EMBO reports

review

The joy of sex pheromones

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Sex pheromones provide an important means of communication to unite individuals for successful reproduction. Although sex pheromones are highly diverse across animals, these signals fulfil common fundamental roles in enabling identification of a mating partner of the opposite sex, the appropriate species and of optimal fecundity. In this review, we synthesize both classic and recent investigations on sex pheromones in a range of species, spanning nematode worms, insects and mammals. These studies reveal comparable strategies in how these chemical signals are produced, detected and processed in the brain to regulate sexual behaviours. Elucidation of sex pheromone communication mechanisms both defines outstanding models to understand the molecular and neuronal basis of chemosensory behaviours, and reveals how similar evolutionary selection pressures yield convergent solutions in distinct animal nervous systems.

Keywords: sex pheromone; receptor; neural circuit; behaviour; evolution

EMBO *reports* (2013) **14,** 874–883; published online 13 September 2013; doi:10.1038/embor.2013.140

See the Glossary for abbreviations used in this article.

Introduction

Nearly 150 years ago, Charles Darwin and the French entomologist Jean-Henri Fabre—although disagreeing on the theory of evolution—both postulated the existence of chemical signals involved in the control of sexual behaviours [1,2]. It was only in the middle of the twentieth century, however, when the German biochemist Adolf Butenandt purified—from half a million scent glands of female silk moths (*Bombyx mori*)—the first sex pheromone: bombykol [3]. Despite this discovery, in many other animals the precise chemical identity and function of sex pheromones in regulating interactions between males and females have been difficult to define. The limited progress might be due, in part, to the inability of our noses to detect the sophisticated chemical conversations of other species [4].

This situation has changed dramatically in the last decade as sex pheromones and receptors have been identified in many species, which have allowed visualization and manipulation of the neural circuits that link these sensory signals with particular behaviours. There is enormous diversity in the chemical nature of sex pheromones, including long-chain hydrocarbons in insects [5], ascaroside (ascr) glycolipids in nematodes [6] and peptides or small proteins in vertebrates (Fig 1; [7,8]). Consistently, the receptors for pheromones have evolved independently in these different animal groups [9,10], and are housed in different types of sensory organ (Fig 1).

Here we consider the crucial roles of sex pheromones and illustrate how diverse animals use and respond to these chemical signals to fulfil them. We focus on the best-described examples of true sex pheromones, defined as chemicals produced by an individual that elicit innate, stereotyped sexual behaviours in conspecifics [11]. We do not cover sexually relevant 'signature mixtures', which are complex combinations of chemicals produced by individuals that can vary within a species and must be learned by the receiver [11], or the many other well-established functions of pheromones—for example, as alarm, trail and aggregation signals [4].

Sex pheromone signalling and gender discrimination

The most important function of sex pheromones is to allow organisms to identify mating partners of the opposite gender. Predictably, chemical signals advertising gender are commonly produced in a sex-specific manner, whether male-specific, female-specific or a combination of the two. Stemming from the identification of bombykol [3], many of the best-characterized insect sex pheromones are female-specific, long-range male attractants [12,13]. This might reflect a historical, rather than taxonomic class, bias: studies of several Hemiptera (true bugs) have revealed principally male-specific sex pheromones [14,15]. Furthermore, in drosophilids, the only identified volatile pheromone is the male-specific cis-vaccenyl acetate (cVA) (Fig 1), which inhibits male–male courtship [16,17]. Non-volatile—or at least less volatile [18]—cuticular hydrocarbon pheromones in insects, detected by contact chemosensation, seem to be combinations of both male-specific and female-specific cues [13]. In *Drosophila melanogaster*, for example, 7,11-heptacosadiene (7,11-HD), an aphrodisiac for males, is a female-specific cuticular hydrocarbon, whilst male hydrocarbons are enriched for 7-tricosene (7-T), an anti-aphrodisiac for other males (Fig 1; [19,20]).

Nematodes have several different mating systems, including dioecy—males and females—as represented by *Panagrellus redivivus*, and androdioecy—self-fertilizing hermaphrodites and males—as represented by *Caenorhabditis elegans*. Although ascr-related sex pheromones are broadly used in nematodes (Fig 1; [6,21]), sex-specific production and responses reflect these different mating systems. For example, *P. redivivus* females, but not males, produce ascr#1, which is repellent for females but a strong attractant for males. *P. redivivus* males produce the dihydroxy ascaroside derivative dhas#18, one of the few characterized female-attractants in worms [22]. *C. elegans* hermaphrodites produce a different pheromone blend to attract

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Received 11 July 2013; accepted 19 August 2013; published online 13 September 2013

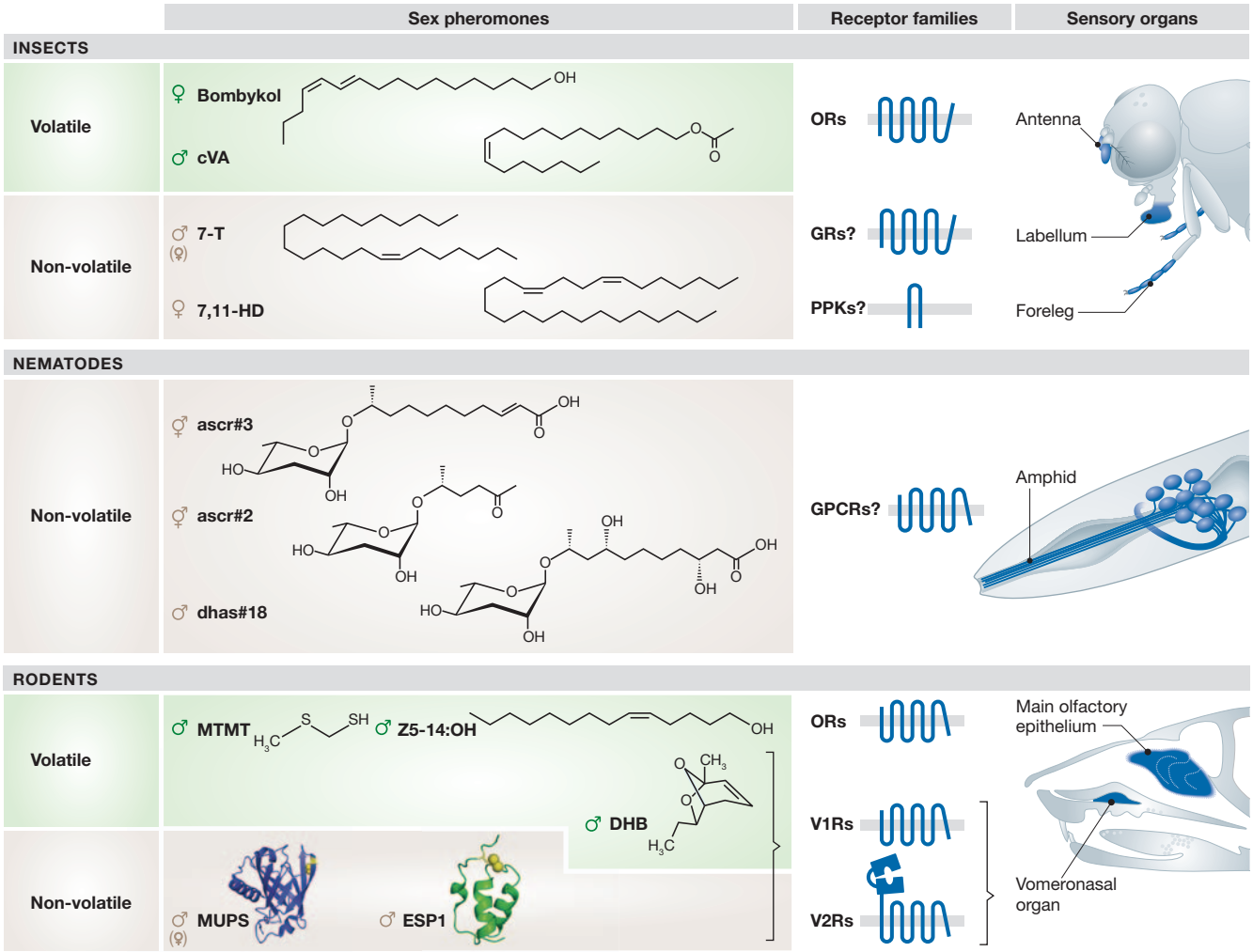

Fig 1 | Diversity of sex pheromones, receptors and sensory organs in insects, nematodes and rodents. In insects, many volatile sex pheromones (for example, bombykol from the silk moth *Bombyx mori* and cVA in drosophilids) are long chain hydrocarbons and are detected by ORs—an unusual class of ionotropic receptors unrelated to GPCRs [108]—which are expressed in the main olfactory organ, the antenna. Non-volatile—or at least less volatile—pheromones (for example, drosophilid 7,11-HD and 7-T) are thought to be detected by GRs (structurally related to ORs) and/or PPK ion channels in the labellum and the forelegs. In nematode worms, sex pheromones are glycolipidic ascarosides and are probably detected by GPCRs expressed in amphid chemosensory neurons, similar to receptors for non-sex pheromone ascarosides. In rodents, many small, volatile sex pheromones, such as DHB, as well as non-volatile protein and peptide pheromones (for example, MUPs and ESP1) might be detected by two different families of GPCRs, V1Rs and V2Rs, respectively, in the vomeronasal organ. A different GPCR family, the ORs, expressed in the main olfactory epithelium detects volatile pheromones, such as MTMT and (*Z*)-5-tetradecen-1-ol (*Z*5-14:OH; [109]). 7,11-HD, 7,11-heptacosadiene; 7-T, 7-tricosene; cVA, *cis*-vaccenyl acetate; DHB, 3,4-dehydro-exo-brevicomin; ESP1, exocrine gland-secreting peptide 1; GPCR, G-protein-coupled receptor; GR, gustatory receptor; MTMT, (methylthio)methanethiol; MUP, major urinary protein; OR, odorant receptor; PPK, Pickpocket; V1R, vomeronasal receptor type 1; V2R, vomeronasal receptor type 2; *Z*5-14:OH, (*Z*)-5-tetradecen-1-ol.

males and, usually, to repel other hermaphrodites. Within this blend, #ascr3—also known as C9 [23]—is behaviourally the most potent [24,25]. In contrast to the dioecious species, however, no male-specific sexual attractants are known. This might be because hermaphrodites represent the overwhelming majority in *C. elegans* (approximately 99.5%, at least in laboratory populations), strongly favouring a male's chances of finding a mate.

In mammals, few definitive cases have been identified in which single pheromone compounds evoke robust sexual behaviours, which might reflect an important contribution of signature mixtures in sexual communication [11,26]. The most compelling example of a sex pheromone is the male-specific exocrine gland-secreting peptide 1 (ESP1; Fig 1). ESP1 is secreted into tear fluids from the extraorbital lacrimal glands [27] and promotes female sexual behaviours, such as lordosis [28]. A second strong sex pheromone candidate is the major urinary protein (MUP) darcin (also known as Mup20), a small protein present in adult male urine ([29]; Fig 1). Darcin can promote attraction of females [29] but also males [30]. In addition, darcin induces spatial learning of other chemical cues, allowing both females and competitor males to relocate sites of previous social interactions [29,30]. Other male pheromones might be volatile chemicals, such as (methylthio)methanethiol (MTMT), 3,4-dehydro-exo-brevicomin (DHB), 2-sec-butyl-4,5-dihydrothiazole (SBT) and (Z)-5-tetradecen-1-ol (Z5-14:OH); these

Glossary

daf	dauer formation
NPR-1	neuropeptide receptor 1
OR	odorant receptor
TGF-β	transforming growth factor beta
Vmn2r116/V2Rp5	vomeronasal 2 receptor 116
V1R	vomeronasal receptor type 1
V2R	vomeronasal receptor type 2

are found in male urine and are attractive to females, either alone or in combination (Fig 1; [31–33]). An ESP1-related peptide of unknown function, ESP36, is expressed only in female tear glands, at least in one mouse strain [34]. However, the best candidate for a female sex pheromone in mammals is (Z)-7-dodecen-1-yl acetate in the Asian elephant, which is released in urine during oestrus. In males this compound elicits flehmen behaviour—curling back of the upper lip and inhalation with the nostrils closed—which is thought to facilitate further chemosensation by the vomeronasal organ [35]. Remarkably, this same molecule is also a female-specific pheromone in many moths, presumably reflecting convergent evolution of volatile hydrocarbon derivatives [35].

Whilst the pheromones that provide gender information are produced sex-specifically, characterization of the cognate receptors and downstream neural circuits has revealed two different strategies as to how these signals are received. In some cases, the pheromone receptor—and the sensory neurons in which it is expressed—are found exclusively in the opposite sex, rendering individuals of the same sex 'blind' to these stimuli. For example, several moth receptors for female pheromones are expressed only in male olfactory organs (antennae), including the *Bombyx mori* bombykol receptor OR1 (Fig 2; [36]). Such sensory dimorphism provides a simple way to couple precisely a pheromone to sex-specific behavioural responses. In *B. mori*, males respond to a female pheromone through a series of behaviours, including wing-flapping, orientation and attempted copulation. Remarkably, artificial activation of BmOR1-expressing neurons—by transgenic manipulation of *B. mori* to express ectopically a pheromone receptor from a different moth species and presentation of its cognate ligand—is sufficient for full induction of these sexual behaviours [37]. Mapping of the neural circuitry responsible for converting detection of a single chemical cue into this range of behaviours will be an exciting, albeit challenging, goal.

In other cases, sex-specific pheromones are detected by both genders, and can elicit distinct behaviours. The *D. melanogaster* male-specific pheromone cVA is detected by OR67d, which is expressed in both male and female antennae (Fig 2; [16,38]). In males, activation of OR67d by cVA inhibits courtship behaviour, thereby preventing males from fruitlessly courting other males. Similarly to *B. mori* OR1-expressing neurons, artificial activation of OR67d neurons is also sufficient to suppress courtship [16]. In females, activation of OR67d neurons is necessary for full sexual receptivity [16]. How cVA can reduce sexual behaviour in males, but increase it in females is an intriguing problem. OR67d-expressing sensory neurons have largely non-dimorphic anatomical and physiological properties. However, higher-order neurons have sexually dimorphic connectivity [39,40], which might underlie sex-specific behavioural responses to the same pheromone (reviewed in [41]).

Discrete sensory neuron populations for *D. melanogaster* cuticular hydrocarbon sex pheromones of females (7,11-HD) and males (7-T) have been identified. These express different combinations of Pickpocket (PPK) receptors (Fig 2; [42–46]). PPKs belong to the degenerin/epithelial sodium channel class of ion channels, but whether these are the pheromone receptors themselves is unclear. A second, apparently distinct, population of sensory neurons for 7-T has been described, which express the gustatory receptors GR32a and GR33a [47,48]. All of these sets of neurons are present in male and female labella as well as in their forelegs, with which flies 'taste' mating partners (Fig 1). Importantly, the PPK-expressing neurons have sexually dimorphic projection patterns (Fig 2; [42–46]). Physiological and behavioural analysis of these neurons has so far been restricted to males [20,42,46–48], but these anatomical observations raise the possibility that females also detect these sex pheromones, but process and respond to them differently. Indeed, females show greater receptivity to males perfumed with additional 7-T [49]. Beyond studies of the roles of individual sex pheromones in *D. melanogaster*, recent work has also begun to explore how volatile and contact chemical cues are integrated to reinforce or refine selection of the right gender for mating [50–52], although the underlying neural basis for sensory integration remains obscure.

Anatomical evidence for sex-specific pheromone processing has also come from studying mouse ESP1. Although exclusively male-specific, this pheromone is recognized by the receptor Vmn2r116—also known as V2Rp5—expressed in vomeronasal organ neurons present in both males and females (Fig 2; [28]). Consistently, ESP1 exposure activates neurons in the vomeronasal organ and several higher centres of the vomeronasal system in both genders [28]. However, at least some ESP1-evoked activity is sexually dimorphic; for example, only females show responses in the posteromedial cortical amygdaloid nucleus and the ventromedial hypothalamic nucleus. ESP1 promotes sexual receptivity in females, notably lordosis behaviour [28]. Although the effect of this peptide on males has yet to be described, the differential activation patterns in the brain are suggestive that this pheromone, as with cVA in *D. melanogaster*, could evoke sex-specific behaviours.

In *C. elegans*, dissection of the neural pathways underlying detection of the hermaphrodite pheromone ascr#3 has revealed a combination of sex-specific sensory detection and central processing properties, which might explain why this pheromone is attractive to males but aversive to hermaphrodites (Fig 2; [23]). ascr#3 stimulates, through an unidentified receptor, the ADL class of sensory neurons in hermaphrodites; these neurons are well-characterized, multi-functional noxious stimuli detectors and their activation promotes avoidance behaviour—although this response can be modulated, as described below [23]. By contrast, males—who also possess ascr#3-sensitive ADL neurons—are attracted by low concentrations of ascr#3 [24]. This marked switch in valence of behavioural response has been related to three sexually dimorphic neural properties. First, ADL physiological responses to pheromone are reduced in magnitude and temporally delayed in males [23]. Second, another class of ascr3#-responsive neurons, ASK, antagonize ADL-mediated avoidance in males, but not hermaphrodites, through the RMG interneuron—a 'hub' that integrates many sensory cues [23,53]. Finally, a third, male-specific neuron, CEM, is necessary for attraction to ascr#3, although it is unknown whether it is directly activated by this pheromone [24]. Thus, both peripheral and central dimorphisms, in part anatomical and in part neurophysiological, seem to underlie the sex-specific behavioural responses to ascr#3.

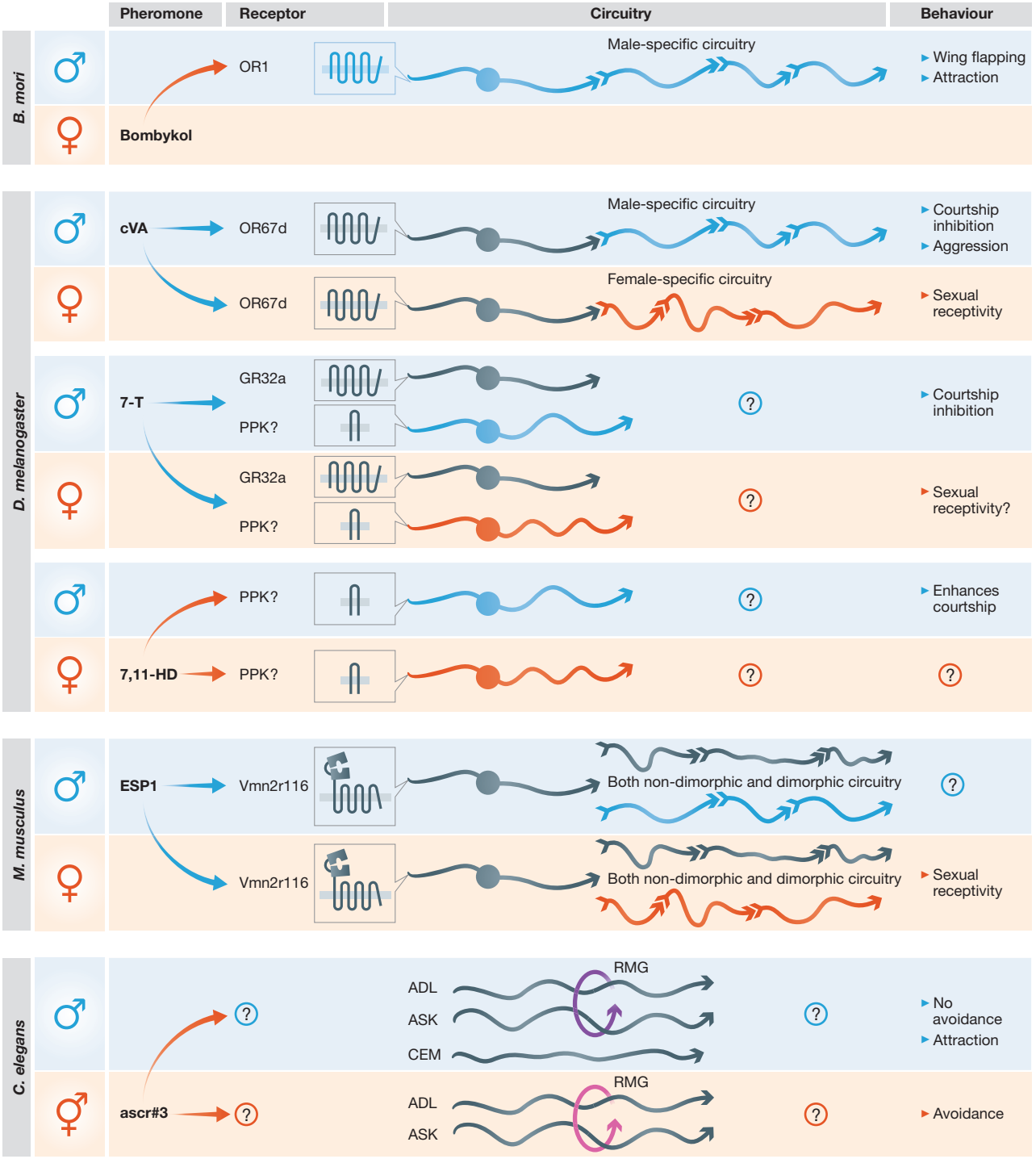

Fig 2 | Sexually dimorphic pheromone processing. Sex pheromones induce gender-specific behaviours through different neural processing strategies. In some cases, such as the female sex pheromone in the silk moth *B. mori*, the cognate receptor is expressed only in the opposite sex. In others, sex-specific pheromones are detected by both sexes but processed differently—either peripherally or centrally—to produce distinct behavioural outputs. cVA, in *Drosophila melanogaster*, and ESP1 in the mouse, are detected by both sexes but might evoke distinct behaviours through sexually dimorphic circuitry (blue: male-specific; red: female-specific) in the central brain. Cuticular hydrocarbon pheromones in *D. melanogaster* (for example, 7,11-HD and 7-T) are detected by circuits that have sexual dimorphisms in sensory neuron projections. In *C. elegans*, ascaroside ascr#3 evokes avoidance in hermaphrodites and attraction in males, in part through a male-specific sensory neuron (CEM) and in part through differential processing of sensory signals from neurons present in both sexes (ASL, ASK) by the RMG interneuron. In many of these examples, the identity of the pheromone receptors is unknown. 7,11-HD, 7,11-heptacosadiene; 7-T, 7-tricosene; ascr, ascaroside; cVA, *cis*-vaccenyl acetate; ESP1, exocrine gland-secreting peptide 1; GR, gustatory receptor; OR, odorant receptor; PPK, pickpocket.

Despite the relatively incomplete knowledge of the identity and function of sex pheromones in any animal species, these examples indicate that gender identification depends on sex-specific pheromonal cues produced by both males and females. These might indicate both whom to and whom not to mate with, through sexually dimorphic peripheral and central neural pathways. Several striking observations suggest that sex-specific behaviours might be founded on relatively limited differences in these sensory circuits. In insects, female tobacco hornworm moths (*Manduca sexta*) grafted with male antennae follow plumes of female pheromones (detected by the male-specific receptors; [54]). In *C. elegans*, mutation of a single developmental regulator, *daf-7*, encoding a TGF-β related protein, is sufficient to render hermaphrodites attracted to pheromones that are normally only attractive to males [55]. Finally, female mice with impaired vomeronasal signal transduction display not only reduced female-specific behaviours but also new behaviours characteristic of males, such as mounting and pelvic thrusting [56]. These studies reveal the existence of 'latent' neural pathways capable of driving behaviours normally shown only by the opposite sex. Such properties might reflect the evolution of sex pheromone-sensing circuits from non-dimorphic pathways through relatively limited developmental modifications.

Discrimination of species, strains and individuals

Successful reproduction also requires the correct matching of conspecifics or, in some cases, individuals of particular strains within species. Many sensory modalities contribute to prevent cross-species and strain mating, including visual and auditory cues, as well as ecological and habitat constraints, which can depend on non-pheromonal chemosensory adaptations [57]. However, sex pheromone signals might be of particular importance to permit species discrimination—both for sexual and non-sexual behaviours—in closely related, morphologically similar species that occupy overlapping niches. Sex pheromones are easily 'mutable', whether by changes in the function or expression of enzymes required for their biosynthesis, or—for peptide/protein pheromones—changes in their sequence. This flexibility can provide a rapid way to evolve private communication between members of the same species, strain or even individuals [57].

Moths offer the best-described examples of sex pheromone-dependent discrimination in phylogenetically close species (reviewed in [57]). Female pheromones typically comprise a species-specific blend of chemicals rather than just a single pheromone. Unique identity is conferred by varying minor components of the blend, or by varying the ratio between components. The latter strategy is exemplified by the European corn borer moth (*Ostrinia nubilalis*), in which two sympatric strains (referred to as E and Z 'races') produce pheromone blends with opposite ratios of isomers of the major female sex pheromone components, E11–14:acetate and Z11-14:acetate (Fig 3; [58]). The genetic basis for this variation has been mapped to allelic variation in a single gene that encodes a fatty-acyl reductase required for pheromone biosynthesis [59]. Such apparently simple changes have a powerful behavioural influence: in the laboratory, these two strains can be mated to produce fertile offspring, but in the wild they do not freely interbreed. The distinct behavioural sensitivity to these pheromone blends is correlated with changes in the peripheral physiological sensitivity of two populations of olfactory sensory neurons that sense these two isomers [60], which could potentially be achieved by a simple switch in OR expression (Fig 3). The molecular basis of this strain specificity remains to be fully worked out. In heterologous cells, several *Ostrinia* ORs respond to pheromone components, such as OR6, which recognizes Z11–14:acetate, and OR3, which is broadly tuned to several pheromone components, including E11–14:acetate [60,61]. However, precise matching of ORs to specific neuronal populations to firmly link *in vitro* and *in vivo* responses awaits. A distinct *Ostrinia* species, the Asian corn borer (*O. furnacalis*), has evolved a pheromone with a small but significant change in the position of the double bond in the hydrocarbon tail (*E*/*Z*12–14:acetate), by using a Δ14-desaturase (Fig 3; [62]). Interestingly, the Asian corn borer OR3 orthologue has drastically reduced sensitivity to the European corn borer pheromone component *E*11–14:acetate, which can be accounted for by a single amino acid polymorphism (A148T; [63]). This receptor mutation might therefore have contributed to the process of speciation by reducing the efficiency of crossbreeding between the ancestors of *O. nubilalis* and *O. furnacalis*.

Species-specific cuticular hydrocarbon blends are well-described in drosophilids. For example, *D. melanogaster* females produce a set of dienes (including 7,11-HD) that are distinct from those found in *D. erecta* females, and essentially absent from other species, such as *D. simulans* [19]. As in moths, such pheromone variation might have a relatively simple genetic basis, with rapidly evolving fatty acid desaturases being prominent candidates [64,65]. Genetic ablation of pheromone-producing cells (oenocytes) in *D. melanogaster* leads to these females being courted inappropriately by *D. erecta* and *D. simulans* males, indicating that these dienes help species discrimination [50]. Artificially perfuming these *D. melanogaster* females with 7,11-HD is sufficient to prevent their courtship by *D. simulans* males, thereby restoring this species barrier [50]. It is noteworthy that this single pheromone seems to act both to promote male courtship within *D. melanogaster* (through PPK-expressing foreleg neurons, as described above) and to inhibit interspecific courtship through an unknown sensory pathway. Formally, this latter function of 7,11-HD in signalling between species is not as a pheromone. A recent study implicated other cuticular hydrocarbon sex pheromones, including 7-T, in preventing male (but not female) *D. melanogaster* from mating with other species, such as *D. virilis* [66]. Here, these signals seem to act through GR32a-expressing neurons in the male forelegs. As described above, this same pheromone and sensory neuron population have also been implicated in preventing inappropriate male–male courtship in *D. melanogaster* [47,48,51]. However, the central neural circuits underlying these behaviours seem to be different [66]. The intertwined relationship of pheromones specifying sex and species discrimination in drosophilids is intriguing and prompts consideration of whether this is a general phenomenon in animals.

Many other insects (such as ants [67] and beetles [68]), as well as various nematodes [21,69] produce species-specific chemical blends. In most cases it is unclear whether these include true pheromones or whether these blends are more appropriately classified as signature mixtures [11]. Regardless, little is known about their role (if any) in determining correct sexual pairing of conspecifics or strains. Insect sex pheromone blends do seem to provide signals to distinguish inbred and outbred individuals: inbred male African butterflies (*Bicyclus anynana*; [70]) and mealworm beetles (*Tenebrio molitor*; [71]) produce chemical signals that are much less attractive to female conspecifics than those produced by outbred animals.

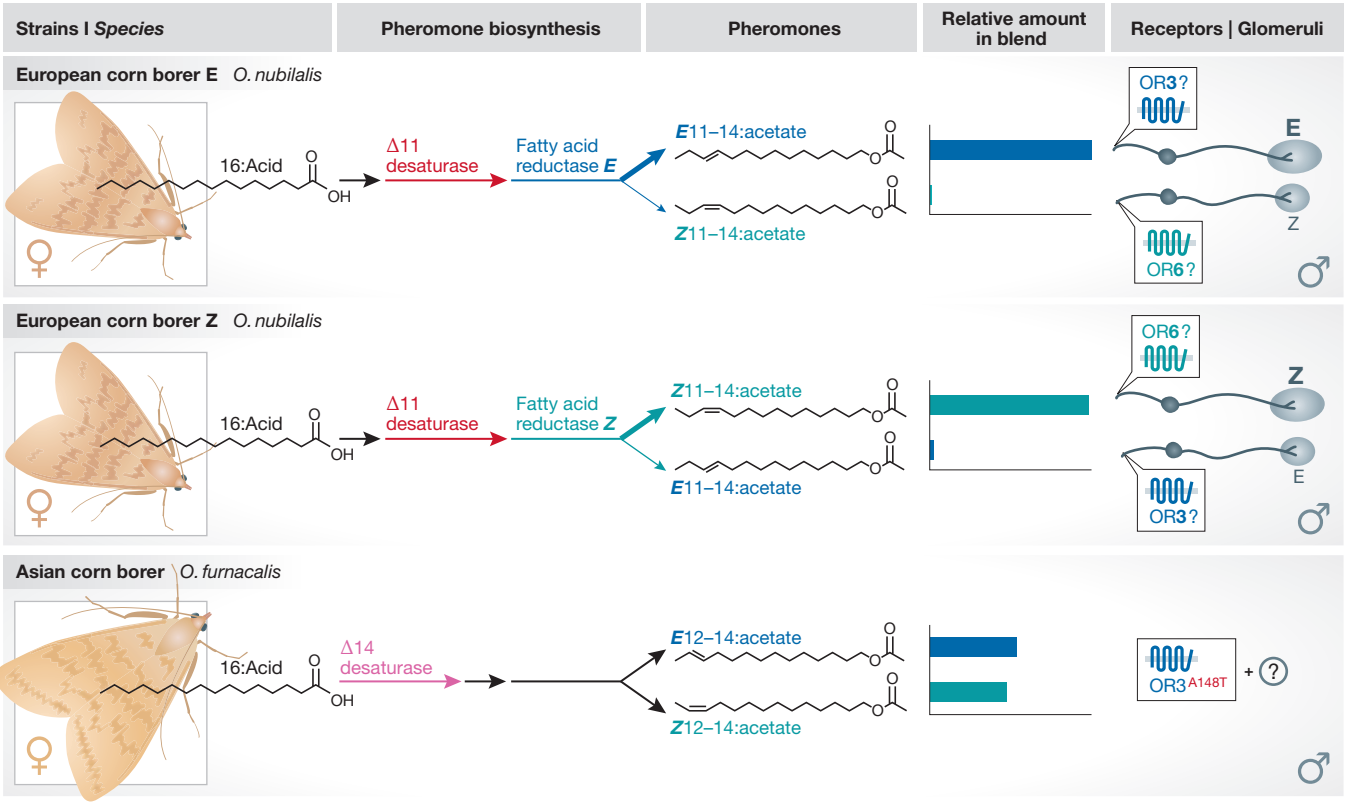

Fig 3 | Species- and strain-specific pheromone recognition. The European corn borer *Ostrinia nubilalis* exists as two distinct strains (or races) 'E' and 'Z'. The female sex pheromone blends of these strains contain different ratios of *E* and *Z* isomers of the 11–14:acetate pheromone component, due to allelic differences in a fatty acid reductase. The underlying molecular basis of pheromone recognition *in vivo* is not clear, but in heterologous cells OR3 responds to several pheromone components (including *E*11–14:acetate), whilst OR6 is selectively tuned to *Z*11–14:acetate. In both strains, the major and minor pheromone components activate the larger and smaller glomeruli, respectively, within the pheromone processing centre in the brain, indicating that a switch in peripheral receptor expression might have occurred between strains. A distinct species, the Asian corn borer *O. furnacalis* uses a different desaturase enzyme during pheromone biosynthesis to produce a structurally distinct *E*/*Z*12–14:acetate pheromone mixture. In this species, the OR3 orthologue has greater specificity for 12–14:acetate pheromone compounds, which can be explained by a single amino acid mutation, A148T. OR, odorant receptor.

Although the mechanism by which inbreeding alters pheromonal blends is unclear, this phenomenon might be advantageous to species by minimizing inbreeding depression within populations [72].

In mammals, clear evidence for sex pheromones in defining species recognition is also lacking. However, the two known male pheromones, ESP1 and the MUP darcin, belong to larger families that display both inter- and intra-species genomic diversity [34,73], raising the possibility that these proteinaceous pheromones could provide this type of information. Indeed, MUPs have been implicated as chemical signals that allow the recognition of genetic heterozygosity—a sign of phenotypic vigour—and enable avoidance of inbreeding, as well as in the distinction of individuals of the same or different species [74–77]. Moreover, some of these proteins can (directly or indirectly) stimulate neurons in the vomeronasal organ [74,78]. Disentangling precisely which MUPs are important, and through which sensory pathways they act, remain important unsolved problems. A third genetically variable family, the major histocompatibility complex (MHC) class I receptors, has also been implicated in defining individual identity [79] (however, by contrast, see [75]). As part of their role in the immune system, these receptors present short peptides (derived from endogenous proteins) on the cell surface. Unexpectedly, such peptides, synthesized *in vitro*, can activate neurons both in the vomeronasal organ and main olfactory epithelium [80,81]. These observations prompted the hypothesis that the MHC genotype of an animal could define a repertoire of associated peptides potentially available to act as pheromonal cues [79]. The natural source of such peptides is, however, unclear as recent proteomic analysis of mouse urine—a rich source of behaviourally relevant chemical cues—identified only one MHC-dependent peptide [82]. Nevertheless, this analysis identified in urine many other genetically variable MHC-independent peptides, which also activated vomeronasal neurons [82]. Although biological functions for such ligands are unknown, the blends of genetically variable peptides—analogous to the chemically diverse hydrocarbon profiles of insects—could potentially provide precise information on species, strain and even individuals during mate choice.

Discrimination of age, fecundity and mating status

Although sex pheromones, by definition, evoke innate, stereotyped sexual behaviours, there can be substantial plasticity in pheromone production and the responses they evoke to provide greater nuance in how these chemicals control interactions between conspecific males and females. The best-documented role for plasticity in sex

Sidebar A | In need of answers

(i) How many different sex pheromones are produced by a given animal and what are their chemical identities? How and where are these signals synthesized, and how is pheromone production modified during development and ageing?

(ii) What are the specific receptors for each sex pheromone, and how are their downstream sensory circuits organized in the brain? How does activation of a specific receptor lead to a particular behavioural response?

(iii) What are the similarities and differences between circuit anatomy and function in males and females, and what are the crucial distinctions that define sexually dimorphic behavioural responses to pheromones? How are these dimorphic properties established during development?

(iv) How do sex pheromone signals integrate with other sensory modalities, such as signature mixtures of chemicals or visual cues, to control behaviour?

(v) Do common strategies in sex pheromone production and detection between organisms reflect evolutionary conservation or convergence? How does the use of distinct strategies between animals reflect their different lifestyles in nature? How do changes in pheromone signalling contribute to speciation?

pheromone signalling is in indicating the age of individuals, a crucial property to ensure mating occurs only after sexual maturation, but before the onset of senescence. For example, in nematodes, sex pheromone production by *Caenorhabditis remanei* females or by *C. elegans* hermaphrodites peaks in young adults [69,83]. Notably, in *C. remanei*, the attraction of male worms to this pheromone also reaches maximum levels at this time [69]. Similarly, in the noctuid moth *Agrotis ipsilon*, male behavioural responses to female pheromones are observed only 3–5 days after eclosion. This maturation correlates with increased physiological responses of olfactory interneurons to pheromones but not to plant volatiles [84,85]. Finally, in mice, ESP1 expression is not observed before four weeks of age [27]—the time when these animals become sexually mature—and initiation (but not the maintenance) of expression is dependent on the male sex hormone testosterone [34].

Pheromone levels can also change in sexually mature adults. In *D. melanogaster*, cuticular hydrocarbon profiles of both males and females vary with age in adults [86,87]. Interestingly, the aphrodisiac 7,11-HD declines over time, which might underlie, in part, a preference of males for younger females [86]. Youthfulness is not always favoured, however: in the European corn borer moth *O. nubilalis*, females have a mating preference for older males [88], and females of the African butterfly *B. anynana* prefer middle-aged (14-day old) over young (three-day old) males [89]. In both cases, these inclinations correlate with changes in proportions of the main components of the male sex pheromone blend. Furthermore, perfuming young *B. anynana* with synthetic 'young' and 'old' male pheromone blends is sufficient to recapitulate the difference in attractiveness, suggesting a direct role for age-dependent pheromone signals in controlling mating success [89].

Ageing also influences production of pheromones, or putative pheromones, in mammals [90–92]. In inbred male mice the urine levels of some androgen-dependent urinary volatiles are increased in middle-aged compared with young adult animals. These changes seem to be behaviourally relevant as female mice are more attracted to the urine of middle-aged animals. The precise pertinent cues are unclear, but MUPs and volatiles bound by MUPs are good candidates, as female preference is abolished when urine is depleted of these proteins [91]. Consistent with this hypothesis, during senescence, male urine becomes less attractive to females and shows declining MUP levels [92], reflecting a potentially honest signal of decreasing fecundity.

In addition to the progressive increases or decreases in sex pheromone signalling that occur with age, shorter-term variation in sex pheromone production and/or responses has been described in several species, including diurnal fluctuations [93–95], seasonal changes [96,97] and, in mammals, during the female reproductive cycle [35,98]. Although specific adaptive advantages of these variations are often easy to accommodate into models reflecting the lifestyle and sexual behaviour of individual species, they are often difficult to prove experimentally as many other phenotypic traits change coordinately over these timescales.

A second major influence on sex pheromone signalling is mating itself. In many species, the chemical profile of the female changes depending on their mating status (reviewed in [99]). This phenomenon has been mostly characterized in insects. For example, in some tortricid moths, mated females suppress production of pheromones that would attract new suitors [100]. In many species, males 'apply' new pheromones to females during mating. In *D. melanogaster*, two male-specific pheromones, cVA and CH503, are transferred to the female during copulation through the seminal fluid, thereby marking her non-virginal status. cVA suppresses further courtship of the mated female by other males through OR67d [16], in the same way as this pheromone prevents male–male courtship (see above). CH503 also inhibits male courtship, through an unknown sensory pathway. Because it perdures longer on females than cVA—perhaps due to its lower volatility—CH503 might suppress remating over a longer time period [101].

Mating experiences can also modify pheromone responses. As with many animals, male *A. ipsilon* moths have a post-ejaculatory refractory period, in order to refill their sex glands [102]. This quiescent period seems to be linked to reduced sensitivity to female sex pheromones. Whilst peripheral pheromone sensation is unchanged, olfactory interneurons show markedly diminished pheromone responsiveness, suggesting a role for neuromodulatory factors in this physiological regulation [102]. A different type of pheromone-sensing plasticity has been observed in *D. melanogaster* males, which show enhanced behavioural sensitivity to cVA after sexual rejection by mated—cVA-scented—females. This 'courtship learning' helps males focus their subsequent mating attempts on more receptive, virgin females [103,104]. Although dopaminergic neuron input to the mushroom body—a site of learning and memory in insects—is required for modulating pheromone responses [104], where and how this intersects with the cVA-sensing circuitry has not yet been determined.

Finally, other social conditions might indirectly modulate sex pheromone-evoked behaviours. In *C. elegans*, behavioural responses to the hermaphrodite sex pheromone ascr#3 are modified by changes in the neuromodulatory state, as revealed by different alleles of the neuropeptide receptor *npr-1* [23]. Low activity alleles of *npr-1*—which are thought to recapitulate a state of metabolic or crowding stress—reduce hermaphrodite avoidance but increase male attraction. NPR-1 functions predominantly in the RMG 'hub' interneuron, which coordinates sexually dimorphic processing of sex pheromones [23]. The adaptive advantage of these changes in pheromone-evoked behaviours is unknown.

Closing remarks

Within the complex universe of chemosensory stimuli that control the interactions of animals with their environment, sex pheromones provide outstanding examples where specific chemical signals are tightly linked to particular species, receptors, sensory circuits and behavioural responses. Fascinatingly, this close relationship has led to sex pheromones being coopted for several other non-sexual functions. For example, larvae of the cotton leafworm moth, *Spodoptera littoralis*, are attracted by an adult sex pheromone, which might help guide them to suitable food sources [105]. Nematophagous fungi sense the ascaroside pheromones produced by their prey as a trigger to set 'traps' [106]. Most famously, sexually deceptive orchids produce remarkably good chemical mimics of female sex pheromone blends of particular insect species in order to attract the corresponding males as unwitting pollinators [107].

Considering the principal roles of sex pheromones, as reviewed here—in uniting conspecific, fecund males and females—there is clearly still much to discover (Sidebar A). Progress will depend on a continued integration of chemistry, molecular genetics, neurophysiology, behavioural analysis and ecology, in both traditional laboratory models and those with better-defined natural histories. Nevertheless, pheromone-sensing pathways have become firmly established as premier models to understand how external information is processed in the nervous system to evoke adaptive behaviours. Current knowledge clearly demonstrates how common functions of sex pheromones are met with common solutions as to how these chemical cues are produced and detected.

ACKNOWLEDGEMENTS

We apologize to our colleagues whose work could not be cited due to space constraints. We thank Jean-Christophe Billeter, Teun Dekker, Darren Logan, Patrick McGrath, Pavan Ramdya, Michael Saina and Kevin Wanner for comments on the manuscript. C.G.-D. was supported by the Postdoctoral Foundation for the Development of Applied Scientific Research and Technology Clarin Programme from the Government of the Principado de Asturias. Research in the laboratory of R.B. is supported by the University of Lausanne, a European Research Council Starting Independent Researcher Grant and the Swiss National Science Foundation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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