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1                    **Evolutionary Importance of Intraspecific Variation in Sex Pheromones**

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12  
13                    **Keywords**

14                    sexual communication, trait variation, intraspecific communication, species interactions,  
15                    signal evolution

25 **Highlights**

26

27 • Sex pheromones have traditionally been viewed as species recognition signals and  
28 thus are expected to show little within-species variation in composition and amount.

29 Current research, however, reveals a high degree of intraspecific variation in sex  
30 pheromone communication channels, suggesting they are molded by multiple  
31 selection forces.

32

33 • Sex pheromone signaling can be costly and plastic, and signals as well as responses  
34 may be affected not only by between-species interactions (e.g. communication  
35 interference, host plants, geographic variation), but also by within-species interactions  
36 (mate choice) in addition to the genetic architecture, physiological state, and previous  
37 experience of individuals.

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39 • As variation in sex pheromone communication can be induced by intrinsic factors, its  
40 evolution may not only follow ecological speciation, but also be the driver of  
41 divergence.

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## 50 **Abstract**

51 As sex pheromones in many insect species are important species-recognition signals that  
52 attract conspecifics and inhibit attraction between heterospecifics; sex pheromones have  
53 predominantly been considered to evolve due to interactions between species. Recent  
54 research, however, is uncovering roles for these signals in mate choice, and that variation  
55 within and between populations can be drivers of species evolution. Variation in pheromone  
56 communication channels arises from a combination of context-dependent, condition-  
57 dependent, or genetic mechanisms in both signalers and receivers. Variation can affect mate  
58 choice and thus gene flow between individuals and populations, affecting species' evolution.  
59 The complex interactions between intraspecific and interspecific selection forces calls for  
60 more integrative studies to understand the evolution of sex pheromone communication.

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## 62 **Sex Pheromones and Their Functions**

63 Pheromones are semiochemicals involved in intraspecific communication, where species-  
64 specific signals are released by a sender that modify the behavior of a receiver [1]. Sex  
65 pheromones signal attraction and selection of potential mates. Sex pheromones are used by a  
66 variety of animal species, but the focus of this Review is on insect sex pheromones, as most  
67 pheromone research has been conducted on this class of animals. The traditional view is that  
68 sex pheromones are important species recognition signals to distinguish between species and  
69 thus under **stabilizing selection** (see Glossary) [2]. As closely related species can have  
70 similar sex pheromone signals that may only differ in the ratio of the different chemical  
71 constituents, the signaler and the receiver need to be finely tuned to recognize each other [3].  
72 Small changes in pheromone release rate or ratio of the chemical constituents can affect  
73 attraction of the receiver [4].

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75 Since sex pheromones are also mate recognition signals acting within species, their roles in  
76 reproductive isolation and speciation processes are important. Reproductive isolation can be  
77 shaped by **reproductive character displacement** [5]. In sex pheromone communication  
78 channels, reproductive character displacement has been found in closely related,  
79 sympatrically occurring taxa [6,7], which lessens communication interference between  
80 closely related and **sympatric species**, but can generate variation between populations that  
81 lead to divergence [5]. Intraspecific variation between geographically distant populations has  
82 been described in many species [8-10], suggesting that such communication interference and  
83 other environmental factors affect variation in pheromone communication.

84

85 Even though intraspecific variation between geographically isolated populations is generally  
86 accepted, variation within populations is still thought to be less common. Johansson & Jones  
87 [11], however, reviewed the role of sex pheromones in mate choice, showing the possibility  
88 of variation in these signals even within populations. We now know that sex pheromones can  
89 even be plastic, and this **plasticity** can be translated into quantitative [12,13] or qualitative  
90 [14] intrapopulation differences. This can take place over an individual's lifespan [12,15],  
91 as a consequence of seasonal development [13], or due to the vicinity of heterospecifics  
92 during development [14]. Since sex pheromones can provide information on the general  
93 condition of the signaler, and the receiver can select based on this variation, intraspecific  
94 variation in sex pheromones must be more common than previously assumed.

95

### 96 **Why Has Intraspecific Variation Been Overlooked?**

97 Although chemical communication is considered to be the oldest form of communication  
98 [16], humans are mostly visually oriented and thus much of the research focused on sexual  
99 signaling has been on variation in visual signaling. Also, it has proven challenging to analyze

100 individual-level variation in chemical extracts. For example, identification of the first moth  
101 sex pheromone required thousands of individuals pooled together [17], naturally obscuring  
102 any intrapopulation variation. In addition, research on sexual selection and sexual conflict  
103 theory is affected by biases of sex roles and assignment to different taxa in sexual selection  
104 research [18]. In moths, females are typically thought to be the signalers and release the sex  
105 pheromone, while males are the responding sex, which could explain the higher volume of  
106 literature on female sex pheromone variation in moths [19-22]. Males, however, also produce  
107 and release sex pheromone [23] to which females may respond, but this aspect has been little  
108 explored (Box I). Finally, female-signaling insect systems are well-represented amongst pest  
109 species, making them economically important. Sex pheromones are commonly used to  
110 monitor the presence and abundance of these pests and to disrupt mating. These applied  
111 aspects of insect chemical ecology likely increased the focus on sex pheromones at the  
112 species level. Thus, it is possible that, to date, our understanding of sex pheromone evolution,  
113 and in particular how intraspecific variation can affect a single species, has been held back by  
114 our research focus.

115

### 116 **Mechanisms Underlying Intraspecific Sex Pheromone Variation**

117 Both sender and receiver are involved in the process of mate attraction, and intraspecific  
118 variation in pheromone communication can either arise as a consequence of physiological  
119 variation in the signaler (sender-specific driver of variation) or the receiver (receiver-specific  
120 driver of variation). To add another layer of complexity, both the sender and the receiver  
121 experience physiological changes within their lifetime, adding within-individual variation to  
122 the between-individual variation at the population level. In addition, senders may become  
123 receivers and vice versa.

124

125 *Sender's Perspective*

126 Diet, age, mating status, and vicinity to conspecifics are among the mechanisms that can  
127 produce physiological changes and contribute to maintenance of intraspecific variation in  
128 pheromone production. These changes can lead to both within- and between-individual  
129 variation. When considering intraspecific sex pheromone variation, it is important to first  
130 consider how they are produced. In some species, pheromone components and precursors are  
131 sequestered from the diet, while in others they are produced *de novo* within the animal [24].  
132 Although both forms of pheromone production rely on the resources available, pheromones  
133 derived from the diet will be more vulnerable to environmental and host-related effects, and  
134 thus to external conditions. In contrast, species that produce their pheromones *de novo* are  
135 probably less susceptible to externally-induced variation, but may exhibit variation due to  
136 internal processes. This is especially likely if there are costs to pheromone production, as this  
137 could result in trade-offs with other processes. Sex pheromones can indeed be costly:  
138 nutritional state influences the amount of pheromone produced in cockroaches [12], smaller  
139 female moths have lower fitness when they are stimulated into pheromone signaling [25], and  
140 pheromone composition predicts the fitness of female moths [26]. The **signaling sex** may  
141 communicate its nutritional state to the receiver [27]. There is also ample evidence that sex  
142 pheromones are affected by suboptimal diet at the larval stage [15,28], in adults [12], or at  
143 both life stages [15]. Pheromone production can be recouped if a higher nutritional diet is  
144 obtained [12], resulting in a dynamic environment-individual interaction. Regardless of how  
145 sex pheromones are produced, their variation can also be a consequence of the internal  
146 physiological state and its changes, such as mating status [29,30] or aging [31,32]. Generally,  
147 a greater investment in pheromone signaling is expected in older unmated females [33]. Older  
148 females may also call earlier in the night to avoid competition with more attractive young  
149 females [34].

150  
151 Other mechanisms that can affect pheromone production and release are pathogens [19],  
152 volatiles from host plants [31], conspecifics [35,36] or prey [37]. Verheggen et al. [37]  
153 recently found that pheromone production in the Asian lady beetles (*Harmonia axyridis*)  
154 (Figure 1A) is conditioned by the presence of prey, as exposure to volatiles of the prey  
155 initiated pheromone production. Also, the female calling behavior of the gregarious beet  
156 webworm moth (*Loxostege sticticalis*) is affected by the vicinity to male conspecifics [38].  
157 Pheromone release can also be increased by crowding and sexual competition, for example in  
158 the American grasshopper (*Schistocerca americana*) [35]. It remains unclear if any of these  
159 changes represent adaptive strategies, i.e. changes in resource allocation depending on  
160 individual status, or are simply non-adaptive responses to environmental triggers. Examples  
161 that do suggest a level of adaptive response to the environment are developmental plasticity  
162 due to the chemical environment, when individuals have been exposed to the chemical  
163 signals of heterospecifics [14], as a consequence of environmental temperature [39], seasonal  
164 variation [13], or nocturnal light pollution [40].

165  
166 Finally, genetic and behavioral mechanisms may contribute to within-population pheromone  
167 variation. For example, a point mutation identified in the tobacco budworm (*Heliothis*  
168 *virescens*) (Figure 1B) translates to a sex pheromone signal with a higher or lower ratio of  
169 two pheromone components [41]. The different ratios of the two components affect the  
170 signal's attractiveness to males, and females releasing a signal with a higher ratio of the two  
171 components are less attractive than females releasing pheromone signal with a lower ratio  
172 [36]. Unattractive females can obtain matings while in close proximity to attractive females  
173 and, therefore, unattractive females are maintained in the population [36]. Similarly, in the  
174 European corn borer (*Ostrinia nubilalis*), two genetically identified sex pheromone strains

175 [42] are maintained because males assortatively mate with females of their own pheromone  
176 strain [43].

177

### 178 *Receiver's Perspective*

179 The traditional view holds that signalers and receivers are finely tuned, where the predicted  
180 shape of the receiver's **preference function** corresponds to the distribution of the female  
181 pheromone signal in the population. Experimental evidence from the 1970-80's suggested  
182 that female pheromone signals experience stabilizing selection [3] and there is a lack of  
183 variation in male responses, because of optimal tuning to specific component ratios [2,4].

184 Variation in either the signaler or receiver, however, can introduce novelty in the  
185 communication channel even in established sender-receiver systems. For example, the rare  
186 receivers in the population that track changes in the pheromone signal [44], which might be  
187 possible through a **saltational event** [45]. New compounds in the signal may remain  
188 unperceived initially and only later do receivers evolve the preference for the new signal [46].  
189 This scenario is known as the asymmetric tracking hypothesis [47]. An alternative and new  
190 conceptual model proposes that the preference trait evolves first in the receiver as a veiled  
191 preference, before the preferred trait is evolved in the signaler, and the receiver starts to select  
192 individuals with the newly preferred trait as soon as the trait arises [48].

193

194 Variation in receivers can take place at different levels, such as between- and within-  
195 individuals, and at different life stages, as a consequence of plasticity in the olfactory system  
196 [49]. In some species, population density can contribute to variation in the morphological  
197 structures of the receiver as a consequence of developmental plasticity. For example, gum-  
198 leaf skeletonizer male moths (*Uraba lugens*) develop longer and more elaborate antennae to  
199 locate females in sparse populations [50]. This plasticity depends on the population density

200 during the developmental stage and suggests that individuals can be more or less sensitive to  
201 pheromone signals.

202

203 Within the lifespan of an individual, plasticity in behavioral responses is well demonstrated in  
204 relation to circadian rhythms, as males of many moth species actively respond to pheromone  
205 sources at restricted times in the photoperiod [51]. A difference in gene expression in odor  
206 receptors seems to be at the base of this behavioral plasticity, linked to both the physiological  
207 state [52] and circadian rhythms [53] with, for example, effects on antennal sensitivity [54].  
208 Responses to sex pheromones can also be modulated by experience, where pre-exposure to  
209 sex pheromones increases long-term sensibility and responsiveness [55,56]. The olfactory  
210 system dynamically adjusts to optimally perceive the surroundings [57] and differential  
211 receptor expression characterizes, for example, mated and unmated individuals [58]. After  
212 matings, some insects become less sensitive to sexual signals and cues for mating sites [59]  
213 either through a reduction in antenna sensitivity [54] or differences in pheromone processing  
214 in the central nervous system in the brain [60]. In other instances, environmental stress can  
215 have an effect on the receiver olfactory system. Sublethal pesticide doses was shown to  
216 increase the peripheral sensitivity of cotton leafworm (*Spodoptera littoralis*) males and  
217 increase their mating success [61]. A recent review [49] reports that the plasticity of the  
218 olfactory system occurs at all levels of the olfactory pathway. When studying the insect  
219 sensory system, it is challenging to integrate the information from the periphery to the brain,  
220 therefore, we have just begun to understand these interactions. As the olfactory system can  
221 plasticly respond to, for example, changes in the environment, physiological state, social  
222 interactions and experience, variation in the olfactory system among individuals is likely to  
223 lead to differences in sensitivity and responses.

224 **Ecological Consequences of Intraspecific Variation: Effect on Attractiveness and**  
225 **Mating Success**

226 Intraspecific variation in sex pheromones can have important consequences in attractiveness,  
227 mating success and mating behavior, even when the source of the variation is entirely  
228 environmental. Furthermore, these consequences can be sex-specific.

229

230 Volatiles produced by host plants can affect the production and response to sex pheromones.

231 This can in turn affect mate location by increasing the calling behavior of signalers, for  
232 example by augmenting the frequency and calling duration [62], or increasing pheromone  
233 release/production [63]. Alternatively, host plant volatiles can enhance receivers' attraction to  
234 pheromone signals [62,64], by attracting a higher number of individuals [62,64] or by  
235 reducing the time to locate the pheromone source [64]. Finally, host plant volatiles can alter  
236 the response to ratios of pheromone components [65]. Thus, in general, host plants can  
237 influence species reproductive success during different stages of the insect lifespan, through  
238 both resource acquisition at the larval stage, and the interaction of plant volatiles with adult  
239 signals and behavior. Oxidative stress and pathogen infections can also affect sex-  
240 pheromone based mate location [66,67]. Curiously, challenged immune systems and  
241 pathogen infections have been found to increase rather than decrease attraction in some  
242 insects, suggesting either terminal investment [67] or a strategy of pathogens to spread  
243 through populations via host manipulation [19]. Immune system challenges can also lead to  
244 sex-specific differences, for example only female mating success might be affected [68]. As  
245 populations vary in parasite load [69], variation in immune challenge could thus impact the  
246 dynamics of both pheromone signaling and sexual selection. In addition to host plants and  
247 pathogens, sex pheromone production and mating success can be affected by seasonal  
248 fluctuations. A good example is the squinting bush brown butterflies (*Bicyclus anynana*), in

249 which males that emerge in the wet season produce more pheromone and have higher mating  
250 success than those that emerge in the dry season [13].

251

252 Mate assessment is based on intraspecific variation in sexually selected traits. Sex  
253 pheromones can be under sexual selection when used as indicators of mate quality. Variation  
254 in sex pheromone composition can be used to avoid mating with siblings [70], which  
255 increases genetic variability and decreases the impact of deleterious alleles. For example, in  
256 *B. anynana*, females avoid mating with inbred males, which is assessed solely on the male-  
257 produced sex pheromone [71]. Sex pheromones are also used as signals to avoid matings that  
258 would lead to a lowered fitness and thus reduce the cost of matings. For example, sexually  
259 immature females of the cotton bollworm (*Helicoverpa armigera*) inhibit male attraction by  
260 temporarily releasing a repellent component in their pheromone signal [72]. Another example  
261 is the haplodiploid parasitic wasp, *Nasonia vitripennis*, in which females are more attracted to  
262 males with more pheromone, which correlates to a full sperm load [73]. As unfertilized eggs  
263 develop into males, this selection thus affects the population sex-ratio.

264

265 Sexual conflicts often arise over mating frequency. For example, in the parasitoid wasp  
266 families Pteromalidae and Braconidae, females tend to mate only once [30,74], while males  
267 mate multiple times. After mating, *Spalangia endius* (Pteromalidae) females actively resist  
268 males by increasing the release of a specific pheromone component, which prevents or  
269 terminates male courtship behavior [74]. Mated *Cotesia glomerata* (Braconidae) females stop  
270 releasing their attractive pheromone component in favor of the repellent only. These changes  
271 likely help mated females to avoid male harassment by reducing the attraction of males, and  
272 are also thought to help males locate virgin females, as their pheromone is distinct from  
273 mated females [30].

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To conclude, intraspecific sex pheromone variation can have important consequences for the attractiveness and mating success of individuals, regardless of the source of this variation. If variation in signals and responses is heritable, there is the potential for different investment or responsive strategies to evolve.

### **Evolutionary Consequences of Intraspecific Sex Pheromone Variation**

Evolution in sexual communication systems can occur when heritable differences in signals and/or responses increase the survival and reproductive output of the individual (Figure 2 & Table 1). Below we explore whether and how such evolution can occur in response to **ecological speciation** or as a driver of population divergence.

#### *Ecological Speciation*

Many herbivore species use chemicals from their host plants as precursors for compounds that make up the pheromone signal. Thus, host plant shifts can directly affect pheromone composition. If this is followed by changes in responses, leading to **assortative mating** and reproductive isolation, ecological speciation can occur [75]. Changes in mating signals following a host plant shift has been experimentally shown in the mustard beetle (*Phaedon cochleariae*) [76], where **cuticular hydrocarbons** used as mate recognition cues differ depending on the host plant on which they feed. Similar changes are hypothesized to have contributed to the speciation process between two parental species of flea beetles (*Altica fragaria* and *A. viridicyanea*) [77]. Pheromone divergence between populations linked to differences in host plants has also been described in the pine and larch strain of larch budmoth (*Zeiraphera diniana*) [78] and the chestnut tortrix (*Cydia splendana*) [79], and may represent the initial step towards reproductive isolation in these species. Changes in host

299 plants, however, do not automatically lead to differences in sex pheromones [80] or to host  
300 shift [81], suggesting that other processes are also involved in speciation events.

301

302 Plants damaged by conspecific herbivores can negatively affect the premating and mating  
303 behavior in the moth species *S. littoralis* [82]. Host plant volatiles released as a result of  
304 herbivory can decrease the calling behavior of the signaler and negatively impact the mating  
305 success. This interaction may result in an avoidance of egg laying on a suboptimal plant that  
306 has high competition for resources or is attractive to the enemies of the herbivore [82]. Thus,  
307 interactions between host plants and pheromone signals and/or responses are complex and not  
308 straightforward, and how these interactions are involved in ecological speciation likely  
309 depends on the plants and insects involved.

310

### 311 *Pheromone Differentiation as Driver of Speciation*

312 Reproductive isolation can also take place without ecological differentiation, through  
313 divergence in mate preference [83]. For example, in the primitive moth *Eriocrania*  
314 *semipurpurella-sangii* species complex, the ratios of components in the female pheromone do  
315 not only reflect geographic differences between populations, but also identify different  
316 species [84]. Since all the species share the same host plant, this is a case in which  
317 reproductive isolation likely has evolved as a result of divergence in sexual communication  
318 [84]. Such divergence could happen if some individuals of the choosing sex have a  
319 preference for a specific pheromone signal and choose those signalers. A good example  
320 comes from *B. anynana*, in which females developed a mating bias towards the pheromone  
321 signal they were exposed to, even when this was a new signal. As this mating bias can be  
322 transmitted to the next generation without the offspring being exposed to the new pheromone  
323 signal [85], such heritable learning can drive the evolution of assortative mating and

324 speciation. The potential of sex pheromones as drivers of speciation has been hypothesized  
325 for some *B. anynana* populations in which sex pheromone differentiation was found to be  
326 higher than genetic differentiation, suggesting that pheromone divergence precedes genetic  
327 divergence [8] (Box II).

328

329 Potential mechanisms that can introduce variation and novelty in sex pheromones lie in the  
330 sequence variability of the genes involved in determining component ratios in pheromone  
331 signals [86], such as gene duplication and amino acid substitution in genes coding for specific  
332 enzymes in the pheromone pathways [87]. Variation in response and preference for  
333 pheromone signals may be due to divergence and evolution in olfactory receptors [88]. For  
334 example, chemosensory divergence in odorant receptors in the peripheral nervous system  
335 associated with reproductive isolation, have been found in two rarely hybridizing *Heliconius*  
336 species [58]. In *O. nubilalis*, however, genetic differences in the central nervous system (i.e.  
337 neurogenesis), instead of the odorant receptors, were recently found to be primarily  
338 responsible for the differential male response [89]. The discovery of the genetic architecture  
339 underlying variation in sexual signals and responses has just recently begun, and so far  
340 mostly focused on Lepidoptera [90]. Recent studies suggest that phenotypic variation in sex  
341 pheromones can be the result of single gene [42] or locus [91] variation or due to a number of  
342 loci [92]. In the moths species studied so far, the genomic regions involved in signaling and  
343 response are unlinked. This makes **runaway selection** unlikely and the lack of **genetic**  
344 **correlation** favors the evolution of complex traits [90]. Thus, how phenotypic variation in  
345 pheromone communication may lead to reproductive isolation and, ultimately, to speciation  
346 is still an evolutionary mystery.

347

348 **How Easily Can Pheromone Communication Channels Evolve?**

349 As insect pheromones are used for pest management tactics, we have some knowledge on the  
350 evolution of sex pheromones in response to anthropogenically induced selection, which  
351 shows that pheromone communication channels can evolve relatively rapidly. Techniques  
352 such as pheromone trapping and **mating disruption** are attractive alternatives to pesticides,  
353 but the continuous use of artificial sex pheromones as disruptants may exert selective  
354 pressure on pest species to change their sexual signals and evolve ‘resistance’ to it [93],  
355 meaning that males are no longer disrupted by the artificial pheromone composition. Cases of  
356 such emerging resistance can be viewed as natural field experiments showing that sexual  
357 communication systems can sometimes evolve quickly. The first field case of male resistance  
358 to pheromone-based traps was reported in Japan, where males of the smaller tea tortrix  
359 (*Adoxophyes honmai*) stopped responding to synthetic lures after about 10 years of treatment,  
360 causing the efficacy of mating disruption to drop from > 96% to < 50% [94]. The strong  
361 selective pressure exerted on this species resulted in the evolution of a resistant population, in  
362 which females changed their pheromone composition and males broadened their pheromone  
363 response [95,96]. This is unlikely to be an isolated case, as intraspecific variation observed in  
364 the cosmopolitan pest, codling moth, *Cydia pomonella*, shows the same potential for a shift in  
365 female sexual signal in response to mating disruption [20].

366

367 The continuous use of artificial pheromones can have an effect on both signalers and  
368 receivers. The signaling sex may evolve a different pheromone signal [95,97] and/or modify  
369 their behavior, by releasing pheromone for a longer time [98]. Pre-exposure to sex  
370 pheromones can affect gene expression of chemosensory genes and olfactory sensitivity [53]  
371 and result either in enhanced [55,56] or reduced [99] receiver sensitivity. All these factors can  
372 contribute to the evolution of resistance to artificial pheromones, and suggest that signaler  
373 and receiver can evolve and change in short time periods. Such quick evolutionary responses

374 to selection indicate that there is a high level of standing genetic variation in populations on  
375 which selection can act.

376

### 377 **Concluding Remarks and Future Perspective**

378 There is an increasing awareness that intraspecific variation in sex pheromone  
379 communication channels, both in the signaler and the receiver, is more common than  
380 previously assumed. There is also an increasing number of studies that point to the existence  
381 of within-population variation, as these signals are used for mate assessment and choice.  
382 Thus, sex pheromone signals and responses can be under multiple selection forces (see  
383 Outstanding Questions) and seem able to evolve relatively quickly. This is particularly well  
384 represented by the fact that wild populations have been found to evolve resistance to  
385 synthetic sex pheromone composition, which suggests a high level of standing genetic  
386 variation within species on which selection can act. Finally, it is important to realize that  
387 individuals in nature can be affected simultaneously by several external factors (e.g.  
388 pathogens, vicinity to conspecifics, predators), which can trade-off with the physiology of the  
389 individual, so that populations always show some degree of variation. We therefore need to  
390 deepen our understanding on how variation in pheromone communication channels affects  
391 and is affected by the interaction between the internal state of signalers and receivers, their  
392 mutual mate choice and their environment. We also need a better understanding of the  
393 mechanisms behind this variation, whether these are genetic or plastic, and which molecular  
394 pathways are involved. With all this information it will be possible to move the field forward  
395 and better understand how the evolution of chemical communication between the sexes  
396 affects speciation.

397

398

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404

405

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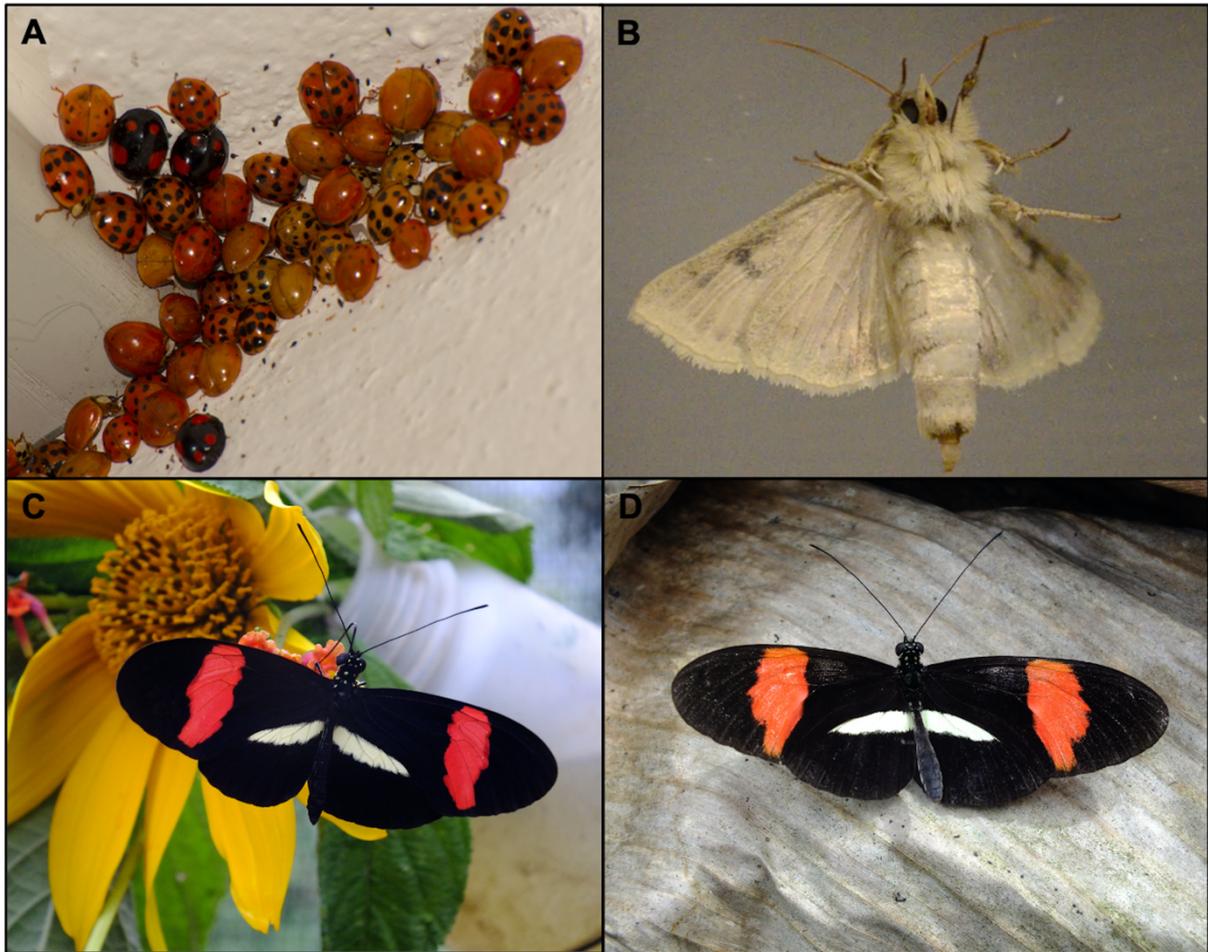
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677 Figure 1. Examples of Some Species for Which Intraspecific Variation in Sex Pheromone

678 Has Been Described in Literature. A) Asian lady beetle (*Harmonia axyridis*) (Photo: Chiara

679 De Pasqual); B) tobacco budworm (*Heliothis virescens*) (Photo: Jan van Arkel/IBED/UvA);

680 C) *Heliconius erato* (C) and *Heliconius melpomene* (D) (Photo: Melanie Brien)



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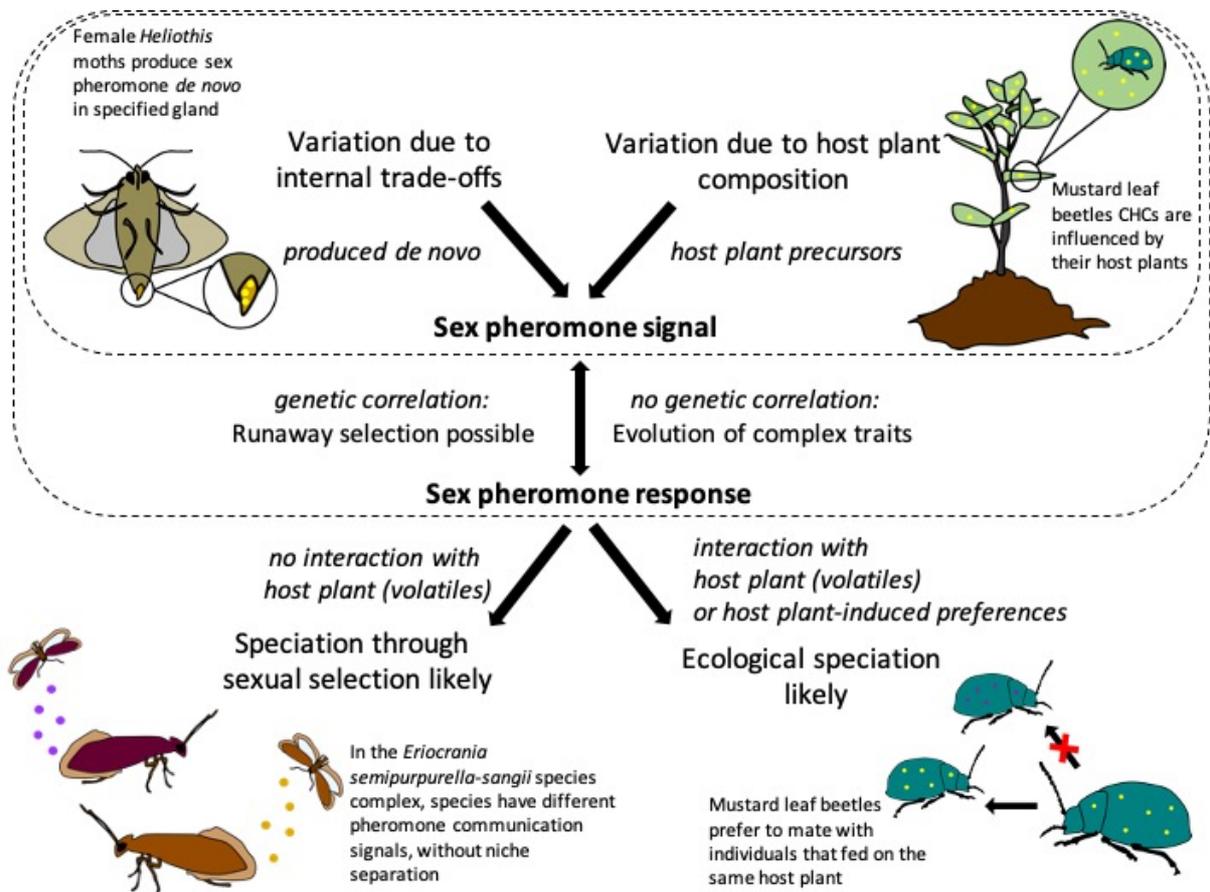
690 Figure 2. Some Possible Routes to Speciation through Variation in Pheromone Production

691 and Perception in Herbivorous Insects. Arrows indicate two of the possible alternative routes

692 in the evolution of sex pheromone signals and responses. Pictures indicate examples

693 discussed in this paper, clockwise from top left (*Heliothis* spp. [24]; mustard leaf beetle

694 (*Phaedon cochleariae*) [75]; *Eriocrania semipurpurella-sangii* [83].



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701 Table 1. Examples of Intraspecific Sex Pheromone Variation for Which Causes and/or  
 702 Consequences of the Variation Has Been Described and Genetic Basis or Heritability is  
 703 Known.

704

Species name	Level of variation <sup>a</sup>		Cause of variation	Consequences of the variation	Genetic basis known or heritability calculated	References
	Quantitative <sup>b</sup>	Qualitative <sup>c</sup>				
Tobacco budworm ( <i>Heliothis virescens</i> )	- <sup>d</sup>	Yes	Single point mutation	Female attractiveness & mating success affected	Genetic basis known	[21,35,40]
	Yes	Yes	Pathogen infection boosted immune system	Sex specific: females mating success affected		[67]
Subflexus straw ( <i>Heliothis subflexa</i> )	-	Yes	Phenotypic plasticity	Increase in <b>assortative attraction</b>	Genetic basis known	[14,99]
Squinting bush brown ( <i>Bicyclus anynana</i> )	Yes	-	Seasonal polyphenism or age	Mating success affected	Heritability known	[13,100]
Parasitic wasp ( <i>Nasonia vitripennis</i> )	Yes	-	Sperm limitation (highly correlated with pheromone titre)	Attractions and mating success affected	Genetic basis known	[72]
Burying beetle ( <i>Nicrophorus vespilloides</i> )	Yes	Yes	Diet or age, or parasite load	Male attractiveness affected	Unknown	[101,102]

705

706 <sup>a</sup> Variation in sex pheromones can take two forms: quantitative and qualitative.

707 <sup>b</sup> Quantitative when the variation is referred to the total amount of the components.

708 <sup>c</sup> Qualitative when the variation is referred to the relative amounts and/or ratio of the components.

709 <sup>d</sup>“-” refers to not-tested or not reported.

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## 712 **Outstanding Questions**

713 1) How do interspecific and intraspecific selection forces interact and cause population  
714 divergence? Individuals often are under multiple selective pressures resulting from abiotic  
715 and biotic sources, which may act in opposite, similar or complex ways.

716

717 2) Is only one sex the signaler and the other sex the receiver, or is there mutual mate choice?

718 As signalers may become receivers in different phases of mate assessment, it is important to

719 determine which signals are being used by which sex at the different phases, and how these

720 signals may interact or evolve separately.

721

722 3) How much of the intraspecific variation is determined by genetic factors and how much

723 plasticity occurs in pheromone signals and responses? Since both sex pheromone signals and

724 responses have been found to be plastic, and plasticity can accelerate as well as impede

725 evolution, we need to gain more knowledge on the fitness consequences and heritability of

726 this plasticity.

727

728 4) When using artificial pheromone blends for mating disruption, could resistance

729 development be delayed by using variable pheromone lures? Knowledge on the level of

730 intraspecific variation in the pheromone signal may be used to develop lures with different

731 pheromone blends to reduce evolution of resistance to pheromone artificial traps.

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738 **Box I Visual vs Chemical signals**

739 In butterflies, mate attraction is typically determined by visual cues, while sex pheromones  
740 are usually involved in short-range attraction. Current research is uncovering many aspects of  
741 sex pheromone roles in butterflies, suggesting that chemical signals are much more involved  
742 than previously expected in diurnal species that commonly rely on visual cues. *Heliconius*  
743 butterflies (Figure 1C,D in main text) are well known for exhibiting Müllerian mimicry, in  
744 which unrelated and chemically defended species mimic each other warning signal to more  
745 efficiently advertise their unpalatability to predators. When two species share identical wing  
746 pattern, such as *H. timareta* and *H. melpomene* in Peru, chemical signals are important to  
747 discriminate against heterospecifics [103]. Sex pheromones have a great importance in  
748 conspecific recognition, act as pre-mating barriers and affect mate choice [104]. As male  
749 pheromones and female-preference have been found to be heritable at least in some  
750 *Heliconius* butterfly species [105], pheromone communication may play a role in their  
751 speciation process. Thus, even in the visually-orientated butterflies, pheromones and other  
752 chemical signals, are used as pre-mating barriers in addition to wing patterns, making these  
753 species multi-modal signalers [106].

754

755 In *Bicyclus anynana*, the male sex pheromone can provide a wealth of information on the  
756 signaler, from the male's age to individual identity [100]. This pheromone is also a plastic  
757 trait as a consequence of a more or less climatic favorable season for their reproductive  
758 success [38], which has implications in their mating success [13]. Their sex pheromone can  
759 be more variable within populations than between populations and possibly acts as precursors  
760 of an ongoing speciation process [8].

761

762 In both *Heliconius* and *Bicyclus* study systems, short-range chemical signals are thus  
763 involved in species recognition and therefore under natural selection, but have also been  
764 shown to act in intraspecific mate assessment and thus under sexual selection. These  
765 examples show that in a male-signaling, female responding system, the progress of our  
766 understanding of the roles of pheromone signals seems to run counter to that in the more  
767 commonly studied females-signaling systems.

768  
769

770 **Box II Ecological and Evolutionary Consequences in the Burying Beetle (*Nicrophorus***  
771 ***vespilloides*)**

772 A comprehensive example of intraspecific sex pheromone variation that merges ecological-  
773 and evolutionary consequences of its variation comes from burying beetles (*Nicrophorus*  
774 *vespilloides*). Intraspecific differences in sex pheromone have been shown to reflect males'  
775 condition, with males being more attractive when they are in better nutritional condition, of  
776 older age, have a larger body size and bear less parasites [101]. Burying beetles start to attract  
777 females once they have found a carcass suitable for reproduction, by releasing a sex  
778 pheromone. Both parents are known to feed on the carcass, so it is beneficial to both the  
779 parents and the offspring to mate only after a carcass has been found. Interestingly, males that  
780 have already performed brood care produce and release a higher amount of sex pheromone,  
781 and attract more females. Because of this positive loop, in which individuals that perform  
782 parental care produce more sex pheromone and become more attractive to females, the  
783 interplay between food source and heightened expression of secondary sexual trait (sex  
784 pheromone) has been suggested to have contributed to the evolution of parental care [102].

785  
786  
787

788 **Glossary**

789 **Assortative attraction or assortative mating:** individuals with similar phenotypes are  
790 attracted and mate with one another more frequently than expected under random mating

791

792 **Cuticular hydrocarbons (CHCs):** cuticular hydrocarbons are molecules on the surface of  
793 adult insects, generally serving as anti-desiccation compounds. In a number of species,  
794 CHCs have also be found to play important roles in insect communication. One of these  
795 roles is attraction and selection of potential mates

796

797 **Ecological speciation:** a form of speciation that arises as a consequence of reproductive  
798 isolation due to a change in ecological factors (e.g. change in the host plant, presence of  
799 predators or parasites and, in general, any environmental factor)

800

801 **Genetic correlation:** two or more sets of genetic loci expected to co-vary as a consequence  
802 of, for example, linkage disequilibrium or pleiotropy

803

804 **Mating disruption:** a pest management technique which prevent individuals to successfully  
805 locate potential mates through the release of a synthetic sex pheromone that mimics the  
806 species sex pheromone

807

808 **Plasticity:** the phenomenon for which organisms with the same genotype manifest alternative  
809 phenotypes in response to environmental conditions, experienced usually during  
810 developmental time

811

812 **Preference function:** along a continuous range of a signaling trait values, most receivers

813 express a preference for intermediate values, while few show preference towards  
814 extreme values

815

816 **Reproductive character displacement:** selection to avoid hybridization between species.

817 When two species have overlapping habitats and (signaling) traits, divergence in one  
818 or more traits evolves in response to unfavorable interactions, e.g. cross-species  
819 attraction. When the divergence is due to selection to avoid hybridization between  
820 races/strains within species, the term 'reinforcement' is used

821

822 **Runaway selection:** due to genetic correlation between the sender and receiver's trait,  
823 changes in the sender's signal are inherited together with changes for the preference  
824 for that signal. This leads to a positive feedback loop, whereby both signal and  
825 preference for the signal become exaggerated over time

826

827 **Saltational event:** evolutionary event which generates greatly or completely different  
828 phenotypes in a few generations, due to a mutation with major phenotypic effect. For  
829 example, a mutation in an enzyme that changes the stereochemistry of a pheromone  
830 component

831

832 **Signaling sex:** the sex releasing a sexual communication signal to attract a potential mate

833

834 **Stabilizing selection:** a form of selection in which the population mean trait converges to  
835 intermediate values, e.g. when the mean pheromone signal is preferred over signals  
836 deviating from the mean

837

838 **Sympatric species:** species with overlapping geographic ranges that are close enough to

839 regularly interact

840