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

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Highlights

- Sex pheromones have traditionally been viewed as species recognition signals and thus are expected to show little within-species variation in composition and amount. Current research, however, reveals a high degree of intraspecific variation in sex pheromone communication channels, suggesting they are molded by multiple selection forces.

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

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

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.

61

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].

74

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] intrapopulational 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

Sender's Perspective

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

Ecological Consequences of Intraspecific Variation: Effect on Attractiveness and

Mating Success

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

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

274

275 To conclude, intraspecific sex pheromone variation can have important consequences for the

276 attractiveness and mating success of individuals, regardless of the source of this variation. If

277 variation in signals and responses is heritable, there is the potential for different investment or

278 responsive strategies to evolve.

279

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

281 Evolution in sexual communication systems can occur when heritable differences in signals

282 and/or responses increase the survival and reproductive output of the individual (Figure 2 &

283 Table 1). Below we explore whether and how such evolution can occur in response to

284 **ecological speciation** or as a driver of population divergence.

285

286 *Ecological Speciation*

287 Many herbivore species use chemicals from their host plants as precursors for compounds

288 that make up the pheromone signal. Thus, host plant shifts can directly affect pheromone

289 composition. If this is followed by changes in responses, leading to **assortative mating** and

290 reproductive isolation, ecological speciation can occur [75]. Changes in mating signals

291 following a host plant shift has been experimentally shown in the mustard beetle (*Phaedon*

292 *cochleariae*) [76], where **cuticular hydrocarbons** used as mate recognition cues differ

293 depending on the host plant on which they feed. Similar changes are hypothesized to have

294 contributed to the speciation process between two parental species of flea beetles (*Altica*

295 *fragaria* and *A. viridicyanea*) [77]. Pheromone divergence between populations linked to

296 differences in host plants has also been described in the pine and larch strain of larch

297 budmoth (*Zeiraphera diniana*) [78] and the chestnut tortrix (*Cydia splendana*) [79], and may

298 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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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)
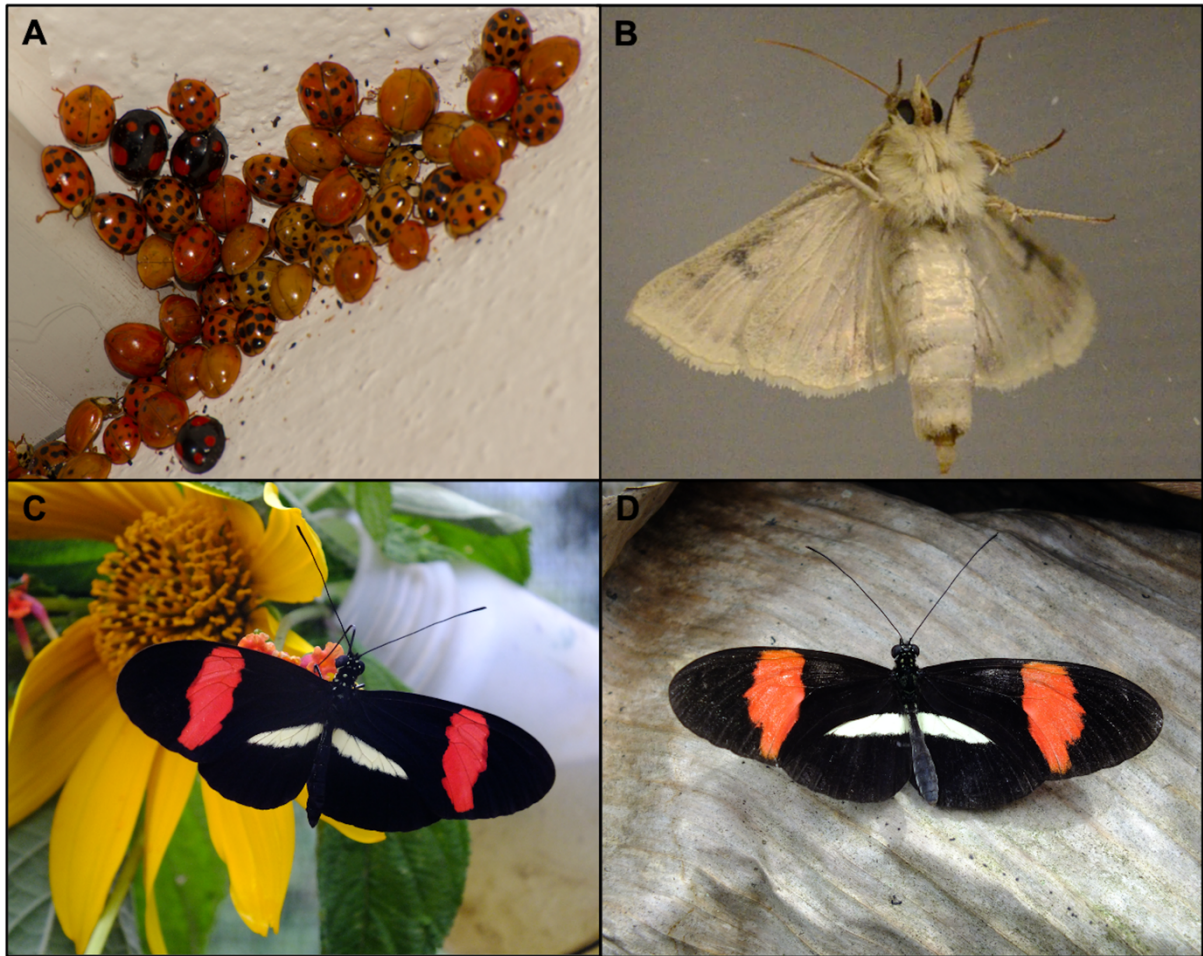

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690 Figure 2. Some Possible Routes to Speciation through Variation in Pheromone Production

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].
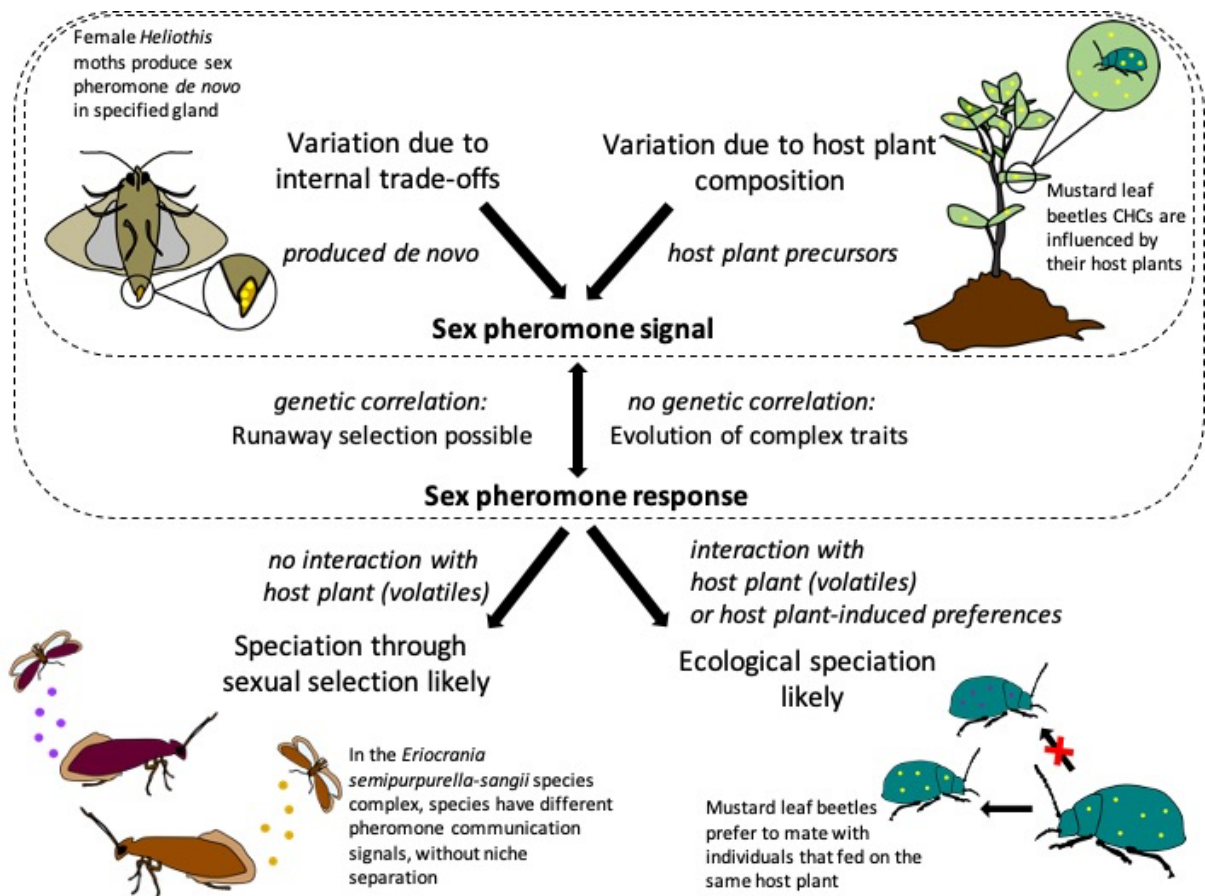
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701 Table 1. Examples of Intraspecific Sex Pheromone Variation for Which Causes and/or

702 Consequences of the Variation Has Been Described and Genetic Basis or Heritability is

703 Known.

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	Level of variation [a]					
Species name	**Quantitative** [b]	**Qualitative** [c]	**Cause of variation**	**Consequences of the variation**	**Genetic basis known or heritability calculated**	**References**
Tobacco budworm (*Heliothis virescens*)	- [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 (*Heliothis subflexa*)	-	Yes	Phenotypic plasticity	Increase in **assortative attraction**	Genetic basis known	[14,99]
Squinting bush brown (*Bicyclus anynana*)	Yes	-	Seasonal polyphenism or age	Mating success affected	Heritability known	[13,100]
Parasitic wasp (*Nasonia vitripennis*)	Yes	-	Sperm limitation (highly correlated with pheromone titre)	Attractions and mating success affected	Genetic basis known	[72]
Burying beetle (*Nicrophorus vespilloides*)	Yes	Yes	Diet or age, or parasite load	Male attractiveness affected	Unknown	[101,102]

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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.

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Outstanding Questions

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

2) Is only one sex the signaler and the other sex the receiver, or is there mutual mate choice? As signalers may become receivers in different phases of mate assessment, it is important to determine which signals are being used by which sex at the different phases, and how these signals may interact or evolve separately.

3) How much of the intraspecific variation is determined by genetic factors and how much plasticity occurs in pheromone signals and responses? Since both sex pheromone signals and responses have been found to be plastic, and plasticity can accelerate as well as impede evolution, we need to gain more knowledge on the fitness consequences and heritability of this plasticity.

4) When using artificial pheromone blends for mating disruption, could resistance development be delayed by using variable pheromone lures? Knowledge on the level of intraspecific variation in the pheromone signal may be used to develop lures with different pheromone blends to reduce evolution of resistance to pheromone artificial traps.

Box I Visual vs Chemical signals

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

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

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 studies 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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Glossary

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

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

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

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

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

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

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