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SHORT COMMUNICATION

# Phenotypic plasticity in sexual communication signal of a noctuid moth

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## Abstract

Variability within sex pheromone signalling systems is generally believed to be low because of strong stabilizing selection; yet the noctuid moth *Heliothis subflexa* (Hs) shows significant intraspecific variation. One possible explanation is that females may alter their sex pheromone blend depending on prevailing olfactory cues in the habitat, which we termed the 'experience hypothesis'. This could be adaptive if Hs females experiencing the pheromone of another species, *Heliothis virescens* (Hv), responded to reduce the frequency of heterospecific matings. We exposed Hs females to no pheromone, Hs pheromone or Hv pheromone in the first 3 days of their adult lives. Hs females in the latter treatment produced significantly more of the acetate Z11-16:OAc, which inhibits the attraction of Hv males. To our knowledge, this is the first study showing adaptive phenotypic plasticity in a moth sex pheromone and suggests that behavioural differentiation may precede genetic divergence in the sexual signals of moths.

## Introduction

The evolution of sexual communication is one of the mysteries in evolutionary biology, because signal and response are generally hypothesized to be under strong stabilizing selection in diverse animal taxa (Löfstedt, 1993; Ritchie, 1996, 2007; Butlin & Trickett, 1997; Coyne *et al.*, 1997; Brooks *et al.*, 2005). As signaller and responder need to be finely tuned to each other for optimal mutual recognition, a population converges to the most attractive signal–response combination. In most nocturnal moths, females are the pheromone signallers and males are the responders. Because males are behaviourally tuned to their species-specific pheromone blend (Cossé *et al.*, 1995; Linn *et al.*, 1997), a mutation that alters the female's pheromone blend is likely to lower her reproductive fitness (Butlin & Trickett, 1997; Zhu *et al.*, 1997). Therefore, the means by which novel signals in sexual communication can evolve, in the face of selection against such change, requires further exploration.

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Directional selection may counteract stabilizing selection, for example through communication interference between sympatric species that use similar premating signals, which may lead to reproductive character displacement. In the past decade, researchers have described patterns in reproductive traits that are in accordance with such displacement, i.e. greater divergence has been found in mate recognition signals of closely related species in areas of sympatry than in areas of allopatry (e.g. Howard & Gregory, 1993; Noor, 1995; Coyne & Orr, 1997; McElfresh & Millar, 1999, 2001; Higgie *et al.*, 2000; Gries *et al.*, 2001; Hobel & Gerhardt, 2003). We recently showed experimentally that communication interference between the moths *Heliothis virescens* (Hv) and *Heliothis subflexa* (Hs) can indeed be a strong directional selection force (Groot *et al.*, 2006).

An alternative possibility to explain variation in sexual communication signals is that these signals are phenotypically plastic. In general, phenotypic plasticity is the ability of an organism to adjust its phenotype to the biotic or abiotic environment (e.g. Via *et al.*, 1995; Agrawal, 2001; Pigliucci, 2001; Price *et al.*, 2003). Response to sexual signals has been found to be plastic in a few recent studies (Anderson *et al.*, 2003; Andersson *et al.*, 2007; Bailey & Zuk, 2008). Phenotypic plasticity in sexual

signals themselves has been explored only in a few *Drosophila* species (Petfield *et al.*, 2005; Kent *et al.*, 2008; Krupp *et al.*, 2008) and a seed beetle (Bashir *et al.*, 2003). In *Drosophila melanogaster* and *Drosophila serrata*, the social environment, i.e. the genetic composition of the group and whether males interacted with males or females, has been shown to affect the composition of cuticular hydrocarbons which play an important role in mate choice. In the seed-eating beetle *Rhyzopertha dominica*, Bashir *et al.* (2003) found varying release rates of aggregation pheromone depending on the type of seed and the presence of females. In moths, pheromone production has been shown to be affected by host plant volatiles (Raina, 1988), sucrose nutrition (as shown for mated *H. virescens* females) (Foster, 2009) and temperature (Raina, 2003). However, these factors have been shown to affect the quantity rather than the quality of the pheromone blend.

Plasticity in female sex pheromone composition in moths can be expected because of a number of characteristics. First of all, females produce their pheromone *de novo* every night (e.g. Jurenka, 2004; Rafaeli, 2005), which may allow adjustment of the pheromone blend. Also, in many moth species females can perceive their own species' female pheromone compounds (Denotter *et al.*, 1978; Schneider *et al.*, 1998; Groot *et al.*, 2005; Hillier *et al.*, 2006), so that they are likely to perceive conspecific as well as heterospecific sex pheromone, at least if their own pheromone blend overlaps with that of the other species. In addition, recently, pheromone receptors have been found in sensilla on the ovipositor of female Hv (Widmayer *et al.*, 2009), suggesting that these receptors might allow a feedback mechanism onto the gland that might affect pheromone emission.

In Hs, one compound in the sex pheromone blend is especially interesting because of its dual function: the acetate Z11-16:OAc not only enhances the attraction of conspecific Hs males but also inhibits the attraction of heterospecfic Hv males (Vickers & Baker, 1997a; Groot *et al.*, 2006). While focusing on the intraspecific variation of sexual communication in both Hv and Hs, we found not only geographical variation in the premating pheromonal signals (Groot *et al.*, 2007, 2009), but also significant variation within a region between years (Groot *et al.*, 2009). Specifically, the acetate Z11-16:OAc was significantly higher in Hs females from North Carolina, where Hv is highly abundant, than in Hs females from the west coast of Mexico, where Hv is virtually absent (Groot *et al.*, 2009). The temporal variation in the sex pheromone of Hs also correlated with Hv abundance: in North Carolina, when Hv was highly abundant (in 2004), Hs females contained significantly more of this acetate than when Hv was much less abundant in 2005 (Groot *et al.*, 2009). These results led us to speculate that females may vary their sex pheromone blend depending on the prevailing olfactory cues in their habitat, which we called the 'experience hypothesis' (Groot *et al.*, 2009).

To explore the presence and possible magnitude of phenotypic plasticity in the sexual communication of Hs, in this study we exposed siblings (i.e. genetically similar groups) to different olfactory environments in their early-adult life in the laboratory to test the 'experience hypothesis'. Specifically, we hypothesized that Hs females would contain higher levels of the acetate Z11-16:OAc when experiencing Hv sex pheromone in the first 3 days of their adult lives than when experiencing no pheromone or conspecific pheromone. We tested this hypothesis by exposing emerging adult females to different pheromone environments and analysing their sex pheromone composition after three days of this exposure.

## Material and methods

### Insects

*Heliothis subflexa* eggs were obtained from a laboratory strain at North Carolina State University and were sent to Max Planck Institute for Chemical Ecology. First instar larvae were reared to adults on artificial diet in a climate chamber (Snijder) with reverse light cycle (L : D 14 h:10 h, 26–27 °C during the photophase, from 17.00 to 7.00 h; 24 °C during scotophase, from 7.00 to 17.00 h) and ~70% relative humidity. Adults were mated in single pair matings and the cups containing offspring larvae were marked according to family to distinguish siblings from nonsiblings. Pupae were sexed and female pupae were used in the bioassay.

### Sex pheromone lures

The sex pheromone blend of Hs females consists of the following 11 compounds: tetradecanal (14:Ald), (*Z*)-9-tetradecenal (Z9-14:Ald), hexadecanal (16:Ald), (*Z*)-7-hexadecenal (Z7-16:Ald), (*Z*)-9-hexadecenal (Z9-16:Ald), (*Z*)-11-hexadecenal (Z11-16:Ald) as the major component, (*Z*)-7-hexadecenyl acetate (Z7-16:OAc), (*Z*)-9-hexadecenyl acetate (Z9-16:OAc), (*Z*)-11-hexadecenyl acetate (Z11-16:OAc), (*Z*)-9-hexadecen-1-ol (Z9-16:OH) and (*Z*)-11-hexadecen-1-ol (Teal *et al.*, 1981; Klun *et al.*, 1982; Heath *et al.*, 1990, 1991; Vickers, 2002; Groot *et al.*, 2007). Red rubber septa (Thomas Scientific, Philadelphia, PA, USA) were ultrasonicated in hexane, allowed to air dry, loaded with the pheromone blend in 100 $\mu$L of hexane, allowed to dry for 1–2 h, loaded with 100 $\mu$L of hexane and finally allowed to dry for 1–2 h. Control lures (no pheromone – nP) received the same treatment as the other lures but were not loaded with pheromone.

Synthetic sex pheromone lures of Hs (HsP) were loaded such that 100% Z11-16:Ald equalled 300 $\mu$g Z11-16:Ald, whereas the additional components were loaded in the following percentages relative to

Z11-16:Ald: 5% 16:Ald, 5% Z7-16:OAc, 10% Z9-16:OAc, 20% Z11-16:OAc and 10% Z11-16:OH. This blend is based on the rubber septum formulation reported by Heath *et al.* (1990), that resulted in high trapping efficacy in the field (Groot *et al.*, 2007). The sex pheromone blend of Hv females consists of a subset of these compounds (Roelofs *et al.*, 1974; Tumlinson *et al.*, 1975; Klun *et al.*, 1979; Vetter & Baker, 1983; Ramaswamy *et al.*, 1985; Teal & Tumlinson, 1986; Vickers & Baker, 1997b). To mimic this sex pheromone blend (HvP), synthetic pheromone lures were loaded similarly as the HsP lures, only this time pheromone components were loaded as follows: 5% 14:Ald, 5% Z9-14:Ald, 10% 16:Ald, 2% Z7-16:Ald, 2% Z9-16:Ald and 1% Z11-16:OH. This loading was based approximately on Teal *et al.* (1986) and resulted in high trap catches of Hv males in the field (A. T. Groot and C. Schal, unpubl. res.). Pheromone compounds were obtained from PHEROBANK (Wageningen, the Netherlands), Shin-Etsu Chemical (Tokyo, Japan) and Bedoukian Research (Danbury, CT, USA).

### Early-adult experience assay

To assess whether females would differ in their pheromone composition when experiencing different pheromone blends as newly eclosed adults, a multichannel olfactometer was devised as follows (see photo Fig. 1). In total, 12 cylinders were connected to an airflow. These cylinders were composed of two single polyoxymethylene copolymer (POC) tubes (diameters: 6 and 5 cm; length: 11 cm each) that were divided by gauze (128 $\mu$m, Sefarpetex) to disperse the air in the downwind tubes. Upwind, compressed air was pushed through a charcoal filter at 10 L per minute, and humidified to about 60% relative humidity by passing through a 0.5-L gas washing bottle filled with distilled water. Water was replaced every other day. The air was split by means of a T-connector, and each half was led through a POC container in which a pheromone lure could be placed. In one container, a rubber septum was placed containing either an Hv (HvP) or an Hs (HsP) synthetic pheromone

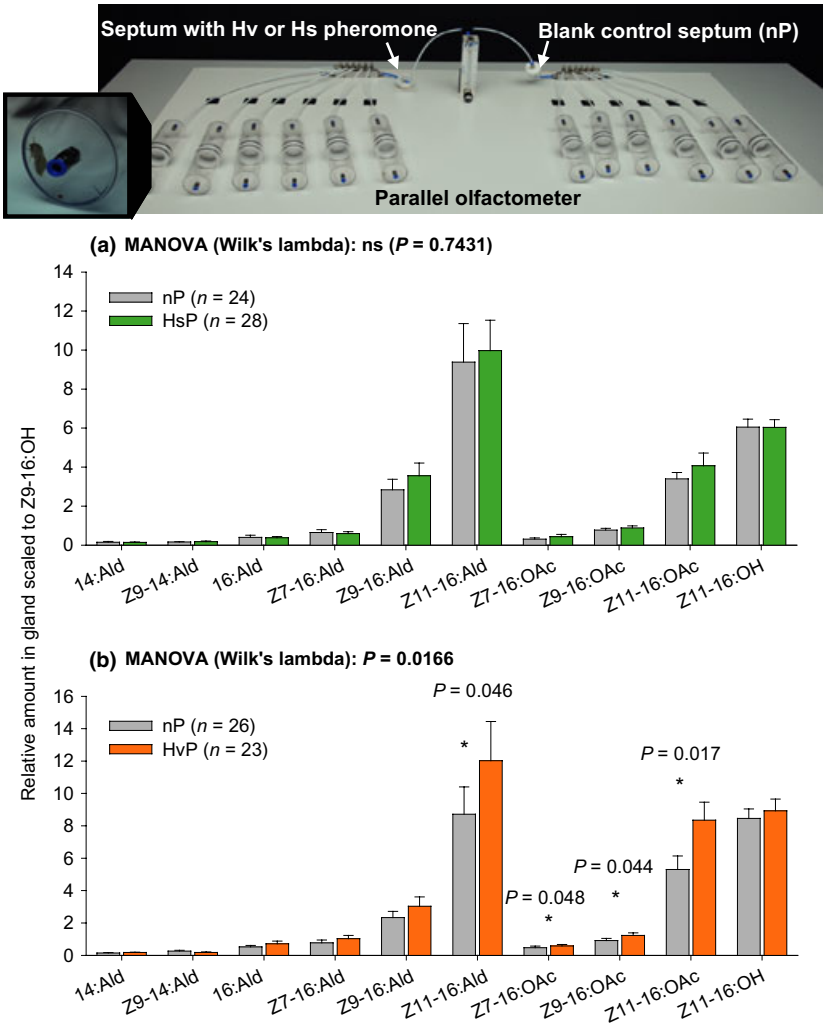


**Fig. 1** Sex pheromone composition of Hs females that emerged and remained for 3 days exposed to either (a) conspecific sex pheromone (HsP) or control odour containing no pheromone (nP) (conspecific experience), or (b) the sex pheromone of Hv (HvP) or control odour (nP) (heterospecific experience).

blend, whereas a control septum loaded with hexane and no pheromone (nP) was placed in the other container. A valve was placed upwind of the containers to avoid possible contamination between the pheromone treatment and the control. Septa were replaced every two weeks.

The pheromone-laden air and the control air were split six-fold each, using a digital flow switch with integrated flow controllers (SMC, Engelsbach, Germany), which allowed adjusting the air flow in each of the six cylinders to ~250 mL per minute (measured at the exit of each tube with a digital air flow meter (SMC, 0.2–10 L $min^{-1}$). Outgoing air was aspirated immediately as the whole set-up was placed in a fume hood. The same reverse light cycle was used as in the climate chambers. All hoses used in this set-up were Teflon®-coated (Jenpneumatik, Jena, Germany). The temperature within the pheromone and nonpheromone tubes was stable between 2 and 27 °C. Physical parameters were checked within the tubes by using a temperature and humidity data logger (EL-USB-2; Lascar electronics, Salisbury, UK).

To confirm that pheromone was only detectable in the appropriate tubes, 3 days after lure exchange we placed a solid phase microextraction (SPME) fibre into a pheromone tube and a second SPME fibre into a nonpheromone tube. Airborne compounds were sampled for 4 h in each tube and desorbed into a gas chromatograph (GC), which confirmed the presence of pheromone in the pheromone tube and its absence in the no-pheromone tube. After 2 weeks, the measurements with the SPME were repeated under the same conditions to verify that the pheromone was still present.

Pupa development time of Hs averages 10–12 days. Female pupae of 5–9 days old were placed in the downwind part of the cylinders to ensure that females would experience the odour upon emergence. To minimize the effect of any possible intraspecific genetic variation, females of the same genetic background (i.e. derived from one single pair mating) were divided over the 12 cylinders. To increase the chance of simultaneous emergence, up to six pupae of one family were placed in each tube. After the first female emerged within one tube, the other pupae were removed immediately. Females were observed with a red light flashlight (LED, 660 nm) every 30 min on random days to check for female calling throughout the experimental period. Emerged females remained in the cylinders for 3 days with a piece of cotton soaked in 10% honey water. After 3 days, the pheromone gland of each female was extracted separately, as described later. The pheromone and control tubes were carefully cleaned with water and 70% ethanol (Merck, Darmstadt, Germany) before new pupae were placed into the tubes. Every other week, the pheromone treatment side and the nonpheromone treatment side were switched to exclude any possible side-specific effects. As we had only one apparatus and wanted to minimize possible cross-contamination, we first tested the HvP/nP treatments until we had extracted the pheromone glands of 25–30 females from each odour treatment (heterospecific experience), and then tested the HsP/nP treatments with a similar number of females (conspecific experience).

### Gland extractions

The sex pheromone glands of each 3-day-old female were separately extracted in the 4th hour of scotophase, as this is the peak of pheromone production in Hs (Heath *et al.*, 1991). Each gland was extruded by pressing the abdomen with forceps and cut with microdissection scissors. Each gland was extracted separately in a glass conical vial in 50 μL of n-hexane (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) containing 25 ng of the internal standard pentadecane. The glass vial was placed in a 4-mL screw top vial (Alltech Grom GmbH, Rottenburg-Hailfingen, Germany) containing 100–200 μL hexane and capped with a solid top polypropylene cap with a TFE (tetrafluoroethylene)/silicone-bonded interseal (Alltech Grom). After 30–40 min, the glands were removed and the extract stored at −20 °C until GC analysis.

### GC analysis

The extracts were reduced to 1–2 μL under a gentle stream of nitrogen and taken up with a 10 μL syringe (701SN 26S GA 2 needle; Hamilton, Reno, NV, USA) together with 1 μL of octane (Fluka, St. Louis, MO, USA) to avoid evaporation. The whole volume of 3–4 μL was transferred to a 0.05-mL Micro-insert (Alltech Grom) that was placed in a spring in 1.5-mL Crimp Neck Vial (Alltech Grom) and capped with 11-mm alucrimp lids which contained a 1-mm clear silicon/clear PTFE (polytetrafluoroethylene) septum (Alltech Grom). The sample was injected with a 7683 automatic injector into the splitless inlet of an HP7890 GC, which was coupled with a high-resolution polar capillary column [DB-WAXetr (extended temperature range); 30 m × 0.25 mm × 0.5 μm] and a flame-ionization detector (FID) using the following program: 60 °C (hold for 2 min) to 180 °C (30 °C $min^{-1}$), followed by an increase of temperature to 230 °C (5 °C $min^{-1}$). The column was then heated to 245 °C at 20°C $min^{-1}$ for 15 min. The FID was kept at 250 °C. To identify the particular compounds, a multicomponent blend (compounds from Pherobank, Wageningen) containing all the compounds of Hs was injected into the GC before or after each daily series of injections. For quantitative analysis, the GC signal was integrated with the software ChemStation (Agilent, Technologies Deutschland GmbH, Böblingen, Germany). For statistical analysis, only those pheromone blends were considered that contained a total pheromone amount > 40 ng, because under this threshold the smaller peaks could not be integrated reliably.

### Statistical analysis

Data were analysed using SAS Version 9.1 (SAS Institute, 2002–2003). First, we conducted a logcontrast transformation by scaling 10 of the 11 compounds relative to the eleventh, and taking the logarithm of each ratio (Aitchison, 1986). These 10 log contrasts are mutually independent and can be analysed using standard multivariate techniques; the price paid for this independence is loss of information on the one compound used to normalize by. We chose to normalize the other compounds relative to the alcohol Z9-16:OH, because (a) this compound has no biological relevance for the attraction or inhibition of either Hv or Hs, to our knowledge, (b) it is produced in amounts that is well above the limit of detection (in contrast to e.g. 14:Ald and Z9-14:Ald) and (c) the total amount of this compound showed the lowest coefficient of variation (CV) in all treatments. We then conducted a multivariate analysis of variance (PROC MANOVA) to assess significant differences between the overall pheromone blends of the different groups of females, i.e. nP vs. HsP (control experiment, with conspecific experience) and nP vs. HvP (heterospecific experience). The results of MANOVA are independent of which compound was chosen to normalize by (Aitchison, 1986). Because we found a significant difference between the two experiments (experiment effect; MANOVA, Wilks' $\lambda$, $F_{10,90} = 3.75$, $P = 0.0003$), we analysed the two experiments separately. Significant differences between each pheromone component were determined by conducting an ANOVA (PROC GLM, SAS Institute, Cary, NC, USA) with a Tukey adjustment for multiple comparisons.

## Results

When Hs females emerged in the conspecific Hs odour (HsP), we found no significant difference in the sex pheromone blend or sex pheromone components between these females and Hs females that had emerged in the absence of any pheromone (nP) (MANOVA, Wilks' $\lambda$, $F_{10,41} = 0.67$, $P = 0.7431$; Fig. 1a). However, females that had emerged in heterospecific Hv odour (HvP) significantly differed in their blend from the females that had emerged in the control odour (MANOVA, Wilks' $\lambda$, $F_{10,38} = 2.60$, $P = 0.0166$; Fig. 1b). Specifically, the females that emerged in the Hv odour contained significantly more of the major component Z11-16:Ald ($F_{1,47} = 4.22$, $P = 0.046$), and all three acetates Z7-16:OAc ($F_{1,47} = 4.11$, $P = 0.048$), Z9-16:OAc ($F_{1,47} = 4.11$, $P = 0.044$) and Z11-16:OAc ($F_{1,47} = 6.13$, $P = 0.017$), than females that had emerged in the control odour.

As the pheromone composition could change depending on whether females have called (i.e. extruded their glands to emit pheromone) before pheromone gland extraction, in a subset of 18 females we assessed whether female calling behaviour was similar in the absence of or in the presence of HvP odour (i.e. in 1b). *Heliothis subflexa* females call most actively between the 4th and 6th hour into scotophase (Heath *et al.*, 1991), which is the reason we extracted the glands in the 4th hour of the scotophase, as stated earlier. In the control odour, three of six observed females called, whereas in the HvP treatment 7 of 12 observed females called, indicating no obvious difference in calling behaviour between the two treatments.

## Discussion

The major finding of this study is that Hs females contained significantly more of the major acetate (Z11-16:OAc) when they emerged and remained in the sex pheromone odour of Hv (HvP) for 3 days compared to Hs females that emerged in control odour (nP) or in the sex pheromone odour of conspecific females (HsP). Thus, early-adult experience of different chemical environments affects the sex pheromone composition in Hs females. This effect may be because of a change in the pupal stage or in the early-adult stage. However, it is unlikely that under field conditions Hs pupae can detect chemical changes in the air because they pupate in the soil, suggesting that modulation of pheromone production occurs after the imaginal moult. To our knowledge, this is the first study showing phenotypic plasticity in production of a moth sex pheromone in response to changes in the pheromonal environment.

As Z11-16:OAc is not present in the sex pheromone blend of Hv, an increase in this compound in Hs accentuates the pheromonal difference between these two species. Additionally, Z11-16:OAc is a strong behavioural antagonist that inhibits the attraction of Hv males to an otherwise attractive pheromone blend (Vickers & Baker, 1997a; Groot *et al.*, 2006). Plasticity in the pheromone production may thus allow a female to produce the compound in higher amounts only when Hv is highly abundant, thus deflecting orientation and courtship by Hv males. This plasticity is clearly adaptive, as interspecific matings incur a fitness cost (Groot *et al.*, 2006) Divergence of communication signals in areas of sympatry has been found in a diverse range of taxa (e.g. Blows & Higgie, 2002; Groning & Hochkirch, 2008; Pfennig & Pfennig, 2009), including Lepidoptera (e.g. Löfstedt *et al.*, 1986; Tóth *et al.*, 1992; McElfresh & Millar, 1999, 2001; Gries *et al.*, 2001; Wang *et al.*, 2008), but so far they were thought to be the product of directional selection over evolutionary time. However, our results imply that differentiation in areas of sympatry can increase through changes in the signaller based on sensory input from its olfactory environment.

Previously, we found geographical variation in the acetate production between Hs females from Mexico and North Carolina (Groot *et al.*, 2009), ranging from 2% in Western Mexico to 35% in North Carolina. This variation was found to be partly genetic (A. T. Groot and

H. Staudacher, unpubl. res.); when crossing and backcrossing Hs-NC with Hs-MXW, we found a major quantitative trait locus (QTL) that explained 40% of the geographical acetate variation, as well as two minor QTL explaining 9% and 7%. The results presented in this study show that another part of the variation may be explained by female's ability to react to increased Hv abundance by increasing the production of Z11-16:OAc. This parallels our previous finding that the temporal variation coincided with the relative abundance of Hv in the field, which led us to hypothesize that females can vary their pheromone blend depending on their experience (Groot *et al.*, 2009). Even though a change in this compound from 15–17% to 22% of the total blend (see Supplementary Fig. S1) may only slightly increase the deterrence of Hv males, this experiment shows the proof of principle that a significant change can occur solely as a result of a change in the chemical environment.

Our results of a significant change in the pheromone blend of Hs when females had emerged in the odour of heterospecific Hv suggest that the presence of heterospecific pheromone might indirectly influence the transcription, translation or activity level of e.g. fatty alcohol acetyltransferases catalysing the conversion from Z11-16:OH to Z11-16:OAc. That a mechanism of chemical plasticity is not only possible, but in fact operational in nature, and is shown by the predatory bolas spider *Mastophorus hutchinsoni* that can mimic the sex pheromone of four different prey moth species, depending on the presence and activity of the moths throughout the night as well as throughout the season (Haynes *et al.*, 2002).

The finding that intraspecific variation in the sex pheromone of the heliothine moth Hs is partly plastic complicates the predictability of the evolutionary fate of a trait (e.g. 'high relative amount of acetates'). In general, plasticity might *retard* the genetic fixation of a trait, as it buffers the action of negative selection: individuals are spared from the force of selection because phenotypic plasticity allows expressing the phenotype that is required in the respective environment (Price *et al.*, 2003; Crispo, 2008). In such a scenario, genetic fixation of the new phenotype and adaptive genetic differentiation is delayed, which is the more traditional view of the interaction between plasticity and evolution (Crispo, 2008). However, plasticity might also *promote* the evolution of a trait as it allows individuals to colonize habitats that would have been inaccessible for nonplastic individuals (Crispo, 2008). In the case of Hs, the spontaneous accentuation of pheromone differences might enable Hs to reproduce in areas with high abundances of Hv because higher amounts of Z11-16:OAc inhibit the attraction of Hv males, with whom Hs can still mate but such hybrid mating produces sterile sons (Laster, 1972). In contrast, nonplastic females might not be able to inhibit heterospecific attraction as successfully. In these areas of high abundances of Hv directional selection may subsequently promote a genetic change that leads to a greater pheromone divergence. The result could be geographical regions with plastic and other regions with nonplastic individuals. However, phenotypic plasticity itself may be costly (Pigliucci, 2001; Price *et al.*, 2003) as it requires the female's ability to detect surrounding pheromone and a regulatory feedback pathway that permits the regulation of gene expression or enzyme activity. In areas with consistently high population sizes of Hv, plasticity may thus get lost again, and the trait 'high acetates' may undergo slow genetic assimilation. In areas with constantly low abundances of Hv, the production of high acetate levels is not necessary because the presence and thus attraction of heterospecific males is minor. In these areas, the trait 'low amounts of acetates' is likely to get genetically fixed.

In conclusion, our results indicate that Hs females can adjust their pheromone composition depending on the odour that they experience in early-adult life. Specifically, the acetate Z11-16:OAc was increased when females experienced Hv odour in the first 3 days of their adult lives, as we predicted. Because a higher acetate level increases the attraction of conspecific males (Groot *et al.*, 2006), this behavioural adjustment may lead to assortative mating. Thus, behavioural differentiation may precede genetic divergence in the sexual signals of moths. Future research on the first evolutionary step in moth sexual communication should further explore the presence and extent of phenotypic plasticity in both signallers and responders.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Sex pheromone composition of Hs females in both experiments when calculating the relative percentages of all compounds, i.e. the sum of all compounds per treatment is 100%.

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