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Artificial night lighting disrupts sex pheromone in a noctuid moth

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Abstract. 1. One major, yet poorly studied, change in the environment is the increase in nocturnal light pollution. Although this strongly alters the habitat of nocturnal species, the ecological consequences are poorly known. Moths are well known to be attracted to artificial light sources, but artificial light may affect them in other ways as well.

2. In this study, female *Mamestra brassicae* moths were subjected to various types of low-intensity artificial night lighting with contrasting spectral compositions (green-rich, red-rich, warm white) or to a dark control treatment and the effects on their sex pheromone production and composition were tested.

3. Artificial night lighting reduced sex pheromone production and altered the chemical composition of the pheromone blend, irrespective of spectral composition. Specifically, amounts of the main pheromone component Z11-16:Ac were reduced, while the deterring compounds Z9-14:Ac, Z9-16:Ac, and Z11-16:OH were increased relative to Z11-16:Ac when females were kept under artificial light. These changes may reduce the effectiveness of the sex pheromones, becoming less attractive for males.

4. These results show for the first time that artificial light at night affects processes that are involved in moth reproduction. The potential for mitigation through manipulation of the spectral composition of artificial light appears limited.

Key words. Disruption, light pollution, *Mamestra brassicae*, sex pheromone composition, sexual communication.

Introduction

As a result of the continuous rise in levels of artificial night lighting (Hölker *et al.*, 2010a), nocturnal animals, representing the majority of the terrestrial fauna (Hölker *et al.*, 2010b), are increasingly confronted with illumination of nightscapes (Cinzano *et al.*, 2001). However, the ecological consequences of artificial night lighting, and possibilities for mitigation of negative effects, remain poorly studied to date (Gaston *et al.*, 2012). Currently, artificial lighting of outdoor public spaces is undergoing a transition from traditional lamp types, such as high- and low-pressure sodium and fluorescence lamps (e.g. mercury vapour and metal halide lamps), towards (cold) white LED lamps (Stone *et al.*, 2012). Depending on the lights that are replaced by the LEDs, this can result in an increased (compared with high- and low-pressure sodium; see Stone *et al.*, 2012; Pawson & Bader, 2014) or a decreased effect (compared with mercury vapour or metal-halide lamps; see Van Grunsven *et al.*, 2014) on insects. However, adjustment of spectral compositions is possible in LED lighting. This allows for application of lamps where harmful parts of the light spectrum are eliminated or strongly reduced in intensity (Gaston *et al.*, 2012) in order to reduce their ecological impact (Poot *et al.*, 2008).

Moths represent a largely nocturnal group of ~140 000 species (New, 2004; Bazinet *et al.*, 2013) that are often attracted to sources of artificial light at night (Van Langevelde *et al.*, 2011; Somers-Yeates *et al.*, 2013). However, the effect of artificial light pollution on aspects of moth ecology is largely unknown

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(Van Geffen *et al.*, 2014, 2015) and should be investigated, as moth populations are declining in western Europe (Conrad *et al.*, 2006; Groenendijk & Ellis, 2011; Fox *et al.*, 2014). These declines are partly ascribed to nocturnal illumination (Conrad *et al.*, 2006; Groenendijk & Ellis, 2011; Fox, 2013), with the proposed mechanism being phototaxis. In this paper, we focus on the influence of light on moth sex pheromone production, which is essential to attract potential mates.

Female moths produce and emit a sex pheromone that consists of a blend of mainly straight chain aldehyde, acetate, and alcohol components. The composition of the sex pheromone blend is highly species-specific: slight differences in relative amounts of different components allow respective males to differentiate between species and thus to identify conspecific females (Löfstedt *et al.*, 1991; Symonds & Elgar, 2008). Males not only have receptors for attractive components, but also for deterring components, often produced by closely related, co-occurring species, to distinguish conspecific from heterospecific females (Den Otter *et al.*, 1989; Christensen *et al.*, 1994; Boo *et al.*, 1995; Vickers & Baker, 1997; Evenden *et al.*, 1999; Eliyahu *et al.*, 2003; Gemeno *et al.*, 2006; Eizaguirre *et al.*, 2007).

The cabbage moth *Mamestra brassicae* (L.) (Noctuidae) is a common species in (sub)urban areas in western Europe. It is a habitat-generalist that is commonly found in cultured and (sub-)urban areas (Waring & Townsend, 2003). Adults of *M. brassicae* are frequently exposed to artificial light at night, as they are nocturnal and attracted to sources of artificial light (Waring & Townsend, 2003). In the Netherlands, populations of *M. brassicae* are declining, with a 50–75% reduction in the reported catches, primarily in light traps, corrected for observation intensity, over the period 1982–2013 (Ellis *et al.*, 2013).

The sex pheromone of *M. brassicae* is well studied, which makes the species suitable as a model species. The pheromone consists of nine different compounds (Struble *et al.*, 1980; Van de Veire & Dirinck, 1986; Den Otter & Van der Haagen, 1989; Renou & Lucas, 1994), most of which are common in co-occurring species (Table 1). The major constituent of the sex pheromone of *M. brassicae* is (*Z*)-11-hexadecenyl acetate (Z11-16:Ac). The attractiveness of this component is increased by small amounts of the two additional components, (*Z*)-11-hexadecenal (Z11-16:Ald) and (*Z*)-11-heptadecenyl acetate (Z11-17:Ac) (Van de Veire & Dirinck, 1986; Renou & Lucas, 1994). Three other compounds found in small amounts in the pheromone blend of *M. brassicae* are known to inhibit attraction of conspecific males: (*Z*)-9-tetradecenyl acetate (Z9-14:Ac), (*Z*)-9-hexadecenyl acetate (Z9-16:Ac), and (*Z*)-11-hexadecenol (Z11-16:OH) (Struble *et al.*, 1980; Renou & Lucas, 1994). Finally, three additional compounds that are present in the pheromone blend but seem not to play a functional role in sexual communication are tetradecanyl acetate (14:Ac), hexadecanyl acetate (16:Ac), and (*E*)-11-hexadecenyl acetate (E11-16:Ac) (Renou & Lucas, 1994).

Sex pheromone production in moths is regulated by pheromone biosynthesis activation neuropeptide (PBAN) (Raina *et al.*, 1989; Jacquin *et al.*, 1994). *Mamestra brassicae* sex pheromone production occurs at night (Noldus & Potting, 1990), and release of PBAN is regulated by light (Tawata &

Table 1. Presence and role of the nine sex pheromone compounds of *Mamestra brassicae* in the pheromone blends of co-occurring noctuids, based on El-Sayed (2012) and references therein.

Species	Habitat	Flight period	14:Ac	Z9-14:Ac	Z11-16:Ald	16:Ac	Z9-16:Ac	E11-16:Ac	Z11-16:Ac	Z11-16:OH	Z11-17:Ac
Mamestra brassicae	Cultured fields	April–October	P	P, D	P, A	P	P, D	P	P, M, A	P, D	P, A
Agrotis ipsilon	Cultured fields	April–October	–	P, M, A	–	P	–	–	P, M, A	P	–
Mamestra oleracea (syn: *Lacanobia oleracea*)	Cultured fields (among others)	April–October	–	–	–	–	–	–	P	P	–
Mythimna pallens	Grasslands, forest edges	May–October	–	–	P	–	–	–	P	–	–
Mythimna unipuncta (syn: *Pseudaletia unipuncta*)	Grasslands	August–November	–	P, A (0.01%) D (1%)	P, D	P	P	–	P, M, A	P, A	–
Ochropleura plecta	Cultured fields	April–October	–	D	P, A	–	–	–	P, M, A	–	–
Trichoplusia ni	Habitat generalist	May–October	–	P, A		–	–	–	P	–	–

P, present in pheromone blend; M, main pheromone component; A, attractor: compound increases attractiveness of main component; D, deterrent: compound decreases attractiveness of main component. Information on habitat and flight period was obtained from Waring and Townsend (2003).

Ichikawa, 2001). Therefore, the production of sex pheromone may be affected by artificial lighting.

In this study, we tested the effect of low levels of artificial night lighting with different spectral compositions on the amount and composition of the sex pheromone produced by *M. brassicae* females. Given the sensitivity of moths to short-wavelength radiation (Agee, 1973; Van Langevelde *et al.*, 2011), we expected the strongest reduction in sex pheromone production under light with higher levels of short wavelengths (e.g. green-rich and white light), and less reduction under light with lower levels of short wavelengths (e.g. red-rich), compared with moths in darkness.

Materials and methods

In a greenhouse, we constructed 20 open-top compartments of $50 \times 50 \times 60\,cm^3$. Each compartment was randomly assigned to one of four artificial light treatments: green-rich (henceforth green), warm white (henceforth white), red-rich (henceforth red) (all 17 ± 1 lx, or 63, 53, and 96 mW m^{-2}, respectively) or no artificial light at night (65 ± 1 mlx). Treatments were divided over five randomised blocks.

Lamps were custom-made by Philips Lighting (Eindhoven, the Netherlands) using LEDs obtained from Farnell (Utrecht, the Netherlands). The spectral compositions of the lamps that were used in this study were specifically designed for potential future application in LED street lighting. Therefore, the lamps had a light quality that allows for colour distinction for the human eye. As a result, lamps were not monochromatic, but comprised wavelengths from the complete spectrum, although highly skewed for some lamps (Fig. 1). Spectral measurements of lamps were performed at 25 °C in a 2-m-diameter Ulbricht sphere (Agilent technologies, Santa Clara, California) equipped with a Cary Varian 17 D digital spectrophotometer. Lamps were allowed to stabilise for 5 min prior to measurements. A photocell was used to measure stability of the lamps and to correct spectral measurements for self-absorption by the lamp units. White lamps consisted of three 60-lumen warm white LEDs (LXML-PWW1-0060), red lamps of two 70-lumen red-orange LEDs (LXM2-PH01-0070) and one 60-lumen warm white LED, and green lamps consisted of one 90-lumen cool white LED (LXML-PWC1-0090), one 23-lumen blue LED (LXML-PB01-0023) and two 80-lumen green LEDs (LXML-PM01-0080). LEDs were mounted in a $15 \times 8 \times 5\,cm^3$ (L × W × H) black plastic housing. Light was mixed in a 10-cm-long, $3.5 \times 3.5\,cm^2$ wide aluminium duct with a standard white diffusor [Polymethyl methacrylate (PPMA) plate] at the end. Green and white lamps had two diffusors to obtain light intensities identical to the red lamps. Lamps were switched on 1 h before the onset of the scotophase (night, starting around 19.00 hours), and switched off 1 h after onset of the photophase (day, starting around 07.00 hours). Daylight was natural, average day and night temperatures were 20 ± 2 and 18 ± 2 °C, respectively. There were no differences in temperature between light treatments [GLM $F_{3,16} = 0.065$, $P = 0.978$; measured in each compartment for two nights with i-buttons (Maxim, Sunnyvale, California)].

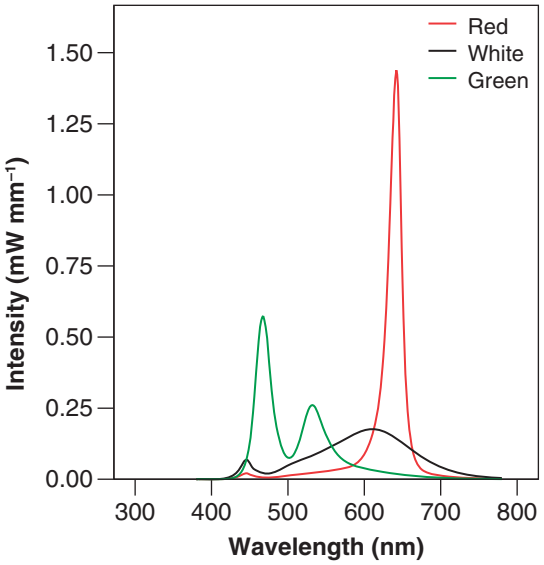

Fig. 1. Visualisation of spectral compositions of lamps used in the experiment. Lines are averages of three lamps per type.

Mamestra brassicae pupae were obtained from a mass rearing program at the Laboratory of Entomology, Wageningen University, the Netherlands (Poelman *et al.*, 2009). In this mass rearing, males and females were stored together in large deposition chambers in a controlled climate room at 20–22 °C, 50–70% RH and under an LD 16:8 h regime. Mating occurred in these chambers, after which females deposited eggs on filter paper. Caterpillars were reared on Brussels sprouts (*Brassica oleracea*) plants. Final-instar caterpillars were placed on an artificial soil medium, in which they pupated. For our experiments, we obtained 100 female *M. brassicae* pupae, 14 ± 1 days old, from this mass-rearing, and placed them individually in 13-cm-high, 7.5-cm-diameter white plastic containers. Each container was filled with a 0.5-cm layer of wood fibre and covered with 1.35 mm^2 white mesh. In each compartment (i.e. under each lamp), we placed five randomly selected containers. There were thus four light treatments with five compartments each, and each compartment contained five pupae, resulting in a nested design. Pupae were checked for emergence every day and freshly emerged moths were provided with a piece of cotton wool soaked in 1:10 sugar:water solution for feeding.

Each night, all three-night-old females were collected for gland extraction. Extractions started at midnight (~5 h after the onset of the scotophase), when females had produced sex pheromone for the coming night and started calling (Noldus & Potting, 1990). Due to variation in emergence date, gland extractions were spread over 15 nights. Glands of 24, 22, 22, and 24 females from green, white, red and dark control treatments, respectively, were successfully extracted.

The pheromone gland extraction and gas chromatography analysis procedures followed were as previously described by Groot *et al.* (2010a). A multi-component reference blend containing the nine previously identified sex pheromone compounds of *M. brassicae* (14:Ac, Z9-14:Ac, Z11-16:Ald, 16:Ac, Z9-16:Ac, E11-16:Ac, Z11-16:Ac, Z11-16:OH, and Z11-17:Ac;

compounds from Pherobank, Wageningen, the Netherlands) (Struble *et al.*, 1980; Van de Veire & Dirinck, 1986; Den Otter & Van der Haagen, 1989; Renou & Lucas, 1994) was injected into the gas chromatograph after the gland extract injections for manual integration of peaks of all components in CHEMSTATION (Agilent Technologies Deutschland GmbH, Böblingen, Germany). The amount of each compound was calculated relative to a 20 ng pentadecane internal standard (see Groot *et al.*, 2010b).

Statistical analyses of pheromone experiment

Artificial light-induced differences in the amount of sex pheromone (ln-transformed) were tested using nested ANOVA at the lamp level (the five moths in one compartment are not independent) with block as a random factor and a Tukey *post hoc* test. For all tests, homoscedasticity and normality of residuals were assessed visually.

As the amount of pheromone compounds can be highly variable, even between females within treatments, changes in pheromone composition are generally given in relative amounts (Heath *et al.*, 1991; Teal & Tumlinson, 1997; Groot *et al.*, 2005, 2009). However, relative amounts introduce the problem of interdependence; if the relative amount of one compound increases, the relative amount of another compound thus decreases. To overcome this interdependence, we performed log-contrast transformation (henceforth log-scaling), following Aitchison (1986) and Groot *et al.* (2010b), prior to analyses on artificial light-induced changes in pheromone blend composition. Differences in log-scaled amounts were analysed with standard parametric statistics. For this log-scaling, we scaled eight out of the nine compounds (14:Ac, Z9-14:Ac, Z11-16:Ald, Z9-16:Ac, E11-16:Ac, Z11-16:Ac, Z11-16:OH, and Z11-17:Ac) relative to the ninth compound (16:Ac) for each female separately, and took the logarithm of each ratio. We sacrificed 16:Ac for log-scaling, because: (i) male antennae are unable to detect this compound (Den Otter & Van der Haagen, 1989; Renou & Lucas, 1994), and hence loss of information on the variation in this compound was most likely not biologically important; (ii) it was the second most abundant pheromone compound, making the integration results reliable; and (iii) it had the lowest coefficient of variation of all pheromone compounds. Subsequently, we used a nested MANOVA to test for differences in sex pheromone blend composition. Variation in the relative amount of each of the log-scaled compounds was analysed with nested ANOVAS and Tukey *post hoc* tests.

Additionally, we calculated ratios between the main attractive component (Z11-16:Ac) and the three compounds that have been shown to decrease attractiveness of this main component: Z9-14:Ac, Z9-16:Ac, and Z11-16:OH (Struble *et al.*, 1980; Renou & Lucas, 1994). We used nested ANOVAS with Tukey *post hoc* tests to test for differences in ratios under the different light treatments (the Z11-16:Ac/Z11-16:Ac ratio was ln-transformed). Statistical analyses were performed in SPSS version 20.

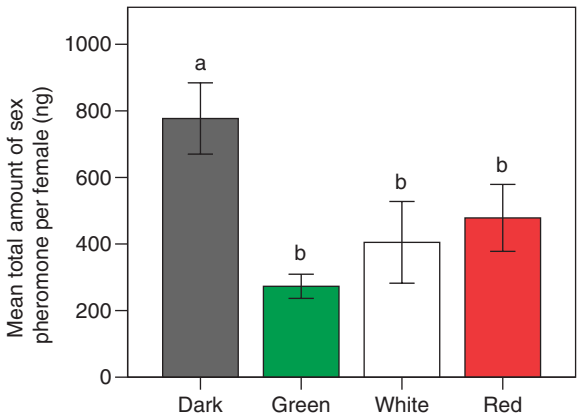

Fig. 2. The amount of sex pheromone produced by *Mamestra brassicae* females under different light treatments. Values are means (±SEM), and different letters indicate significant differences (Tukey, $\alpha = 0.05$).

Results

The total amount of sex pheromone per moth varied between 154 and 2901 ng. Artificial light strongly reduced sex pheromone production ($F_{3,16.4} = 8.427$, $P = 0.001$), irrespective of spectral composition (Fig. 2). Also, the composition of the sex pheromone blend was significantly altered by artificial light (Wilks' λ, $F_{24,186} = 2.903$, $P < 0.001$; Fig. 3a,b). Specifically, we found a relative reduction of the major sex pheromone component, Z11-16:Ac, for females subjected to artificial light, as compared with darkness ($F_{3,16.4} = 8.230$, $P = 0.001$). Z9-16:Ac, a minor pheromone compound that may act as a deterrent (Renou & Lucas, 1994), was reduced in females under green light compared with females in darkness when log-scaled to 16:Ac ($F_{3,16.4} = 5.123$, $P = 0.011$; Fig. 3a). However, relative to the amount of the main attractive component (Z11-16:Ac), the amount of Z9-16:Ac increased up to 1.6-fold (2.2%, under white artificial light) compared with darkness (1.4%) ($F_{3,16.5} = 3.724$, $P = 0.032$; Fig. 4a). The ratios between the two other deterrents and the main attractive pheromone component, Z11-16:Ac, also increased under artificial light: the ratio Z9-14:Ac/Z11-16:Ac was increased in females under artificial light (irrespective of spectral composition) compared with females in darkness ($F_{3,16.5} = 8.587$, $P = 0.001$; Fig. 4b), and the ratio of Z11-16:OH/Z11-16:Ac was significantly higher in females under green (1.8%) and white (1.7%) light than in females under red light (1.2%) and in darkness (0.9%) ($F_{3,16.2} = 6.819$, $P = 0.003$; Fig. 4c).

Discussion

We found that artificial light at night strongly reduced the total amount of sex pheromone produced by female *M. brassicae*, possibly through light inhibition of PBAN (Tawata & Ichikawa, 2001), the neuropeptide that regulates sex pheromone production (Raina *et al.*, 1989; Jacquin *et al.*, 1994). PBAN is produced in the suboesophageal ganglion, probably continuously, so that it accumulates during the photophase (Rafaeli, 1994), while its

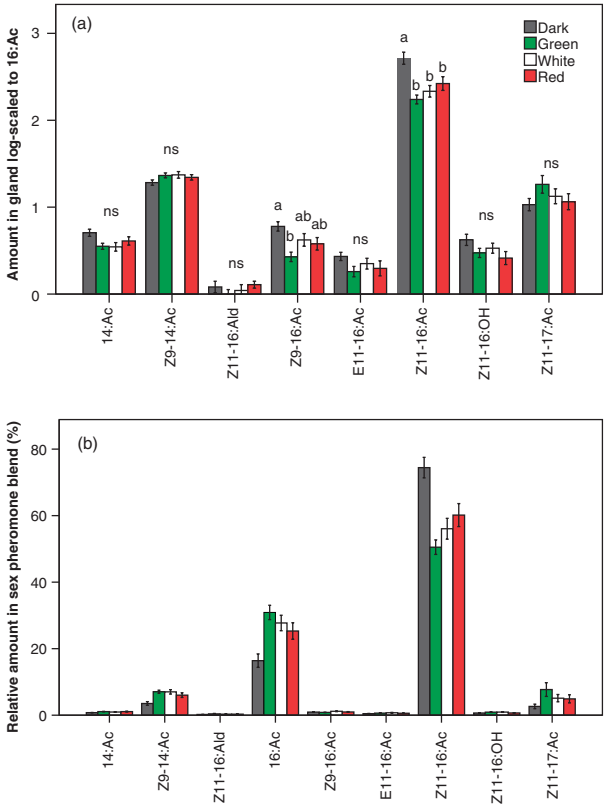

Fig. 3. (a) Mean (± SEM) relative amounts of sex pheromone compounds, log-scaled to 16:Ac, in the sex pheromone blend of moths under the different light treatments. Letters indicate significant differences between treatments per component (Tukey, $\alpha = 0.05$). (b) Mean (± SEM) untransformed relative amounts of the nine sex pheromone compounds in the glands of females under different light treatments.

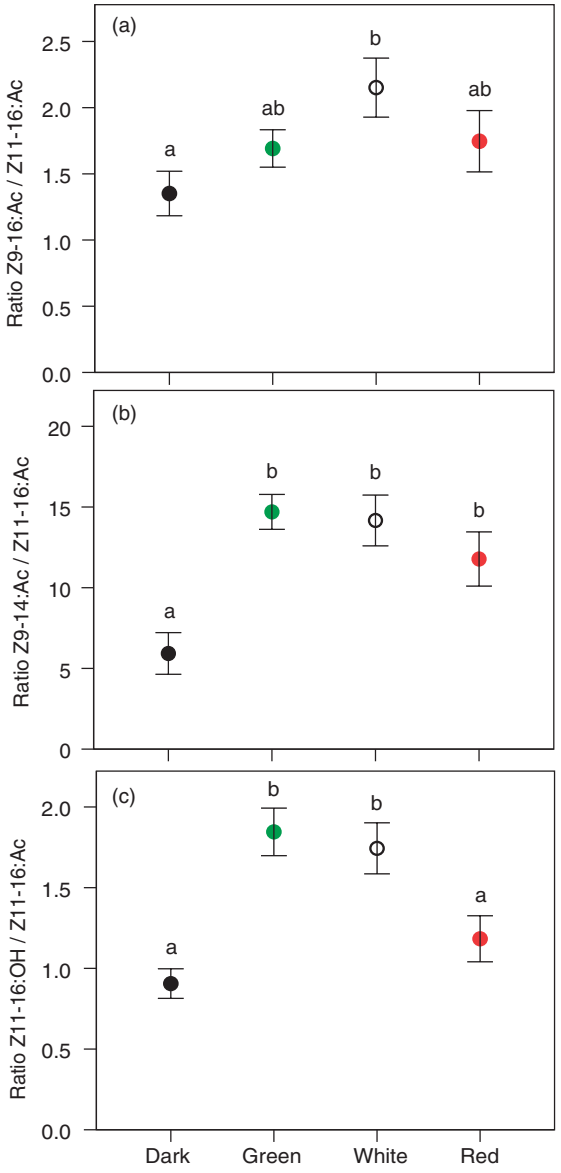

Fig. 4. Ratio of Z9-16:Ac/Z11-16:Ac (a), Z9-14:Ac/Z11-16:Ac (b), and Z11-16:OH/Z11-16:Ac (c) under the different light treatments. Dots represent means, bars are 1 SEM, and letters indicate significant differences between treatments (Tukey, $\alpha = 0.05$).

release from the corpus cardiacum into the haemolymph is regulated by a circadian clock (Rafaeli, 1994), and coincides with the daily rhythm of sex pheromone production in *M. brassicae* (Iglesias *et al.*, 1999). How the release of PBAN is regulated by light is currently unknown (see also Groot (2014)).

Surprisingly, artificial light changed the chemical composition of the pheromone blend, reducing the relative amount of the main attractive component and increasing the relative amount of inhibitory compounds relative to the main attractive component. As the chemical composition of the sex pheromone blend is an important determinant of the attractiveness of sex pheromone signals, and key for recognition of conspecific females by males, slight changes in composition can reduce male response, or even change species specificity (Löfstedt *et al.*, 1991; Symonds & Elgar, 2008). Species specificity is largely the result of strict differences in the relative contribution of different compounds in the pheromone blends. In the case of *M. brassicae*, many co-occurring species share important sex pheromone compounds (see Table 1). For example, Z11-16:Ac is the main attractive component in the blend of *Ochropleura plecta*, *Agrotis ipsilon*, and *Mythimna unipuncta.* These species all share the same habitat and flight period with *M. brassicae*. The difference between these species is that *O. plecta* and *M. brassicae* females also contain Z11-16:Ald as a (minor) attractive component in their pheromone blend, whereas it is absent, non-active, or deterrent in the other species (Table 1). *Agrotis ipsilon* and *M. unipuncta* have Z9-14:Ac and Z11-16:OH in their blends, while both compounds are deterrent for *M. brassicae* males, at least at higher doses (Descoins *et al.*, 1978; Struble *et al.*, 1980). In *M. brassicae*, this alcohol is most likely a precursor and not emitted from the gland (Bestman *et al.*, 1988), as this compound has been shown to be deterrent at levels as low as 0.1% (Descoins *et al.*, 1978). Hence, the greater than two-fold increase in the ratio of Z11-16:OH/Z11-16:Ac that we found may not affect the emission ratios, but, if so, even the smallest amount

of Z11-16:OH will reduce the attraction of *M. brassicae* males. The other inhibitory compound for *M. brassicae*, Z9-14:Ac, is also produced by *M. brassicae* females. This compound has been shown to be attractive when present at 0.1% of the blend (Subchev *et al.*, 1987), but inhibitory when 1–2% is added to the blend (Descoins *et al.*, 1978; Struble *et al.*, 1980). Thus, the nearly three-fold increase in this compound that we found under the different light conditions is likely to affect the attraction of *M. brassicae* males negatively. The small differences in pheromone blend composition require males to be highly sensitive to subtle changes in pheromone composition. However, actual testing of how the artificial light-induced compositional changes in sex pheromone blends affect the attraction of moths is an important next step to undertake.

The range of variation that we found in the pheromone composition of *M. brassicae* is similar to or even higher than that found in the geographic variation of, for example, *Heliothis virescens*, *Heliothis subflexa* (Groot *et al.*, 2009), *Agrotis ipsilon* (Gemeno *et al.*, 2000), *Agrotis segetum* (Wu *et al.*, 1999), and *Cydia pomonella* (Dumenil *et al.*, 2014). However, the variation that we measured was due only to the different light conditions that the females had experienced for three nights. Such a plasticity in the pheromone blend has been reported only once before (Groot *et al.*, 2010b) and shows that moth sex pheromone blends may be more plastic and variable than generally considered. In general, moth sex pheromones are hypothesised to be under stabilising selection, because moth sexual communication is important for species recognition and thus should not vary, as this may directly decrease fitness. Our study shows that varying only one environmental factor, in this case light, can change the sex pheromone blend significantly. Recently, we also demonstrated that artificial light may lead to reduced mating success under field conditions in the geometrid moth *Operophtera brumata* (Van Geffen *et al.*, 2015).

It should be pointed out that we limited ourselves in this study to the sex pheromone composition present in the pheromone gland of individual females, as analysing pheromone release rates of individual females is technically very challenging. However, in general, a one-to-one correspondence is found between the composition in the pheromone gland and the emitted blend (Du *et al.*, 1987; Heath *et al.*, 1991; Bäckman *et al.*, 1997; Svensson *et al.*, 1997), indicating that the pheromone composition in the gland reflects the blend that is released. The only exception is Z11-16:OH, as alcohols serve as immediate precursors to their aldehyde and acetate derivatives (Tillman *et al.*, 1999; Rafaeli, 2002; Jurenka, 2004).

Whereas compositional changes in the pheromone blend were strongest under green and white light, the reduction in pheromone quantity was also significant in females under red light (see Fig. 2). These results indicate that spectral alterations may mitigate negative effects of artificial light on moths, but only to a limited extent. This contrasts with the current perception that spectral alterations are a promising tool to reduce negative effects of artificial night lighting (Gaston *et al.*, 2012), but is in line with a recent study by Pawson and Bader (2014), who demonstrated that shifts in colour temperature (i.e. slight changes in spectral composition) of white LEDs did not influence the number of insects that were attracted to the light. Other studies on moths, concerning attraction to artificial light (Van Langevelde *et al.*, 2011; Somers-Yeates *et al.*, 2013; Van Grunsven *et al.*, 2014), development and life-history (Van Geffen *et al.*, 2014), and reproduction under field conditions (Van Geffen *et al.*, 2015), show that the negative consequences of artificial light may be mitigated at least partly by application of light that is poor in short-wavelength radiation. Compared with darkness, however, red light still often has significant negative effects (Van Geffen *et al.*, 2014, 2015), despite the insensitivity of moth eyes to longer-wavelength radiation (Agee, 1973). Possibly, these negative effects of red light are driven by low amounts of short-wavelength radiation in the red light that is tested in these studies (Fig. 1). Completely removing short-wavelength light or using monochromatic light might effectively mitigate negative effects, but would result in very poor colour recognition and thus render a light source impractical for most applications. Therefore, adjustment of spectral composition could possibly play a role in mitigating the effects of artificial light, but the possibilities of mitigation with lights that are still of use for general application is limited compared with other modern light sources that do not emit UV.

We conclude that artificial light disrupts sex pheromone production in *M. brassicae* moths, both quantitatively and qualitatively. The significant differences in the pheromone blend after being in different experimental conditions shows that the pheromone composition is more plastic than previously assumed (Löfstedt, 1993; Butlin & Trickett, 1997; Groot *et al.*, 2010b). Such shifts can have serious consequences for reproduction, because male response can be disrupted, which may lead to reduced mating success (Van Geffen *et al.*, 2015). Such mating reductions could have important implications for moth populations on illuminated nights.

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References

Agee, H.R. (1973) Spectral sensitivity of the compound eyes of field-collected adult bollworms and tobacco budworms. *Annals of the Entomological Society of America*, **66**, 613–615.

Aitchison, J. (1986) *The Statistical Analysis of Compositional Data*. The Blackburn Press, Caldwell, New Jersey.

Bäckman, A.C., Bengtsson, M. & Witzgall, P. (1997) Pheromone release by individual females of codling moth, *Cydia pomonella*. *Journal of Chemical Ecology*, **23**, 807–815.

Bazinet, A.L., Cummings, M.P., Mitter, K.T. & Mitter, C.W. (2013) Can RNA-Seq resolve the rapid radiation of advanced moths and butterflies (Hexapoda: Lepidoptera: apoditrysia)? An exploratory study. *PLoS ONE*, **8**, e82615.

Bestman, H.J., Erler, J. & Vostrowsky, O. (1988) Determination of diel periodicity of sex pheromone release in three species of Lepidoptera by 'closed-loop-striping'. *Experientia*, **44**, 797–799.

Boo, K.S., Park, K.C., Hall, D.R., Cork, A., Berg, B.G. & Mustaparta, H. (1995) (Z)-9-Tetradecenal – a potent inhibitor of pheromone-mediated communication in the Oriental tobacco budworm, *Helicoverpa assulta*. *Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology*, **177**, 695–699.

Butlin, R. & Trickett, A.J. (1997) Can population genetic simulations help to interpret pheromone evolution?. *Insect Pheromone Research: New Directions* (ed. by R. T. Carde and A. K. Minks), pp. 548–562. Chapman and Hall, New York, New York.

Christensen, T.A., Lashbrook, J.M. & Hildebrand, J.G. (1994) Neural activation of the sex pheromone gland in the moth *Manduca sexta* – real-time measurement of pheromone release. *Physiological Entomology*, **19**, 265–270.

Cinzano, P., Falchi, F. & Elvidge, C.D. (2001) The first World Atlas of the artificial night sky brightness. *Monthly Notices of the Royal Astronomical Society*, **328**, 689–707.

Conrad, K.F., Warren, M.S., Fox, R., Parsons, M.S. & Woiwod, I.P. (2006) Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. *Biological Conservation*, **132**, 279–291.

Den Otter, C.J. & Van der Haagen, M.M. (1989) Sex pheromone attractants and inhibitors in the cabbage armyworm *Mamestra brassicae* L. (Lep.: Noctuidae): electrophysiological discrimination. *Insect Science and Application*, **10**, 235–242.

Den Otter, C.J., Bruins, A.P., Hendriks, H. & Bijpost, S.C.A. (1989) Palmitic acid as a component of the male-to-male inhibitory pheromone of the summerfruit tortrix moth, *Adoxophyes orana*. *Entomologia Experimentalis et Applicata*, **52**, 291–294.

Descoins, C., Priesner, E., Gallois, M., Am, H. & Martin, G. (1978) Sur la secretion pheromonale des femelles vierges de *Mamestra brassicae* L. et *Mamestra oleracea* L. (Lepidoptera, Noctuidae, Hadeninae). *Comptes Rendus de l'Académie des Sciences, Serie D*, **286**, 77–80.

Du, J.W., Lofstedt, C. & Lofqvist, J. (1987) Repeatability of pheromone emissions from individual female ermine moths *Yponomeuta padellus* and *Yponomeuta rorellus*. *Journal of Chemical Ecology*, **13**, 1431–1441.

Dumenil, C., Judd, G.J.R., Bosch, D., Baldessari, M., Gemeno, C. & Groot, A.T. (2014) Intraspecific variation in female sex pheromone of the codling moth *Cydia pomonella*. *Insects*, **5**, 705–721.

Eizaguirre, M., Albajes, R., Lopez, C., Sans, A. & Gemeno, C. (2007) Inhibition of pheromone response in *Sesamia nonagrioides* by the pheromone of the sympatric corn borer, *Ostrinia nubilalis*. *Pest Management Science*, **63**, 608–614.

Eliyahu, D., Nagalakshmi, V., Applebaum, S.W., Kubli, E., Choffat, Y. & Rafaeli, A. (2003) Inhibition of pheromone biosynthesis in *Helicoverpa armigera* by pheromonostatic peptides. *Journal of Insect Physiology*, **49**, 569–574.

Ellis, W.N., Groenendijk, D., Groenendijk, M., Huijgens, T., Jansen, M., Van der Meulen, J. *et al.* (2013) *Nachtvlinders belicht: dynamisch, belangrijk, bedreigd*. De Vlinderstichting en werkgroep vlinderfaunistiek, Wageningen, The Netherlands.

El-Sayed, A.M. (2012) *The pherobase: database of insect pheromones and semiochemicals*.

Evenden, M.L., Judd, G.J.R. & Borden, J.H. (1999) Mating disruption of two sympatric, orchard-inhabiting tortricids, *Choristoneura rosaceana* and *Pandemis limitata* (Lepidoptera: Tortricidae), with pheromone components of both species' blends. *Journal of Economic Entomology*, **92**, 380–390.

Fox, R. (2013) The decline of moths in Great Britain: a review of possible causes. *Insect Conservation and Diversity*, **6**, 5–19.

Fox, R., Oliver, T.H., Harrower, C., Parsons, M.S., Thomas, C.D. & Roy, D.B. (2014) Long-term changes to the frequency of occurrence of British moths are consistent with opposing and synergistic effects of climate and land use changes. *Journal of Applied Ecology*, **51**, 949–957.

Gaston, K.J., Davies, T.W., Bennie, J. & Hopkin, J. (2012) Reducing the ecological consequences of night-time light pollution: options and developments. *Journal of Applied Ecology*, **49**, 1256–1266.

Gemeno, C., Lutfallah, A.F. & Haynes, K.F. (2000) Pheromone blend variation and cross-attraction among populations of the black cutworm moth (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, **93**, 1322–1328.

Gemeno, C., Sans, A., Lopez, C., Albajes, R. & Eizaguirre, M. (2006) Pheromone antagonism in the European corn borer moth *Ostrinia nubilalis*. *Journal of Chemical Ecology*, **32**, 1071–1084.

Groenendijk, D. & Ellis, W.N. (2011) The state of the Dutch larger moth fauna. *Journal of Insect Conservation*, **15**, 95–101.

Groot, A.T. (2014) Circadian rhythms of sexual activities in moths: a review. *Frontiers in Ecology and Evolution, Section Chemical Ecology*, **2**, 1–21. DOI: 10.3389/fevo.2014.00043.

Groot, A.T., Fan, Y., Brownie, C., Jurenka, R.A., Gould, F. & Schal, C. (2005) Effect of PBAN on pheromone production in mated *Heliothis virescens* and *Heliothis subflexa* females. *Journal of Chemical Ecology*, **31**, 15–28.

Groot, A.T., Inglis, O., Bowdridge, S., Santangelo, R.G., Blanco, C., López, J.D. Jr. *et al.* (2009) Geographic and temporal variation in moth chemical communication. *Evolution*, **63**, 1987–2003.

Groot, A.T., Blanco, C.A., Classen, A., Inglis, O., Santangelo, R.G., Lopez, J.D. *et al.* (2010a) Variation in sexual communication of the tobacco budworm, *Heliothis virescens*. *Southwestern Entomologist*, **35**, 367–372.

Groot, A.T., Classen, A., Staudacher, H., Schal, C. & Heckel, D.G. (2010b) Phenotypic plasticity in sexual communication signal of a noctuid moth. *Journal of Evolutionary Biology*, **23**, 2731–2738.

Heath, R.R., McLaughlin, J.R., Proshold, F. & Teal, P.E.A. (1991) Periodicity of female sex pheromone titer and release in *Heliothis subflexa* and *H. virescens* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, **84**, 182–189.

Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C.C., Henckel, D. *et al.* (2010a) The dark side of light: a transdisciplinary research agenda for light pollution policy. *Ecology and Society*, **15**, 13.

Hölker, F., Wolter, C., Perkin, E.K. & Tockner, K. (2010b) Light pollution as a biodiversity threat. *Trends in Ecology & Evolution*, **25**, 681–682.

Iglesias, F., Jacquin-Joly, E., Marco, M.-P., Camps, F. & Fabrias, G. (1999) Temporal distribution of PBAN-like immunoreactivity in the hemalymph of Mamestra brassciae females in relation to sex pheromone production and calling behavior. *Archives of Insect Biochemistry and Physiology*, **40**, 80–87.

Jacquin, E., Jurenka, R.A., Ljungberg, H., Nagnan, P., Lofstedt, C., Descoins, C. *et al.* (1994) Control of sex pheromone biosynthesis in the moth *Mamestra brassicae* by the pheromone biosynthesis activating neuropeptide. *Insect Biochemistry and Molecular Biology*, **24**, 203–211.

Jurenka, R. (2004) Insect pheromone biosynthesis. *In Chemistry of Pheromones and Other Semiochemicals I*, Vol. **239** (ed. by S. Schulz), pp. 97–131. Springer-Verlag, Berlin-Heidelberg, Germany.

Löfstedt, C. (1993) Moth pheromone genetics and evolution. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, **340**, 167–177.

Löfstedt, C., Herrebout, W.M. & Menken, S.B.J. (1991) Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths (Yponomeutidae). *Chemoecology*, **2**, 20–28.

New, T.R. (2004) Moths (Insecta: Lepidoptera) and conservation: background and perspective. *Journal of Insect Conservation*, **8**, 79–94.

Noldus, L. & Potting, R.P.J. (1990) Calling behavior of *Mamestra brassicae* – effect of age and photoperiod. *Entomologia Experimentalis et Applicata*, **56**, 23–30.

Pawson, S.M. & Bader, M.K.F. (2014) LED lighting increases the ecological impact of light pollution irrespective of color temperature. *Ecological Applications*, **24**, 1561–1568.

Poelman, E.H., Van Dam, N.M., Van Loon, J.J.A., Vet, L.E.M. & Dicke, M. (2009) Chemical diversity in *Brassica oleracea* affects biodiversity of insect herbivores. *Ecology*, **90**, 1863–1877.

Poot, H., Ens, B.J., de Vries, H., Donners, M.A.H., Wernand, M.R. & Marquenie, J.M. (2008) Green light for nocturnally migrating birds. *Ecology and Society*, **13**, 47.

Rafaeli, A. (1994) Pheromonotropic stimulation of moth pheromone gland cultures in-vitro. *Archives of Insect Biochemistry and Physiology*, **25**, 287–299.

Rafaeli, A. (2002) Neuroendocrine control of pheromone biosynthesis in moths. *International Review of Cytology – A Survey of Cell Biology*, Vol. **213** (ed. by K. W. Jeon), pp. 49–91. Academic Press, London, U.K.

Raina, A.K., Jaffe, H., Kempe, T.G., Keim, P., Blacher, R.W., Fales, H.M. *et al.* (1989) Identification of a neuropeptide hormone that regulates sex pheromone production in female moths. *Science*, **244**, 796–798.

Renou, M. & Lucas, P. (1994) Sex pheromone reception in *Mamestra brassicae* L. (Lepidoptera): responses of olfactory receptor neurones to minor components of the pheromone blend. *Journal of Insect Physiology*, **40**, 75–85.

Somers-Yeates, R., Hodgson, D., McGregor, P., Spalding, A. & Ffrench-Constant, R.H. (2013) Shedding light on moths: shorter wavelengths attract noctuids more than geometrids. *Biology Letters*, **9**, 20130376.

Stone, E.L., Jones, G. & Harris, S. (2012) Conserving energy at a cost of biodiversity? Impacts of LED lighting on bats. *Global Change Biology*, **18**, 2458–2465.

Struble, D.L., Arn, H., Buser, H.R., Städler, E. & Freuler, J. (1980) Identificantion of 4 sex pheromone components isolated from calling fermales of *Mamestra brassicae*. *Zeitschrift für Naturvorschung B - A Journal of Chemical Science*, **35**, 45–48.

Subchev, M.A., Stanimovora, L.S. & Milkova, T.S. (1987) The effect of compounds related to cis-ll-hexadecenyl acetate on its attractiveness to the males of *Mamestra brassicae* L. (Lepidoptera: Noctuidae) and some other noctuid species. *Folia Biologica*, **35**, 143–150.

Svensson, M.G.E., Bengtsson, M. & Lofqvist, J. (1997) Individual variation and repeatability of sex pheromone emission of female turnip moths Agrotis segetum. *Journal of Chemical Ecology*, **23**, 1833–1850.

Symonds, M.R.E. & Elgar, M.A. (2008) The evolution of pheromone diversity. *Trends in Ecology & Evolution*, **23**, 220–228.

Tawata, M. & Ichikawa, T. (2001) Circadian firing activities of neurosecretory cells releasing pheromonotropic neuropeptides in the silkmoth, *Bombyx mori*. *Zoological Science*, **18**, 645–649.

Teal, P.E.A. & Tumlinson, J.H. (1997) Effects of interspecific hybridization between *Heliothis virescens* and *Heliothis subflexa* on the sex pheromone communication system. *Insect Pheromone Research: New Directions* (ed. by R. T. Cardé and A. K. Minks), pp. 535–547. Chapman and Hall, New York, New York.

Tillman, J.A., Seybold, S.J., Jurenka, R.A. & Blomquist, G.J. (1999) Insect pheromones – an overview of biosynthesis and endocrine regulation. *Insect Biochemistry and Molecular Biology*, **29**, 481–514.

Van de Veire, M. & Dirinck, P. (1986) Sex pheromone components of the cabbage armyworm *Mamestra brassicae*. Isolation, identification and field experiments. *Entomologia Experimentalis et Applicata*, **41**, 153–155.

Van Geffen, K.G., Van Grunsven, R.H.A., Van Ruijven, J., Berendse, F. & Veenendaal, E.M. (2014) Artificial light at night causes diapause inhibition and sex-specific life history changes in a moth. *Ecology and Evolution*, **4**, 2082–2089.

Van Geffen, K.G., Van Eck, E., De Boer, R.H.A., Van Grunsven, R.H.A., Salis, L., Berendse, F. *et al.* (2015) Artificial light at night inhibits mating in a Geometrid moth. *Insect Conservation and Diversity*, . DOI: 10.1111/icad.12116..

Van Grunsven, R.H.A., Donners, M., Boekee, K., Tichelaar, I., Van Geffen, K.G., Groenendijk, D. *et al.* (2014) Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. *Journal of Insect Conservation*, **18**, 225–231.

Van Langevelde, F., Ettema, J., Donners, M., Wallis de Vries, M.F. & Groenendijk, D. (2011) Effect of spectral composition of artificial light on the attraction of moths. *Biological Conservation*, **144**, 2274–2281.

Vickers, N.J. & Baker, T.C. (1997) Chemical communication in heliothine moths. 7. Correlation between diminished responses to points source plumes and single filaments similarly tainted with a behavioral antagonist. *Journal of Comparative Physiology A Sensory Neural and Behavioral Physiology*, **180**, 523–536.

Waring, P. & Townsend, M. (2003) *Nachtvlinders, veldgids met alle in Nederland en België voorkomende soorten*. Tirion Uitgevers B.V., Baarn, The Netherlands.

Wu, W., Cottrell, C.B., Hansson, B.S. & Löfstedt, C. (1999) Comparative study of pheromone production and response in Swedish and Zimbabwean populations of turnip moth, *Agrotis segetum*. *Journal of Chemical Ecology*, **25**, 177–196.

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