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### When sexual signallers are choosers too

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## 6 | General discussion

Naomi L. Zweerus

### 1. Mutual mate choice may explain how changes in sexual communication can occur

Sexual selection acts on variation in sexual signals within and between populations and may drive communication divergence (Maan & Seehausen, 2011; Wilkins et al., 2013; De Pasqual et al., 2021). Since hybridization with other species has negative fitness consequences, species recognition is a central function of sexual signals (Ryan & Rand, 1993; Löfstedt, 1993; Price, 1998; Cardé & Haynes, 2004; Ritchie, 2007; Symonds & Elgar, 2008). The selection for the mean signal in a population can minimize cross-attraction of heterospecific individuals (Paterson, 1985; Butlin et al., 1985; Löfstedt, 1993; Droney et al., 2012; Moran et al., 2020). Species recognition signals are thus under stabilizing selection. Since stabilizing selection for species recognition would limit within-species variation, it is unclear how sexual communication systems that function as species recognition signals can evolve.

Sexual signals may not only be used for species recognition but also to select partners within a species. In moths, males may not only choose females (Löfstedt, 1993; Bonduriansky, 2001; Johansson & Jones, 2007), but females may also choose males once males have been attracted. Selection pressures are probably fundamentally different when males not only select females but females also choose males. If the signalling sex is also choosy, the numbers and interactions of possible selection pressures increase. Extending the conventional perspective of one signalling and one choosing sex to two signallers and two choosers may help to understand how changes in sexual communication can occur.

While female signals in moths are widely studied (Löfstedt, 1993; Vickers & Baker, 1997; Baker & Vickers, 1997; Wyatt, 2003; Cardé & Haynes, 2004; El-Sayed, 2011), female mate choice has been mostly neglected (for exceptions see Phelan & Baker, 1986; Iyengar & Eisner, 1999b; Jang & Greenfield, 2000; Iyengar et al., 2001). In this thesis, I investigated female mate choice in the tobacco budworm *Chloridea virescens* (Lepidoptera: Noctuidae) and determined the male signal underlying female mate choice. Importantly, I found experimental evidence for female mate choice (chapter 2). Interestingly, I found that females select males based on size and not based on the traditional male hairpencil pheromone (chapters 2 and 4). Moreover, females discriminate between males in a relative manner (chapters 2 and 4). While I demonstrated that the traditional male hairpencil pheromone does not underlie female choice (chapter 2), I found that the nutrition-derived compounds MeSA and  $\delta$ -decalactone in the male hairpencil may determine male attractiveness (chapter 4). Finally, I investigated how female mating status may affect female sexual behaviour and mate choice. By comparing sexual behaviour and mate preference between virgin and mated females, I showed that mating status affects the strength of female preference (chapter 3, see also Lu et al., 2017). Since males choose females based on their sex pheromone and this thesis shows that females are choosers too, my main conclusion is that the communication system of *C. virescens* is based on mutual mate choice. Below, I discuss the implications of female choice and mutual mate choice on the evolution of sexual communication systems in general and on the evolution of sexual communication systems in moths in particular.

## 2. Aspects of female mate choice in *C. virescens*

### a. Females choose males based on quality

Females may increase individual fitness by selecting high-quality partners (Parker, 1983). An increase in fitness by having more offspring (traditionally referred to as 'direct' benefits) or offspring with higher fitness ('indirect' benefits; Kokko et al., 2003). In chapter 2, I showed that *C. virescens* females benefit from mating with larger males, because these matings lead to higher reproductive output. Consistent with this finding, I showed that female *C. virescens* prefer larger males in two-choice experiments (chapters 2, 3 and 4). It was beyond the scope of this thesis to determine the heritability of male traits and the effects of female mate choice in offspring. However, male and female body size in Lepidopterans can be heritable (e.g., Iyengar & Eisner, 1999a; Davis & Landolt, 2012) and body size is a common indicator of female fecundity in insects (Honěk, 1993). The preference for larger males could thus result in more attractive sons and more fecund daughters.

### b. Females can make relative choices between males

I consistently found that the difference in male pupal mass explained female choice (chapters 2, 3 and 4) and thus, that females compare and choose males relative to one another. Empirical evidence for relative choice was found also in other systems, such as crickets (*G. bimaculatus*, Bateman et al., 2001), fish (*Cottus bairdii* (Brown, 1981) and *Poecilia reticulata* (Pitcher et al., 2003; see also Jennions & Petrie, 1997)), and certain *Drosophila* species (Hoikkala & Aspi, 1993). While absolute choice would select against all the males that do not match a certain criterion (e.g., are smaller than a threshold value in body size), relative choice does not require any absolute threshold value. Hence, the criterion to choose for a male is that he is larger than the other male while the actual size is irrelevant. Consequently, selection by relative choice is less stringent than selection by absolute choice. In context of this thesis, relative female choice for larger males is particularly interesting since *C. virescens* females attract males by signalling and also mate multiple times (see chapter 3). Thus, females may directly compare attracted males relative to one another or possibly also to previous mating partners.

### c. Mate choice or cryptic female choice?

Multiple matings in females in combination with sperm storage allows for postcopulatory female choice (cryptic female choice (Thornhill, 1983; Eberhard, 1996; Arnqvist, 2014; Firman et al., 2017) or paternity choice (Birkhead & Møller, 1993; Andersson & Simmons, 2006)). Females may introduce bias in paternity by selectively using sperm for fertilisation, which has been demonstrated in various Lepidopterans (Thornhill, 1983; LaMunyon & Eisner, 1993; Gage, 1994; Wedell & Cook, 1998; Solensky & Oberhauser, 2009; Xu & Wang, 2010; Mongue et al., 2015). To better understand how and when female choice affects paternity, it is necessary to determine how spermatophore content varies between males of different size and also within a male between multiple matings and how this ultimately translates into paternity patterns.

In Lepidoptera, spermatophores contain two sperm types: eupyrene (fertilizing) and apyrene (non-fertilizing) sperm (Proshold, 1975; Silberglied et al., 1984; Cook & Wedell, 1999; LaMunyon, 2000; LaMunyon & Huffman, 2001). The ratio of eupyrene to apyrene sperm number

in *C. virescens* is generally about 1:10 and this ratio seems not to change with a male's mating history (LaMunyon & Huffman, 2001). Male pupal mass correlates positively with spermatophore size in *C. virescens* (LaMunyon, 2000). Additionally, adult feeding affects spermatophores as nectar feeding is associated with larger spermatophores (Blanco et al., 2009). However, spermatophore size does not correlate with the number of sperm in the spermatophore (LaMunyon & Huffman, 2001). Hence, large spermatophores do not necessarily mean a lot of sperm. Besides, females store the equivalent to one ejaculate of eupyrene sperm, while this volume can consist of sperm from different males (LaMunyon, 2000). Interestingly, twice-mated females store almost two ejaculates' worth of apyrene sperm (LaMunyon, 2000). Besides, larger males pass on more apyrene sperm to virgins than to mated females (LaMunyon & Huffman, 2001) and apyrene sperm number decreases with the number of times a male has mated (LaMunyon, 2000). Apyrene sperm might thus be a nuptial gift. As a promising next step, I suggest to test the hypothesis that females could bias paternity towards larger males based on the absolute amount of apyrene sperm perceived.

**d. The amount of  $\delta$ -decalactone may signal male quality**

While I found that male quality is reflected in male size (chapter 2), it remained unclear how females can gauge male size. Since males also emit pheromone from elaborate structures called hairpencils (Grant, 1970; Teal & Tumlinson, 1989; Birch et al., 1990), it was proposed that the male hairpencil pheromone underlies female choice (Teal et al., 1981; Birch & Hefetz, 1987; Hillier & Vickers, 2004). However, in my quest to identify the male signal underlying female choice, I found that the previously identified male pheromone compounds are not related to female choice (chapter 2 and 4). However, two nutrition-derived chemicals (methyl salicylate and  $\delta$ -decalactone) were recently discovered as part of the male hairpencil pheromone (Liu et al., in prep). Therefore, I tested the hypothesis that methyl salicylate (MeSA) and/or  $\delta$ -decalactone are used by females to assess males (chapter 4). I showed that  $\delta$ -decalactone is correlated to male size, and thus this compound may inform females about male size and underly female choice. This is especially interesting because  $\delta$ -decalactone is nutrition-derived, so that variation in the amount of this compound in males may arise by differences in their acquisition at the larval stage. Furthermore, adult males fed with sugar-water showed higher amounts of  $\delta$ -decalactone than males that were fed with plain water (chapter 4). Resource acquisition at the larval and adult stage may thus play a central role in male sexual attraction, because larval diet and adult feeding have a significant effect on male attractiveness (chapter 2 and 4).

**e. Female might consider additional chemical signal components for mate assessment**

While  $\delta$ -decalactone can be an indicator of male size in *C. virescens* (chapter 4), it might not be the only signal that females assess. Additional chemical signal components may come into play for female mate choice at short-range. The moths' antennae are prominent structure to detect chemicals (Almaas & Mustaparta, 1990; Krieger et al., 2002, 2004; Hillier et al., 2006; Wang et al., 2011). Surprisingly, I found that females mate just as well without antenna (chapter 2), which suggests that antennal detection of the male signal is non-essential for female mate acceptance.

However, chemical signals can also be perceived by other body parts, because olfactory receptors have also been found on insect legs, the proboscis, mouth parts and on the abdomen (Krieger et al., 2002, 2004; Jacquin-Joly & Merlin, 2004; Vogel et al., 2010; Gomez-Diaz & Benton, 2013). In analysing courtship behaviour in high frame-rate video recordings, I found that males and females have physical contact during courtship, especially with their legs in a behaviour I termed 'grubbing' (chapter 2). *C. virescens* females may thus perceive sexual signals via their legs or other sensitive body parts. Additional olfactory signals that comprise information about male size may be perceived at the point in courtship when the male leg is interlaced with a female leg (chapter 2).

A possible chemical signal component that *C. virescens* females may use for mate assessment are cuticular hydrocarbons (CHCs). CHCs are used as sexual signals by many insect species (Ginzel & Blomquist, 2016). Several beetles, such as the rustic borer *Xylotrechus colonus* (Ginzel et al., 2003), leaf beetles *Chrysochus sp.* (Peterson et al., 2007) and the mustard leaf beetle *Phaedon cochleariae* (Geiselhardt et al., 2009, 2012; Otte et al., 2015), display sex pheromones on their cuticles. Short-range contact pheromones also play a central role in mate choice of Hawaiian swordtail crickets *Laupala spp.* (Mullen et al., 2007) and the model organism *Drosophila melanogaster* (Ferveur et al., 2005). In chapter 5, I investigated the chemical composition on legs of males and females in three heliothine species and found two long-chain carbon compounds in leg extracts of all species (chapter 5). Interestingly, these long-chain carbon compounds show intersex differences in all species (chapter 5). However, since the amounts of these compounds were consistently higher on female legs than on male legs (chapter 5), it is unlikely that females use these CHCs for mate assessment. Butyrate esters, however, have been found on moth legs and male legs have higher amounts of these compounds than female legs (Choi et al., 2016). *C. virescens* females could thus use butyrate esters on male legs to choose among males.

#### f. Female might consider other, non-chemical signals for mate assessment

Sexual signals can be composed of several components. Such a multicomponent signal can have the same sensory modality (i.e., unimodal) or different ones (i.e., multimodal) (Candolin, 2003). Evidence is accumulating that various species use multimodal signals for sexual attraction (see Jennions & Petrie, 1997; Candolin, 2003; Hebets & Papaj 2005; Barry et al., 2010). For instance, male praying mantids *Pseudomantis albofimbriata* use chemical and visual cues to assess females (Barry et al., 2010, 2015) and female field crickets *Teleogryllus oceanicus* assess males based on acoustic and contact-based chemical signals (Thomas & Simmons, 2009; Gray et al., 2014). Similarly, female European tree frogs *Hyla arborea* choose males based on a combination of acoustic and visual signals (Gomez et al., 2009).

In moths, females may use other modalities than chemicals alone for mate assessment. Several sensory modalities could be considered in a multimodal signal for female choice in *C. virescens*. First, the leg-to-leg contact during grubbing may also transmit tactile stimuli that inform about male size in a morphometric manner. Since leg size is positively correlated with body size in *C. virescens* (chapter 2), a larger male has longer legs. Male leg size might thus be measured by

females in leg-to-leg contact. Second, male acoustic signals may be produced during courtship, as demonstrated in other noctuid moths, including the heliothine moth *Heliothis zea* (Nakano et al., 2009a; 2009b). Studies on the production of acoustic signals in *C. virescens* are, to my knowledge, unexplored so far. Since tympanal ears to detect ultrasonic sound are present in all noctuids (Eggers, 1919; Ghiradella, 1971; Surlykke, 1984; Surlykke et al., 1988; Nakano et al., 2015), acoustic signals can be perceived and may be part of the sexual communication in *C. virescens* at short-range. Sound production correlates with body size in variety of sound-producing animals (e.g., frogs, crickets and cicadas (Prestwich, 1994; Verburt & Ferguson, 2010; Bertram et al., 2011; Thomson et al., 2014)) and larger size is associated with lower acoustic frequencies (Bennet-Clark, 1998). If wing vibrations generate acoustic signals, I expect wing size to determine the frequencies of the signal. Likewise, if the insect body functions as resonance body, larger male may produce lower frequency sounds, which has been demonstrated in crickets (Brown et al., 1996; Deb et al., 2012). Finally, the size of the approaching male could simply be seen by the female. Vision is omnipresent in insects and Lepidoptera possess image-forming compound eyes (Langer et al., 1979; Warrant et al., 2003; Land & Nilsson, 2012; Yan et al., 2014; Sondhi et al., 2021). Female moths may thus perceive visual stimuli for mate assessment. Not only male body size but also the size of the hairpencils could be gauged this way. Hence, hairpencils might be ornaments themselves, with larger males showing off larger hairpencils. The perception of a multicomponent and/or multimodal signal about male size for female mate choice in *C. virescens* is thus plausible. Multiple signals may be easier to detect than a single signal (Hebets & Papaj, 2005; Groot & Vedenina, 2021). A multicomponent signal can allow for a more accurate perception because multiple signal aspects inform about the sender (Candolin, 2003; Hebets & Papaj, 2005).

#### **g. Can females learn about the “best” male?**

Since *C. virescens* is polygamous, females might not only assess multiple males for one mating but compare males between different matings. Remembering the information of previously encountered or mated males would be relevant for a polygamous species like *C. virescens*. The comparison of previously encountered or mated males requires some sort of learning and memorizing of their characteristics. Evidence from several moth species, including *C. virescens*, shows that moths are capable of associative learning (Hartlieb, 1996; Fan et al., 1997; Daly et al., 2004; Skiri et al., 2005; Jørgensen et al., 2007; Blackiston et al., 2008). A multimodal signal can enhance the neuronal response and thus, creates a longer-lasting impression (Hebets & Papaj, 2005). If the different sensory modalities of a multimodal signals are processed simultaneously, the process requires that the signals get transduced by multisensory neurons (Hebets & Papaj, 2005). Interestingly, a multisensory neuron has been described in *C. virescens* (Zhao et al., 2013). This neuron is most likely responsible for the simultaneous integration of acoustic and olfactory signals (Zhao et al., 2013). An interesting hypothesis I would like to propose is therefore that polygamous *C. virescens* females assess a combined chemical-acoustic-morphometric male signal. In conclusion, a multimodal signal could be advantageous for this polygamous species because it can allow for a or more accurate comparison between males of different mating events.

### 3. The function of male hairpencils and their pheromone

Now that I showed that the traditional male hairpencil pheromone is not used by females to choose among males, the obvious question is what these large structures are used for instead. In addition to female choice, male-male competition may affect the evolution of male traits. The male hairpencils disperse pheromone (Hendricks & Shaver, 1975; Teal & Tumlinson, 1989; Birch et al., 1990) and are thus an ornament. A previous study showed that males hairpencils apply the pheromone compounds on females and are effective in chemical mate guarding, because they inhibit mating by other males (Hosseini et al., 2016). Chemical mate guarding is an aspect of intrasexual competition and the involved chemicals evolve through intrasexual selection (Andersson, 1994; Berglund et al., 1996; Rico-Guevara & Hurme, 2019). Larger males probably have larger hairpencils and therefore may perfume more and/or a larger area on the female abdomen, thereby leaving a stronger or more persistent chemical mate-guarding mark. Larger males may thus be more successful in preventing other males from mating.

Intra-male competition could also manifest itself as a form of combat. While I never observed direct male-male interactions, I did observe male harassment of mating pairs: single males start courting a pair *in copula* by everting the hairpencils and moving around the copulating pair. Since males perceive their own pheromone and the pheromone is emitted when males evert their hairpencils (Hillier et al., 2007), I suppose that single males are trying to outcompete the already mating male. Harassment of mating pairs can thus be part of male-male competition in form of direct confrontation of the rivals. Both the chemical mate guarding and the harassment suggest a role of the male hairpencil pheromone in intrasexual competition, and is thus likely a male armament instead of an ornament.

### 4. Sexual signals in *C. virescens* are shaped by selection pressures on both sexes

The main result of my research is that males and females in *C. virescens* are reciprocal signallers and mutual choosers. Since the previously identified male pheromone compounds from the hairpencils share the same biosynthetic pathway with the female sex pheromone components (Tillman et al., 1999; Jurenka 2003, 2004), they are genetically linked. Selection for the female long-range sex pheromone could thus affect the pheromone composition in male hairpencils. Delta-decalactone, however, is an additional pheromone compound and does not share the biosynthesis of the traditional pheromone compounds (Liu et al., in prep.). As I showed in chapter 4,  $\delta$ -decalactone may indicate male size and affects female mate choice. Interestingly,  $\delta$ -decalactone is not found in the female sex pheromone (Roelofs et al., 1974; Tumlinson et al., 1975; Vetter & Baker, 1983; pers. observation). Hence, the actual trait that underlies male mate choice and female mate choice are independent from each other. If the male and the female sexual signal are not genetically linked, the male and the female choice systems are decoupled (Servedio & Lande, 2006). Hence, both sexes impose selection on the opposite sex but the evolution of the male and female signals must be considered separately.

### 5. Mutual mate choice opens a range of possible selection forces and interactions

If mutual mate choice is based on different signals for males and females, selection can be stabilizing on one signal and directional on the other signal. Moreover, mate choice may interact

with intrasexual competition in each sex. Males compete with other males in form of sperm competition (LaMunyon, 2000, 2001). The fact that larger males may pass on larger spermatophores and more apyrene sperm than smaller males (LaMunyon, 2000; LaMunyon & Huffman, 2001) suggest that larger males are more likely to sire offspring when females mated multiply. Female mate choice for larger males has been demonstrated in this thesis. Whether females are involved in intrasexual competition has yet to be determined. The fact that female preference is not related to the male preference suggests that selection by female mate choice may help in maintaining variation in sexual signals. Thus, diversification of sexual signals is possible by mutual mate choice based on different signals in each sex.

#### **6. Natural selection and sexual selection may interact**

In this thesis, I focused on sexual selection but pheromone compounds may also be subject to natural selection. For example, I found that two compounds present on the legs of heliothine moths, 16:Ald and 16:OH, possess antimicrobial properties. This new and interesting finding suggests that these leg compounds may protect moth eggs from bacterial threats. If so, then these antibacterial compounds on moth legs are under natural selection (chapter 5). It is likely that larger individuals can deposit more of these protective substances than smaller individuals, for instance because body size correlates positively with production and/or because a larger leg helps to deposit a larger amount.

In addition to the protective function of 16:Ald, parasitoids might home in on the same compound (Boo & Yang, 2000; chapter 5). Oviposition sites of larger individuals might thus be easier detected, putting eggs and offspring of larger individuals at higher parasitisation risk. Opposing natural selection forces may thus act on this pheromone compound. The possible trade-offs between body size, antibacterial effects, and the chance of attracting parasitoids will determine the optimum amount of 16:Ald in males, which is probably different depending on e.g., bacterial communities on the plants and/or parasitoid densities in the environment.

#### **7. How the combination of selection pressures can drive divergence in sexual communication**

Considering all my findings, I would like to explore in this last part how the results can contribute to our understanding of the evolution of sexual communication systems. Mate choice can lead to reproductive isolation based on non-random mating. Since I found that male size explains female mate choice and that diet and feeding has a significant effect on male attractiveness (chapter 2, 3, and 4), food sources are likely central to any shift in sexual communication. Food sources are expected to correlate with environmental conditions and other external factors, such as competition or predation (reviewed for example in Martin, 1987; van Veen et al., 2006; Rodrigues et al., 2016). Hence, traits that are involved in feeding are likely subject to natural selection. I propose that sexual selection drives divergence in sexual signals if factors affecting male size (e.g., diet) are reinforced by natural selection. In the three evolutionary scenarios sketched below, I elaborate on how mutual mate choice in combination with other findings of this thesis could contribute to speciation.

**Scenario 1: Oviposition and larval feeding impacts mate attraction.**

Since I found that females choose bigger, higher-quality males and may assess a nutrition-derived compound ( $\delta$ -decalactone) in the male hairpencil pheromone for mate choice, I hypothesize that the feeding habits of larvae and host-plant choice affect sexual selection and can drive speciation. The male hairpencil pheromone is likely more variable in natural populations than in lab populations, because larvae of this generalist moth feed on a vast variety of plants. However, despite this ability to develop on a broad range of host plants (Blanco et al., 2007; Fitt, 1989; Sheck & Gould, 1993; Waldvogel & Gould, 1990), some strains develop better on certain plant species over others (Blanco et al., 2008). Moreover, *C. virescens* females prefer to oviposit on the host plant on which they hatched from the egg (Karpinski et al., 2014). Since host preference and possibly also mate preference in *C. virescens* is at least partly genetically determined (Schneider & Roush, 1986; Sheck & Gould, 1995; Karpinski et al., 2014), these preferences could be the starting point of divergence. Hence, if the male pheromone is affected by the host plant they were feeding on as larvae and if females prefer to mate with males that come from the same host plant they originate from, then local host plant specialization is likely to occur (Karpinski et al., 2014). Such an effect of larval feeding on moth pheromone has been found in the European corn borer (*Ostrinia nubilalis*), where the plant species a larva develops on determines the attractiveness of its adult pheromone blend and adults prefer to mate with individuals from the same host plant species (Thomas et al., 2003; Malausa et al., 2005). Similarly, host-plant specificity can be reflected in hydrocarbons that may be used as contact pheromones (Ginzel & Blomquist, 2016). For example, mustard leaf beetles can feed on various cabbage (*Brassica spp.*) and radish species (*Raphanus spp.*), but males prefer to mate with females that were reared on the same plant species, a factor that is reflected in their CHC profile (Geiselhardt et al., 2012; Ginzel & Blomquist, 2016). Thus, changes in pheromone composition based on host-plant specificity may lead to assortative mating and reproductive isolation (Benthenod et al., 2005; Groot et al., 2008; Ginzel & Blomquist, 2016).

**Scenario 2: Synchrony with partners from host plants of similar nutritional value.**

Larger, more attractive males might not only originate from a specific host plant but they must also be available at the right time. Body size of insects is correlated with development time (Blanckenhorn, 2000; Beukeboom, 2018) and development time is related to the energetic and nutritional quality of food (Edgar, 2006; Chown & Gaston, 2010; Beukeboom, 2018). Larvae that develop on plant species with high nutritional value and/or high protein content do have a higher chance to grow bigger and to develop faster than larvae developing on plant species with low nutritional value and/or low protein content. Adult eclosion may thus become synchronized among individuals developing on host-plants with identical nutritional value and/or protein content. Empirical evidence confirms that larval performance and generation time in *C. virescens* varies with host plants (Blanco et al. 2008). Protein content of the diet correlates positively with development and thus, generation time (Blanco et al., 2008). Interestingly, plants belonging to the Fabaceae (legumes) are richer in protein than Malvaceae (mallows) (Kotkar et al., 2009) and *C. virescens* individuals develop faster on legumes than on mallows (Blanco et al., 2008). Hence, females developing on legumes and are more likely to mate with other fast-developing males

from the same host and these males match the female preference for larger males. If host plants affect developmental time and moth body size, temporal associations between hatching, mate attraction, and mate preference may occur. Temporal synchronisation of hatching related to the host plant combined with sexual selection might thus drive host-plant specialization.

### **Scenario 3: Selection for larval food with the highest nutritional value.**

While protein content of host-plants differs (Blanco et al., 2008; Kotkar et al., 2009), there is an alternative food source which provides by far the highest nutritional density and protein, namely conspecifics (Polis, 1981). In fact, *C. virescens* larvae are highly cannibalistic (Joyner & Gould, 1985; reviewed in Richardson et al., 2010) and cannibalism is heritable in *C. virescens* (Gould et al., 1980). The advantages of cannibalistic males are twofold; first, the high-protein content of the diet allows them to develop faster and second, they are likely to become bigger adults. Thus, females not only attract these early hatching males but also select them because of their large size. Since females prefer larger males, cannibalism could be sexually selected.

Although cannibalism is likely to affect male size in a positive way, how does it affect male attractiveness determined by nutrition-derived pheromone compounds (such as  $\delta$ -decalatone, chapter 4)? Feeding on conspecifics surely provides protein, which facilitates developing into a large male but might not serve as pheromone compounds. However, fatty-acids as pheromone precursors could be obtained by cannibalistic feeding. Moreover, conspecific larvae have fed on plant tissue and thus, may deliver nutrition-derived compounds (e.g.,  $\delta$ -decalactone) indirectly as they have previously sequestered the compound. If this is the case, cannibalism and an attractive male pheromone are not mutually exclusive. Populations could thus diverge because of host limitation and concurrent selection for cannibalism.

## **8. Future research**

To make any realistic prediction about the evolution of the male signals underlying female mate choice, it would be important to clarify whether selection on male signals is co-dependent on the selection of female preferences. Two avenues for future research to investigate how female mate choice may impact the evolution of moth sexual communication further seem particularly promising. First, I suggest to assess other possible modalities in the male signal. As discussed earlier, females might consider additional signal components for mate assessment. Especially acoustic signals have been reported to play a role in courtship of other moth species (Nakano et al., 2009). Therefore, I propose to determine whether *C. virescens* males and possibly also females produce acoustic signals during courtship. If so, investigating this sexual monologue or dialogue could provide valuable insights into the selection on the multiple signal components. Also, butyrate esters or CHCs on moth legs are strong candidates for sexual signals (chapter 5, Choi et al., 2016). Investigating their role in each sex can shed light into how and what kind of additional signals define precopulatory interactions.

Second, the effects of female mate choice on the next generation needs to be evaluated if we want to make predictions on the evolutionary consequences of sexual selection. While I could show that mating with larger males leads to more offspring (chapter 2), I did not investigate the next generation. To better understand sexual selection via female mate choice, it

is important how a preference for larger males affects the body size distribution of offspring. Additionally, it would be relevant to clarify how the effect of mating with a preferred partner translates into a particular sex pheromone composition in female offspring and the quantity of nutrition-derived pheromone compounds in male offspring, and whether and how parental preferences are correlated to the preference in offspring.

### **9. Concluding remarks**

In this thesis, I investigated female mate choice in a species with prominent female sexual signals and male mate choice. I showed that females choose males based on a male signal that is associated with male size. The results dismiss the traditional male hairpencil pheromone as the signal underlying female mate choice. Delta-decalactone content in the male hairpencil, however, may be the chemical signal for male assessment. These results show that the male signal is not related to the female signal. Hence, there is mutual mate choice, but the reciprocal choice is based on different types of sexual signals. The selection pressures arising from male and female mate choice are therefore decoupled. Mutual mate choice on decoupled signals opens a variety of possible selection pressures. The different selection pressures and their interactions are likely to maintain variation in male and female sexual signals. While earlier research focused predominantly on female sexual signals and male mate choice and therefore fell short in explaining how the evolution of sexual communication may result in a high diversity of moth species, the integrative perspective of two signallers and two choosers opens new avenues in evolutionary scenarios on how moth sexual communication channels can diverge.

## REFERENCES

- Almaas T, Mustaparta H. Pheromone reception in tobacco budworm moth, *Heliothis virescens*. *Journal of chemical ecology*. 1990;16(4):1331–47.
- Andersson M. Sexual selection. Vol. 72. Princeton University Press; 1994.
- Andersson M, Simmons LW. Sexual selection and mate choice. *Trends in ecology & evolution*. 2006;21(6):296–302.
- Arnqvist G. Cryptic female choice. In: Shuker DM, Simmons LW, editors. *The evolution of insect mating systems*. Oxford University Press Oxford; 2014. p. 204–20.
- Baker TC, Vickers NJ. Pheromone-mediated flight in moths. In: *Insect Pheromone Research*. Springer; 1997. p. 248–64.
- Barry KL, Holwell GI, Herberstein ME. Multimodal mate assessment by male praying mantids in a sexually cannibalistic mating system. *Animal Behaviour*. 2010;79(5):1165–72.
- Barry KL, White TE, Rathnayake DN, Fabricant SA, Herberstein ME. Sexual signals for the colour-blind: cryptic female mantids signal quality through brightness. *Functional Ecology*. 2015;29(4):531–9.
- Bateman PW, Gilson LN, Ferguson J. Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Animal Behaviour*. 2001;61(3):631–7.
- Bazinet AL, Cummings MP, Mitter KT, Mitter CW. Can RNA-Seq resolve the rapid radiation of advanced moths and butterflies (Hexapoda: Lepidoptera: Apoditrysia)? An exploratory study. *PLoS one*. 2013;8(12):e82615.
- Bennet-Clark H. Size and scale effects as constraints in insect sound communication. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*. 1998;353(1367):407–19.
- Berglund A, Bisazza A, Pilastro A. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biological Journal of the Linnean Society*. 1996;58(4):385–99.
- Bertram SM, Thomson I, Auguste B, Dawson J, Darveau CA. Variation in cricket acoustic mate attraction signalling explained by body morphology and metabolic differences. *Animal behaviour*. 2011;82(6):1255–61.
- Bethenod M, Thomas Y, Rousset F, Frérot B, Pélozuelo L, Genestier G, et al. Genetic isolation between two sympatric host plant races of the European corn borer, *Ostrinia nubilalis* Hübner. II: assortative mating and host-plant preferences for oviposition. *Heredity*. 2005;94(2):264–70.
- Beukeboom LW. Size matters in insects—an introduction. *Entomologia Experimentalis et Applicata*. 2018;166(1):2–3.
- Birch MC, Hefetz A. Extrusible organs in male moths and their role in courtship behavior. *Bulletin of the ESA*. 1987;33(4):222–9.
- Birch M, Poppy G, Baker TC. Scents and eversible scent structures of male moths. *Annual review of entomology*. 1990;35(1):25–54.
- Birkhead T, Møller A. Female control of paternity. *Trends in Ecology & Evolution*. 1993;8(3):100–4.
- Blackiston DJ, Silva Casey E, Weiss MR. Retention of memory through metamorphosis: can a moth remember what it learned as a caterpillar? *PLoS One*. 2008;3(3):e1736.
- Blanckenhorn WU. The evolution of body size: what keeps organisms small? *The quarterly review of biology*. 2000;75(4):385–407.
- Blanco CA, Rojas MG, Groot AT, Morales-Ramos J, Abel CA. Size and chemical composition of *Heliothis virescens* (Lepidoptera: Noctuidae) spermatophores. *Annals of the Entomological Society of America*. 2009;102(4):629–37.
- Blanco CA, Terán-Vargas AP, Abel CA, Portilla M, Rojas MG, Morales-Ramos JA, et al. Plant host effect on the development of *Heliothis virescens* F. (Lepidoptera: Noctuidae). *Environmental Entomology*. 2008;37(6):1538–47.
- Blanco CA, Terán-Vargas AP, López Jr JD, Kauffman JV, Wei X. Densities of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) in three plant hosts. *Florida Entomologist*. 2007;90(4):742–50.
- Bonduriansky R. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biological Reviews*. 2001;76(3):305–39.
- Boo K, Yang J. Kairomones used by *Trichogramma chilonis* to find *Helicoverpa assulta* eggs. *Journal of Chemical Ecology*. 2000;26(2):359–75.
- Brown L. Patterns of female choice in mottled sculpins (Cottidae, Teleostei). *Animal Behaviour*. 1981;29(2):375–82.
- Brown WD, Wideman J, Andrade MC, Mason AC, Gwynne DT. Female choice for an indicator of male size in the song of the black-horned tree cricket, *Oecanthus nigricornis* (Orthoptera: Gryllidae: Oecanthinae). *Evolution*. 1996;50(6):2400–11.

- Butlin R, Hewitt G, Webb S. Sexual selection for intermediate optimum in *Chorthippus brunneus* (Orthoptera: Acrididae). *Animal Behaviour*. 1985;33(4):1281–92.
- Candolin U. The use of multiple cues in mate choice. *Biological reviews*. 2003;78(4):575–95.
- Cardé RT, Haynes KF. Structure of the pheromone communication channel in moths. In: Cardé RT, Millar JG, editors. *Advances in insect chemical ecology*. Cambridge, UK: Cambridge University Press; 2004. p. 283–332.
- Choi MY, Ahn SJ, Park KC, Vander Meer R, Cardé RT, Jurenka R. Tarsi of male heliothine moths contain aldehydes and butyrate esters as potential pheromone components. *Journal of chemical ecology*. 2016;42(5):425–32.
- Chown SL, Gaston KJ. Body size variation in insects: a macroecological perspective. *Biological reviews*. 2010;85(1):139–69.
- Cook PA, Wedell N. Non-fertile sperm delay female remating. *Nature*. 1999;397(6719):486–486.
- Daly KC, Christensen TA, Lei H, Smith BH, Hildebrand JG. Learning modulates the ensemble representations for odors in primary olfactory networks. *Proceedings of the National Academy of Sciences*. 2004;101(28):10476–81.
- Davis TS, Landolt PJ. Body size phenotypes are heritable and mediate fecundity but not fitness in the lepidopteran frugivore *Cydia pomonella*. *Naturwissenschaften*. 2012;99(6):483–91.
- De Pasqual C, Groot AT, Mappes J, Burdfield-Steel E. Evolutionary importance of intraspecific variation in sex pheromones. *Trends in Ecology & Evolution*. 2021;
- Deb R, Bhattacharya M, Balakrishnan R. Females of a tree cricket prefer larger males but not the lower frequency male calls that indicate large body size. *Animal Behaviour*. 2012;84(1):137–49.
- Dronney DC, Musto CJ, Mancuso K, Roelofs WL, Linn CE. The response to selection for broad male response to female sex pheromone and its implications for divergence in close-range mating behavior in the European corn borer moth, *Ostrinia nubilalis*. *Journal of Chemical Ecology*. 2012;38(12):1504–12.
- Eberhard W. *Female control: sexual selection by cryptic female choice*. Vol. 69. Princeton University Press; 1996.
- Edgar BA. How flies get their size: genetics meets physiology. *Nature Reviews Genetics*. 2006;7(12):907–16.
- Eggers F. *Das thoracale bitympanale Organ einer Gruppe der Lepidoptera heterocera*. 3rd ed. Vol. 41. G. Fischer; 1919.
- El-Sayed AM. The pherobase: database of insect pheromones and semiochemicals. 2011. Available from: <http://www.pherobase.com>
- Fan RJ, Anderson P, Hansson B. Behavioural analysis of olfactory conditioning in the moth *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *The Journal of experimental biology*. 1997;200(23):2969–76.
- Ferveur JF. Cuticular hydrocarbons: their evolution and roles in *Drosophila* pheromonal communication. *Behavior genetics*. 2005;35(3):279–95.
- Firman RC, Gasparini C, Manier MK, Pizzari T. Postmating female control: 20 years of cryptic female choice. *Trends in Ecology & Evolution*. 2017;32(5):368–82.
- Fitt GP. The ecology of *Heliothis* species in relation to agroecosystems. *Annual review of entomology*. 1989;34(1):17–53.
- Gage MJ. Associations between body size, mating pattern, testis size and sperm lengths across butterflies. *Proceedings of the Royal Society of London Series B: Biological Sciences*. 1994;258(1353):247–54.
- Geiselhardt S, Otte T, Hilker M. The role of cuticular hydrocarbons in male mating behavior of the mustard leaf beetle, *Phaedon cochleariae* (F.). *Journal of chemical ecology*. 2009;35(10):1162–71.
- Geiselhardt S, Otte T, Hilker M. Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. *Ecology letters*. 2012;15(9):971–7.
- Ghiradella H. Fine structure of the noctuid moth ear. I. The transducer area and connections to the tympanic membrane in *Feltia subgothica* Haworth. *Journal of morphology*. 1971;134(1):21–45.
- Ginzel MD, Blomquist GJ. *Insect Hydrocarbons: Biochemistry and Chemical Ecology*. In: Cohen E, Moussian B, editors. *Extracellular composite matrices in arthropods*. Berlin: Springer; 2016.
- Ginzel MD, Blomquist GJ, Millar JG, Hanks LM. Role of contact pheromones in mate recognition in *Xylotrechus colonus*. *Journal of chemical ecology*. 2003;29(3):533–45.
- Gomez D, Richardson C, Lengagne T, Plenet S, Joly P, Léna JP, et al. The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog (*Hyla arborea*). *Proceedings of the Royal Society B: Biological Sciences*. 2009;276(1666):2351–8.
- Gomez-Diaz C, Benton R. The joy of sex pheromones. *EMBO reports*. 2013;14(10):874–83.

- Gould F, Holtzman G, Rabb R, Smith M. Genetic variation in predatory and cannibalistic tendencies of *Heliothis virescens* strains. *Annals of the Entomological Society of America*. 1980;73(3):243–50.
- Grant G. Evidence for a male sex pheromone in the noctuid, *Trichoplusia ni*. *Nature*. 1970;227(5265):1345–6.
- Gray B, Bailey NW, Poon M, Zuk M. Multimodal signal compensation: do field crickets shift sexual signal modality after the loss of acoustic communication? *Animal behaviour*. 2014;93:243–8.
- Groot AT, Marr M, Schöfl G, Lorenz S, Svatos A, Heckel DG. Host strain specific sex pheromone variation in *Spodoptera frugiperda*. *Frontiers in Zoology*. 2008;5(1):1–13.
- Groot AT, Vedenina VY, editors. *Multimodal Mating Signals: Evolution, Genetics and Physiological Background*. Lausanne: Frontiers Media SA; 2021.
- Hartlieb E. Olfactory conditioning in the moth *Heliothis virescens*. *The Science of Nature*. 1996;2(83):87–8.
- Hebets EA, Papaj DR. Complex signal function: developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology*. 2005;57(3):197–214.
- Hendricks D, Shaver T. Tobacco budworm: male pheromone suppressed emission of sex pheromone by the female. *Environmental Entomology*. 1975;4(4):555–8.
- Hillier N, Kelly D, Vickers N. A specific male olfactory sensillum detects behaviorally antagonistic hairpencil odorants. *Journal of Insect Science*. 2007;7(1):4.
- Hillier N, Kleineidam C, Vickers NJ. Physiology and glomerular projections of olfactory receptor neurons on the antenna of female *Heliothis virescens* (Lepidoptera: Noctuidae) responsive to behaviorally relevant odors. *Journal of Comparative Physiology A*. 2006;192(2):199–219.
- Hillier N, Vickers N. The role of heliothine hairpencil compounds in female *Heliothis virescens* (Lepidoptera: Noctuidae) behavior and mate acceptance. *Chemical Senses*. 2004;29(6):499–511.
- Hoikkala A, Aspi J. Criteria of female mate choice in *Drosophila littoralis*, *D. montana*, and *D. ezoana*. *Evolution*. 1993;47(3):768–77.
- Honěk A. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*. 1993;483–92.
- Hosseini SA, Van Wijk M, Ke G, Goldansaz SH, Schal C, Groot AT. Experimental evidence for chemical mate guarding in a moth. *Scientific reports*. 2016;6(1):1–6.
- Iyengar VK, Eisner T. Female choice increases offspring fitness in an arctiid moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences*. 1999b;96(26):15013–6.
- Iyengar VK, Eisner T. Heritability of body mass, a sexually selected trait, in an arctiid moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences*. 1999a;96(16):9169–71.
- Iyengar VK, Rossini C, Eisner T. Precopulatory assessment of male quality in an arctiid moth (*Utetheisa ornatrix*): hydroxydanaidal is the only criterion of choice. *Behavioral Ecology and Sociobiology*. 2001;49(4):283–8.
- Jacquín-Joly E, Merlin C. Insect olfactory receptors: contributions of molecular biology to chemical ecology. *Journal of Chemical Ecology*. 2004;30(12):2359–97.
- Jang Y, Greenfield MD. Quantitative genetics of female choice in an ultrasonic pyralid moth, *Achroia grisella*: variation and evolvability of preference along multiple dimensions of the male advertisement signal. *Heredity*. 2000;84(1):73–80.
- Jennions MD, Petrie M. Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*. 1997;72(2):283–327.
- Johansson BG, Jones TM. The role of chemical communication in mate choice. *Biological Reviews*. 2007;82(2):265–89.
- Jørgensen K, Strandén M, Sandoz JC, Menzel R, Mustaparta H. Effects of two bitter substances on olfactory conditioning in the moth *Heliothis virescens*. *Journal of Experimental Biology*. 2007;210(14):2563–73.
- Joyner K, Gould F. Developmental consequences of cannibalism in *Heliothis zea* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*. 1985;78(1):24–8.
- Jurenka R. Biochemistry of female moth sex pheromones. In: *Insect pheromone biochemistry and molecular biology*. Elsevier; 2003. p. 53–80.
- Jurenka R. Insect pheromone biosynthesis. *The chemistry of pheromones and other semiochemicals I*. 2004;97–132.
- Karpinski A, Haenniger S, Schöfl G, Heckel DG, Groot AT. Host plant specialization in the generalist moth *Heliothis virescens* and the role of egg imprinting. *Evolutionary ecology*. 2014;28(6):1075–93.
- Kokko H, Brooks R, Jennions MD, Morley J. The evolution of mate choice and mating biases. *Proceedings of the Royal Society of London Series B: Biological Sciences*. 2003;270(1515):653–64.

- Kotkar HM, Sarate PJ, Tamhane VA, Gupta VS, Giri AP. Responses of midgut amylases of *Helicoverpa armigera* to feeding on various host plants. *Journal of Insect Physiology*. 2009;55(8):663–70.
- Krieger J, Grosse-Wilde E, Gohl T, Dewer Y, Raming K, Breer H. Genes encoding candidate pheromone receptors in a moth (*Heliothis virescens*). *Proceedings of the National Academy of Sciences*. 2004;101(32):11845–50.
- Krieger J, Raming K, Dewer YM, Bette S, Conzelmann S, Breer H. A divergent gene family encoding candidate olfactory receptors of the moth *Heliothis virescens*. *European Journal of Neuroscience*. 2002;16(4):619–28.
- LaMunyon CW. Sperm storage by females of the polyandrous noctuid moth *Heliothis virescens*. *Animal Behaviour*. 2000;59(2):395–402.
- LaMunyon CW. Determinants of sperm precedence in a noctuid moth *Heliothis virescens*: a role for male age. *Ecological Entomology*. 2001;26(4):388–94.
- LaMunyon CW, Eisner T. Postcopulatory sexual selection in an arctiid moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences*. 1993;90(10):4689–92.
- LaMunyon CW, Huffman TS. Determinants of sperm transfer by males of the noctuid moth *Heliothis virescens*. *Journal of Insect Behavior*. 2001;14(2):187–99.
- Land MF, Nilsson DE. *Animal eyes*. 2nd ed. Oxford, England: OUP Oxford; 2012. (OABS Oxford Animal Biology Series).
- Langer H, Hamann B, Meinecke C. Tetrachromatic visual system in the moth *Spodoptera exempta* (Insecta: Noctuidae). *Journal of Comparative Physiology A*. 1979;129(3):235–9.
- Liu Y, Heath M, Zhang S, Wanga G, Buellesbach J, Van Wijk M, et al. A plant-derived male sex pheromone in a moth and its receptor. 2022
- Löfstedt C. Moth pheromone genetics and evolution. *Philosophical Transactions of the Royal Society of London B*. 1993;340(1292):167–77.
- Lu Q, Huang LY, Liu FT, Wang XF, Chen P, Xu J, et al. Sex pheromone titre in the glands of *Spodoptera litura* females: circadian rhythm and the effects of age and mating. *Physiological Entomology*. 2017;42(2):156–62.
- Maan ME, Seehausen O. Ecology, sexual selection and speciation. *Ecology letters*. 2011;14(6):591–602.
- Malausa T, Bethenod MT, Bontemps A, Bourguet D, Cornuet JM, Ponsard S. Assortative mating in sympatric host races of the European corn borer. *Science*. 2005;308(5719):258–60.
- Martin TE. Food as a limit on breeding birds: a life-history perspective. *Annual review of ecology and systematics*. 1987;18(1):453–87.
- Mongue AJ, Ahmed MZ, Tsai MV, de Roode JC. Testing for cryptic female choice in monarch butterflies. *Behavioral Ecology*. 2015;26(2):386–95.
- Moran PA, Hunt J, Mitchell C, Ritchie MG, Bailey NW. Sexual selection and population divergence III: Interspecific and intraspecific variation in mating signals. *Journal of Evolutionary Biology*. 2020;33(7):990–1005.
- Mullen SP, Mendelson TC, Schal C, Shaw KL. Rapid evolution of cuticular hydrocarbons in a species radiation of acoustically diverse Hawaiian crickets (Gryllidae: Trigonidiinae: Laupala). *Evolution*. 2007;61(1):223–31.
- Nakano R, Takanashi T, Fujii T, Skals N, Surlykke A, Ishikawa Y. Moths are not silent, but whisper ultrasonic courtship songs. *Journal of Experimental Biology*. 2009;212(24):4072–8.
- Nakano R, Ishikawa Y, Tatsuki S, Skals N, Surlykke A, Takanashi T. Private ultrasonic whispering in moths. *Communicative & Integrative Biology*. 2009;2(2):123–6.
- Nakano R, Takanashi T, Surlykke A. Moth hearing and sound communication. *Journal of Comparative Physiology A*. 2015;201(1):111–21.
- Otte T, Hilker M, Geiselhardt S. The effect of dietary fatty acids on the cuticular hydrocarbon phenotype of an herbivorous insect and consequences for mate recognition. *Journal of chemical ecology*. 2015;41(1):32–43.
- Parker G. Mate choice. In: Bateson P, editor. *Mate quality and mating decisions*. Cambridge University Press; 1983. p. 141–66.
- Paterson HE. The recognition concept of species. In: Ereshefsky M, editor. *The Units of Evolution: essays on the nature of species*. Cambridge Massachusetts: The MIT Press; 1985.
- Peterson MA, Dobler S, Larson EL, Juárez D, Schlarbaum T, Monsen KJ, et al. Profiles of cuticular hydrocarbons mediate male mate choice and sexual isolation between hybridising *Chrysochus* (Coleoptera: Chrysomelidae). *Chemoecology*. 2007;17(2):87–96.

- Phelan P, Baker TC. Male-size-related courtship success and intersexual selection in the tobacco moth, *Ephesia elutella*. *Experientia*. 1986;42(11):1291–3.
- Pitcher TE, Neff BD, Rodd FH, Rowe L. Multiple mating and sequential mate choice in guppies: females trade up. *Proceedings of the Royal Society of London Series B: Biological Sciences*. 2003;270(1524):1623–9.
- Polis GA. The evolution and dynamics of intraspecific predation. *Annual Review of Ecology and Systematics*. 1981;12(1):225–51.
- Prestwich K. The energetics of acoustic signaling in anurans and insects. *American Zoologist*. 1994;34(6):625–43.
- Price T. Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*. 1998;353(1366):251–60.
- Proshold F, LaChance L, Richard R. Sperm production and transfer by *Heliothis virescens*, *H. subflexa*, and the sterile hybrid males. *Annals of the Entomological Society of America*. 1975;68(1):31–4.
- Richardson ML, Mitchell RF, Reagel PF, Hanks LM. Causes and consequences of cannibalism in noncarnivorous insects. *Annual review of entomology*. 2010;55:39–53.
- Rico-Guevara A, Hurme KJ. Introsexually selected weapons. *Biological Reviews*. 2019;94(1):60–101.
- Ritchie MG. Sexual selection and speciation. *Annu Rev Ecol Evol Syst*. 2007;38:79–102.
- Rodrigues LR, Duncan AB, Clemente SH, Moya-Laraño J, Magalhães S. Integrating competition for food, hosts, or mates via experimental evolution. *Trends in Ecology & Evolution*. 2016;31(2):158–70.
- Roelofs WL, Hill AS, Carde RT, Baker TC. Two sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. *Life sciences*. 1974;14(8):1555–62.
- Ryan MJ, Rand AS. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution*. 1993;47(2):647–57.
- Schneider JC, Roush RT. Genetic differences in oviposition preference between two populations of *Heliothis virescens*. In: Huettel MD, editor. *Evolutionary genetics of invertebrate behavior*. Springer; 1986. p. 163–71.
- Servedio MR, Lande R. Population genetic models of male and mutual mate choice. *Evolution*. 2006;60(4):674–85.
- Sheck A, Gould F. The genetic basis of host range in *Heliothis virescens*: larval survival and growth. *Entomologia Experimentalis et Applicata*. 1993;69(2):157–72.
- Sheck A, Gould F. Genetic analysis of differences in oviposition preferences of *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae). *Environmental Entomology*. 1995;24(2):341–7.
- Silberglieud RE, Shepherd JG, Dickinson JL. Eunuchs: the role of apyrene sperm in Lepidoptera? *The American Naturalist*. 1984;123(2):255–65.
- Skiri H, Strandén M, Sandoz JC, Menzel R, Mustaparta H. Associative learning of plant odorants activating the same or different receptor neurones in the moth *Heliothis virescens*. *Journal of Experimental Biology*. 2005;208(4):787–96.
- Solensky M, Oberhauser K. Male monarch butterflies, *Danaus plexippus*, adjust ejaculates in response to intensity of sperm competition. *Animal Behaviour*. 2009;77(2):465–72.
- Sondhi Y, Ellis EA, Bybee SM, Theobald JC, Kawahara AY. Light environment drives evolution of color vision genes in butterflies and moths. *Communications biology*. 2021;4(1):1–11.
- Surlykke A. Hearing in notodontid moths: a tympanic organ with a single auditory neurone. *Journal of Experimental Biology*. 1984;113(1):323–35.
- Surlykke A, Larsen ON, Michelsen A. Temporal coding in the auditory receptor of the moth ear. *Journal of Comparative Physiology A*. 1988;162(3):367–74.
- Symonds MR, Elgar MA. The evolution of pheromone diversity. *Trends in ecology & evolution*. 2008;23(4):220–8.
- Teal P, McLaughlin J, Tumlinson J. Analysis of the reproductive behavior of *Heliothis virescens* (F.) under laboratory conditions. *Annals of the Entomological Society of America*. 1981;74(3):324–30.
- Teal P, Tumlinson J. Isolation, identification, and biosynthesis of compounds produced by male hairpencil glands of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). *Journal of chemical ecology*. 1989;15(1):413–27.
- Thomas M, Simmons L. Sexual selection on cuticular hydrocarbons in the Australian field cricket, *Teleogryllus oceanicus*. *BMC Evolutionary Biology*. 2009;9(1):162.
- Thomas Y, Bethenod MT, Pelozoelo L, Frérot B, Bourguet D. Genetic isolation between two sympatric host-plant races of the European Corn Borer, *Ostrinia Nubilalis* Hubner I. Sex pheromone, moth emergence timing, and parasitism. *Evolution*. 2003;57(2):261–73.

- Thomson IR, Darveau CA, Bertram SM. Body morphology, energy stores, and muscle enzyme activity explain cricket acoustic mate attraction signaling variation. *PLoS One*. 2014;9(3):e90409.
- Thornhill R. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *The American Naturalist*. 1983;122(6):765–88.
- Tillman JA, Seybold SJ, Jurenka RA, Blomquist GJ. Insect pheromones—an overview of biosynthesis and endocrine regulation. *Insect biochemistry and molecular biology*. 1999;29(6):481–514.
- Tumlinson J, Hendricks D, Mitchell E, Doolittle R, Brennan M. Isolation, identification, and synthesis of the sex pheromone of the tobacco budworm. *Journal of Chemical Ecology*. 1975;1(2):203–14.
- van Veen F, Morris RJ, Godfray HCJ. Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. *Annu Rev Entomol*. 2006;51:187–208.
- Verburgt L, Ferguson J. Mate choice in field crickets: can females acoustically detect male body size? *Journal of ethology*. 2010;28(1):141–51.
- Vetter RS, Baker TC. Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female sex pheromone glands. *Journal of Chemical Ecology*. 1983;9(6):747–59.
- Vickers NJ, Baker TC. Chemical communication in heliothine moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist. *Journal of Comparative Physiology A*. 1997;180(5):523–36.
- Vogel H, Heidel AJ, Heckel DG, Groot AT. Transcriptome analysis of the sex pheromone gland of the noctuid moth *Heliothis virescens*. *BMC genomics*. 2010;11(1):1–21.
- Waldvogel M, Gould F. Variation in oviposition preference of *Heliothis virescens* in relation to macroevolutionary patterns of heliothine host range. *Evolution*. 1990;44(5):1326–37.
- Wang G, Vasquez G, Schal C, Zwiebel L, Gould F. Functional characterization of pheromone receptors in the tobacco budworm *Heliothis virescens*. *Insect molecular biology*. 2011;20(1):125–33.
- Warrant E, Kelber A, Kristensen NP. *Lepidoptera, moths and butterflies*. Kristensen NP, editor. Vol. 2. Berlin & New York: Walter de Gruyter; 2003.
- Wedell N, Cook PA. Determinants of paternity in a butterfly. *Proceedings of the Royal Society of London Series B: Biological Sciences*. 1998;265(1396):625–30.
- Wilkins MR, Seddon N, Safran RJ. Evolutionary divergence in acoustic signals: causes and consequences. *Trends in ecology & evolution*. 2013;28(3):156–66.
- Wyatt TD. *Pheromones and animal behaviour*. Vol. 626. Cambridge University Press, Cambridge; 2003.
- Xu J, Wang Q. Mechanisms of last male precedence in a moth: sperm displacement at ejaculation and storage sites. *Behavioral Ecology*. 2010;21(4):714–21.
- Yan S, Zhu J, Zhu W, Zhang X, Li Z, Liu X, et al. The expression of three opsin genes from the compound eye of *Helicoverpa armigera* (Lepidoptera: Noctuidae) is regulated by a circadian clock, light conditions and nutritional status. *PLoS one*. 2014;9(10):e111683.
- Zhao XC, Pfuhl G, Surlykke A, Tro J, Berg BG. A multisensory centrifugal neuron in the olfactory pathway of heliothine moths. *Journal of Comparative Neurology*. 2013;521(1):152–68.