Accepted: 5 November 2021

DOI: 10.1111/eea.13145

MINI REVIEW

Special Issue: Insect Chemical Communication

Entomologia Experimentalis et Applicata

A unique sense of smell: development and evolution of a sexually dimorphic antennal lobe – a review

Aidan T. Williams | **Eveline C. Verhulst** | **Alexander Haverkamp**

Laboratory of Entomology, Wageningen University, PO Box 9101, Wageningen, 6700 HB, The Netherlands

Correspondence Alexander Haverkamp, Laboratory of Entomology, Wageningen University, PO Box 9101, 6700 HB, Wageningen, The Netherlands.
Email: alexander.haverkamp@wur.nl

Funding information
Nederlandse Organisatie voor Wetenschappelijk Onderzoek, Grant/Award Number: 016.Veni.192.116 and 016.Vidi.189.099

Abstract

Pheromones are pivotal to sexual communication in insects. These chemical signals are processed by sexually dimorphic circuitries in the antennal lobe (AL) of the insect brain. However, there is limited understanding of how these circuitries form during AL development. Our review addresses this issue by comparing how circuitries develop throughout the growth processes of peripheral and deutocerebral neurons in various insect orders. Olfactory sensory neurons (OSNs) expressing novel pheromone receptors are eligible candidates to initiate new sexually-dimorphic circuitries when these OSNs survive programmed cell death and match the physiological properties of pheromone-sensing sensilla. The probability of these OSNs forming new glomeruli is largely determined by the degree of glia-OSN interactions and projection neuron (PN) prepatterning. The relative contribution of either of these processes determines the degree of evolutionary neuroplasticity, which is particularly prevalent in those species with complex ALs lacking specific macroglomerular structures. The extent of sexual dimorphism is determined by sex-determination genes, such as *Doublesex* and *Fruitless*, that regulate factors inducing OSN programmed cell death. Currently, these mechanisms are largely unexplored. This review, therefore, aims to provide a solid foundation for ongoing research into the evolution of AL sexual dimorphism and formation of pheromone circuitries in the light of insect sex determination.

KEYWORDS
antennal lobe, chemical signals, *Doublesex*, *Fruitless*, insect brain, neuroplasticity, olfactory sensory neuron, olfactory system, pheromones, programmed cell death, sex determination, sexual communication

INTRODUCTION

Well-defined neural circuitries in the invertebrate nervous system have long served as model systems to address fundamental questions in neuroscience (Kandel, 2001; Masse et al., 2009). The insect antennal lobe (AL), in particular, possesses highly specialized and well-defined neural circuitries ideal for examination. In this review, we will assess how developmental processes in this brain region contribute to the evolution of sexually dimorphic pheromone circuitries within insects.

Insect neurobiologists have long been fascinated by the curious anatomy of the AL, not only by its synaptic organization, but also by the functional properties of its neurons. Each AL neuron has a defined role in processing information. When combined, these neurons reconstruct the complex olfactory world of an insect (Hansson & Anton, 2000). The extensive variety in neuronal composition and AL

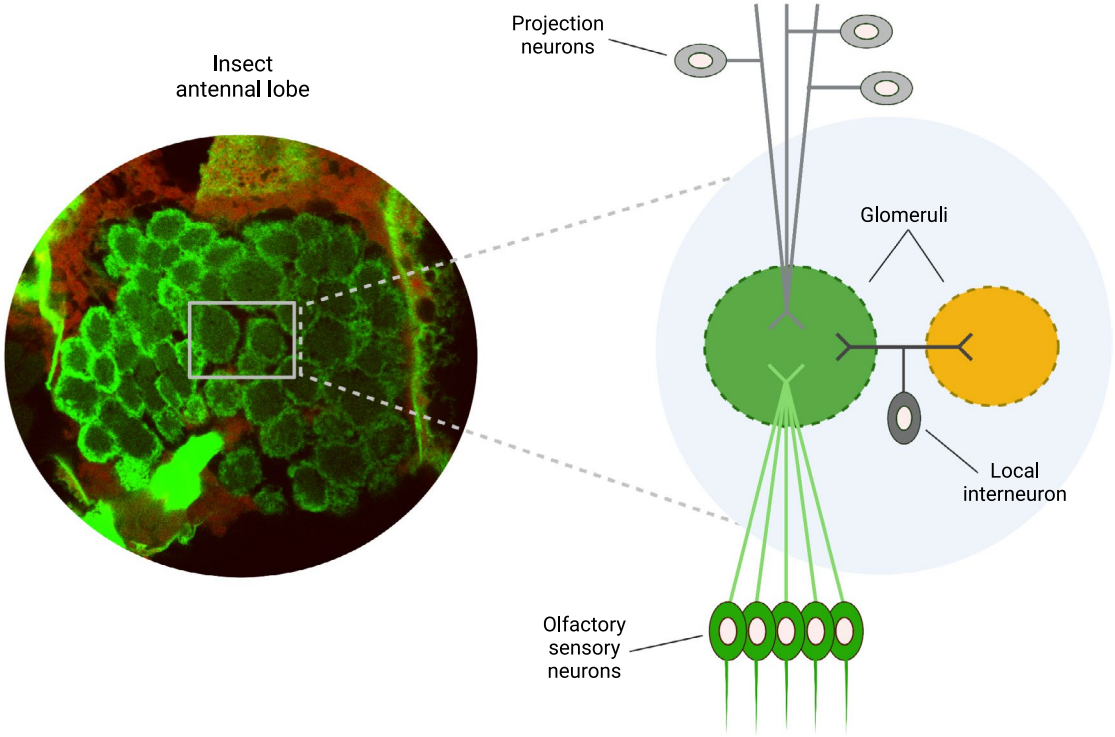

FIGURE 1 Overview of the insect antennal lobe (AL) and the neuronal composition of olfactory glomeruli. A glomerulus is composed of olfactory sensory neurons (OSNs), projection neurons (PNs), and local neurons (LNs)

organization reflects numerous lifestyle and behavioural adaptations to chemical environments (Boeckh & Tolbert, 1993; Chou et al., 2010; Kollmann et al., 2016). The AL is the primary olfactory-processing centre where olfactory sensory neurons (OSNs) from the antennae form synapses with projection neurons (PNs) of higher brain centres to produce spherical neuropils, called olfactory glomeruli (Figure 1). These can range from hundreds of microglomeruli as found in locusts (Ignell et al., 2001) to voluminous glomerular islets as present in moths (Anton & Homberg, 1999). In addition to these inter-taxonomic variations, insect ALs also show sexual dimorphism in the structure, number, and volume of glomeruli, which in turn adds to the diversification of insect species (Arnold et al., 1985; Rospars & Hildebrand, 2000; Kondoh et al., 2003; Roselino et al., 2015). Sex-specific pheromonal glomeruli, for example, are finely tuned to process spatiotemporal information of a limited number of compounds (Argawal & Isacoff, 2011). The question remains how these sexually dimorphic differences evolve within the conventional blueprint of AL development.

Records show that the insect brain and, more specifically, the process of insect olfaction have been the source of intrigue among biologists for at least the past 200 years. The French biologist Félix Dujardin was not only the first to identify the mushroom bodies (*corpora pedunculata*) in Hymenoptera and to consider them as the seat of insect intelligence, he also produced revealing illustrations of the honeybee brain that include globular structures we now know as antennal lobes (Dujardin, 1850). These dense AL masses at the base of insect antennae were later described by Leydig (1864) and Rabl-Rückhard (1875) as non-nucleated cells. However, it was Dietl (1876) who more correctly classified these structures as olfactory bodies. In an unpublished communication (1874) to the Kielers Physiologische Vereins, the German amateur neurobiologist Johann Flögel also drew attention to the fact that these structures are indeed not cells. Flögel was the first to discover that the ALs are composed of small ball-like elements (Flögel, 1878) or, in his words, scent bodies (*Geruchskörper*). He also identified tracts connecting the ALs to the calyces of the mushroom bodies and observed a proportional relationship between the size of the calyx and the number of AL scent bodies. Four years later, Bellonci (1883) went on to name these 'scent bodies' olfactory glomeruli (*Glomeruli olfactorii*) and also made the significant assertion that the olfactory lobes and glomeruli in lower vertebrates are homologous to the ALs and glomeruli in insects.

The term 'glomerulus' was next mentioned in the illustrated studies of French neuroanatomist Henri Viallanes, who used the word in his paper on the locust brain (Viallanes, 1887). He proposed a new classification of the insect brain, appointing the AL and the antennal and accessory nerves to the deutocerebrum. Viallanes also confirmed Flögel's observations of neuronal tracts connecting the AL to the cups of the mushroom bodies. A decade later, F.C. Kenyon verified these connections by using Golgi's method of silver chromate staining and determined the

production of olfactory glomeruli through tuftlike terminations of afferent antennal fibres (Kenyon, 1896). These are the first steps in identifying glomeruli as distinct olfactory-processing units in the insect AL.

The earliest accounts of AL sexual dimorphism in insects coincide with the discovery of enlarged glomeruli. In 1924, F. Bretschneider was the first to describe conspicuous sex-specific glomeruli in moths. He observed that male oak egger moths display several enlarged glomeruli where the antennal nerve fibres enter the AL (Bretschneider, 1924). Koontz & Schneider (1987) later identified these enlarged glomeruli in males as processing neuropils for female sex pheromones. Since Bretschneider's discovery, similar enlarged glomeruli have been determined in other insect species: cockroaches (Jawlowski, 1948; Neder, 1959; Boeckh et al., 1987), bees (Arnold et al., 1984; Brockmann & Brückner, 2001), ants (Kleineidam et al., 2005; Nishikawa et al., 2008), flies (Kondoh et al., 2003), and moths (Anton & Homberg, 1999). More recently, this glomerular mass was coined 'macroglomerulus' for a single glomerulus (Boeckh and Boeckh, 1979) and 'macroglomerular complex' (MGC) for multiple glomeruli (Hildebrand et al., 1980). Functional subdivisions have been identified in the MGC as separate pheromone-processing regions (Hansson et al., 1991), demonstrating how sex-specific glomeruli have evolved to process pheromone blends.

Glomerular development depends on the growth processes of OSNs and deutocerebral neurons, following a genetically fixed programme. Olfactory sensory neurons expressing specific pheromone receptors project their axons to sexually dimorphic glomeruli or glomerular clusters (Hansson et al., 1992). Projection neurons, on the other hand, connect the glomeruli through conserved tracts to the higher brain centres in the lateral protocerebrum. This connectivity occurs almost universally in insects and constitutes a genetic ground plan for connecting glomeruli (Strausfeld et al., 2009), although the precise molecular mechanisms underlying the development of sexually dimorphic AL circuitries have yet to be fully determined. Research, however, has revealed that sex-specific glomeruli develop in response to sex-determining transcription factors, such as *Doublesex* (*Dsx*) and *Fruitless* (*Fru*) (Kimura et al., 2008; Cachero et al., 2010; Zhou et al., 2014). These sex-specific mechanisms are integrated in AL development and could drive the evolution of novel sexually dimorphic circuitries.

In this review, we examine and evaluate the current consensus on the evolution of AL sexual-dimorphism and pheromone processing. We analyse developmental principles, from receptor expression and sensillum specialization to AL synaptic innervation, and compare their relative contribution to the glomerular development in various species. We assess how specialized sensilla give rise to novel OSN-glomeruli associations and how OSN-afferents are sorted through pioneering sensory neurons. We subsequently look at how the intricate interactions between OSN sorting, glia proliferation, and PN patterning are related to glomerular development. Each OSN expresses olfactory receptors (ORs) that form functional sensing units with the highly conserved olfactory receptor co-receptor (*Orco*). The role of the latter in glomerular development is also discussed in this review. Sex-determining mechanisms are finally evaluated to present a framework for ongoing research into these processes at different levels of AL development.

SEXUALLY DIMORPHIC ANTENNAE

It can be a daunting task for a diminutive invertebrate to navigate a complex chemical world, continuously being confronted by an infinite number of odour blends. As a result, insects have evolved remarkable peripheral adaptations to filter background noise and transduce relevant cues to reliable signals (Hansson & Stensmyr, 2011). The olfactory receptor repertoire has evolved from very few receptors in primitive arthropods to a highly divergent receptor superfamily that is specifically adapted to an insect's chemical environment (Clyne et al., 1999; Robertson et al., 2003; Missbach et al., 2014). An insect's distinct environment is perceived by specific receptor repertoires, which aid insects in fulfilling behaviours crucial to their survival (Stensmyr et al., 2003; Gardiner et al., 2008; Carey et al., 2010), such as finding mates, foraging for food, and selecting suitable oviposition sites. Olfactory receptors are expressed in OSNs that are encapsulated by specialized sensory organs, the antennal sensilla (Zacharuk, 1980). These small hairlike structures protruding from the antennal cuticle are efficiently equipped to capture and transport chemical compounds to the OSN membrane (Steinbrecht, 1997).

The striking morphological diversity of insect sensilla makes it possible to classify them into types, the most common of which are placoid (platelike) (Figure 2A,D), trichoid (hairlike) (Figure 2B,C), basiconic (conelike) (Figure 2D), and coeloconic (peglike) (Schneider & Steinbrecht, 1968). Chemosensing sensilla are further subdivided according to the presence of pores (Steinbrecht, 1997), where uniporous sensilla are responsible for contact chemoreception or gustation and multiporous sensilla for olfaction. The diverse shapes and distributional patterns of sensilla reflect diverse sensory modalities and occasionally take the form of extreme specialized morphologies (Schneider, 1964). Sensillum specialization for pheromone detection can sometimes be visible, even to the naked eye, and gives rise to conspicuous sexual dimorphisms in the shape and size of antennae (Steinbrecht, 1987). However, there can also be distinct sexually dimorphic differences in the number and distribution of sensillum types (Figure 2A,B), which exemplify sexual differences in olfactory life histories – or, simply put, a sex-specific sensillum repertoire to perform sex-specific behaviours.

Antennal sexual dimorphism is epitomized in certain moth species. Male saturniid moths, for example, possess

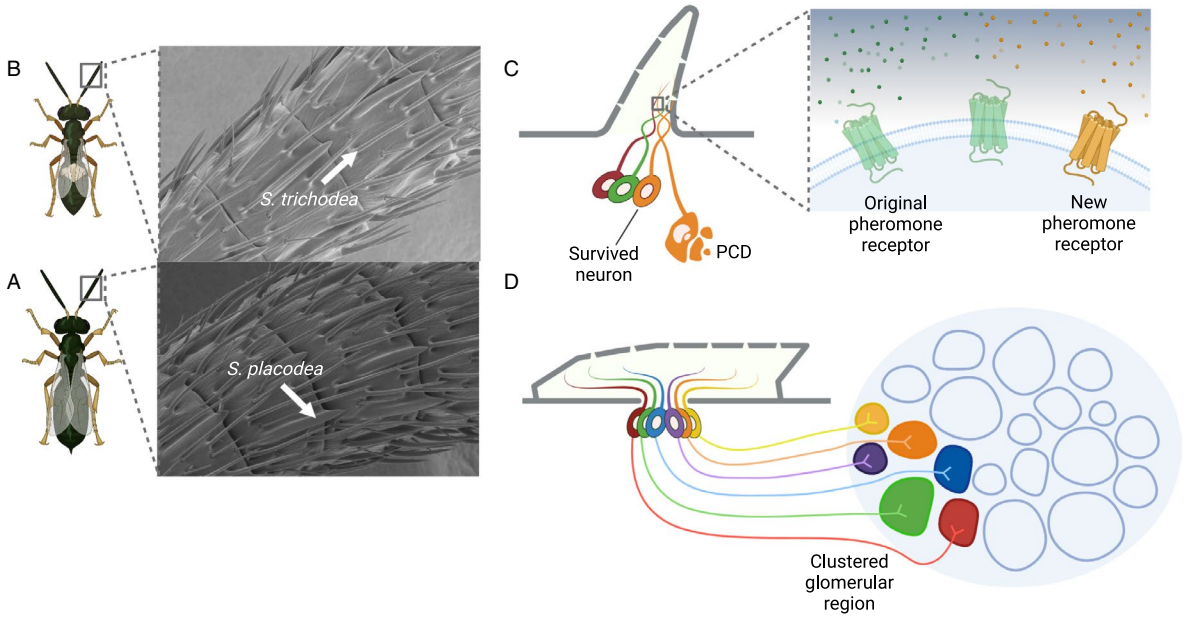

FIGURE 2 Sexual dimorphism in olfactory sensilla on the antennae of a parasitoid wasp. (A) Olfactory sensilla of a female parasitoid wasp highlighting the high abundance of placoid sensilla. (B) Olfactory sensilla of a male parasitoid wasp highlighting the presence of trichoid sensilla. (C) Cross section of a trichoid sensillum composed of three functional olfactory sensory neurons (OSNs). Excess OSNs are eliminated through programmed cell death (PCD). Olfactory sensory neurons surviving PCD are eligible candidates to evolve novel pheromone receptors through gene duplication. (D) Cross section of a placoid sensillum composed of six functional OSNs. The OSNs from specific sensilla project to clustered regions in the insect antennal lobe (AL)

conspicuous trichoid sensilla to reflect their predisposition to trace odours of female mates. Up to 50 000 of these pheromone-detecting sensilla utilize much of the antennal surface area to maximize detection of the female's volatile pheromone (Boeckh et al., 1960). A predominance of a certain sensillum increases the number of receptors tuned to the specific pheromone and improves antennal sensitivity by lowering the response threshold to the pheromone molecules (Kaissling & Priesner, 1970). Similar quantitative adaptations, though less visually apparent, have also been observed in Hymenoptera species, as in the greatly increased number of pore plates in drone honeybees (Esslen & Kaissling, 1976). These pore plates contain OSNs expressing general food receptors as well as specific receptors for the queen substance and scent-gland odours (Nasonov's gland) (Kaissling & Renner, 1968; Vareschi, 1971). The pore plates bear a close structural relationship with trichoid sensilla ('sensilla trichodea curvata') in *Formica* ants, which are basically pore plates lifted from the antennal cuticle (Walther, 1981). It would appear that in Hymenoptera both types can transmute into each other and therefore share common developmental features. Such a bimodal relationship could be regulated through a sex-differentiating switch mechanism, repressing one morphological state while activating the other. Silencing transcription factors such as *Dsx* or changing this gene's sex-specific splicing could therefore display a reversal to either placoid or trichoid morphology (AT Williams, HM Smid & EC Verhulst, in prep.).

Sensillum sexual dimorphism, although numerically conspicuous in one taxon, can be obscurely visible in others and still determine important chemosensory properties. Male and female *Bombyx mori* (L.) moths differ slightly in the number of trichoid sensilla (Steinbrecht, 1970), but differ quite significantly in the properties of the two OSNs in the trichoids. In males, these OSNs respond to the pheromones bombykol and bombykal, whereas in females these OSNs are unresponsive to these substances (Kaissling et al., 1978). Transgenetic studies have expressed the moth's bombykol receptor in 'empty neurons' from *Drosophila* basiconic sensilla, but observed a low sensitivity compared to the native trichoid sensilla on the moth's antennae (Syed et al., 2010). However, expression of the moth's bombykol receptor in the *Drosophila* trichoid system yielded a sensitivity comparable to the moth's native trichoids, suggesting this to be a more suitable habitat for the moth's bombykol receptor. Pheromone sensitivity of OSNs therefore seems to be closely associated with the structural, biochemical, and biophysical properties of their corresponding sensillum. Specialized subpopulations of pheromone-sensing neurons are located in specialized sensilla specifically programmed for pheromone detection (Ha & Smith, 2006). These sensillum-specific neural subpopulations not only differ between sexes, but also show a functional and topographical segregation in the AL (Figure 2D).

A SPECIALIZED SENSILLUM SUBSYSTEM

Antennal lobe architecture depends on the variety of sensillum morphologies. Chemosensing sensilla and

their corresponding OSNs are often distinctly distributed on antennal segments (Vosshall et al., 1999; De Bruyne et al., 2001; Stocker, 2001) and can be traced simultaneously to distinct regions of the AL (Gao et al., 2000; Couto et al., 2005; Grabe et al., 2016). The major pheromone-sensing neurons in *Drosophila* are located in specific trichoid sensilla, which are marginally more abundant in males (Xu et al., 2005). The pheromone-sensing neurons of these trichoids respond to the courtship volatile *cis*-vaccenyl acetate (cVA) (Van der Goes van Naters & Carlson, 2007) and project their axons to two sexually dimorphic glomeruli (Datta et al., 2008), which are also significantly larger in males (Kondoh et al., 2003). In the cockroach *Periplaneta americana* (L.), basiconic sensilla project OSNs to an MGC composed of two immediately adjacent glomeruli (Watanabe et al., 2012). Likewise, in the hawkmoth *Manduca sexta* (L.), OSNs from male-specific trichoid sensilla project their axons exclusively towards the MGC (Christensen et al., 1995). The axons from trichoids on the dorsal side of the moth's antennae are projected towards the medial region, whereas axons from ventral trichoids project towards lateral areas. Finally, social Hymenoptera species typically utilize a sensillum subsystem for perceiving colony cues and pheromones, as well as odours associated with foraging (Ozaki et al., 2005; Kropf et al., 2014; Couto et al., 2017). Sensory neurons capable of detecting these compounds are typically located in basiconic sensilla usually present in only one of the sexes.

Whatever strategy is used, it is evident that numerous insects employ a specialized sensillum subsystem containing distinctive physiological features that corresponds to spatially segregated glomeruli in the AL, whether these form an MGC or a cluster of regular glomeruli. This functional correspondence between a sensillum, OSN, and glomerulus results in a functional segregation of pheromonal and non-pheromonal afferents in the antennae, although further research needs to be carried out to determine how this segregation actually takes place and which molecular mechanisms are responsible for it. Before entering the AL, OSNs are actively sorted in the antennae through interactions with peripheral glia cells (Sen et al., 2005). In *Drosophila*, the majority of these glia cells originate from pioneering sensory neurons specified by the *Atonal* transcription factor. These sensory neurons are the first to permeate the antennal lobe during development (Jhaveri et al., 2000). The glia cells wrap the OSN tracts into three distinct fascicles before exiting the antennae and entering the AL. Loss of *Atonal* function disrupts OSN segregation from all chemosensing sensilla (ca. 1000 neurons in *Drosophila*) and glomerular patterning fails to materialize (Jhaveri & Rodrigues, 2002). Once firmly established, excess OSN afferents are eliminated from a sensillum through programmed cell death (Sen et al., 2004) (Figure 2C). Any neuron that has survived this process has the potential to integrate into pre-existing olfactory circuitries and form novel sensillum-receptor glomerular associations (Prietro-Godina et al., 2020). These surviving neurons have the potential to evolve into novel sexually dimorphic pheromone circuitries.

SEXUALLY DIMORPHIC ANTENNAL LOBE

In terms of task assignment, structure, and composition, the AL is surprisingly unique; in terms of its development, it is rather ambiguous. Its uniqueness is manifested in the diversity of distinct glomeruli, with each having its own olfactory identity. In moths, honeybees, and fruit flies, each glomerulus has its own identifiable shape and position in the fixed organizational structure of the AL (Rospars, 1988; Rospars & Chambille, 1989). Rospars & Chambille were the first to determine this fixed organizational structure in cockroach species (Chambille et al., 1980; Rospars & Chambille, 1981; Chambille & Rospars, 1985). In addition to a unique appearance, each glomerulus possesses a unique neuronal composition based on the total number of innervating OSNs and the synapses they form with local interneurons (LNs) and PNs (Grabe et al., 2016). This unique neuronal composition determines glomerular size, which in turn determines sensory specialization and the contribution of processed odour compounds to behavioural relevance (Dekker et al., 2006; Linz et al., 2013). Highly significant ecological cues, such as sex pheromones, are usually processed by labelled lines: single glomeruli that receive few lateral inputs from LNs, but display a relatively high number of PNs (Grabe et al., 2016). The low degree of lateral processing in these glomeruli enables fast innate responses towards sex pheromones. General odours, however, elicit broader AL response patterns, called combinatorial codes, which require computational input from multiple interconnected glomeruli. In this way, distinct processing channels are formed from different neuronal compositions, being either broadly tuned or narrowly tuned to odour compounds (Sachse et al., 1999; Christensen & Hildebrand, 2002; Haverkamp et al., 2018). It remains unclear, however, how these coding systems are established throughout AL development, how developmental changes can result in novel labelled lines, and to what extent combinatorial coding contributes to pheromone processing in different insect orders.

Pheromone processing in the AL is not an isolated task. Insect pheromones interact with additional cues in the AL to modulate important reproductive decisions. Food odours trigger *Drosophila* males to deposit the pheromone 9-tricosene on rotting fruit, which promotes aggregation and mediates female oviposition decisions (Lin et al., 2015). Food odours also enhance male attractiveness as females become more receptive when food is sensed in conjunction with the male pheromone cVA (Lebreton et al., 2015). At the AL level, food odours enhance activation of the cVA-responsive glomerulus (DA1) and this effect is mediated through lateral excitation from neighbouring glomeruli (Das et al., 2017). Similar interactions are also found

in the AL between moth pheromones and plant volatiles (Reddy & Guerrero, 2004), the latter of which are capable of masking or synergizing the pheromone response from a male moth's MGC (Namiki et al., 2008; Chaffiol et al., 2012; Deisig et al., 2012). Although extensive interaction in flies and moths occurs at the glomerular level, pheromone processing in the AL of these insects still follows a dedicated labelled-line system (Haverkamp et al., 2018). Hymenoptera, however, form an exception to insect orders that use a specialized labelled-line system.

In species such as ants, honeybees, and parasitoid wasps, pheromones can play a role other than sexual attraction and may favour a combinatorial-coding strategy instead (Carcaud et al., 2015). These species utilize olfactory subsystems, consisting of glomerular clusters innervated by uniglomerular PNs (Kropf et al., 2014). Honeybees possess two olfactory subsystems, innervated separately by two different PN tracts (Galizia & Rössler, 2010). While queen and brood pheromones are processed by either of these tracts, all pheromone components are capable of eliciting a combinatorial code in both subsystems (Carcaud et al., 2015). Ants, bees, and wasps also use a conserved cluster innervated by basiconic-associated OSNs for the coding of colony cues, pheromones, and odours associated with foraging (Ozaki et al., 2005; Nakanishi et al., 2010; Couto et al., 2017). The parasitoid wasp *Nasonia vitripennis* (Walker) possesses a highly sexually dimorphic AL (ca. 185 glomeruli in males and ca. 225 glomeruli in females), which is arranged in sex-specific clusters likely to be used for processing pheromones and/or host odours (AT Williams, HM Smid & EC Verhulst, in prep.). These dimorphic clusters are currently being investigated in the light of sex-differentiating mechanisms to ascertain whether silencing sex-determination genes leads to a sex-reversal in glomerular organization. Silencing these genes in *Drosophila* does not lead to a significant change in glomerular organization (Stockinger et al., 2005). However, because Hymenoptera use a different strategy to process pheromones, it is likely that glomerular development is more intricate in these species.

A PREPATTERNED OUTLINE?

To understand the developmental evolution of pheromone-processing glomeruli, we need to dissect their neurophysiological properties and elucidate neurogenetic mechanisms. Across almost all insect taxa, the number of AL glomeruli approximates the number of genes that encode olfactory-receptor proteins. Most OSNs express only one or rarely two OR genes (Clyne et al., 1999; Vosshall et al., 1999; Silbering et al., 2011), and the axons of neurons expressing the same OR gene converge in the same glomerulus (Gao et al., 2000; Vosshall et al., 2000; Couto et al., 2005; Fishilevich & Vosshall, 2005; Silbering et al., 2011). These functional relationships have been coined the one-neuron-one-receptor and one-receptor-one-glomerulus principles. Each OR has a specific chemoreceptive field, being broadly or narrowly tuned to specific odour compounds, and these tuning properties are inherited by their corresponding glomeruli (Hallem and Carlson, 2006). Convergent projections to specific glomeruli therefore create a topographic olfactory map, as witnessed in *Drosophila*, and the identity of a glomerulus should therefore be closely associated with OSNs expressing a specific OR gene. However, the genetic mechanisms for glomerular development are not the same as those generating different types of ORs: silencing an OR gene does not eliminate OSN connectivity or glomerular formation in *Drosophila* (Dobritsa et al., 2003; Elmore et al., 2003; Goldman et al., 2005; Ray et al., 2007). In fact, a prototypic AL has already formed in the fly well before ORs are expressed and before OSNs have innervated their corresponding glomeruli (Jefferis et al., 2004).

Projection neurons are the first to reach the AL and their dendrites develop to establish protoglomeruli. In *Drosophila*, PNs are prespecified by lineage and birth order to form synapses with specific incoming OSNs and therefore to transmit specific olfactory information (Jefferis et al., 2001). Glomerulus-specific PNs prepattern their target region at the onset of *Drosophila* pupation before OSN arrival (Figure 3), suggesting olfactory circuitries are formed through synaptic recognition of OSNs by the resident PNs (Jefferis et al., 2004). This stage of prespecification may be used to hardwire glomerular identity, enabling stereotypical behaviours towards sex pheromones. Within 18 h of puparium formation, after pioneering sensory neurons permeate the AL, OSNs converge on their target glomerulus and connect with PNs. Molecular mechanisms, such as the adhesion molecules N-cadherin and Dscam, enable class-specific OSN axon sorting (Zhu & Luo, 2004; Goyal et al., 2019) and synaptogenesis is initiated after OSNs recognize PN dendritic cues (Zhu et al., 2006). Although PNs are necessary for correct patterning of the AL in *Drosophila*, it has been demonstrated that glomeruli in *Manduca* also form after individual PN clusters are surgically removed (Oland & Tolbert, 1998). In this species, the AL template of protoglomeruli is stabilized through glia-cell proliferation.

GLIA AND OSNS: EFFECTIVE TEAM PLAYERS?

Glia cells play a crucial role in the induction of glomeruli formation through reciprocal interaction with innervating OSNs (Oland & Tolbert, 2011). The glia cells first form a ring around the immature AL neuropil, after which they are stimulated by ingrowing axons to migrate around the protoglomeruli and form glomerular envelopes (Oland & Tolbert, 1987). These glia-cell walls act as important boundaries to the protoglomeruli for incoming OSNs to initiate synaptic interactions with PNs. Research on the relative contribution of glia towards glomerular development has focused specifically on the comparison between *Manduca* and *Drosophila*. In the former, glia cells form thick glomerular boundaries that are essential for the formation of stable glomeruli (Tolbert et al., 2004). Delayed or incomplete development of glial cells in the *Manduca* AL can result in the misrouting

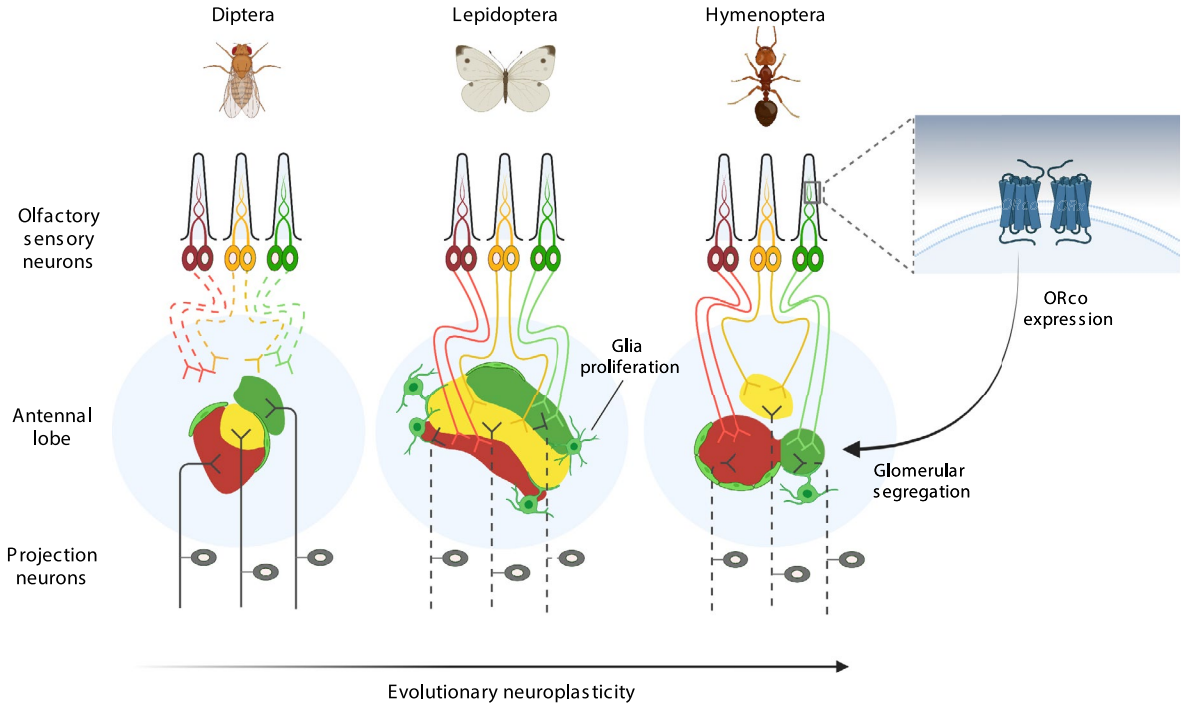

FIGURE 3 Conceptional diagram of antennal lobe (AL) development in three insect orders. Known pheromone-processing glomeruli are portrayed for Diptera (DA1, VA1D, and VA1v in *Drosophila*) and Lepidoptera [macroglomerular complex (MGC) of *Manduca*]. In *Drosophila*, projection neuron (PN) prepatterning is crucial for forming stable glomeruli, whereas refinement through glia proliferation and olfactory sensory neuron (OSN) innervation play a lesser role. In Lepidoptera, however, glomeruli are also able to form after PNs are surgically removed and after transplanting the antennae between sexes. Glia proliferation and OSN innervation play a bigger role in glomerular refinement in these species. In Hymenoptera, especially ants, OSN innervation is crucial for refining glomerular structure. *Olfactory receptor co-receptor* (*Orco*) knock-out in these species leaves a latent PN-prepatterned structure. The process of OSNs refining glomerular development makes modifications in glomerular structure more likely to occur and therefore increases the degree of evolutionary neuroplasticity

of pheromone-specific OSNs and incomplete glomerular segregation of the MGC (Rössler et al., 2000). Glia cells in *Drosophila*, however, form rather weak boundaries and are unlikely to play a significant role in either axonal sorting or glomerular isolation (Oland et al., 2008). Moreover, glia processes are also extended well after glomeruli have formed and therefore play a minor role in glomerular stabilization (Jefferis et al., 2004; Oland et al., 2008). Taken together, the glomerular structure in *Drosophila* appears to be determined primarily by a prespecified PN pattern, whereas in *Manduca* OSN-glia interactions appear to be paramount for forming stable glomeruli, reshaping the innervation patterns of the initial PNs (Jefferis et al., 2004; Oland & Tolbert, 2011) (Figure 3). Locusts, to give a further example, employ a microglomerular design (Ignell et al., 2001) and add extra glomeruli to the AL after each moulting event (Ochieng et al., 1998), whereas PNs remain constant as the locust ages (Anton et al., 2002). It would be interesting to investigate how glia affect AL formation of these species and how signals from innervating OSNs cause further proliferation.

TRANSPLANTING INSECT ANTENNAE

A good example of primary glomerular development is illustrated in the experiments using antennae transplanted between different sexes of the hawkmoth *M. sexta* (Schneiderman et al., 1982; Rössler et al., 1999; Kalberer et al., 2010). These experiments involve removing the antennal imaginal discs of late-instar caterpillars of one sex and replacing them with an antennal imaginal disc of the opposite sex. During metamorphosis, the insects that have undergone this procedure then develop antennae according to the sex of the donor insect. Interestingly, the OSNs from these transplanted antennae also significantly reshape the AL of the recipient. In Lepidoptera, the male AL is characterized by the MGC, which consists of axons from pheromone-sensing neurons (PSNs) arriving from the antenna, LNs that process the antennal signals, and PNs that transfer the pheromone signal to higher brain centres (Hansson et al., 1991; Reisenman et al., 2011). The female AL, on the other hand, contains two lateral large female-specific glomeruli (latLFG), which respond to different plant volatiles (Reisenman et al., 2004). If a male antennal imaginal disc is transplanted onto a female *M. sexta*, the male pheromone-sensing neurons will induce the formation of a male MGC instead of the latLFG, stimulating the female LNs and PNs, which are already present in the AL, to adapt to these altered glomeruli (Schneiderman et al., 1982; Rössler et al., 1999).

In a number of cases, these gynandromorphic females also show oviposition behaviour in response to

stimulation with an artificial pheromone blend, indicating that the newly formed connections between the male OSNs and the female PNs actually trigger the same behavioural circuitries as would normally be activated by the OSNs innervating the latLFG (Schneiderman et al., 1986). However, these results also indicate that there is no OSN/PSN-specific recognition by the PNs. Which OSN will connect to the waiting PNs simply depends on the specific location to which the neurons are guided by glia cells. This of course begs the question which mechanisms are employed by the glia cells to sort the arriving OSNs. Activation of the epidermal growth factor receptor (EGFR) along with Fasciclin II and Neuroglian, members of the immunoglobulin superfamily of cell-adhesion molecules (IgSF CAMs) in the axons of the arriving OSNs, play an important role in guiding these neurons to the right location in the developing AL (Gibson & Tolbert, 2006). On the glia side, fibroblast growth factor receptors (FGFRs) are crucial for the development of the axon sorting zone (Gibson et al., 2012). However, it has yet to be established how the axons of specific OSNs are guided to a certain protoglomerulus. Interestingly, the olfactory receptor co-receptor (*Orco*), which is required for normal activity of the olfactory receptors, is not necessary for the correct development of the AL in *Manduca* or *Drosophila* (Fandino et al., 2019; Ryba et al., 2020).

Independent of the specific mechanism, the role glia cells play in the formation of the olfactory glomeruli does pose a potential problem for the evolution of new olfactory circuitries: novel OSN types can arise through duplication events in OR genes and through the survival of OSNs during metamorphosis, which would otherwise be destined for programmed cell death (Prieto-Godino et al., 2020) (Figure 2C). How then are glia cells able to recognize these new OSN types in order to induce the formation of a new glomerulus? In *Drosophila*, OSNs expressing genetically similar ORs often project to neighbouring glomeruli, which would suggest that OSN types arising through gene duplication might initially be sorted to the same or adjacent locality in the AL (Couto et al., 2005; Prieto-Godino et al., 2020). From an evolutionary perspective, glia cells would ultimately have to recognize the novel OSN populations as such and initiate a budding process, through which glomeruli with a similar response spectrum would form in close proximity to each other, comparable to that of the pheromone-detecting glomeruli in the majority of insect species (Hansson & Stensmyr, 2011). However, given the fact that the total number of OSNs will be limited by the surface area of the antenna, and assuming that the number of PNs does not arise proportionally with the number of novel OSN types, new glomeruli will lead to a reduction in the size of the existing AL structures. Over time, pheromone components could increase and take on additional roles beyond sexual communication, which would then result in rewiring of PSNs to make a switch from a labelled-line system to combinatorial coding scheme. This pheromone-coding switch ultimately leads to a loss of distinct MGCs in the AL, which might help to explain the absence of these structures in many Hymenoptera species.

OLFACTORY RECEPTOR CO-RECEPTOR AND THE LOSS OF GLOMERULI

Although *Drosophila* glomerular formation is generally not affected by genetically eliminating OR genes, it appears the opposite is true in Hymenoptera where *Orco* is concerned (Trible et al., 2017). Insect OR proteins form a complex with *Orco*, a highly conserved co-receptor, which localizes the OR to the dendritic membrane (Jones et al., 2005). The colocalized OR-*Orco* complex is essential for signal transduction, enabling OSN polarization after receptor binding of olfactory ligands (Larsson et al., 2004). Studies carried out on *Orco* null mutants of ants revealed that these ants showed a loss of OSNs and a dramatic reduction (by ca. 82%) in the number of glomeruli (Trible et al., 2017). As a consequence, these mutant ants demonstrated significant deficiencies in social behaviour, including an inability to: recognize nestmates, follow pheromone trails, recognize general odorants, and forage for food. Conspecific communication was similarly impaired, resulting in unsuccessful mating and a significantly reduced fitness (Yan et al., 2017). *Olfactory receptor co-receptor* expression in OSNs is therefore required for the development of pheromone-detecting glomeruli in the Hymenoptera AL. All Hymenoptera employ two AL olfactory subsystems, each of which has a PN tract that projects to either the mushroom body or the lateral horn (Rössler & Zube, 2011; Couto et al., 2016). Both subsystems can process information from pheromones, but extract different odorant properties before transmitting signals to the higher brain centres (Brill et al., 2013; Rössler & Brill, 2013; Carcaud et al., 2015). This demonstrates that pheromone processing requires that both subsystems in the AL cooperate in parallel (Carcaud et al., 2015). Glomeruli in the *Orco* mutant ants were indeed lacking glomerular clusters from both subsystems, which explains the malfunction of social behaviour in these ants.

Loss of *Orco* in non-Hymenoptera species leads to impaired pheromone sensitivity, but does not cause severe anatomical changes in glomerular organization (Ryba et al., 2020). Knocking out *Orco* diminishes pheromone detection in *M. sexta* (Fandino et al., 2019), *B. mori* (Liu et al., 2017), *Spodoptera littoralis* (Boisduval) (Koutroumpa et al., 2016), and *Locusta migratoria* (L.) (Li et al., 2016), but has little effect on the structure of the AL. Loss of *Orco* also has no significant impact on the *Drosophila* AL, with essentially no difference in the number and organization of glomeruli (Ryba et al., 2020). In Hymenoptera, however, ORs and *Orco* are expressed before the onset of glomerular formation. It is therefore likely that, through *Orco*, OSNs play a more important role in refining glomerular identity after PNs have prepatterned the glomerular structure. The remaining glomeruli in the *Orco* mutant ants failed

to segregate normally (Yan et al., 2017), indicating the significance of *Orco* and OSNs in elaborating AL complexity (Figure 3). Hymenoptera have evolved an expansive receptor repertoire, which is reflected in large ALs and elaborate mushroom bodies. A parasitic or social lifestyle might have favoured flexible developmental processes, which enabled combinatorial coding adaptations for cognitive-demanding tasks, such as spatial learning and host foraging. Pheromone processing might have initially followed a labelled-line strategy in ancestral species, but eventually evolved and developed into a combinatorial coding strategy that favours elaborate ALs. Research should therefore be carried out to investigate this transition by making phylogenetic comparisons and elucidating neurogenetic mechanisms.

SEX-DETERMINATION AND THE BIRTH OF NEURONS

Molecular basis of sex determination in insects

In most insects, sexual differentiation is initiated by the action of two highly conserved transcription factors: *Dsx* (reviewed in Verhulst & van de Zande, 2015) and *Fru* (reviewed in Sato & Yamamoto, 2020). In *D. melanogaster* and many other holometabolous insects, the transcripts of both genes are sex-specifically spliced into the female splice-variant by the upstream splicing factors *Transformer* (*Tra*) and *Transformer-2* (*Tra-2*), whereas in males *Tra* is not functionally present to splice *Dsx* and *Fru* and both are spliced by default into the male-specific splice-variants (Cline & Meyer, 1996; Ryner et al., 1996; Heinrichs et al., 1998; Verhulst et al., 2010; Geuverink & Beukeboom, 2014; Bopp et al., 2014). Both male- and female-specific *Dsx* isoforms are required for sexual differentiation (Baker & Ridge, 1980), whereas only the male-specific *Fru* isoforms are required for the development of neural pathways for male courtship behaviour (Gill, 1963; Ito et al., 1996; Ryner et al., 1996) and the female-specific *Fru* isoforms are assumed to have no function (Taylor et al., 1994). Von Philipsborn et al. (2014) have investigated all four major *Drosophila Fru* splice variants and attempted to correlate the cellular and behavioural function of each *Fru* isoform. Here, however, we refer to all male-specific *Fru* protein isoforms as *Fru*.

In previous overviews of sex determination and differentiation in *Drosophila*, *Dsx* is shown to be required for morphological differentiation and *Fru* to be the master control gene required for shaping the brain centres for male sexual behaviour (Ito et al., 1996; Ryner et al., 1996; Demir & Dickson, 2005; Manoli et al., 2005). We now know, however, that this distinction is not that strict and that *Dsx* and *Fru* are both needed to regulate courtship behaviour in males (Villella & Hall, 1996; Billeter et al., 2006; Shirangi et al., 2006; Rideout et al., 2007), whereas for sexual behaviour in females only *Dsx* is required (Zhou et al., 2014). The role of *Fru* in controlling brain organization for male sexual behaviour in other insects is largely unknown. Despite this, recent research suggests that in basal hemimetabolous insects, *Fru* is not sex-specifically spliced and is not likely to be involved in neural sex determination (Watanabe, 2019). However, even in *Drosophila*, all neuronal clusters expressing *Dsx* are either sex-specific or sexually dimorphic and none are sexually monomorphic (Nojima et al., 2021). This suggests that AL development and the resulting behaviours in males and females could be predominantly controlled by *Dsx*.

Fruitless and/or *Doublesex* regulate neuronal growth and development

Sex-specific splicing of *Fru* is highly complex in both Diptera and Hymenoptera (Heinrichs et al., 1998; Bertossa et al., 2009), due to the use of different promoters and alternative splicing. Only transcripts originating from the first, most distal, promoter are sex-specifically spliced by *Tra* and *Tra-2*, whereas transcripts from the other promoters are non-sex-specifically spliced (Heinrichs et al., 1998; Bertossa et al., 2009). In *Drosophila*, both male and female *Fru* mRNA expression is observed in the central nervous system (CNS) (Usui-Aoki et al., 2000), with about 2% of the CNS cells expressing male-specific *Fru* at peak expression approximately 2 days into pupal development (Lee et al., 2000; reviewed in Baker et al., 2001). Only in males, however, are *Fru* proteins expressed in the CNS, whereas in females *Fru* proteins are not expressed here (Usui-Aoki et al., 2000). In males, these *Fru* expressing cells are organized into ca. 20 groups (Lee et al., 2000). One of these clusters, termed mAL, is located just above the AL and contains 30 neurons in males and five neurons in females (Kimura et al., 2005), and is thought to be involved in pheromone input integration (Yamamoto, 2007). The cell-death inducing *grim*, *head involution defective* (*hid*), and *reaper* (*rpr*) genes appear to regulate the number of interneurons in the mAL under repressive control of *Fru* (Figure 4). In females, the majority of these mAL neurons are destined to die due to the absence of *Fru*, as ectopic *Fru* protein expression in females inhibits the death of these mAL neurons (Kimura et al., 2005). In summary, in *Drosophila Fru* inhibits cell death in males and regulates male-specific morphology of the mAL neurites, whereas in females, absence of *Fru* leads to programmed cell death and female-specific development of the few remaining mAL neurites (Kimura et al., 2005; reviewed in Yamamoto, 2007).

Programmed cell death is not the only mechanism employed in *Drosophila* to regulate the number of neurons. *Dsx* is expressed earlier than *Fru* in the CNS (Lee et al., 2002) and controls abdominal neural stem-cell proliferation on a sex-specific basis (Taylor & Truman, 1992). Four of these abdominal neural stem cells express *Dsx*, but female-specific *Dsx* induces programmed cell death of these neural stem cells in conjunction with the Hox gene *Abdominal-B* (Ghosh et al., 2019). Male-specific *Dsx*, however, regulates

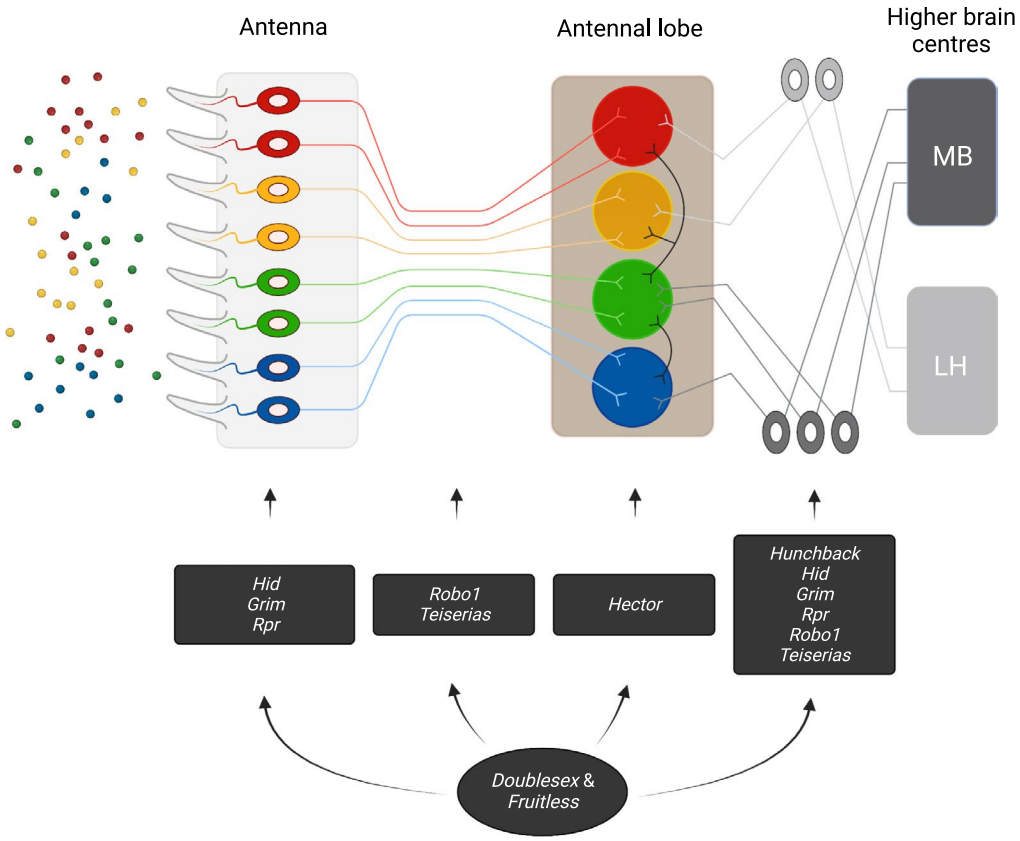

FIGURE 4 Conceptional diagram showing the target genes of *Doublesex* (*Dsx*) and *Fruitless* (*Fru*) transcription factors affecting developmental processes at various regions of the olfactory system, starting from the antenna and the antennal lobe to the higher brain centres such as the mushroom body (MB) and the lateral horn (LH)

a continued proliferation into late third-instars, resulting in neurons crucial for male mating behaviour (Taylor & Truman, 1992; Billeter et al., 2006).

A combined action of *Fru* and *Dsx* has been shown in the birth and death of the P1 neuronal cluster (Kimura et al., 2008). In males, this P1 cluster expresses *Fru* in about 20 neurons and extends from the AL to the lateral horn. *Fruitless* is needed for correct neurite arborization of these neurons in the lateral horn to initiate courtship (Kimura et al., 2008). Both male-specific *Dsx* and *Fru* are required for the correct number of P1 cells, as loss of either of them leads to a reduction in these cells (Kimura et al., 2008). In females, female-specific *Dsx* is involved in the active removal of the P1 cluster from the female brain by programmed cell death, again involving *Grim*, *Hid*, and *Rpr* (Kimura et al., 2008). Three other neuronal clusters express *Dsx* and are candidates for female-specific behaviour (Zhou et al., 2014). Two of these clusters (pCd and PC1) respond to the male-specific pheromone, cVA, to promote female receptivity (Zhou et al., 2014). Overall, *Dsx* and *Fru* appear to regulate the birth and death of olfactory neurons by targeting the programmed-cell-death-inducing factors *Grim*, *Hid*, and *Rpr*. The same mechanisms potentially regulate OSN survival rate and the formation of novel OSN circuitries described by Prietro-Godina et al. (2020).

Fruitless target genes involved in neuron differentiation and signalling

Fruitless target genes are involved in changes in dendritic arborization: the ipsilateral neurite in the mAL is present in males but absent in females, and the branching of the contralateral neurite differs between males and females (Kimura et al., 2005). The potential *Fru* downstream target, the transcription factor *Hunchback*, has been shown to be required for male-specific branching of the contralateral neurites (Goto et al., 2011), whereas the *Fru* target gene *Robo1*, an axon guidance protein, normally inhibits ipsilateral neurite development in females. Its expression, however, is repressed by *Fru* in males, resulting in ipsilateral neurite development (Ito et al., 1996). Recently, another *Fru* target gene has been identified, *Teiresias*, which is required for feminization of the neurite patterns, possibly by interacting with *Robo1* in females, and is repressed by *Fru* in males (Sato et al., 2020). The potential *Fru* target gene *Hector*, a novel putative G-protein coupled receptor (GPCR), is found in a subset of AL glomeruli that are innervated by *Fru*-expressing PNs. Signalling through this GPCR in PNs is essential for male courtship behaviour (Li et al., 2011). It would be interesting to investigate whether the combined action of *Robo1*, *Teiresias*, and *Hector* similarly regulate the correct targeting of OSN axons to the AL (Figure 4).

Fruitless and/or *doublesex* rewire olfactory brain circuitry

The male pheromone cVA has opposite effects in male and female *Drosophila*. It inhibits male-male mating behaviour, but promotes courtship and receptivity in females through the action of the Or67d receptor (Kurtovic et al., 2007). It has been shown that this single OSN class is capable of switching sex-specific behaviour in response to cVA (Kurtovic et al., 2007). The Or67d OSNs project to the DA1 glomerulus in the AL of both males and females (Manoli et al., 2005; Stockinger et al., 2005). The OSNs innervating DA1 then synapse male-specifically with PNs that project to the lateral horn under control of *Fru*, whereas in females, DA1 PNs show a female pattern of axonal arbours in the lateral horn (Datta et al., 2008). The Or67d OSNs and the PNs both express *Fru*, but show no sexual dimorphism (Kurtovic et al., 2007; Datta et al., 2008). This would suggest that the olfactory input can be the same for both sexes, but the processing of this information in the lateral horn occurs sex-specifically. Kohl et al. (2013) builds on the work of Chachero et al. (2010) and shows that indeed two populations of lateral-horn neurons, aSP-f and aSP-g, have sex-specific dendritic arborization and re-route pheromone information to create a bidirectional switch. Male-specific *Fru* protein is required for the male form of the circuitry switch by connecting the aSP-f neurons to the circuitry and disconnecting the aSP-g neurons (Kohl et al., 2013). A similar circuitry controlled by *Dsx* and *Fru* switches between courtship and aggression in *Drosophila* males (Koganezawa et al., 2016). More recently, an additional circuitry was identified in *Drosophila* that processes distinct sex-specific inputs, which converges on the *Dsx*-expressing anterior dorsal neuron (aDN) cluster, which in turn relays them to sex-specific outputs (Nojima et al., 2021). This cluster has sexually dimorphic dendritic arborizations, receiving inputs from visual PNs in males, and predominantly olfactory PNs in females. The aDNs in females connect to an egg-laying circuitry, and in males aDNs play a role in motion detection during courtship, enabling the males to locate and track a moving female (Nojima et al., 2021). In conclusion, only minimal sex-specific changes to higher brain order neurons would seem to be sufficient to elicit dramatic differences in sex-specific behaviour (Kohl et al., 2013; Nojima et al., 2021). Taken together, *Dsx* and *Fru* are essential for connecting and disconnecting AL PNs with higher brain order neurons to form circuitries for sex-specific behaviours.

CONCLUSIONS

Antennal lobe sexual dimorphism is determined by genetically fixed growth processes of peripheral and deutocerebral neurons, but the relative contribution of these processes vary across insect orders. The first stages of inducing sexually dimorphic pheromone circuitries are most likely set at the periphery through the expression of novel pheromone receptors in specialized sensilla. Neurons that succeed in surviving programmed cell death are eligible candidates for novel pheromone circuitries. If these functional 'surviving' neurons match the right biochemical properties of their specific sensillum, a new olfactory pathway might be maintained if the novel OSN types are matched by similar expansions in the number of novel pheromone compounds. Pioneering sensory neurons sort the newly formed afferents of these surviving neurons to the AL and corresponding glomerulus. From an evolutionary perspective, an increase in the number of sensilla in one sex would not only simultaneously increase the number of these functional surviving neurons, but also the volume of the corresponding glomerulus and sensitivity to the pheromone component. It would be interesting to see whether the same processes of OSN-programmed cell death contribute to the reduction of glomerular volume and to the loss of redundant glomeruli in one sex, amplifying the degree of sexual dimorphism over evolutionary times. Such a phenomenon could occur in species that have evolved extreme differences in sex-specific tasks, such as in colony-forming insects, or differences in reproductive roles.

The probability for the establishment of sexually dimorphic glomeruli seems to be largely determined by the degree of glia-OSN interactions and PN prepatterning. The relative contribution of either of these two processes determines the degree of evolutionary neuroplasticity. Transplanting male antennae onto female moths will form a male MGC even in the presence of female PNs, suggesting that signals induced by OSNs play a significant role in glia proliferation in these species. It remains to be investigated, however, what these signals are composed of and how glia cells sort incoming OSNs. Although genetically similar, novel OSNs might initially be sorted to the same glomerulus in the AL, but ultimately can be guided to a different location by glia cells and evolve into new glomeruli. The greatest degree of evolutionary neuroplasticity in this process is expected to have occurred in species with complex ALs lacking any specific macroglomerular structures. Hymenoptera integrate pheromone processing into combinatorial codes composed of glomerular clusters instead of MGCs that represent labelled lines. New glomeruli in these species may have initially emerged from a macroglomerulus and evolved into glomerular clusters after pheromone blends increased in complexity. Pheromone receptors would be excellent candidates to investigate this latter instance, given that previous studies have already determined the significant role the olfactory co-receptor *Orco* plays in glomerular formation.

Different outcomes of sexual dimorphism are determined through genetic sex-determination mechanisms, which are able to operate at various levels throughout AL development. The transcription factors *Dsx* and *Fru* can specify which neuron types are expressed and are needed for correct neurite arborization. Differential regulation of factors inducing programmed cell death determines the

birth and death of olfactory neurons. Surviving neurons with beneficial properties can be favoured over evolutionary time and upregulated through sex-determining transcription factors by inhibiting the process of programmed cell death. Understanding the full significance of these transcription factors on AL development is still very much in its infancy, and the large majority of research has been carried out on *Drosophila*. This review can therefore serve as a foundation on which to initiate research into sexual dimorphism in the AL in light of sex-determining transcription factors.

In addition to providing a deeper understanding of the evolutionary processes involved, specific knowledge of pheromones and pheromone circuitries can also have broad environmental and societal benefits. In agriculture and horticulture, sex pheromones are widely used as a tool to control insect pests and disease vectors, for example through mate disruption, but also serve as a potential means to attract biological control agents. Research into neuronal mechanisms of pheromone communication in parasitoid Hymenoptera is still in its infancy, whereas these species are crucial for maintaining ecosystem stability. In the same way, pollinating Hymenoptera fulfil essential ecosystem services and utilize complex pheromone-coding systems that require further research. Sex determination processes play an important role in these and other insect species and may have significant developmental consequences for pheromone-processing circuitries. Integrating sex determination in AL development therefore provides an opportunity to research these concepts and their numerous beneficial applications.

ACKNOWLEDGMENTS

We are grateful to the staff of the Wageningen Electron Microscopy Centre for the production of scanning electron microscopy images of insect antennal sensilla. This work is partly financed by a VIDI grant (016.Vidi.189.099) of the Dutch Research Council (NWO) to ECV and an NWO VENI grant (016.Veni.192.116) to AH.

AUTHOR CONTRIBUTIONS

Aidan Williams: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (supporting); Investigation (lead); Visualization (lead); Writing – original draft (lead); Writing – review & editing (equal). Eveline C Verhulst: Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Funding acquisition (equal); Supervision (equal); Visualization (supporting); Writing – original draft (supporting); Writing – review & editing (equal). Alexander Haverkamp: Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Funding acquisition (equal); Investigation (supporting); Supervision (equal); Validation (supporting); Visualization (supporting); Writing – original draft (supporting); Writing – review & editing (equal).

ORCID

Aidan T. Williams https://orcid.org/0000-0001-5997-8001
Eveline C. Verhulst https://orcid.org/0000-0002-4781-535X
Alexander Haverkamp https://orcid.org/0000-0003-3512-9659

REFERENCES

Agarwal G & Isacoff E (2011) Specializations of a pheromonal glomerulus in the *Drosophila* olfactory system. *Journal of Neurophysiology 105*: 1711–1721.

Anton S & Homberg U (1999) Antennal lobe structure. *Insect Olfaction* (ed. by BS Hansson), pp. 97–124. Springer, Heidelberg, Germany.

Anton S, Ignell R & Hansson BS (2002) Developmental changes in the structure and function of the central olfactory system in gregarious and solitary desert locusts. *Microscopy Research and Technique 56*: 281–291.

Arnold G, Masson C & Budharugsa S (1984) Demonstration of a sexual dimorphism in the olfactory pathways of the drones of *Apis mellifica* L. (Hymenoptera, Apidae). *Experientia 40*: 723–725.

Arnold G, Masson C & Budharugsa S (1985) Comparative study of the antennal lobes and their afferent pathway in the worker bee and the drone (*Apis mellifera*). *Cell and Tissue Research 242*: 593–605.

Baker BS & Ridge KA (1980) Sex and the single cell. I. On the action of major loci affecting sex determination in *Drosophila melanogaster*. *Genetics 94*: 383–423.

Baker BS, Taylor BJ & Hall JC (2001) Are complex behaviors specified by dedicated regulatory genes? Reasoning from *Drosophila*. *Cell 105*: 13–24.

Bellonci G (1883) Intorno alla struttura e alle connessioni dei lobi olfattorii negli Artropodi superiori e nei Vertebrati. Atti della Reale Accademia dei Lincei 1882-1883.

Bertossa RC, van de Zande L & Beukeboom LW (2009) The *Fruitless* gene in *Nasonia* displays complex sex-specific splicing and contains new zinc finger domains. *Molecular Biology and Evolution 26*: 1557–1569.

Billeter JC, Villella A, Allendorfer JB, Dornan AJ, Richardson M et al. (2006) Isoform-specific control of male neuronal differentiation and behavior in *Drosophila* by the fruitless gene. *Current Biology 16*: 1063–1076.

Boeckh J & Boeckh V (1979) Threshold and odor specificity of pheromone-sensitive neurons in the deutocerebrum of *Antheraea pernyi* and *A. polyphemus* (Saturnidae). *Journal of Comparative Physiology A 132*: 235–242.

Boeckh J, Ernst KD & Selsam P (1987) Neurophysiology and neuroanatomy of the olfactory pathway in the cockroach. *Annals of the New York Academy of Sciences 510*: 39–43.

Boeckh J, Kaissling KE & Schneider D (1960) Sensillen und Bau der Antennengeißel von *Telea polyphemus*. *Zoologische Jahrbücher/Abteilung für Anatomie und Ontogenie der Tiere 78*: 559–584.

Boeckh J & Tolbert LP (1993) Synaptic organization and development of the antennal lobe in insects. *Microscopy Research and Technique 24*: 260–280.

Bopp D, Saccone G & Beye M (2014) Sex determination in insects: variations on a common theme. *Sexual Development 8*: 20–28.

Bretschneider F (1924) Über die Gehirne des Eichenspinners und des Seidenspinners (*Lasiocampa quercus* L. und *Bombyx mori* L.). *Jenaische Zeitschriften für Naturwissenschaften 60*: 563–578.

Brill MF, Rosenbaum T, Reus I, Kleineidam CJ, Nawrot MP & Rössler W (2013) Parallel processing via a dual olfactory pathway in the honeybee. *Journal of Neuroscience 33*: 2443–2456.

Brockmann A & Brückner D (2001) Structural differences in the drone olfactory system of two phylogenetically distant *Apis* species, *A. florea* and *A. mellifera*. *Naturwissenschaften 88*: 78–81.

Cachero S, Ostrovsky AD, Yu JY, Dickson BJ & Jefferis GSXE (2010) Sexual dimorphism in the fly brain. *Current Biology 20*: 1589–1601.

Carcaud J, Giurfa M & Sandoz JC (2015) Differential combinatorial coding of pheromones in two olfactory subsystems of the honey bee brain. *Journal of Neuroscience 35*: 4157–4167.

Carey AF, Wang G, Su CY, Zwiebel LJ & Carlson JR (2010) Odorant reception in the malaria mosquito *Anopheles gambiae*. *Nature 464*: 66–71.

Chaffiol A, Kropf J, Barrozo RB, Gadenne C, Rospars JP & Anton S (2012) Plant odour stimuli reshape pheromonal representation in neurons of the antennal lobe macroglomerular complex of a male moth. *Journal of Experimental Biology 215*: 1670–1680.

Chambille I, Masson C & Rospars JP (1980) The deutocerebrum of the cockroach *Blaberus craniifer* Burm. Spatial organization of the sensory glomeruli. *Journal of Neurobiology 11*: 135–157.

Chambille I & Rospars JP (1985) Neurons and identified glomeruli of antennal lobes during postembryonic development in the cockroach *Blaberus craniifer* Burm. (Dictyoptera: Blaberidae). *International Journal of Insect Morphology and Embryology 14*: 203–226.

Chou YH, Spletter ML, Yaksi E, Leong JC, Wilson RI & Luo L (2010) Diversity and wiring variability of olfactory local interneurons in the *Drosophila* antennal lobe. *Nature Neuroscience 13*: 439.

Christensen TA, Harrow ID, Cuzzocrea C, Randolph PW & Hildebrand JG (1995) Distinct projections of two populations of olfactory receptor axons in the antennal lobe of the sphinx moth *Manduca sexta*. *Chemical Senses 20*: 313–323.

Christensen TA & Hildebrand JG (2002) Pheromonal and host-odor processing in the insect antennal lobe: how different? *Current Opinion in Neurobiology 12*: 393–399.

Cline TW & Meyer BJ (1996) Vive la difference: males vs females in flies vs worms. *Annual Review of Genetics 30*: 637–702.

Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J & Carlson JR (1999) A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron 22*: 327–338.

Couto A, Alenius M & Dickson BJ (2005) Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. *Current Biology 15*: 1535–1547.

Couto A, Lapeyre B, Thiéry D & Sandoz JC (2016) Olfactory pathway of the hornet *Vespa velutina*: new insights into the evolution of the hymenopteran antennal lobe. *Journal of Comparative Neurology 524*: 2335–2359.

Couto A, Mitra A, Thiéry D, Marion-Poll F & Sandoz JC (2017) Hornets have it: a conserved olfactory subsystem for social recognition in hymenoptera? *Frontiers in Neuroanatomy 11*: 48.

Das S, Trona F, Khallaf MA, Schuh E, Knaden M et al. (2017) Electrical synapses mediate synergism between pheromone and food odors in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA 114*: E9962–E9971.

Datta SR, Vasconcelos ML, Ruta V, Luo S, Wong A et al. (2008) The *Drosophila* pheromone cVA activates a sexually dimorphic neural circuit. *Nature 452*: 473–477.

De Bruyne M, Foster K & Carlson JR (2001) Odor coding in the *Drosophila* antenna. *Neuron 30*: 537–552.

Deisig N, Kropf J, Vitecek S, Pevergne D, Rouyar A et al. (2012) Differential interactions of sex pheromone and plant odour in the olfactory pathway of a male moth. *PloS One 7*: e33159.

Dekker T, Ibba I, Siju KP, Stensmyr MC & Hansson BS (2006) Olfactory shifts parallel superspecialism for toxic fruit in *Drosophila melanogaster* sibling, *D. sechellia*. *Current Biology 16*: 101–109.

Demir E & Dickson BJ (2005) *fruitless* splicing specifies male courtship behavior in *Drosophila*. *Cell 121*: 785–794.

Dietl MJ (1876) Die Organisation des Arthropodengehirns. *Zeitschrift für Wissenschaftliche Zoologie 27*: 488–517.

Dobritsa AA, Van der Goes van Naters W, Warr CG, Steinbrecht RA & Carlson JR (2003) Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron 37*: 827–841.

Dujardin F (1850) Mémoire sur le système nerveux des insectes. *Annales des Sciences Naturelles Biodiversity 14*: 195–206.

Elmore T, Ignell R, Carlson JR & Smith DP (2003) Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. *Journal of Neuroscience 23*: 9906–9912.

Esslen J & Kaissling KE (1976) Zahl und Verteilung antennaler Sensillen bei der Honigbiene (*Apis mellifera* L.). *Zoomorphologie 83*: 227–251.

Fandino RA, Haverkamp A, Bisch-Knaden S, Zhang J, Bucks S et al. (2019) Mutagenesis of odorant coreceptor *Orco* fully disrupts foraging but not oviposition behaviors in the hawkmoth *Manduca sexta*. *Proceedings of the National Academy of Sciences of the USA 116*: 15677–15685.

Fishilevich E & Vosshall LB (2005) Genetic and functional subdivision of the *Drosophila* antennal lobe. *Current Biology 15*: 1548–1553.

Flögel JHL (1878) Über den einheitlichen Bau des Gehirns in den verschiedenen Insektenordnungen. *Zeitschrift für Wissenschaftliche Zoologie 30*: 556–592.

Galizia CG & Rössler W (2010) Parallel olfactory systems in insects: anatomy and function. *Annual Review of Entomology 55*: 399–420.

Gao Q, Yuan B & Chess A (2000) Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nature Neuroscience 3*: 780–785.

Gardiner A, Barker D, Butlin RK, Jordan WC & Ritchie MG (2008) *Drosophila* chemoreceptor gene evolution: selection, specialization and genome size. *Molecular Ecology 17*: 1648–1657.

Geuverink E & Beukeboom LW (2014) Phylogenetic distribution and evolutionary dynamics of the sex determination genes *doublesex* and *transformer* in insects. *Sexual Development 8*: 38–49.

Ghosh N, Bakshi A, Khandelwal R, Rajan SG & Joshi R (2019) The Hox gene *Abdominal-B* uses Doublesex^{F} as a cofactor to promote neuroblast apoptosis in the *Drosophila* central nervous system. *Development 146*: dev175158.

Gibson NJ & Tolbert LP (2006) Activation of epidermal growth factor receptor mediates receptor axon sorting and extension in the developing olfactory system of the moth *Manduca sexta*. *Journal of Comparative Neurology 495*: 554–572.

Gibson NJ, Tolbert LP & Oland LA (2012) Activation of glial FGFRs is essential in glial migration, proliferation, and survival and in glia-neuron signaling during olfactory system development. *PloS One 7*: e33828.

Gill KS (1963) A mutation causing abnormal courtship and mating behavior in males of *Drosophila melanogaster*. *American Zoologist 3*: 507.

Goldman AL, Van der Goes van Naters W, Lessing D, Warr CG & Carlson JR (2005) Coexpression of two functional odor receptors in one neuron. *Neuron 45*: 661–666.

Goto J, Mikawa Y, Koganezawa M, Ito H & Yamamoto D (2011) Sexually dimorphic shaping of interneuron dendrites involves the hunchback transcription factor. *Journal of Neuroscience 31*: 5454–5459.

Goyal G, Zierau A, Lattemann M, Bergkirchner B, Javorski D et al. (2019) Inter-axonal recognition organizes *Drosophila* olfactory map formation. *Scientific Reports 9*: 11554.

Grabe V, Baschwitz A, Dweck HK, Lavista-Llanos S, Hansson BS & Sachse S (2016) Elucidating the neuronal architecture of olfactory glomeruli in the *Drosophila* antennal lobe. *Cell Reports 16*: 3401–3413.

Ha TS & Smith DP (2006) A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in *Drosophila*. *Journal of Neuroscience 26*: 8727–8733.

Hallem EA & Carlson JR (2006) Coding of odors by a receptor repertoire. *Cell 125*: 143–160.

Hansson BS & Anton S (2000) Function and morphology of the antennal lobe: new developments. *Annual Review of Entomology 45*: 203–231.

Hansson BS, Christensen TA & Hildebrand JG (1991) Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. *Journal of Comparative Neurology 312*: 264–278.

Hansson BS, Ljungberg H, Hallberg E & Löfstedt C (1992) Functional specialization of olfactory glomeruli in a moth. *Science 256*: 1313–1315.

Hansson BS & Stensmyr MC (2011) Evolution of insect olfaction. *Neuron 72*: 698–711.

Haverkamp A, Hansson BS & Knaden M (2018) Combinatorial codes and labeled lines: how insects use olfactory cues to find and judge food, mates, and oviposition sites in complex environments. *Frontiers in Physiology 9*: 49.

Heinrichs V, Ryner LC & Baker BS (1998) Regulation of sex-specific selection of *fruitless* 5′ splice sites by *transformer* and *transformer-2*. *Molecular and Cellular Biology 18*: 450–458.

Hildebrand JG, Matsumoto SG, Camazine SM, Tolbert LP, Blank S et al. (1980) Organisation and physiology of antennal centres in the brain of the moth *Manduca sexta*. *Insect Neurobiology and Pesticide Action* (Neurotox 79), pp. 375–382. Society of Chemical Industry, London, UK.

Ignell R, Anton S & Hansson BS (2001) The antennal lobe of Orthoptera–anatomy and evolution. *Brain, Behavior and Evolution 57*: 1–17.

Ito H, Fujitani K, Usui K, Shimizu-Nishikawa K, Tanaka S & Yamamoto D (1996) Sexual orientation in *Drosophila* is altered by the satori mutation in the sex-determination gene *fruitless* that encodes a zinc finger protein with a BTB domain. *Proceedings of the National Academy of Sciences of the USA 93*: 9687–9692.

Jawlowski H (1948) Studies on the insect brain. *Annales Universitatis Mariae Curie-Sklodowska C 3*: 1–30.

Jefferis GS, Marin EC, Stocker RF & Luo L (2001) Target neuron prespecification in the olfactory map of *Drosophila*. *Nature 414*: 204–208.

Jefferis GS, Vyas RM, Berdnik D, Ramaekers A, Stocker RF et al. (2004) Developmental origin of wiring specificity in the olfactory system of *Drosophila*. *Development 131*: 117–130.

Jhaveri D, Sen A & Rodrigues V (2000) Mechanisms underlying olfactory neuronal connectivity in *Drosophila*—the *atonal* lineage organizes the periphery while sensory neurons and glia pattern the olfactory lobe. *Developmental Biology 226*: 73–87.

Jhaveri D & Rodrigues V (2002) Sensory neurons of the *Atonal* lineage pioneer the formation of glomeruli within the adult *Drosophila* olfactory lobe. *Development 129*: 1251–1260.

Jones WD, Nguyen TAT, Kloss B, Lee KJ & Vosshall LB (2005) Functional conservation of an insect odorant receptor gene across 250 million years of evolution. *Current Biology 15*: R119–R121.

Kaissling KE, Kasang G, Bestmann HJ, Stransky W & Vostrowsky O (1978) A new pheromone of the silkworm moth *Bombyx mori*. *Science of Nature 65*: 382–384.

Kaissling KE & Priesner E (1970) Die Riechschwelle des Seidenspinners. *Naturwissenschaften 57*: 23–28.

Kaissling KE & Renner M (1968) Antennale Rezeptoren für Queen Substance und Sterzelduft bei der Honigbiene. *Zeitschrift für Vergleichende Physiologie 59*: 357–361.

Kalberer NM, Reisenman CE & Hildebrand JG (2010) Male moths bearing transplanted female antennae express characteristically female behaviour and central neural activity. *Journal of Experimental Biology 213*: 1272–1280.

Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. *Science 294*: 1030–1038.

Kenyon FC (1896) The brain of the bee. A preliminary contribution to the morphology of the nervous system of the Arthropoda. *Journal of Comparative Neurology 6*: 133–210.

Kimura Ki, Hachiya T, Koganezawa M, Tazawa T & Yamamoto D (2008) *Fruitless* and *Doublesex* coordinate to generate male-specific neurons that can initiate courtship. *Neuron 59*: 759–769.

Kimura KI, Ote M, Tazawa T & Yamamoto D (2005) *Fruitless* specifies sexually dimorphic neural circuitry in the *Drosophila* brain. *Nature 438*: 229–233.

Kleineidam CJ, Obermayer M, Halbich W & Rössler W (2005) A macroglomerulus in the antennal lobe of leaf-cutting ant workers and its possible functional significance. *Chemical Senses 30*: 383–392.

Koganezawa M, Kimura KI & Yamamoto D (2016) The neural circuitry that functions as a switch for courtship versus aggression in *Drosophila* males. *Current Biology 26*: 1395–1403.

Kohl J, Ostrovsky AD, Frechter S & Jefferis GS (2013) A bidirectional circuit switch reroutes pheromone signals in male and female brains. *Cell 155*: 1610–1623.

Kollmann M, Schmidt R, Heuer CM & Schachtner J (2016) Variations on a theme: antennal lobe architecture across Coleoptera. *PloS One 11*: e0166253.

Kondoh Y, Kaneshiro KY, Kimura KI & Yamamoto D (2003) Evolution of sexual dimorphism in the olfactory brain of Hawaiian *Drosophila*. *Proceedings of the Royal Society of London B 270*: 1005–1013.

Koontz MA & Schneider D (1987) Sexual dimorphism in neuronal projections from the antennae of silk moths (*Bombyx mori*, *Antheraea polyphemus*) and the gypsy moth (*Lymantria dispar*). *Cell and Tissue Research 249*: 39–50.

Koutroumpa FA, Monsempes C, François MC, De Cian A, Royer C et al. (2016) Heritable genome editing with CRISPR/Cas9 induces anosmia in a crop pest moth. *Scientific Reports 6*: 29620.

Kropf J, Kelber C, Bieringer K & Rössler W (2014) Olfactory subsystems in the honeybee: sensory supply and sex specificity. *Cell and Tissue Research 357*: 583–595.

Kurtovic A, Widmer A & Dickson BJ (2007) A single class of olfactory neurons mediates behavioural responses to a *Drosophila* sex pheromone. *Nature 446*: 542–546.

Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H & Vosshall LB (2004) *Or83b* encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron 43*: 703–714.

Lebreton S, Trona F, Borrero-Echeverry F, Bilz F, Grabe V et al. (2015) Feeding regulates sex pheromone attraction and courtship in *Drosophila* females. *Scientific Reports 5*: 13132.

Lee G, Foss M, Goodwin SF, Carlo T, Taylor BJ & Hall JC (2000) Spatial, temporal, and sexually dimorphic expression patterns of the *fruitless* gene in the *Drosophila* central nervous system. *Journal of Neurobiology 43*: 404–426.

Lee G, Hall JC & Park JH (2002) *Doublesex* gene expression in the central nervous system of *Drosophila melanogaster*. *Journal of Neurogenetics 16*: 229–248.

Leydig F (1864) *Vom Bau des thierischen Körpers: Handbuch der vergleichenden Anatomie (Vol. 1)*. Verlag der H. Laupp'schen Buchhandlung, Tübingen, Germany.

Li Y, Hoxha V, Lama C, Dinh BH, Vo CN & Dauwalder B (2011) The *hector* G-protein coupled receptor is required in a subset of *fruitless* neurons for male courtship behavior. *PLoS One 6*: e28269.

Li Y, Zhang J, Chen D, Yang P, Jiang F et al. (2016) CRISPR/Cas9 in locusts: successful establishment of an olfactory deficiency line by targeting the mutagenesis of an odorant receptor co-receptor (*Orco*). *Insect Biochemistry and Molecular Biology 79*: 27–35.

Lin CC, Prokop-Prigge KA, Preti G & Potter CJ (2015) Food odors trigger Drosophila males to deposit a pheromone that guides aggregation and female oviposition decisions. *eLife 4*: e08688.

Linz J, Baschwitz A, Strutz A, Dweck HK, Sachse S et al. (2013) Host plant-driven sensory specialization in *Drosophila erecta*. *Proceedings of the Royal Society B 280*: 20130626.

Liu Q, Liu W, Zeng B, Wang G, Hao D & Huang Y (2017) Deletion of the *Bombyx mori* odorant receptor co-receptor (*BmOrco*) impairs olfactory sensitivity in silkworms. *Insect Biochemistry and Molecular Biology 86*: 58–67.

Manoli DS, Foss M, Villella A, Taylor BJ, Hall JC & Baker BS (2005) Male-specific *fruitless* specifies the neural substrates of *Drosophila* courtship behaviour. *Nature 436*: 395–400.

Masse NY, Turner GC & Jefferis GS (2009) Olfactory information processing in *Drosophila*. *Current Biology 19*: R700–R713.

Missbach C, Dweck HK, Vogel H, Vilcinskas A, Stensmyr MC et al. (2014) Evolution of insect olfactory receptors. *eLife 3*: e02115.

Nakanishi A, Nishino H, Watanabe H, Yokohari F & Nishikawa M (2010) Sex-specific antennal sensory system in the ant *Camponotus japonicus*: glomerular organizations of antennal lobes. *Journal of Comparative Neurology 518*: 2186–2201.

Namiki S, Iwabuchi S & Kanzaki R (2008) Representation of a mixture of pheromone and host plant odor by antennal lobe projection neurons of the silkmoth *Bombyx mori*. *Journal of Comparative Physiology A 194*: 501–515.

Neder R (1959) Allomerrisches Wachstum von Hirnteilen bei drei verschieden grossen Schabenarten. *Zoologische Jahrbücher – Abteilung für Anatomie und Ontogenie der Tiere 4*: 411–464.

Nishikawa M, Nishino H, Misaka Y, Kubota M, Tsuji E et al. (2008) Sexual dimorphism in the antennal lobe of the ant *Camponotus japonicus*. *Zoological Science 25*: 195–204.

Nojima T, Rings A, Allen AM, Otto N, Verschut TA et al. (2021) A sex-specific switch between visual and olfactory inputs underlies adaptive sex differences in behavior. *Current Biology 31*: 1175–1191.

Ochieng SA, Hallberg E & Hansson BS (1998) Fine structure and distribution of antennal sensilla of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). *Cell and Tissue Research 291*: 525–536.

Oland LA, Biebelhausen JP & Tolbert LP (2008) Glial investment of the adult and developing antennal lobe of *Drosophila*. *Journal of Comparative Neurology 509*: 526–550.

Oland LA & Tolbert LP (1987) Glial patterns during early development of antennal lobes of *Manduca sexta*: a comparison between normal lobes and lobes deprived of antennal axons. *Journal of Comparative Neurology 255*: 196–207.

Oland LA & Tolbert LP (1998) Glomerulus development in the absence of a set of mitral-like neurons in the insect olfactory lobe. *Journal of Neurobiology 36*: 41–52.

Oland LA & Tolbert LP (2011) Roles of glial cells in neural circuit formation: insights from research in insects. *Glia 59*: 1273–1295.

Ozaki M, Wada-Katsumata A, Fujikawa K, Iwasaki M, Yokohari F et al. (2005) Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science 309*: 311–314.

von Philipsborn AC, Jörchel S, Tirian L, Demir E, Morita T et al. (2014) Cellular and behavioral functions of *fruitless* isoforms in *Drosophila* courtship. *Current Biology 24*: 242–251.

Prieto-Godino LL, Silbering AF, Khallaf MA, Cruchet S, Bojkowska K et al. (2020) Functional integration of 'undead' neurons in the olfactory system. *Science Advances 6*: eaaz7238.

Rabl-Rückhard H (1875) Studien über Insektengehirne. Reichert und Du Bois Reymond's Archiv für Anatomie, pp. 488–489.

Ray A, van der Goes van Naters W, Shiraiwa T & Carlson JR (2007) Mechanisms of odor receptor gene choice in *Drosophila*. *Neuron 53*: 353–369.

Reddy GV & Guerrero A (2004) Interactions of insect pheromones and plant semiochemicals. *Trends in Plant Science 9*: 253–261.

Reisenman CE, Christensen TA, Francke W & Hildebrand JG (2004) Enantioselectivity of projection neurons innervating identified olfactory glomeruli. *Journal of Neuroscience 24*: 2602–2611.

Reisenman CE, Dacks AM & Hildebrand JG (2011) Local interneuron diversity in the primary olfactory center of the moth *Manduca sexta*. *Journal of Comparative Physiology A 197*: 653–665.

Rideout EJ, Billeter JC & Goodwin SF (2007) The sex-determination genes *fruitless* and *doublesex* specify a neural substrate required for courtship song. *Current Biology 17*: 1473–1478.

Robertson HM, Warr CG & Carlson JR (2003) Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA 100*: 14537–14542.

Roselino AC, Hrncir M, da Cruz Landim C, Giurfa M & Sandoz JC (2015) Sexual dimorphism and phenotypic plasticity in the antennal lobe of a stingless bee, *Melipona scutellaris*. *Journal of Comparative Neurology 523*: 1461–1473.

Rospars JP (1988) Structure and development of the insect antennodeutocerebral system. *International Journal of Insect Morphology and Embryology 17*: 243–294.

Rospars JP & Chambille I (1981) Deutocerebrum of the cockroach *Blaberus craniifer* Burm. Quantitative study and automated identification of the glomeruli. *Journal of Neurobiology 12*: 221–247.

Rospars JP & Chambille I (1989) Identified glomeruli in the antennal lobes of insects: in variance, sexual variation and postembryonic development. *Neurobiology of Sensory Systems* (ed. by RN Singh & NJ Strausfeld), pp. 355–375. Springer, Boston, MA, USA.

Rospars JP & Hildebrand JG (2000) Sexually dimorphic and isomorphic glomeruli in the antennal lobes of the sphinx moth *Manduca sexta*. *Chemical Senses 25*: 119–129.

Rössler W & Brill MF (2013) Parallel processing in the honeybee olfactory pathway: structure, function, and evolution. *Journal of Comparative Physiology A 199*: 981–996.

Rössler W, Oland LA, Higgins MR, Hildebrand JG & Tolbert LP (1999) Development of a glia-rich axon-sorting zone in the olfactory pathway of the moth *Manduca sexta*. *Journal of Neuroscience 19*: 9865–9877.

Rössler W, Tolbert LP & Hildebrand JG (2000) Importance of timing of olfactory receptor-axon outgrowth for glomerulus development in *Manduca sexta*. *Journal of Comparative Neurology 425*: 233–243.

Rössler W & Zube C (2011) Dual olfactory pathway in Hymenoptera: evolutionary insights from comparative studies. *Arthropod Structure and Development 40*: 349–357.

Ryba AR, McKenzie SK, Olivos-Cisneros L, Clowney EJ, Pires PM & Kronauer DJC (2020) Comparative development of the ant chemosensory system. *Current Biology 30*: 3223–3230.

Ryner LC, Goodwin SF, Castrillon DH, Anand A, Villella A et al. (1996) Control of male sexual behavior and sexual orientation in *Drosophila* by the *fruitless* gene. *Cell 87*: 1079–1089.

Sachse S, Rappert A & Galizia CG (1999) The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code. *European Journal of Neuroscience 11*: 3970–3982.

Sato K, Ito H & Yamamoto D (2020) *teiresias*, a *Fruitless* target gene encoding an immunoglobulin-superfamily transmembrane protein, is required for neuronal feminization in *Drosophila*. *Communications Biology 3*: 1–10.

Sato K & Yamamoto D (2020) The mode of action of *Fruitless*: is it an easy matter to switch the sex? *Genes, Brain and Behavior 19*: e12606.

Sen A, Kuruvilla D, Pinto L, Sarin A & Rodrigues V (2004) Programmed cell death and context dependent activation of the EGF pathway regulate gliogenesis in the *Drosophila* olfactory system. *Mechanisms of Development 121*: 65–78.

Sen A, Shetty C, Jhaveri D & Rodrigues V (2005) Distinct types of glial cells populate the *Drosophila* antenna. *BMC Developmental Biology 5*: 1–11.

Schneider D (1964) Insect antennae. *Annual Review of Entomology 9*: 103–122.

Schneider D & Steinbrecht RA (1968) Checklist of insect olfactory sensilla. *Symposia of the Zoological Society of London 23*: 279–297.

Schneiderman AM, Hildebrand JG, Brennan MM & Tumlinson JH (1986) Trans-sexually grafted antennae alter pheromone-directed behaviour in a moth. *Nature 323*: 801–803.

Schneiderman AM, Matsumoto SG & Hildebrand JG (1982) Trans-sexually grafted antennae influence development of sexually dimorphic neurones in moth brain. *Nature 298*: 844–846.

Shirangi TR, Taylor BJ & McKeown M (2006) A double-switch system regulates male courtship behavior in male and female *Drosophila melanogaster*. *Nature Genetics 38*: 1435–1439.

Silbering AF, Rytz R, Grosjean Y, Abuin L, Ramdya P et al. (2011) Complementary function and integrated wiring of the evolutionarily distinct *Drosophila* olfactory subsystems. *Journal of Neuroscience 31*: 13357–13375.

Steinbrecht RA (1970) Zur Morphometrie der Antenne des Seidenspinners, *Bombyx mori* L.: Zahl und Verteilung der Riechsensillen (Insecta, Lepidoptera). *Zeitschrift für Morphologie der Tiere 68*: 93–126.

Steinbrecht RA (1987) Functional morphology of pheromone-sensitive sensilla. *Pheromone Biochemistry* (ed. by GD Prestwich & GJ Blomquist), pp. 353–384. Academic Press, New York, NY, USA.

Steinbrecht RA (1997) Pore structures in insect olfactory sensilla: a review of data and concepts. *International Journal of Insect Morphology and Embryology 26*: 229–245.

Stensmyr MC, Giordano E, Balloi A, Angioy AM & Hansson BS (2003) Novel natural ligands for *Drosophila* olfactory receptor neurones. *Journal of Experimental Biology 206*: 715–724.

Stocker RF (2001) *Drosophila* as a focus in olfactory research: mapping of olfactory sensilla by fine structure, odor specificity, odorant

receptor expression, and central connectivity. *Microscopy Research and Technique 55*: 284–296.
Stockinger P, Kvitsiani D, Rotkopf S, Tirián L & Dickson BJ (2005) Neural circuitry that governs *Drosophila* male courtship behavior. *Cell 121*: 795–807.
Strausfeld NJ, Sinakevitch I, Brown SM & Farris SM (2009) Ground plan of the insect mushroom body: functional and evolutionary implications. *Journal of Comparative Neurology 513*: 265–291.
Syed Z, Kopp A, Kimbrell DA & Leal WS (2010) Bombykol receptors in the silkworm moth and the fruit fly. *Proceedings of the National Academy of Sciences of the USA 107*: 9436–9439.
Taylor BJ & Truman JW (1992) Commitment of abdominal neuroblasts in *Drosophila* to a male or female fate is dependent on genes of the sex-determining hierarchy. *Development 114*: 625–642.
Taylor BJ, Villella A, Ryner LC, Baker BS & Hall JC (1994) Behavioral and neurobiological implications of sex-determining factors in *Drosophila*. *Developmental Genetics 15*: 275–296.
Tolbert LP, Oland LA, Tucker ES, Gibson NJ, Higgins MR & Lipscomb BW (2004) Bidirectional influences between neurons and glial cells in the developing olfactory system. *Progress in Neurobiology 73*: 73–105.
Trible W, Olivos-Cisneros L, McKenzie SK, Saragosti J, Chang N-C et al. (2017) *orco* mutagenesis causes loss of antennal lobe glomeruli and impaired social behavior in ants. *Cell 170*: 727–735.
Usui-Aoki K, Ito H, Ui-Tei K, Takahashi K, Lukacsovich T et al. (2000) Formation of the male-specific muscle in female *Drosophila* by ectopic *fruitless* expression. *Nature Cell Biology 2*: 500–506.
Van der Goes van Naters W & Carlson JR (2007) Receptors and neurons for fly odors in *Drosophila*. *Current Biology 17*: 606–612.
Vareschi E (1971) Duftunterscheidung bei der Honigbiene—Einzelzell-Ableitungen und Verhaltensreaktionen. *Zeitschrift für Vergleichende Physiologie 75*: 143–173.
Verhulst EC & van de Zande L (2015) Double nexus—*Doublesex* is the connecting element in sex determination. *Briefings in Functional Genomics 14*: 396–406.
Verhulst EC, van de Zande L & Beukeboom LW (2010) Insect sex determination: it all evolves around *transformer*. *Current Opinion in Genetics and Development 20*: 376–383.
Viallanes H (1887) Etudes histologiques et organologiques sur les centres nerveux et les organs des sens des animaux articulés. I. Le cerveau du criquet (*Oedipoda coerulescence* et *Caloptenus italicus*). *Annales des Sciences Naturelles*, Zoologie 7e série *2*: 1-98.
Villella A & Hall JC (1996) Courtship anomalies caused by *doublesex* mutations in *Drosophila melanogaster*. *Genetics 143*: 331–344.
Vosshall LB (2000) Olfaction in *Drosophila*. *Current Opinion in Neurobiology 10*: 498–503.
Vosshall LB, Amrein H, Morozov PS, Rzhetsky A & Axel R (1999) A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell 96*: 725–736.
Walther JR (1981) Die Morphologie und Feinstruktur der Sinnesorgane auf den Antennengeisse ln der Männchen, Weibchen und Arbeiterinnen der roten Waldameise *Formica rufa* Linne 1758 mit einem Vergleich der antennalen Sensillenmuster weiterer Formicoidea (Hymenoptera). PhD Dissertation, Free University, Berlin, Germany.
Watanabe T (2019) Evolution of the neural sex-determination system in insects: does *fruitless* homologue regulate neural sexual dimorphism in basal insects? *Insect Molecular Biology 28*: 807–827.
Watanabe H, Haupt SS, Nishino H, Nishikawa M & Yokohari F (2012) Sensillum-specific, topographic projection patterns of olfactory receptor neurons in the antennal lobe of the cockroach *Periplaneta americana*. *Journal of Comparative Neurology 520*: 1687–1701.
Xu P, Atkinson R, Jones DN & Smith DP (2005) *Drosophila* OBP LUSH is required for activity of pheromone-sensitive neurons. *Neuron 45*: 193–200.
Yamamoto D (2007) The neural and genetic substrates of sexual behavior in *Drosophila*. *Advances in Genetics 59*: 39–66.
Yan H, Opachaloemphan C, Mancini G, Yang H, Gallitto M et al. (2017) An engineered *orco* mutation produces aberrant social behavior and defective neural development in ants. *Cell 170*: 736–747.
Zacharuk RY (1980) Ultrastructure and function of insect chemosensilla. *Annual Review of Entomology 25*: 27–47.
Zhou C, Pan Y, Robinett CC, Meissner GW & Baker BS (2014) Central brain neurons expressing *doublesex* regulate female receptivity in *Drosophila*. *Neuron 83*: 149–163.
Zhu H & Luo L (2004) Diverse functions of N-cadherin in dendritic and axonal terminal arborization of olfactory projection neurons. *Neuron 42*: 63–75.
Zhu H, Hummel T, Clemens JC, Berdnik D, Zipursky SL & Luo L (2006) Dendritic patterning by *Dscam* and synaptic partner matching in the *Drosophila* antennal lobe. *Nature Neuroscience 9*: 349–355.

How to cite this article: Williams AT, Verhulst EC & Haverkamp A (2022) A unique sense of smell: development and evolution of a sexually dimorphic antennal lobe – a review. *Entomologia Experimentalis et Applicata* 170: 303-318. https://doi.org/10.1111/eea.13145