

Review



Cite this article: Adams SA, Tsutsui ND. 2020 The evolution of species recognition labels in insects. *Phil. Trans. R. Soc. B* **375**: 20190476. <http://dx.doi.org/10.1098/rstb.2019.0476>

Accepted: 27 February 2020

One contribution of 22 to a theme issue ‘Signal detection theory in recognition systems: from evolving models to experimental tests’.

Subject Areas:
evolution, behaviour

Keywords:
chemical ecology, insect, pheromone, speciation, reproductive isolation, mating

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The evolution of species recognition labels in insects

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The evolution of pre-zygotic reproductive isolation is a key step in the process of speciation. In many organisms, particularly insects, chemical labels are used as pheromones for species-specific mate recognition. Although an enormous body of knowledge exists regarding the patterns of pheromone chemical ecology, much less is known about the evolutionary processes that underlie the origin of new mating pheromones. Here, we examine case studies that have illuminated the origins of species-specific mating pheromones and suggest future directions for productive research.

This article is part of the theme issue ‘Signal detection theory in recognition systems: from evolving models to experimental tests’.

1. Introduction

The organization of life into a hierarchy of selves is one of the most remarkable natural phenomena, laying the foundation for complex entities to arise from more simple components [1,2]. The most obvious selves—cells, bodies, societies and species—are each demarcated by sophisticated self/non-self recognition systems. A new self can evolve when the existing self/non-self recognition system is repressed, eliminated or recalibrated, and a new boundary develops to encompass and define the new self. The origin of new species is an evolutionary process of fundamental importance and, consequently, has been the subject of enormous attention. Of particular interest is how the selective forces that are generated during adaptation, genetic divergence and hybridization can promote the evolution of greater reproductive isolation [3].

Although reproductive isolation can evolve in a number of different ways, species-specific mate recognition is always a key element. The evolution of species recognition systems facilitates the selection of appropriate mates and reduces the occurrence of potentially deleterious heterospecific mating. Thus, the formation and evolution of new self/non-self recognition systems is fundamental to understanding the formation and evolution of species and their reproductive boundaries. However, few examples exist in which we have a detailed picture of the process by which these recognition systems evolve or how new recognition systems are formed. Here, we examine the state of knowledge regarding species recognition system evolution, focusing on chemical recognition labels and limiting our review to insects, from where the majority of our collective knowledge is derived. Although these examples reveal some general principles, many questions also remain regarding the origins of the recognition systems and the typical sequence of events.

Chemosensation is the most ancient and widespread mode of perception and is the major mode of communication in many organisms. Pheromones are semiochemicals or mixtures of semiochemicals that are released by one organism and that affect the behaviour of conspecific individuals. A thorough understanding of the role of pheromones in speciation requires knowledge of both pattern and process. Most research on the chemical ecology of speciation and reproductive isolation is directed toward the former. Examples of such pattern-oriented studies in chemical ecology are those that catalogue the semiochemicals possessed by an organism, describe the distribution of

semiochemicals within or among groups, test for correlations between candidate chemicals and a phenotype of interest, identify the neurological circuitry of chemosensation or catalogue the genes involved in producing or detecting semiochemicals. There is an enormous number of such studies on the topic of the chemical ecology of speciation, many of which report the number and identity of mate recognition pheromones, how these pheromones are synthesized or acquired by the organism, the mechanics of pheromone perception or the behavioural responses to prospective conspecific or heterospecific mates. These studies provide the crucial foundation of knowledge for understanding the processes of speciation and are some of the necessary first steps in understanding the chemical basis of speciation, but alone they do not address the *processes* of speciation. Studies that reveal fundamental processes are much more rare. In addition to the foundational knowledge of patterns described above, an understanding of evolutionary processes typically requires functional manipulative experiments, an understanding of evolutionary timelines and, ideally, knowledge of the order of events in the evolutionary progression.

At the most general level, the processes involved are familiar to all students of evolutionary biology and include adaptation, genetic and phenotypic divergence through time and space, the origin of genetic incompatibilities, the evolution of assortative mating, etc. However, the consideration of systems in which chemical ecology plays a central role reveals a layer of more specific questions:

1. From where do new mate recognition pheromones originate? Do they arise from pre-existing semiochemicals or appear *de novo* as products of sudden large changes? If the former, how is the original function of the semiochemicals reconciled with their new role as pheromones? Also, if the former, does this mean that the accidental, incidental odorants that organisms emit as byproducts of their metabolism and physiology are all potential sex pheromones? Or are there biases that predispose some to take on this function?
2. Do the pheromones for mate recognition arise prior to the ability to perceive them or does the ability to perceive exist first? Are there general rules that determine which is likely to evolve first?

Here, we focus on these process-oriented questions and highlight examples from the literature that have effectively answered them. We recognize that several conceptual models have been developed to explain the evolutionary processes of how sex pheromones and perception may evolve. These include the gradual coevolution of signal and perception through a series of incremental changes, sensory bias, synchronous evolution of signal and perception via genetic linkage, and asymmetric tracking, in which the direction and intensity of selection is correlated with the magnitude of mate competition (reviewed in [3–5]). However, our goal here is not to provide a review that matches empirical studies with models nor provide a comprehensive overview of all empirical examples, but instead, to highlight cases that exemplify particular approaches to studying the processes underlying the evolution of species recognition labels. These examples are diverse and different but, at the same time, possess some fundamental similarities. Thus, we hope that highlighting these examples will be instructive for

scientists who wish to pursue research that reveals broader evolutionary processes, in systems with some solid foundational knowledge of chemical ecological patterns as well as less well-studied non-model systems.

2. Environmental constraints on *Drosophila* cuticular hydrocarbons

Cuticular hydrocarbons (CHCs) are some of the most well-studied insect chemicals, and there are several examples of CHC pheromones arising from ancestral CHCs that performed non-communicative roles. CHCs are widespread in arthropods and well-studied as molecules for communication and recognition [6]. However, CHCs likely evolved initially for desiccation resistance and prevention of microbial infection and only later took on functions as pheromones [6]. Thus, when existing CHCs take on new functions as pheromones, there is potential conflict between these new communication functions and their initial non-pheromonal roles.

CHCs typically take the form of a waxy film on the exoskeleton (cuticle) [7,8]. Different types of CHCs have different physical properties and, therefore, vary in efficacy as barriers to water loss [9–11]. The melting temperature (T_m) of a particular hydrocarbon, for example, is a crucial property because the transition from solid to liquid results in a substantial increase in cuticular permeability, and consequently, water loss. The T_m of different CHCs is, in turn, strongly affected by their molecular structure. Linear n-alkanes have the highest T_m , increasing by 1–3°C with each additional carbon [10]. However, T_m is substantially reduced (by 20–50°C) when CHCs possess double bonds or methyl branches [10]. Because methyl-branching reduces the T_m , selection for increased desiccation resistance should select for fewer methyl-branched alkanes in the CHC mixture [12]. However, the same molecular features that reduce the efficacy of CHCs as desiccation barriers likely enhance their potential as pheromones, by making numerous permutations available and thus increasing potential information content. The ability to produce many different variants is particularly important when CHCs are used for species-specific mate recognition because so many different species produce CHCs, which can increase the risk of accidental hybridization.

The interplay between natural selection for these different functions can lead to the environment constraining the universe of possible CHC molecules that are available for communication roles. For example, the comparison of widely distributed *Drosophila melanogaster* populations has shown that males from cooler regions have the shorter chain monoene 7-tricosene (7-T) as one of their main hydrocarbons whereas males from populations in warmer environments have more of the longer chain 7-pentacosene (7-P) [13]. Consistent with the higher T_m of longer CHCs, flies with higher levels of 7-P have greater desiccation resistance and thus enjoy higher fitness than 7-T flies in higher temperature environments [13]. Furthermore, when flies from a single population with a gradient of 7-P and 7-T profiles were reared at different temperatures in a laboratory selection experiment, after only a few generations, flies reared at the higher temperature produced more 7-P whereas the cooler temperature-reared flies produced more 7-T and started to exhibit partial sexual isolation [13].

This interplay between the environment and behaviour has been explored in more natural settings as well [12]. The closely

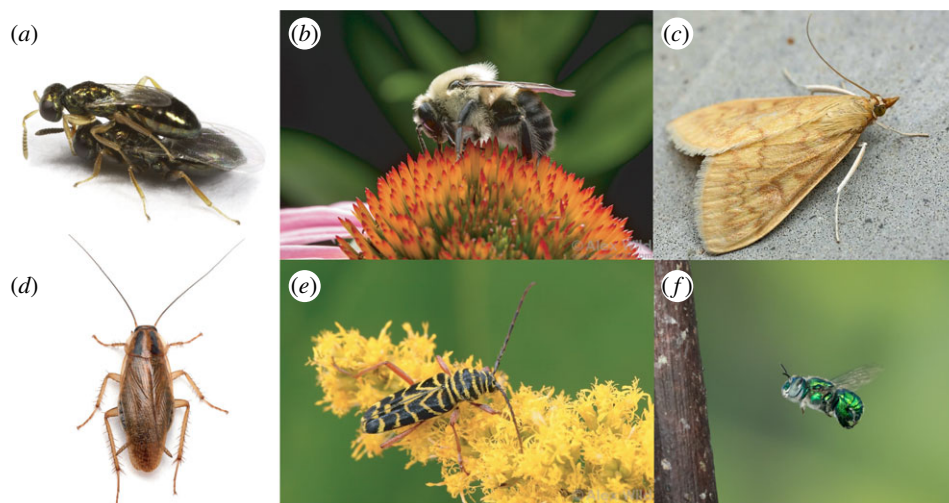


Figure 1. Gallery of some of the insect taxa discussed. (a) The jewel wasp, *Nasonia vitripennis* (photo: Joshua Gibson and Elizabeth Cash), (b) a bumblebee, *Bombus griseocollus* (Alexander Wild), (c) the European corn borer, *Ostrinia nubilalis* (@entomart), (d) the German cockroach, *Blatella germanica* (Alexander Wild), (e) a cerambycid beetle, *Megacyllene caryae* (Alexander Wild), and (f) an orchid bee, *Euglossa dilemma* (Thomas Eltz). (Online version in colour.)

related fruit flies *Drosophila serrata* and *Drosophila birchii* occupy partially overlapping ranges in eastern Australia. *Drosophila birchii* is adapted to more moist conditions and is found in humid rainforest habitats. *Drosophila serrata*, on the other hand, is significantly more desiccation resistant and is found in drier habitats outside and around the rainforest [12]. These two species exhibit strong pre-mating isolation in which chemical cues are used for mate recognition [14]. Examination of CHCs reveals that *D. serrata* has relatively high amounts of methyl-branched CHCs (mbCHCs) while *D. birchii* expresses only trace amounts of mbCHCs in its profile [15]. Methyl-branched CHCs are known to play a role in mate choice while also conferring resistance to desiccation. To investigate whether mbCHCs are involved in both desiccation resistance and species recognition, Chung *et al.* [15] used RNA interference (RNAi) to silence the gene encoding the mbCHC-specific *microsomal fatty acid synthase* (*mFAS*)—a key gene in the biosynthesis of mbCHCs, but not n-alkanes or alkenes. The *mFAS*-silenced *D. serrata* exhibited significant reductions in both desiccation resistance and male mating success. However, the application of synthetic mbCHCs to these flies partially restored their desiccation resistance and the application of a specific mbCHC, 2Me-C26, increased the mating success of *mFAS*-silenced males as well as wild-type *D. serrata*. Overall, this system provides a nice example of how the acquisition of a new biological function can produce molecules that have dual functions, resulting in interesting potential conflicts between the two roles.

3. The evolution of a new sex pheromone in *Nasonia*

It is easy to imagine the evolution of new mating pheromones through gradual changes in the components of a blend, perhaps in allopatry, along with correlated changes in the chemosensory system. However, it is less clear how wholly new mating pheromones could originate *de novo*. Jewel wasps in the genus *Nasonia* (figure 1a) are one model system in which important steps in the origin and evolution of a new pheromone have been reconstructed. In this case, male *Nasonia vitripennis*

attract conspecific virgin females by emitting a blend of three chemicals: 4(R),5(S)-5-hydroxy-4-decanolide (abbreviated 'RS'), 4(R),5(R)-5-hydroxy-4-decanolide (RR) and 4-methylquinazoline (MQ). When the expression of these three semiochemicals was examined in a phylogenetic context, it became clear that the ancestral sex pheromone blend only included RS and MQ; RR appears to have evolved recently in the lineage leading to *N. vitripennis* [16]. Since RS and RR are stereoisomers, a likely scenario for the origin of RR is a simple change in the existing biosynthetic pathway for RS that leads to an altered RR product. Crucially, virgin females of the sister species *Nasonia giraulti* exhibit no discernible behavioural difference toward pheromone blends with or without RR whereas female *N. vitripennis* show significantly stronger attraction to blends that contain RR [16]. This suggests that when RR first appeared, perhaps as a result of a chance mutation, it was neither preferred nor avoided by females, thus allowing it to persist until a behavioural preference subsequently evolved. Quantitative trait locus association studies and functional manipulations with RNAi allowed the authors to identify candidate biosynthetic genes that likely underlie the origin of the RR form [16]. Thus, the order of events in this evolutionary sequence shows that a pre-existing sensory bias is unnecessary for the origin and spread of a new mating pheromone. Although this represents one of the most well-understood examples of sex pheromone evolution, important work remains to be done in clarifying how, exactly, female behavioural preferences for RR evolved after this semiochemical appeared.

4. Genomic mechanisms generate pheromone diversity in *Bombus*

In some cases, proliferation of biosynthesis genes may enable the production of new semiochemical variants, which can then take on functions as pheromones. Advances in genomics and functional molecular characterization are revealing some of these processes. In bumblebees (*Bombus*, figure 1b), for example, the mating pheromone blend of males includes a number of different fatty alcohols, and the composition of

these mixtures varies across closely related species [17]. Fatty acyl reductases (FARs) are key enzymes in the production of these fatty alcohols, and recent functional studies have shown that the expression of specific FAR genes produces predicted pheromone blends in yeast expression systems [18]. Interestingly, FAR genes have undergone an expansion in the lineage containing bumblebees (*Bombini*) and the related stingless bees (*Meliponini*), from one or two gene copies in the ancestors up to 8–25 copies in this lineage [18]. Surprisingly, the genomic region where these FAR genes occur is significantly enriched for transposable elements (TEs). This suggests a mechanism for the proliferation of FAR genes in association with TE expansion in the common ancestor. In the future, it is likely that similar processes that promote genomic instability, rearrangement or gene duplication in other taxa will be found to drive the evolution of recognition label diversity and, thus, facilitate speciation.

5. Evolution of pheromone production and perception in *Ostrinia*

The chemical ecology of lepidopteran mating systems has been the subject of enormous research attention, and one of the iconic systems in this order is the *Ostrinia* moths (figure 1c). The genus *Ostrinia* is a globally distributed group of more than 20 closely related species and, as prominent agricultural pests, their life history, chemical ecology, behaviour, genetics and neurobiology have been examined in great detail [19,20]. Although there are still gaps in our knowledge of the exact sequence of events, much is known about both label production and pheromone perception in this system.

The European corn borer, *Ostrinia nubilalis*, is a polyphagous moth native to Europe, northern Africa and western Asia and is invasive in North America after inadvertent introductions in the early 1900s [19]. Populations of *O. nubilalis* across the globe can vary in the host plant that they prefer (C3 versus C4 plants), the number of reproductive generations per year (voltinism) and their sex pheromone communication system [19]. There are two main strains that exist: the E-strain, in which females produce pheromone blends of (E)-11- and (Z)-11-tetradecenyl acetate (11-14: OAc) at a 99:1 ratio, and the Z-strain, in which females produce these pheromones at a 3:97 ratio. In both cases, males display preferential attraction to the pheromone blend produced by females of the same strain [19,20]. Pheromone production is controlled by the autosomal locus *Pher*, which corresponds to the pheromone gland *fatty acyl reductase* gene *pgfar*. A fixed single-nucleotide polymorphism between *pgfar-e* and *pgfar-z* alleles causes differences in the biosynthesis of isomeric components of the female pheromone blends [21]. Both strains produce the same ratio of E and Z pheromone precursors, but the E-strain enzyme preferentially produces the E isomer and the Z-strain the Z isomer, thus producing the respective ratios of (E)-11-14: OAc and (Z)-11-14: OAc [21]. Using this marker, researchers were able to genotype light- or pheromone-trapped individuals and found complete fixation of the *pgfar-z* allele in the midwestern United States where only Z-strain pheromone populations are found. When moths were collected and genotyped at 11 sympatric sites, reproductive isolation between pheromone strains was found to be stringent in some sites but up to 42% of individuals caught from other sites were found to be hybrids [22]. This indicates that assortative

mating via pheromone cues occurs in some locations but not in others and suggests that genetic differentiation between strains may depend on a combination of different pre-zygotic reproductive barriers—in this case sexual recognition cues and voltinism [20,22].

In addition to the knowledge of pheromone biosynthesis, the pheromone perception side of the mate recognition system is also well known in *O. nubilalis*. The genes responsible for male chemosensory response, *Resp*, are located within a region of the sex-linked Z-chromosome that encodes a suite of genes suggested to be involved in neural development and function [20,22]. The olfactory neurons in male *O. nubilalis* that respond to both the major and minor female pheromone components are physically linked to glomeruli of differing sizes in the antennal lobe [19,23]. These neural connections are identical except for the fact that they are physically reversed between the E- and Z-strain [23]. However, among F₁ hybrids that prefer a more equal ratio of the E and Z pheromone isomers, the macroglomeruli for the major and minor pheromone components are of approximately equal sizes, and the inheritance of glomerulus size was found to be co-dominant and sex-linked [19,23]. Recent genetic work by Koutroumpa *et al.* [24] further showed that the major factors influencing male pheromone response and discrimination between the E- and Z-strains are not necessarily odorant receptors or differences in pheromone detection, but instead are genes that control nervous system structure and the development of neural pathways that convey information from the antennae.

Interestingly, several studies have shown that *Ostrinia* populations can harbour rare males that exhibit chemosensory sensitivity to specific novel odorants. Thus, it is plausible that this species has evolved new mating pheromones through an evolutionary sequence similar to that in *Nasonia* wasps: new semiochemicals are generated by chance mutations to existing biosynthetic pathways and initially remain neutral or nearly neutral. Then, rare sensory variants arise that can detect them, and they spread as a consequence of the increased fitness conferred by assortative mating.

6. Evolution of sex pheromone perception in *Drosophila*

In contrast with the ‘pheromone first’ patterns seen in *Nasonia*, *Bombus* and *Ostrinia*, there are also examples of pheromone perception evolving first. In some cases, the ability to perceive new pheromones can emerge from an existing sensory repertoire that evolved for other, non-reproductive functions. For example, when heightened sensitivity to particularly salient environmental cues evolves, this may produce a ‘sensory drive’ in which semiochemicals that are similar to the environmental odours are predisposed to be acquired as mate recognition labels. While much of the work conducted on *Drosophila* pheromones has focused on compounds found on the cuticle of the flies that act as contact cues, recent work has found aldehyde pheromones that appear to be involved in more long-distance species recognition. Lebreton *et al.* [25] found that the pheromone (Z)-4-undecenal (Z4-11Al) elicits flight attraction in both sexes of *D. melanogaster* but a close relative, *Drosophila simulans*, did not respond to this compound. The biosynthetic precursor for Z4-11Al is the cuticular hydrocarbon (Z,Z)-7,11-heptacosadiene (7,11-HD), a compound found in cosmopolitan populations of *D.*

melanogaster and used for conspecific recognition. Interestingly, *D. melanogaster* from a Zimbabwe population mainly produce a different precursor, 5,9-heptacosadiene (5,9,-HD), and only small amounts of 7,11-HD, and they do not respond to Z4-11Al. Furthermore, twin olfactory receptors, Or69aA and Or69aB, are co-expressed in the same olfactory sensory neuron in these cosmopolitan *D. melanogaster* flies. Or69aA is tuned to food odorants whereas Or69aB is tuned to Z4-11Al. Moreover, it appears that the species-specific Or69aB was derived from Or69aA, which is a receptor found more widely in many *Drosophila* species. This could be evidence for sensory drive where the environment, in this case food odours, shapes sensory preferences that affect mate preference and the evolution of mating signals.

Recent evidence suggests that the *desaturase 1* gene (*desat1*) may be involved in both the production and reception of pheromones involved in mate and species recognition in *Drosophila*. The *desat1* gene has five regulatory regions that yield five alternative transcripts that all give rise to a $\Delta 9$ -desaturase enzyme [26]. The *desat1* gene is involved in the production of the female cuticular hydrocarbon 7,11-HD and male cuticular hydrocarbon 7-T in cosmopolitan populations of *D. melanogaster*, but interestingly, the *desat1* gene is also involved in the perception of these compounds through expression in several brain centres that are involved in chemosensory behaviours relating to reproduction [26]. Alterations of *desat1* transcription were shown to affect male courtship without affecting pheromone discrimination [27]. Furthermore, most cosmopolitan populations of *D. melanogaster* carry a non-functional copy of the *desat2* gene which sits in tandem to the *desat1* gene in their genome. A functional copy of the *desat2* gene is found in African populations of *D. melanogaster* and is the reason for some of the differences found in the pheromone compounds used in mate recognition. It is believed that these *desat* genes are heavily involved in the pheromone communication system and have rapidly evolved to create differences in both pheromone composition and perception in these populations of *D. melanogaster*, as well as in other species of *Drosophila* [26].

7. Permissive mate acceptance in *Blattella*

In many cases, an organism's environment and habitat may shape the specificity of mate recognition. An interesting example where this may be occurring is the German cockroach (*Blattella germanica*; figure 1d), which is a widely distributed human commensal and pest insect. As in many insect species, females of the German cockroach emit a volatile pheromone (blattaquinone) for long-distance attraction of males and produce a low-volatility, short-distance pheromone mixture that arriving males detect by contact chemoreception [28]. The female contact sex pheromone has been identified as a mixture of at least six different cuticular lipids that can trigger male courtship behaviour either individually or in combination [29]. When males detect these pheromones, or detached female antennae that have the pheromones on their surface, they exhibit a stereotypical courtship behaviour. Interestingly, experiments using detached antennae demonstrate that male German cockroaches responded positively to 5 of 20 roach species, some of which are very distantly related [30]. The authors suggest that this permissive mate acceptance behaviour may have evolved, in part, as a byproduct

of relaxed selective pressure in the anthropogenic environment where German cockroaches live. Because closely related species are largely absent, the reduced fitness of accidental hybridization may be rarely experienced, thereby allowing permissive mate acceptance behaviour to spread. At the same time, distantly related species may have separately converged on sex pheromones that are sufficiently similar to those of *B. germanica* to trigger apparent mate acceptance behaviour. It is easy to imagine how new pheromones could become incorporated into an existing repertoire of sex pheromones when the perceiver has such broad, permissive recognition.

8. Evolution of signals and perception in cerambycid beetles

An enormous body of knowledge exists on the chemical ecology of cerambycid beetles (figure 1e), including the identification of numerous compounds for long-distance attraction and short-distance recognition [31]. In many cases, the function of these pheromones is supported by robust behavioural assays, often in the realistic conditions of field trials, and using synthetic versions of the candidate compounds [31]. Although it is puzzling to note that many species produce somewhat similar pheromone mixtures, and pheromone traps can yield multiple different species, closer examination reveals that spatial and temporal context of pheromone release can add the necessary specificity to allow pre-zygotic reproductive isolation [32].

Complementary research revealing the identity, functionality and phylogenetic relationship of chemosensory receptors has contributed insights into the role of chemical ecology in speciation. For example, Mitchell *et al.* [33] characterized an odorant receptor, McOr20, from the cerambycid beetle *Megacyllene caryae* that showed varying sensitivity to different stereoisomers of 2,3-hexanediol—a pheromone component produced by numerous species of cerambycids. The authors found that McOr20 was more sensitive to (2S,3R)-2,3-hexanediol than it was to the other isomers, but that it was still sensitive to the other isomeric forms at a low level. They suggested that this low, but present, sensitivity could be a function retained from an ancestral receptor for diols and hydroxyalkanones. Thus, as seen in the German cockroach above, the breadth of this sensory window could provide opportunities to acquire new species-specific pheromones.

9. Simultaneous evolution of signal and perception in *Euglossa*

One fascinating variation on this theme is well documented in the neotropical *Euglossa* orchid bees (figure 1f). In these bees, males collect fragrances from their environment, especially odorants from orchids and other flowers [34]. These substances are stored in specialized cavities in the hindlegs of the males and later emitted when the males court females [34]. In Central America, *Euglossa dilemma* and *Euglossa viridissima* exist in partially overlapping ranges and appear to have diverged approximately 150 000 years ago [35]. Each of these species collects a unique compound that is not present in the other species' repertoire: *E. dilemma* collects a substance known as HNDB (2-hydroxy-6-non-1,3,dienyl benzaldehyde), but *E.*

viridissima does not. *Euglossa viridissima* collects L97, a derivative of linoleic acid, but *E. dilemma* does not [36]. Interestingly, genomic analyses identified a single region of extremely high differentiation, and a single odorant receptor (OR) gene within this region appears to have evolved under strong positive selection. This sensory evolution has likely been a major contributor to the divergence of these two species by driving differential preferences for the respective, species-specific semiochemicals [36].

A remarkable feature of this system that sets it apart from other examples is that changes in a single sensory gene can drive simultaneous changes in both the label and the preference. When new olfactory sensitivities arise through changes in ORs, the mating signals produced by males can change, as males may then collect new types of fragrances that they later present to females. However, if the same ORs are expressed in females and also enable detection of the new semiochemicals, then female preferences may simultaneously expand to include the new fragrance components. It will be interesting to see future work that connects these changes in the peripheral nervous system to both male and female behaviour.

10. Conclusion

What do these examples tell us about how to build a greater understanding of the evolutionary processes that underlie the origin and evolution of species recognition pheromones? First, the wealth of existing knowledge about the identity and occurrence of potential semiochemicals provides a strong foundation to build upon. One clear shortcoming, however, is the uneven taxonomic distribution of these data. Even among the many descriptive pattern-oriented studies, the vast majority of research has been performed in the Diptera, Hymenoptera, Coleoptera and Lepidoptera, and taxon sampling within these orders is also biased toward certain families, genera and species. Moreover, the wealth of taxonomic and behavioural diversity that exists in non-insect arthropods is woefully understudied. In part, these biases arise from the historical emphasis on the chemical ecology of agriculturally significant insects, as a means of developing potential pheromone-based controls. An unfortunate consequence of this is that our current understanding of pheromone evolution and speciation is derived from insects with particular, perhaps non-representative, life histories. To fully understand the complexity of speciation, it is imperative that a variety of taxa be studied to examine how alternative lifestyles and evolutionary history may affect the processes of pheromone-mediated speciation.

It is clear that attention to both the pheromone production and perception sides of species-specific mate recognition is necessary to provide clarity on the sequence of events during pheromone-mediated speciation. The most widely held model is that genetic differentiation between populations can lead to reduced fitness in 'hybrid' offspring, through a variety of mechanisms, including Dobzhansky–Muller incompatibilities. These can act as post-zygotic reproductive isolating mechanisms by reducing gene flow between the populations, perhaps only slightly, and thus create selective pressure that favours the spread of assortative mating. A number of questions remain unanswered, however, about how exactly this works. In terms of sensory

perception, does this selection operate primarily on the peripheral nervous system (detection) or on behavioural preferences (responses)? Answers to this question will come from behavioural experiments in which different pheromone blends can be tested for their attraction within and among species and populations. In addition, combining molecular genetic tools, such as delta halo (empty neuron) *Drosophila* lines, CRISPR-cas9 gene editing and heterologous gene expression systems with neurophysiological and behavioural approaches, will enable *in vivo* functional testing of chemosensory receptors that are used in the perception of mating pheromones, revealing how changes in sensory perception are involved in this process.

On the signal production side, what are the features of signals that predispose them to be adopted as species recognition labels? As described above, one emerging pattern is that FAR and FAS genes appear to be commonly involved in the origin of new pheromones, as changes in these genes can allow new pheromones to emerge out of the diverse fatty acid metabolic pathways. In these cases, as well as when pheromones arise from other chemical families, understanding the biosynthetic pathways that underlie their production will reveal the underlying genetic and protein machinery that changes in response to selection for assortative mating.

By accumulating such knowledge about the patterns of chemical communication and placing it in a phylogenetic context, we will more clearly understand the sequence of evolutionary events that are behind the origins of pheromones and their roles in reproductive isolation and speciation. Of particular value are careful analyses of closely related species, ideally sister species and close relatives, in which the presence and absence of pheromones can be mapped to reconstruct the timing of their origin. In addition, studies of non-model organisms will diversify our knowledge of pheromone evolution and will likely reveal unanticipated mechanisms and new surprises.

Finally, as we build a more robust body of empirical knowledge about the evolutionary processes involved in species-specific mate recognition, we will be better able to link data to theory and, perhaps, develop new conceptual models. Within the literature on social evolution, the influential work of Reeve [37] and others has facilitated research on social recognition systems, thus providing a framework for generating new hypotheses and providing predictions about the structure, function and evolutionary dynamics of societal recognition. In the same way that Reeve stimulated the field of social recognition by clearly setting forth the signal production and perception components of societal self/non-self recognition systems and how their interactions drive the evolution of societal boundaries, similar attention to the signal production and perception components of species boundaries will likely prove to be equally fruitful.

Data accessibility. This article has no additional data.

Authors' contributions. This paper was conceived and co-written by both authors equally.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by the U.S. National Science Foundation (IOS-1557934/1557961), USDA National Institute of Food and Agriculture (2016-67013-24749) and USDA Hatch Project (CA-B-INS-0087-H).

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