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Title: Evolutionary importance of intraspecific variation in sex pheromones

Year: 2021

Version: Accepted version (Final draft)

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Please cite the original version:

De Pasqual, C., Groot, A. T., Mappes, J., & Burdfield-Steel, E. (2021). Evolutionary importance of intraspecific variation in sex pheromones. *Trends in Ecology and Evolution*, 36(9), 848-859.

<https://doi.org/10.1016/j.tree.2021.05.005>

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

399 **Acknowledgments**

400 We would like to thank Thomas Blankers at the University of Amsterdam, the *plantaginis*
401 research group at the University of Jyväskylä and three reviewers for their useful comments
402 on a previous version of the manuscript. CDP and JM were funded by the Academy of
403 Finland (project no. 320438).

404

405

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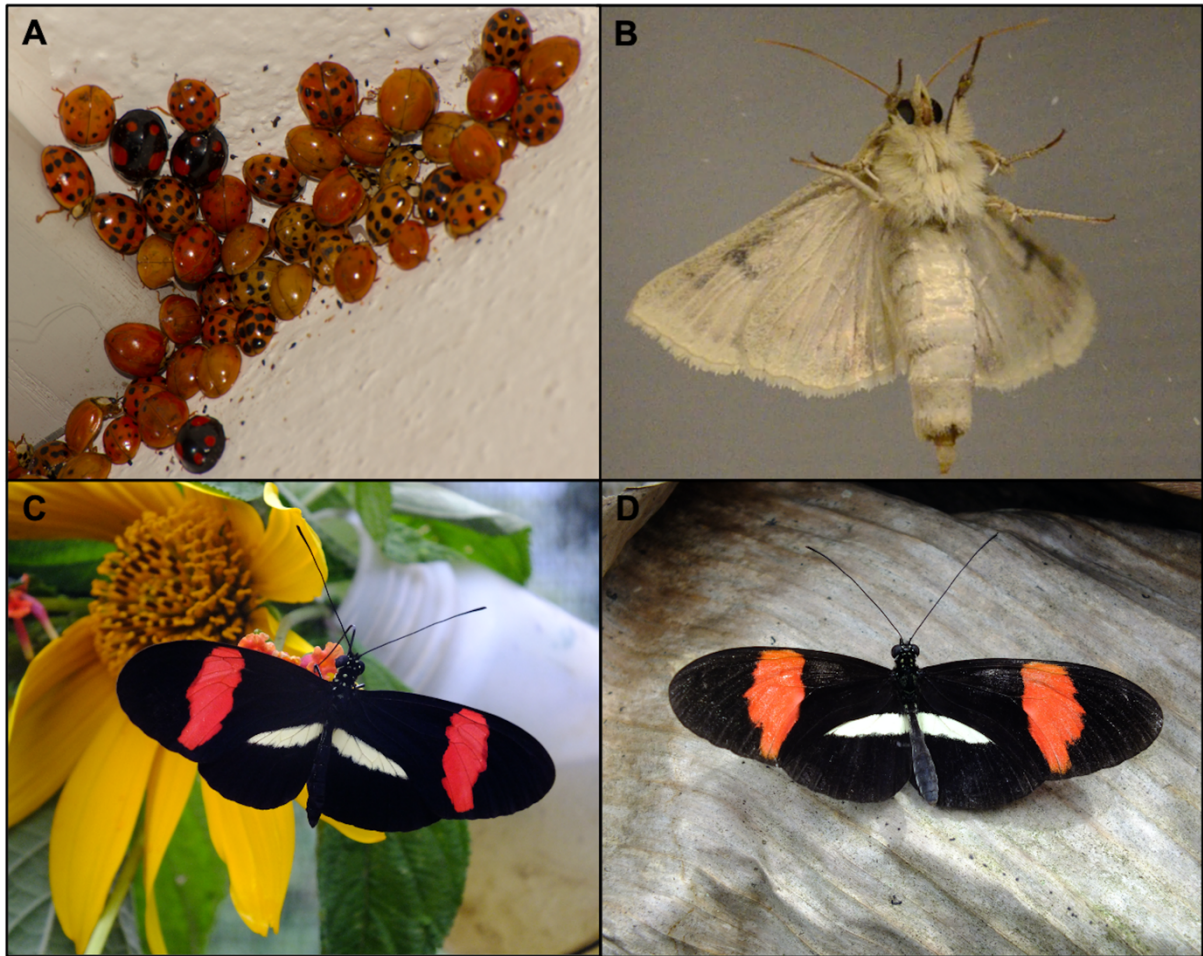
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677 Figure 1. Examples of Some Species for Which Intraspecific Variation in Sex Pheromone

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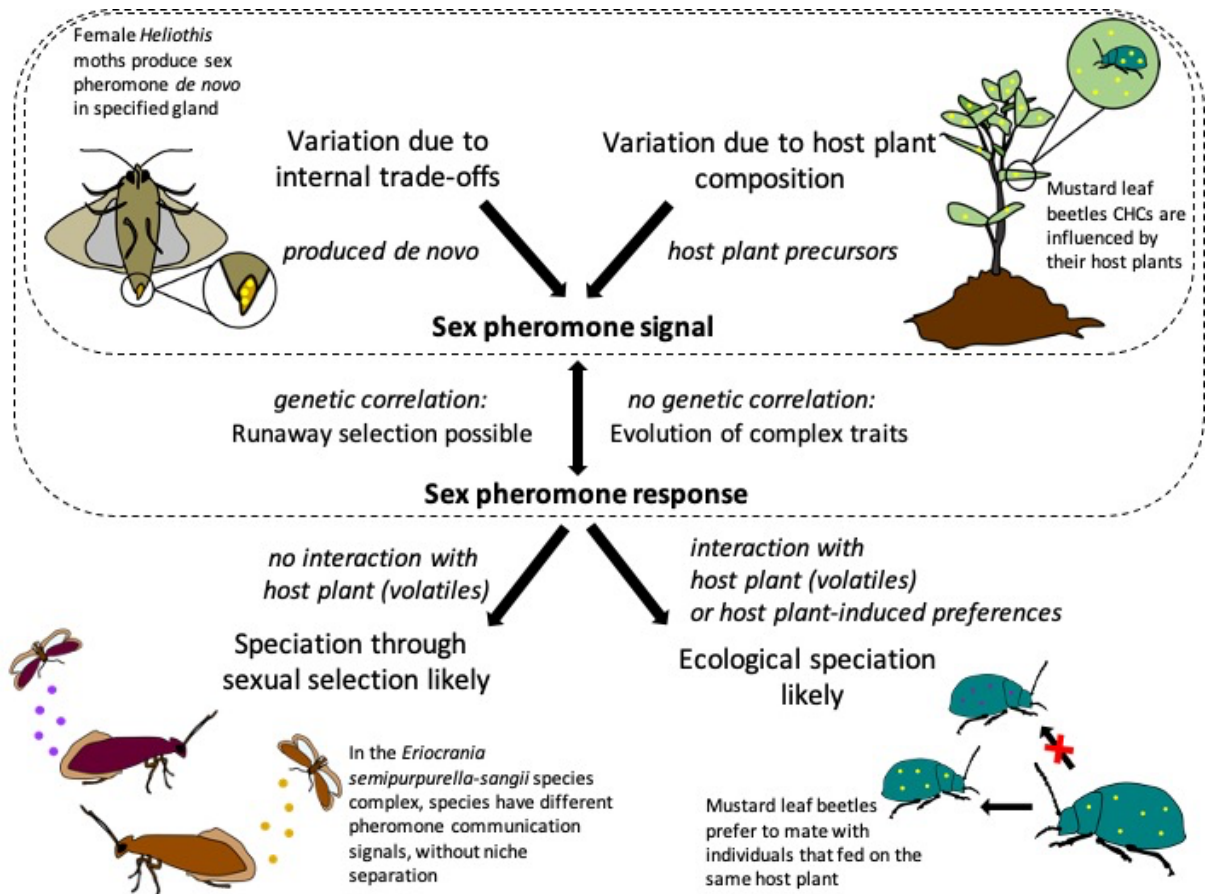
690 Figure 2. Some Possible Routes to Speciation through Variation in Pheromone Production

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 ^a		Cause of variation	Consequences of the variation	Genetic basis known or heritability calculated	References
	Quantitative ^b	Qualitative ^c				
Tobacco budworm (<i>Heliothis virescens</i>)	- ^d	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 assortative attraction	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 ^a Variation in sex pheromones can take two forms: quantitative and qualitative.

707 ^b Quantitative when the variation is referred to the total amount of the components.

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

709 ^d“-” refers to not-tested or not reported.

710

711

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.

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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].

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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