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# Diversity of olfactory structures: A comparative study of antennal sensilla in Trichoptera and Lepidoptera

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ABSTRACT

The antenna is the main sensory organ of insects, housing different types of sensilla dedicated to detect chemical cues, motion, humidity and temperature. Sensilla are divided into different types based on their wall structure and morphology. Among the olfactory sensilla, there is an enormous variation in the numbers and morphological types present in different insect taxa. The reasons for this variation remain obscure, though there may be a correlation between sensillum morphology and the characteristics of the stimulus that the olfactory sensory neurons inside the sensillum detect. Here, we report the first comparative analysis of the morphology and ultrastructure of sensilla from *Rhyacophila nubila* (Rhyacophilidae: Trichoptera) and three species of Lepidoptera, *Eriocrania semipurpurella* (Eriocraniidae), *Lampronia capitella* (Prodoxidae), and *Bicyclus anynana* (Nymphalidae), which use different chemical types of pheromones. Our results, together with a thorough literature review, suggest a shift in major types of olfactory sensilla, from a high proportion of sensilla placodea or auricillica in Trichoptera and the most basal moth lineages (including Eriocraniidae), respectively, to sensilla trichodea in the more derived Lepidoptera (including Prodoxidae and the Ditrysia clade), which parallels the change in the types of sex pheromones used.

## 1. Introduction

Olfaction plays a critical role in insects, underlying behaviors such as host-seeking, mate-finding and enemy-avoidance. The olfactory system of an adult insect consists of two pairs of main olfactory appendages on the head, the antennae and the palps. The antennae are usually covered with scales and other structures, often hair-like, called sensilla, involved in the detection of chemical, mechanical and thermal stimuli (Hansson and Stensmyr, 2011; Schneider, 1964). Based on their wall structure (i.e., pores and wall properties) and external appearance, sensilla are classified into different morphological types, e.g. trichodea, basiconica, chaetica, coeloconica, ascoidea, vesiculoclada, auricillica, placodea, styloconica, ampullacea, squamiforma, campaniforma, and Böhm's bristles (Hallberg and Hansson, 2003; Keil, 1999). Different insect species possess various subsets of these types, and in different relative abundances. Also, the different types of sensilla are used for distinctive functions, for instance sensilla trichodea, basiconica, coeloconica, vesiculoclada, auricillica, placodea and ascoidea are used for olfaction, sensilla chaetica for taste, sensilla styloconica and ampullacea for thermo- or hygroreception, and sensilla squamiforma, campaniforma, and Böhm's bristles for mechanoreception (Hallberg and Hansson, 2003). The olfactory sensilla of insects generally contain specialized structures, including multiple cuticular pores, facilitating detection of odor molecules (Schneider, 1964). The pores in the sensillum wall are connected to pore-tubules suspended in the sensillum lymph, which might possibly serve as a route for the odor molecules to reach the odorant receptors (ORs) in the dendrites of the olfactory sensory neurons (OSNs) (Carlson, 1996; Larter et al., 2016; Steinbrecht, 1996). The OSNs are responsible for the detection of different sets of odor molecules. Insect olfactory sensilla typically contain 2–3 OSNs (Andersson et al., 2009; Hallberg and Hansson, 1999; Ljungberg et al., 1993; Yuvaraj et al., 2013) but sometimes more depending on taxon (reviewed in Keil, 1999).

The number of sensilla on the antenna varies widely between species, for instance, psyllids (Sternorrhyncha: Psyllidae) may only have four olfactory sensilla (Kristoffersen et al., 2006; Yuvaraj et al., 2013), whereas moths and butterflies (Lepidoptera) may have tens of thousands sensilla, belonging to several morphological types (Steinbrecht, 1970; Ansebo et al., 2005; Hallberg and Hansson, 2003; Wee et al., 2016). The difference in antennal architecture may be the result of adaptation to different ecological niches (Hallberg and Hansson, 1999; Hansson and Stensmyr, 2011), although evidence supporting this suggestion remains elusive. The different morphology of the sensilla may also be advantageous for specific tasks. For instance, long sensilla

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trichodea can be organized to create basket-like sieves to capture sex pheromone molecules in male moths (Steinbrecht, 1996). Indeed, sensilla trichodea house the OSNs tuned to pheromones in *Drosophila* and many moth species (Ansebo et al., 2005; Clyne et al., 1997; Hallberg et al., 1994; Ljungberg et al., 1993; Pophof et al., 2005). On the other hand, the ORs that detect sex pheromones (Yuvaraj et al., 2017) in the basal moth *Eriocrania semipurpurella* (Lepidoptera: Eriocraniidae) are located in sensilla auricillica, as suggested by single sensillum recordings (Larsson et al., 2002).

In most species of moths, females produce long-range sex pheromones to attract males for mating (Ando et al., 2004; Löfstedt et al., 1991). Moth pheromone communication has been well characterized in terms of pheromone biosynthesis, and to some extent olfactory reception (Ando et al., 2004; Jurenka, 2004; Löfstedt et al., 2016; Zhang and Löfstedt, 2013). Moth pheromones are divided into different types based on their site of production, chemical structure and biosynthetic origin (Löfstedt et al., 2016). Type 0 pheromones are short-chain secondary alcohols or ketones, which are similar to general plant volatiles (Kozlov et al., 1996; Löfstedt et al., 2016). This type is used by a few old lineages of Lepidoptera, as well as the sister group Trichoptera (caddisflies; Fig. 1). Type I pheromones are $C_{10}$-$C_{18}$ acetates, alcohols, and aldehydes, which are used by approximately 75% of the moth species (Ando et al., 2004; Löfstedt et al., 2016).

Because olfactory sensilla are key elements of olfaction by allowing the odor molecules to enter the internal environment, it is possible that major changes in pheromone signalling, such as the transition from structurally dissimilar Type 0 to Type I pheromones in Lepidoptera (Löfstedt et al., 2016), have been associated with modifications of sensillum morphology or alterations in the relative abundance of sensillum types. The antennal morphology and sensillar ultrastructure have been investigated in many families within the Lepidoptera (Supplementary information), and most of the species from these families use Type I sex pheromones (Löfstedt et al., 2016). To our knowledge, the antennal morphology of moth species using Type 0 pheromones has so far only been studied in a single species, namely *E. semipurpurella* (Larsson et al., 2002). No studies have compared the antennal architecture or diversity of olfactory sensilla between species using Type 0 or Type I sex pheromones.

In this study, we describe and compare the antennal morphology and sensillar structures of two moth species representing two of the most basal lepidopteran lineages (*E. semipurpurella*: Eriocraniidae; *Lampronia capitella*: Prodoxidae), one species of butterfly (*Bicyclus anynana*; Lepidoptera: Nymphalidae), and one species of Trichoptera (*Rhyacophila nubila*; Rhyacophilidae), using scanning and transmission electron microscopy. We hypothesize that the use of different pheromone types among these species may be associated with differences in the types of sensilla present, or sensilla frequencies. The caddisfly, *R. nubila* belongs to the sister group of Lepidoptera, and uses similar Type 0 pheromone compounds as *E. semipurpurella* (Löfstedt et al., 1994). The currant shoot borer moth, *L. capitella*, belongs to the first lepidopteran lineage using female-produced Type I sex pheromone compounds (Fig. 1; Löfstedt et al., 2016). Butterflies form a relatively derived monophyletic clade within Lepidoptera, but unlike moths, they do not use female-produced sex pheromones. In contrast, butterflies use male-produced pheromones for short-range courtship behaviour, which makes them interesting for the present comparison. Some of the male-produced pheromone compounds of the tropical butterfly *B. anynana* and several of its congeners, are structurally similar to typical pheromone compounds in moths (Nieberding et al., 2008; Bacquet et al., 2015). We also present an overview of antennal morphological studies from Lepidoptera and Trichoptera, and summarize previously reported electrophysiological data recorded from the various morphological sensillum types. We observe variation in the occurrence of different types of sensilla among the four species studied and throughout the lepidopteran phylogeny.

## 2. Material and methods

### 2.1. Insects

Pupae of *R. nubila* were collected from fresh water streams near Sjöbo, Sweden (55°41′13.2″N, 13°21′24.6″E), and adults were allowed to emerge in the laboratory. Adult males of *E. semipurpurella* were collected, using pheromone traps, from a birch forest in Skrylle, close to Lund, Sweden (55°38′51.0″N, 13°41′28.1″E). Male and female *L. capitella* adults were collected by hand from black currant fields near Roskilde, Denmark (55°36′26.8″N, 11°58′35.2″E). The squinting bush browns, *Bicyclus anynana*, were derived from a lab-reared population maintained at the University of Cambridge, UK, originally founded from 80 gravid females collected in Malawi in 1988 (courtesy of Dr. Oskar Brattström).

### 2.2. Scanning electron microscopy (SEM)

The antennae from live insects were dissected and immersed in a freshly prepared fixative solution, containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M $l^{-1}$ cacodylic buffer (pH 7.4) for 24 h at 4 °C. The antennae were then dehydrated in a graded ethanol series followed by critical-point drying (BAL-TEC CPD 030). The dried specimens were carefully glued onto SEM stubs, and sputter-coated with gold (Cesington 108 auto, 45 s, 20 mA). The preparations were viewed using a scanning electron microscope (SEM; Hitachu SU3500) at 5 kV.

### 2.3. Transmission electron microscopy (TEM)

The fixated antennae (see above) were post-fixed in 1% osmium tetroxide in 0.1 M $l^{-1}$ cacodylic buffer for 2 h at 4 °C. The specimens were dehydrated in a graded ethanol series and embedded in epoxy resin (Agar 100) via acetone. Semi-thin sections (1.5 µm) were made using a Leica EM UC7 ultratome with a glass knife and stained with Richardson's solution (Richardson et al., 1960) to examine the orientation of the tissue in the trimmed block. Ultra-thin sections (50 nm) were made using a Leica EM UC7 ultratome with a diamond knife. The sections were mounted on cupper grids and stained with 2% uranyl acetate (30 min) and lead citrate (4 min), and then examined using a JEOL JEM 1400 Plus transmission electron microscope. The images captured from both SEM and TEM were edited using Adobe Photoshop and Illustrator software (www.adobe.com/).

### 2.4. Data analysis

The different types of sensilla were discriminated based on their morphological features defined in the literature (Hallberg and Hansson, 1999; Ivanov and Melnitsky, 2016). The abundance of different types of sensilla along the antennae of *R. nubila* varied and thus we counted the sensilla on segments from the proximal, middle and distal parts of the antenna, i.e., segment number 7, 20 and 30, respectively. For *E. semipurpurella* and *L. capitella*, the numbers of different types of sensilla were counted on a middle segment of the antennae, as the different types show a homogenous distribution along the antennae. In *B. anynana*, the sensilla were only present on the club-shaped distal segments and therefore the sensilla on the middle segment of the club were counted. The absolute number and relative abundance (percent of total sensillum count) of the different sensillum types are presented as means of three replicates, four in the case of *L. capitella*. There was no difference in sensillum numbers between male and female *B. anynana*, hence counts from male and female antennae were pooled.

### 2.5. Antennal morphology of other lepidopteran species

We compiled data on previously reported antennal morphology, sex pheromone compounds, and electrophysiological recordings from species

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