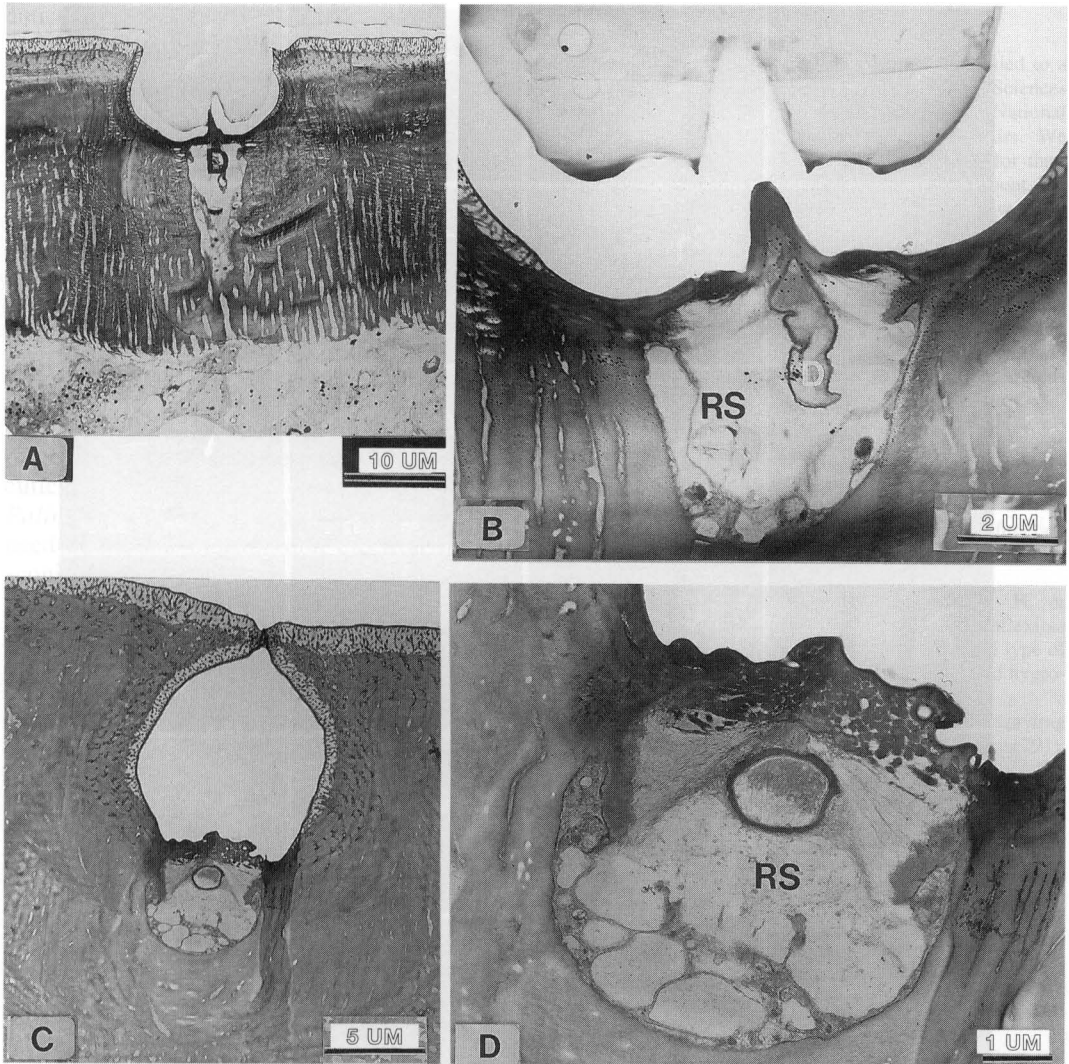


Fig. 3. Large sensory receptors including innervation and details of wing cuticle. Abbreviations: D, denrite; RS, receptors sinus (transmission electron micrographs).

relatively small areas in search of females. The motion of the male wings is best described as kind of a sculling motion where the male appears to maximize the exposure of the wings to the water surface immediately in front and to the sides of the male (Fig. 4). Males were very agile fliers and they could literally turn around on the spot.

Generally males only sought to mate with females that were still in contact with the water surface. Females that escaped the water surface were largely ignored by the frenzied males.

DISCUSSION

The presence of large coeloconic peg receptors only on male imago wings (females only possess much smaller receptors), penetration of crystal violet into pegs, and the unique flight behavior of males (especially when females are emerging) suggest that these receptors are chemosensorily involved in locating females. Further observation with scanning and transmission electron microscopy are necessary to confirm the presence of pores in the pegs. Fresh specimens specially cleaned to remove such



Fig. 4. Photographs of successive video frames of a male imago searching for females. The counter in the right bottom of each frame shows the time in hours (P.M.), minutes and seconds.

cuticular contaminating substances as waxes and debris are often necessary before pores can be observed in scanning electron microscopy (KAPOOR, 1985). Recent work of LANDOLT *et al.* (1996) showed equivalent attraction of live male imagoes to pentane washes of female adults and to intact females. Final confirmation of the potential chemosensory role of these structures awaits electrophysiological studies of these receptors.

If pores are not found after further study, then it is possible that these large receptors are hygroreceptive or thermoreceptive (see reviews in ALTNER *et al.*, 1983; ALTNER & LOFTUS, 1985). These receptors often consist of poreless and unsocketed sensilla (peg) in a cuticular pit which is a description that fits *Palingenia*'s wing receptors. However we need additional transmission electron microscopic work in order to compare the ultrastructure of *Palingenia*'s wing receptors with hygroreceptive structures described in the literature (ALTNER *et al.*, 1983; ALTNER & LOFTUS, 1985). It is possible also that the smaller coeloconic peg receptors found on both the male imago and female subimago are involved in orienting to the water surface, which seems to be especially important to the Palingeniidae.

A complete determination of what senses/structures are important in mate location for the male imago means that receptors on the whole body need to be looked for. This would include the antenna, legs, forceps (pores have been observed by us on the sensilla of the forceps in *Palingenia longicauda*) and the body surface. Vision is probably important at relatively long distances, whereas chemosensory structures, and hygroreceptive structures might be effective at short distances. In *Palingenia longicauda* males seem to intensely focus their efforts on the water surface within the sculling reach of their fore and hind wings which are studded on the principal concave wing veins with large coeloconic peg receptors. It would be interesting to determine if the portion of the compound eyes which surveys this same zone has relatively high acuity as determined by the presence of fovea, i.e. an area of the eye in which interommatidial angles between adjacent ommatidia are small.

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REFERENCES

- ALTNER, H. & LOFTUS, R. 1985. Ultrastructure and function of insect thermo- and hygroreceptors. *Ann. Rev. Entomol.* 30: 273-295.
- ALTNER, H., SCHALLER-SELZER, L., STETTER, H. & WOHLRAB, I. 1983. Poreless sensilla with inflexible sockets. A comparative study of a fundamental type of insect sensilla probably comprising thermo- and hygroreceptors. *Cell Tissue Res.* 234: 279-307.
- ANDRIKOVICS, S., FINK, T.J. & CSER, B. 1992. Tiszavirág monografia, *Palingenia longicauda* (OLIVIER, 1791). Tisza Klub Fuzetek 2: 1-35.
- KAPOOR, N.N. 1985. External morphology and distribution of the antennal sensilla of the stonefly, *Paragnetina media* (WALKER) (Plecoptera: Perlidae). *Int. J. Insect Morphol. & Embryol.* 14: 273-280.
- LANDOLT, P., SARTORI, M., ELPERS, C. & TOMKA, I. Biological studies of *Palingenia longicauda* (OLIVIER) (Ephemeroptera: Palingeniidae) in one of its last European refuges-feeding habits, ethological observations and egg structure. In: CORKUM, L.D. & CIBOROWSKI, J.H. (Eds) *Current Directions in Research on Ephemeroptera*, pp. 273-281, Canadian Scholars' Press Inc., Toronto.
- LANDOLT, P., SARTORI, M. & STUEDEMANN, D. 1997. *Palingenia longicauda* (Ephemeroptera, Palingeniidae): from mating to the larval stage. In: LANDOLT, P. & SARTORI, M. (Eds). *Ephemeroptera & Plecoptera: Biology-Ecology-Systematics*. pp. 15-20. MTL, Fribourg.
- RUSSEV, B.K. 1987. Ecology, life history and distribution of *Palingenia longicauda* (OLIVIER) (Ephemeroptera). *Tijdschrift. voor Entomol.* 130: 109-127.
- SARTORI, M., LANDOLT, P., LUBINI, V. & RUFFIEUX, L. 1995. Biological studies of *Palingenia longicauda* (OLIVIER) (Ephemeroptera: Palingeniidae) in one of its last European refuges-abiotic characteristics and description of the habitat. In: CORKUM, L.D. & CIBOROWSKI, J.H. (Eds) *Current Directions in Research on Ephemeroptera*, pp. 263-272, Canadian Scholars' Press Inc., Toronto.
- SLIFER, E.H. 1960. A rapid and sensitive method for identifying permeable areas in the body wall of insects. *Entomol. News* 71: 179-182.
- SLIFER, E.H. 1977. Sense organs on the antennal flagellum of mayflies (Ephemeroptera). *J. Morph.* 153: 355-362.