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► **To cite this version:**

Stephen P Foster, Karin G Anderson, Jérôme Casas. The Dynamics of Pheromone Gland Synthesis and Release: a Paradigm Shift for Understanding Sex Pheromone Quantity in Female Moths. *Journal of Chemical Ecology*, 2018, 44 (6), pp.525-533. 10.1007/s10886-018-0963-z . hal-02573239

HAL Id: hal-02573239

<https://hal.science/hal-02573239>

Submitted on 14 May 2020

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**The dynamics of pheromone gland synthesis and
release: a paradigm shift for understanding sex
pheromone quantity in female moths**

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Abstract

Moths are exemplars of chemical communication, especially with regard to specificity and minute amounts used. Yet, little is known about how females manage synthesis and storage of pheromone to maintain release rates attractive to conspecific males and why such small amounts are used. We developed, for the first time, a quantitative model, based on an extensive empirical data set, describing the dynamical relationship among synthesis, storage (titer) and release of pheromone over time in a moth (*Heliothis virescens*). The model is compartmental, with one major state variable (titer), one timevarying (synthesis), and two constant (catabolism and release) rates. The model was a good fit, suggesting it accounted for the major processes. Overall, we found the relatively small amounts of pheromone stored and released were largely a function of high catabolism rather than a low rate of synthesis. A paradigm shift may be necessary to understand the low amounts released by female moths, away from the small quantities synthesized to the (relatively) large amounts catabolized. Future research on pheromone quantity should focus on structural and physicochemical processes that limit storage and release rate quantities. To our knowledge, this is the first time that pheromone gland function has been modeled for any animal.

Key words: chemical communication; tracer-tracee analysis; compartmental model; Lepidoptera; Noctuidae

Introduction

Pheromones mediate a wide range of intraspecific behaviors in animals, including mating, aggregation, sociality, alarm, trail following and resource partitioning. These pheromones are made and/or stored in an exocrine gland before secretion to the body surface, or directly to the environment, where they are detected by conspecifics (Johansson and Jones 2007; Wyatt 2014). While much work has focused on characterizing

the specificity of pheromones, the proximate mechanisms that modulate the quantities of pheromone synthesized, stored and secreted over time and space have largely been ignored (Foster 2016; Johansson and Jones 2007; Umbers et al. 2015).

Probably the most widely studied example of pheromones in animals is that of the sex pheromones of moths (Allison and Cardé 2016a; Johansson and Jones 2007; Wyatt 2014), which are exemplars of highly specific communication involving minute chemical quantities (Cardé and Baker 1984; Foster 2016; Greenfield 1981; Umbers et al. 2015). Typically, it is female moths that release the sex pheromone that may elicit flight responses from conspecific males over distances of tens to hundreds of meters (Cardé 2016). Synthesis and release occur in an exocrine gland, usually located between the 8th and 9th abdominal segments (Foster 2016; Jurenka 2017; Ma and Ramaswamy 2003), over a specific period of the day (Groot 2014). Although the two physiologies temporally overlap (Groot 2014; Jurenka 2017), they are controlled by distinct mechanisms. In many species, synthesis is controlled by release of the pheromone biosynthesis activating neuropeptide (PBAN) (Jurenka 2017),

whereas release, usually associated with an overt behavior termed “calling” (Allison and Cardé 2016b), appears to be controlled either directly from terminal nerve input (Christensen et al. 1994) or, indirectly through rhythmic muscular squeezing of the gland forcing pheromone to the cuticular surface (Raina et al. 2000; Solari et al. 2007). The small quantities of pheromone released by female moths must relate to the quantities of pheromone synthesized and available for release; yet little is known about the quantitative relationship between these physiologies for any species of moth (Cardé and Baker 1984; Foster 2016; Umbers et al. 2015). In part, this is due to the widespread use of gland titer, the amount stored in or on the gland, as an interchangeable proxy (e.g., Symonds et al. 2012) for both pheromone synthesis and release (Foster 2016). While titer must relate to both, as it results from the differential between cumulative synthesis and usage over time, any relationship is likely to be both time-dependent and non-linear.

Recently (Foster and Anderson 2011), we determined pheromone synthesis rate in a moth for the first time, by feeding female *Heliothis virescens* (Fabricius) (family:

Noctuidae) U-¹³C-glucose and determining pheromone enrichment over time by mass isotopomer distribution analysis (MIDA) (Hellerstein and Neese 1992; Wolfe and Chinkes 2005). Since determination of the kinetics required measurements over ca. two hours, we reasoned we could measure this, as well as release rate and gland titer, over the combined diel synthesis and calling periods [hereafter referred to as the “sexually active period”; ca. 8–10 h in *H. virescens* (Heath et al. 1991)] in order to determine the dynamical relationship among these processes. However, since it is known that pheromone is catabolized while stored in the gland (Ding and Prestwich 1986; Foster 2000), we also needed to measure and account for the rate of this process.

In this paper, we develop a compartmental model, based on an extensive empirical data set, describing the dynamical relationship among synthesis, titer, catabolism and release of sex pheromone in the moth *H. virescens*. In contrast to the assumption that costs of pheromone synthesis may limit the quantity of pheromone released (Harari et al. 2011; Johansson and Jones 2007; Umbers et al. 2015), we show that most pheromone synthesized is actually

catabolized in the gland. Consequently, it is the amount catabolized, not synthesized that principally shapes gland titer, and limits release rate, across the sexually active period.

Methods and Materials

Conceptual Model and Biological System. We incorporated all major known processes and state variables of import to moth sex synthesis and release into our conceptual model, namely rate of synthesis (Foster and Anderson 2011; Foster et al. 2017), titer (Foster 2016), catabolism (Ding and Prestwich 1986), and release rate (Heath et al. 1991). Thus, we developed a compartmental model with one major state variable, gland titer, one input, synthesis rate, and two outputs, catabolic and release rates (Fig. 1a). We chose *H. virescens* (Fabricius) as our subject because it has a typical moth sex pheromone system, utilizing the fatty acid-derived “Type 1” moth sex pheromone components, (Z)-11hexadecenal (Z11-16:Ald) and (Z)-9-tetradecenal (Z9-14:Ald) (Heath et al. 1991; Roelofs et al. 1974), and it is the only moth for which rates of pheromone synthesis have been quantified (Foster and Anderson 2011; Foster et al. 2017).

Insects. Our *H. virescens* colony was established from one previously at USDA-ARS, Fargo, and later supplemented with insects supplied by Dr. F. Gould, North Carolina State University. Larvae were maintained at 25°C under a 16:8 L:D photoperiod (i.e., a 480 min scotophase) and fed on a wheatgerm/casein diet. Pupae were sexed and female

pupae placed in separate containers under the same environmental conditions as larvae. Each day, newly emerged adult females were collected and held without access to food until used in experiments. For model development, we used 2-d-old females (i.e., 2 d after eclosion), while for model testing we used 1- and 3-d-old females.

Titer. The gland of an individual female was dissected, and extracted in *n*-heptane, along with 25 ng of (Z)-11-tetradecenal as internal standard, for at least 1 h at ambient temperature. We quantified only Z11-16:Ald, since this is >90% of the total pheromone (Heath et al. 1991), and we wished to model quantity, not blend ratio. For titer determination, we used gas chromatography/mass spectrometry (GC/MS; see supplementary) and monitored *m/z* 192 and 220 for Z11-14:Ald (internal standard), and Z11-16:Ald, respectively.

We analyzed 2-d-old females every 120 min (N = 10 for each time point), starting 120 min before, through to the end of, the scotophase. Females were also analyzed 360 min into the following photophase, when they are not producing significant amounts of pheromone (Foster and Anderson 2011). We also analyzed 1- and 3-d-old females (N = 10 for each time point) at the beginning and middle of the scotophase.

Synthesis rate. Females were fed U-¹³C-glucose (99% enriched, Cambridge Isotope Laboratories, Cambridge, MA), and synthesis rate of Z11-16:Ald determined using MIDA (Hellerstein and Neese 1992; Wolfe and Chinkes 2005), as previously described

(Foster and Anderson 2011); see supplementary material for a more detailed description. Briefly, females that ingested 25 μ l of a 10% (w/v) U- ^{13}C -glucose solution were analyzed (for titer) at various times over the next 120 min (including before feeding; i.e., at $t=0$). U- ^{13}C -glucose is rapidly absorbed, glycolyzed and oxidized to acetyl CoA, which is used for de novo pheromone biosynthesis (Foster and Anderson 2011). For MIDA, we monitored m/z 220, 222, and 224, representing unlabeled (M+0), singly labeled (M+1) (one $^{13}\text{C}_2$ unit) and doubly labeled (M+2) isotopomers of Z11-16:Ald. Enrichment of pheromone reached equilibrium 90 min after feeding for all time periods; therefore, we used precursor enrichment of individual females 90 min after feeding to calculate individual fractional synthetic rates (FSR; see supplementary). Synthesis rate for each time period was calculated by multiplying FSR by pool size (titer at $t=0$ for each time period).

For 2-d-old females, synthesis rate was determined every 120 min of the scotophase (i.e., starting at min 0, 120, 240 and 360) and also starting at min 360 of the photophase. For 1- and 3-d-old females, synthesis rate was determined at min 0 and 240 of the scotophase only. For each time point, 5–10 individual females were analyzed. Errors were calculated as standard errors of the product of two random variables, titer and FSR, following Lynch and Walsh (1998).

Release rate. Just prior to the scotophase start, two females of the same age (1, 2 or 3 d) were placed inside a glass chamber (400 ml) through which charcoal-filtered air flowed at $300 \text{ ml}\cdot\text{min}^{-1}$. Pheromone released was collected on Tenax TA (400 mg; 60-80 mesh,

Supelco, Bellefonte, PA), inside a Pasteur pipet with glass wool at the ends. Pheromone was collected for 120 min periods throughout the scotophase, commencing at the beginning (with all four time periods collected for the same pair of females). In addition, for 2-d-old females, we collected for 120 min, starting at min 60 and 180 of the scotophase (again both times from the same pair). Pheromone was desorbed with *n*hexane, and analyzed by GC/MS (see titer method). Nine pairs of females were sampled for each 120 min period.

Glandular catabolism. Females (2 d only; 6–15 per time point) were decapitated midway through the scotophase (when titer was high) and titer determined by GC/MS at various times over the next 240 min. To confirm that decapitation also stops pheromone release (the other possible fate of pheromone), we collected pheromone released from pairs of females (N =4) immediately after decapitation (120 min into the scotophase) over the next 240 min.

Calling periodicity. Individual females (N = 20 for each of 1, 2 and 3 d-old) were placed in clear glass vials (25 x 75 mm) just prior to the start of the scotophase, and observed every 30 min over the entire scotophase for whether or not they exhibited calling behavior. A red-filtered light (10 lux) was used to aid observations. Data were summarized as proportions of females exhibiting calling during each 60 min period.

Model. In addition to our major state variable (titer), input (pheromone synthetic rate), and outputs (catabolic and release rates) (Fig. 1a), we defined further state variables, the integrals of synthesis, release and catabolic rates. According to the usual nomenclature of

compartmental models (DiStefano III 2013), we called a and d the release and catabolic rate constants, with time^{-1} units. Thus, a pheromone release or catabolic rate (mass per unit time) refers to the appropriate rate constant multiplied by titer, T . By contrast, the time-dependent pheromone synthetic rate is given directly in mass per unit time, as we have no estimate for the upstream compartment amounts. All rates are expressed as $\text{ng}\cdot\text{min}^{-1}$ Z11-16:Ald.

The equations in the model were:

(1) Titer: $dT/dt = b(t) - (d \cdot T) - (a \cdot T)$

(2) Synthesis: $dS/dt = b(t)$

(3) Catabolism: $dD/dt = d \cdot T$

(4) Release: $dR/dt = a \cdot T$

In the implementation of the model using R (R Core Team 2013), a linear interpolation method was used (function `approx` in Soetaert, Cash & Mazzia 2012, page 57), due to the different time bases (spans) in the experiments. For example, titer was an instantaneous measure (e.g., at $t = 0$ min), whereas synthesis and release rate measurements were made over 2 h periods (e.g., from $t = 0$ –120 min). Since we had to choose the same instant in time for the multiplication of titer and FSR to estimate synthetic rate, we chose the midpoint of these determinations (in our example, $t = 60$ min) as the nominal value and used titer at the start of that period (i.e., before label had been ingested). We also used a mid-time point for pheromone release. In practice, this is reasonable for FSR, since we used precursor enrichment at $t = 90$ min and the rate of change of enrichment over the period of 0–60 min for the calculation (see supplementary); however, it did introduce some inaccuracy in titer (real titer at $t = 0$ is

different from real titer at 60 min). Since titer data were more variable than other data sets, we screened for outliers using the Shapiro test at 0.05 level and quantile plots for normal distributed values; two outliers were excluded from the analysis.

Our model is composite and dynamical, so the usual measures of overall goodness of fit do not operate; therefore, one has to use more qualitative assessments, such as the presence of biases, qualitative trends, behavior of the variances, consistency of assumptions, structural robustness or independent data sets (Goriely 2018, Haefner 2005, Weisberg 2013). In this respect, the pheromone release rates of days 1 and 3 are true independent data sets for testing the model. The cumulative released amounts were well predicted for these days, a strong test of the model.

We did not conduct a systematic sensitivity analysis of the model. Rather, we explored the consequences of changing parameter values to represent some biologically interesting scenarios. The outputs of these simulations are reported in appropriate places in the Discussion.

Results

Synthesis rate. Synthesis rate in 2-d-old females showed a rapid rise around the start of the scotophase, maintained its level, before declining rapidly at the end of the scotophase (Fig. 1b). It likely maintained a low (basal) rate for most of the subsequent photophase, as nominally 420 min into the photophase, the synthesis rate was 12.6 ng.h⁻¹ (data not used in model). While our data had limited time points, due to each requiring ca. 2 h

for determination, the pattern suggests a mostly stepwise on/off (or very low) synthesis, consistent with diel release of PBAN controlling the activity of an enzyme(s) in the pathway (Jurenka 2017).

For the two periods measured (nominally 60 and 300 min into the scotophase) for each of 1- and 3-d-old females, there was a similar increase in synthesis rate as observed for 2 d females over the same times, although synthesis rate declined with increasing age. The data used to calculate synthetic rate are given in supplementary

Table 1.

For the model, we fitted three different hyperbolic Michaelis-Menten equations to the observed rates for each of the three days (Fig. 1b). The number of sampling times is too low to apply non-linear fits (four sampling points for day 2, and only two for days 1 and day 3), so we used a linear fit on linearized data, as done in Crawley (2007, page 203). Note that this technique required us to assume that the plateau was reached after 5 h in the scotophase and forces the fit through this point. Hence, no goodness of fit can be used.

Release rate. Release rate by 2-d-old females increased through the first hours of the scotophase.

However, after reaching a peak nominally 180 min into the scotophase, release rate declined slightly and held steady for the remainder of the scotophase (when measurements ceased) (Fig. 1c). Three-d-old females showed a similar release rate pattern over time to that of 2-d-old females. By contrast, 1 d-old females had a peak release rate somewhat later (ca. 360 min) in the scotophase. In general, females released pheromone at a lower rate with increasing age (Fig. 1c).

The plotted estimate of the release rate constant of 2 d females showed high variability and no clear trend, so we estimated it at 0.0028 min^{-1} (S.E. 0.0005, $n=4$), and assumed it constant over the three days.

Glandular catabolism. Following decapitation, titer stayed briefly at the same level before declining very rapidly, such that 240 min after decapitation, it was roughly 10% that prior to decapitation (Fig. 1d). Decapitated females did not release detectable levels of pheromone over the 4 h following decapitation.

An exponential decline [$y=\log(-0.012x+4.24)$, 92 d.f., $P<0.0001$, residual standard error: 0.8095] for the data of decapitated females (Fig. 1d) gave a good fit. The residual

analysis shows, however, a sharper decline very early on, not captured by the exponential decrease. The degradation rate constant $= 0.012 \text{ min}^{-1}$ (S.E. 0.0013), and was assumed to be constant over the three days.

Titer. Two d-old females showed a typical titer pattern, observed in this (Foster 2005; Raina et al. 1986) and other species (Groot 2014) of moths, consisting of a low (basal) titer prior to the start of the scotophase, rising to a peak around midscotophase and then dropping rapidly back to the basal level by the end of the scotophase and through the following photophase (Fig. 2b; supplementary Table 1).

The dynamics of titer in the model showed a convex curve when titer was increasing and a concave one when decreasing. Only two time points (0 and 240 min in the scotophase) were measured for both 1 and 3 d-old females, and these showed a similar pattern to 2 d-old females (Fig. 2a,c), Titer generally decreased with increasing age (Fig. 2a,b,c). Titer showed greater change throughout the scotophase than did synthesis or release rates.

Model testing and exploration. The predicted titers (Fig. 2a-c), as well as cumulative pheromone released over each of the three days, closely followed the

observed values, such that we are confident that the model contains the major processes contributing pheromone quantity management in the gland. A comparison of the rate of synthesis with the rate of disappearance (usage), defined as the sum of the rates of release and catabolism, shows that synthesis is greater than usage, particularly through the first half of the scotophase (supplementary Fig. 1), allowing the titer increase observed during this period (Fig. 2). Toward the end of the scotophase, when synthesis had stopped, the large negative differential resulted in the sharp decrease in titer observed (Fig. 2). Figure 3

(along with Fig. 2d), summarizes all the predicted processes, enabling a clear comparison of fluxes and age-related processes; note the large proportion of pheromone degraded compared to that released.

Calling periodicity. Females of all three ages showed similar patterns of calling through the first 300 min of the scotophase. Basically, a small proportion of females called early, with most calling between 120-300 min (Fig. 4). Peak calling occurred earlier in the sexually active period with increasing age, a phenomenon noted in other species (e.g., Webster and Cardé 1982). A high proportion of 1-d-old females called past hour 5 of the scotophase, in contrast to the other two ages.

Discussion

To maximize fecundity, a female moth must mate soon after becoming sexually mature, as delays can be deleterious (Umbers et al. 2015). The synthesis and release of sex pheromone are crucial for ensuring rapid mating of sexually mature females (Allison and

Cardé 2016a). For the first time, we have collected and modeled dynamic data on pheromone synthesis, storage, catabolism and release in a moth, to understand how the system manages attractive pheromone release rates across a daily calling period. The data fit was good, suggesting that we accounted for the major processes influencing gland quantity management.

Pheromone catabolism modulates gland titer. Catabolism is the primary use of pheromone, accounting for roughly 80% of pheromone produced over the course of a sexually active period. Consequently, it has a much greater affect on shaping pheromone titer over time than does release, and effectively limits peak titer. Without catabolism, model simulations predict 1 d females attaining a peak titer of 410 ng, a value well in excess of any we have observed. However, we observed two 1 d females with titers of ca. 200 ng (these appeared to fit a different distribution and were excluded as outliers from our analyses). Interestingly, the model predicts a similar value (170 ng) for 1 d females if they catabolize, but do not release, pheromone. While, these unusual titers could also result from reduced catabolism or increased synthesis, this prediction suggests that some females, which on the basis of titer might be considered “high releasers”, may not in fact actually release (much) pheromone.

In endocrine systems, hormone titers are controlled by a combination of mechanisms (Molina 2013). For example, in insects, juvenile hormone titers are controlled by feedback mechanisms controlling synthesis, catabolism, sequestration, and protection from catabolism (Goodman and Cusson 2012; Nijhout and Reed 2008). In comparison, titer in the exocrine pheromone system of *H. virescens* is controlled predominantly by

usage (release and catabolism), with the system appearing to lack feedback control over synthesis, as evidenced by a fairly constant synthesis rate regardless of titer profile. Indeed, the only control of synthesis appears to be the circadian release/non-release of PBAN, which probably defines the period of synthesis but not the degree (Groot 2014; Jurenka 2017). The lack of fine control of synthesis throughout this period may follow from the pheromone's target being exogenous (i.e., to males), rather than endogenous, to the producer (female).

Why modulate titer? It is generally accepted that the release of greater quantities of pheromone should result in females attracting more males over greater distances, and hence is likely to enhance fitness (Baker and Roelofs 1981; Cardé 2016; Foster and Johnson 2011; Greenfield 1981; Symonds et al. 2012; Umbers et al. 2015). Why, then, do females need a titer control that lowers amounts of pheromone available for release? One possibility is that high titers of pheromone might be deleterious to gland cell function. This was suggested for species that use aldehyde components (like *H. virescens*) following the identification of non-specific oxidases that converts alcohols to aldehydes in the cuticular layer of the gland (Fang et al. 1995; Teal and Tumlinson 1988). However, it fails to explain why species that do not use aldehyde components also catabolize pheromone [e.g., (Foster 2000)]. We think symmorphosis a more likely explanation for titer modulation; i.e., that the gland is structurally incapable of release rates substantially greater than the maximal ones observed (in 1 d females). Catabolism thus prevents the gland from accumulating excess pheromone, which it cannot release during the calling period. For example, without catabolism, our model predicts that 1 d females would have a titer of 315 ng at the end of the calling period. Such large amounts of pheromone

remaining after calling had ceased might result in (accidental) further release, subjecting refractory and reproducing females to continued harassment by males.

Assuming a passive release of pheromone (e.g., Solari et al. 2007), the evaporation rate of a given component (of specific vapor pressure) will depend upon flux to the cuticular surface and the area of the gland over which pheromone is distributed, as well as environmental factors such as temperature and windspeed (Nielsen et al. 1995). Little is known about pheromone movement through the gland or its distribution on the surface, precluding calculations of theoretical release rates based on vapor pressures. However, one or both could be limiting, meaning that increased synthesis or indeed reduced catabolism might result in increased titer but not in increased release rate. Studies quantifying pheromone glandular fluxes and surface distribution, in combination with both theoretical and empirical determinations of release, are needed to understand how gland structure may determine pheromone release rate.

A relatively high rate of synthesis, combined with catabolism, allows titer to build up rapidly when synthesis starts and decline rapidly once synthesis stops, helping synchronize pheromone availability/non-availability with calling periodicity. A rough synchronization of pheromone availability with calling periodicity is suggested by our data. At the start of the scotophase, when pheromone is being synthesized and titer increasing, high proportions of females of all three ages call. However, near the end of the scotophase, when synthesis has stopped, only 1 d females have high titers, and only they are still calling in high proportions. The slightly earlier peak in calling with increasing age is consistent with other studies (Umbers et al. 2015). We note, however, that females were not fed in this experiment. Just as starvation can influence pheromone

titer (Foster 2009; Foster and Johnson 2010), it might also influence calling behavior and release rate.

Conclusion. A reason often cited for why moths release small amounts of chemicals is that excess synthesis represents some ‘cost’ to a female (Cardé and Baker 1984; Harari and Steinitz 2013; Johansson and Jones 2007; Symonds et al. 2012). Our work demonstrates that catabolism, not release, is the primary fate of most pheromone synthesized by *H. virescens* females and probably for many other moth species. This suggests a paradigm shift may be necessary to understand the low amounts released, away from focusing on the small quantities synthesized to focus on the (relatively) large amounts catabolized. Our model helps explain how producing a large excess of pheromone over that released is not the outcome of a maladaptive process, but is constitutive to the design of a control mechanism that both limits storage and synchronizes availability/non-availability of pheromone with need (release through calling). Our model forms the basis of future work looking at neglected gland processes that determine release rate, including catabolism and cell to surface pheromone flux, as well as structural limitations including pore diameter and density and cuticular pheromone distribution. To our knowledge, this is the first model containing the major known physiological processes of a pheromone gland in any animal, making it a framework for the quantitative functioning of exocrine glands in species from moths to mammals.

Acknowledgments

This work was funded in part by a United States Department of Agriculture Hatch Project ND02388 (to SPF). The purchase of the GC/MS system was funded in part by a United States Department of Agriculture–National Institute of Food and Agriculture Instrument Grant, 2015-07238 (to SPF). We thank Le Studium Loire Valley Institute for Advanced Studies and Dr. D. Giron for supporting the visit of SPF to Tours, where this collaboration was initiated. We also thank Dr. T. Zemb (ICSM, Site de Marcoule) and the INOV group at IRBI for discussions and comments on the work.

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Captions for onlineversion

Figure1. Model for the pheromone gland of *Heliothis virescens* and parameter estimations for associated physiological processes through the scotophase (0-480 min). The compartmental model (a) has three state variables: gland titer, release and catabolism. The pheromone synthesis rate is the only time varying parameter. The observed rates of synthesis (b) and release (c) are shown for females age 1 (open circles), 2 (closed squares) and 3 (stars) days, respectively (mean \pm S.E.M). In (b) and (c), the shaded area shows when synthesis stops (illustrated by the low rate at 420 min). (d) Time course of gland titer (plus fitted curve) after decapitation (of 2 d females) at 240 min into the scotophase (arrow shows when decapitation occurred).

Figure2. Model testing. (a-c) Observed (open circles) titers of individual females and predicted titers (lines) over the scotophase (time 0-480 min), and (d) mean observed and predicted cumulative amounts of pheromone released by females for each of the three days (1 d black, 2 d red, 3 d blue). The shaded area shows when pheromone synthesis stops.

Figure3. Model exploration. Predicted mean amounts (a) cumulatively synthesized, (b) stored in the gland, and (c) cumulatively catabolized over the scotophase (min 0-480) for each of the three days (1 d black, 2 d red, 3 d blue). The shaded area shows when pheromone synthesis stops.

Figure4. Calling frequency of females of different age. While high proportions of females of all ages (1 d black, 2 d red, 3 d blue) call in the first part of the scotophase (min 0-480), only 1 d females call in high proportions in the latter part. The shaded area shows when pheromone synthesis stopped. The colored arrows indicate approximate peaks of calling for the three age groups.

Captions for print version

Figure1. Model for the pheromone gland of *Heliothis virescens* and parameter estimations for associated physiological processes through the scotophase (0480 min). The compartmental model (a) has three state variables: gland titer, release and catabolism. The pheromone synthesis rate is the only time varying parameter. The observed rates of synthesis (b) and release (c) are shown for females age 1 (open circles), 2 (closed squares) and 3 (stars) days, respectively (mean \pm S.E.M). In (b) and (c), the shaded area shows when synthesis stops (illustrated by the low rate at 420 min). (d) Time course of gland titer (plus fitted curve) after decapitation (of 2 d females) at 240 min into the scotophase (arrow shows when decapitation occurred).

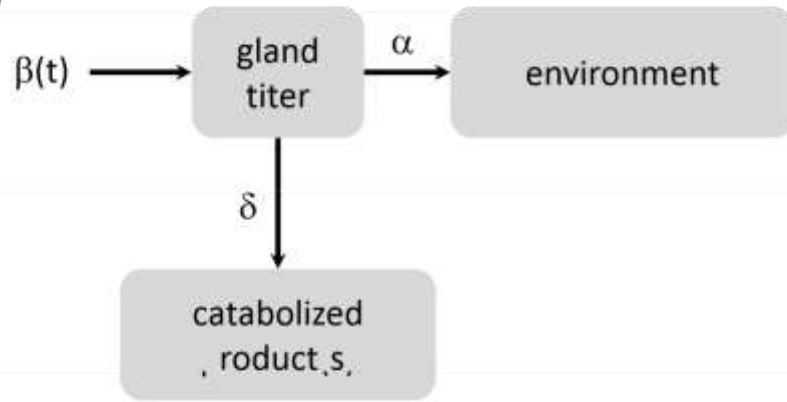
Figure2. Model testing. Observed (open circles) titers of individual females and predicted titers (lines) over the scotophase (time 0–480 min) for (a) 1-d-old, (b), 2d-old, and (c) 3-d-old; (d) mean observed and predicted cumulative amounts of pheromone released by females for each of the three days (1 d = dotted line, 2 d = solid line, 3 d = dashed line). The shaded area shows when pheromone synthesis stops.

Figure3. Model exploration. Predicted amounts (a) cumulatively synthesized, (b) stored in the gland, and (c) cumulatively catabolized over the scotophase (min 0–480) for each of the three days (1 d = dotted line, 2 d = solid line, 3 d = dashed line). The shaded area shows when pheromone synthesis stops.

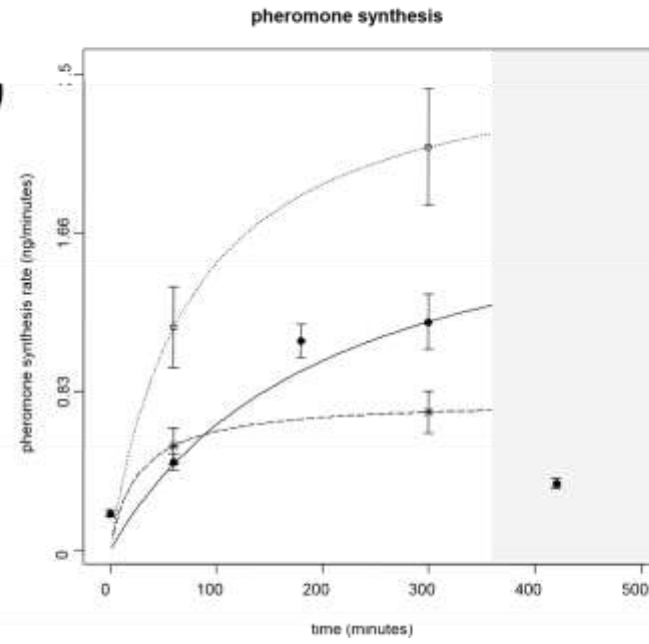
Figure4. Calling frequency of females of different age. While high proportions of females of all ages (1 d = dotted line, 2 d = solid line, 3 d = dashed line) call in the first part of the scotophase (min 0–480), only 1 d females call in high proportions in the latter part. N = 20 for each time point. The shaded area shows when pheromone synthesis stopped. The colored arrows indicate approximate peaks of calling for the three age groups.

Figure 1

(a)

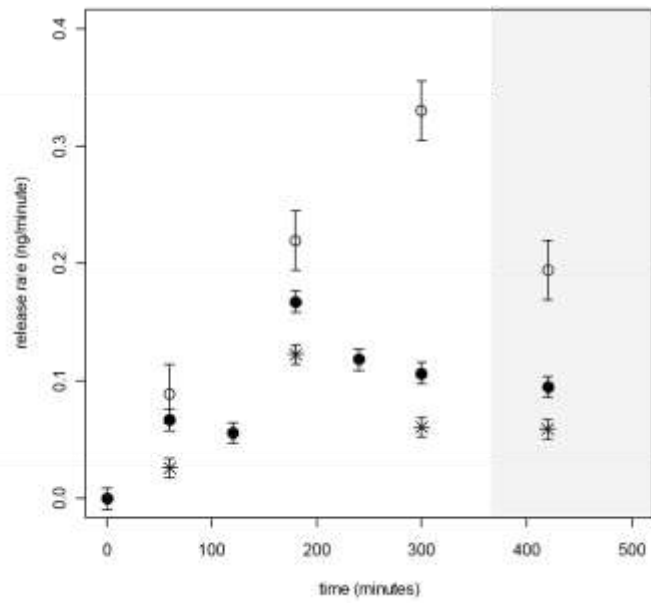


(b)



pheromone release

(c)



pheromone catabolism degradation

(d)

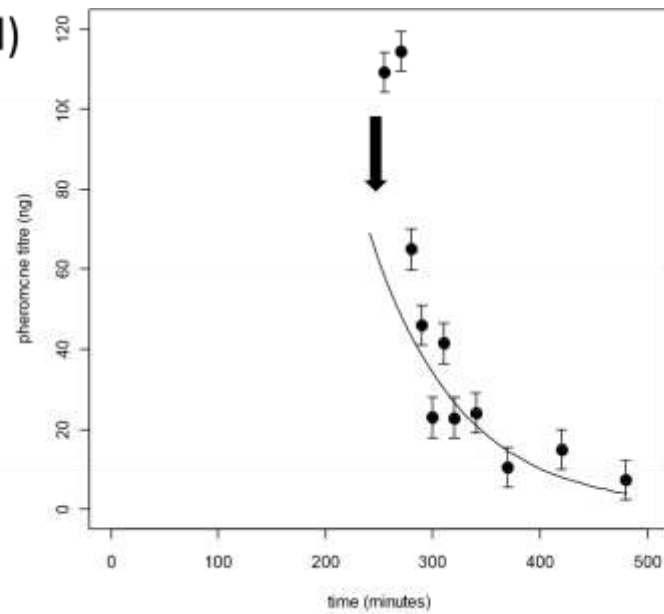


Figure 2

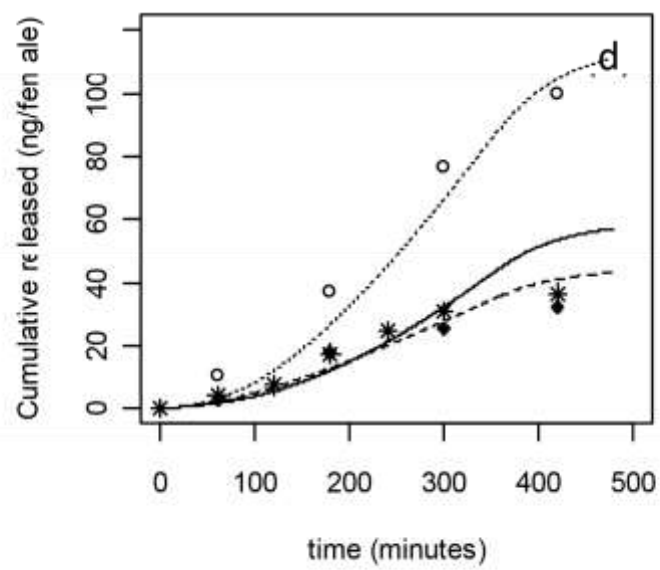
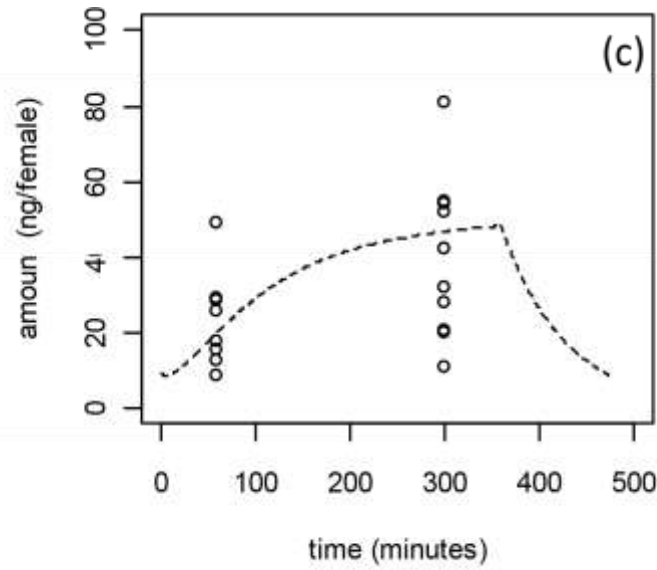
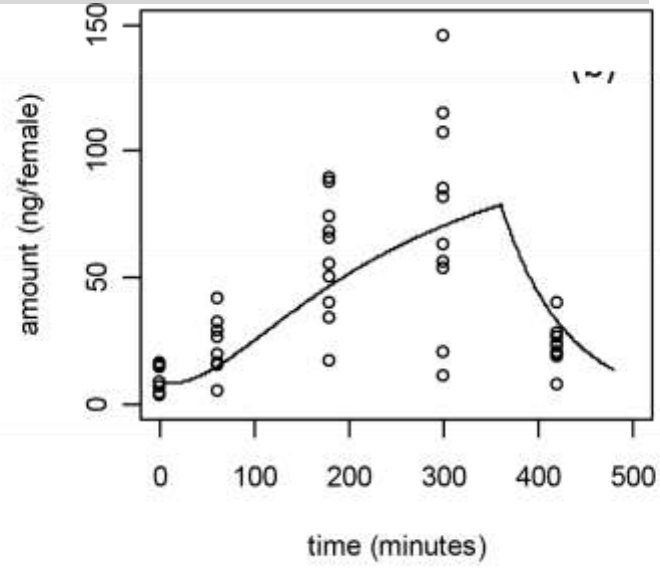
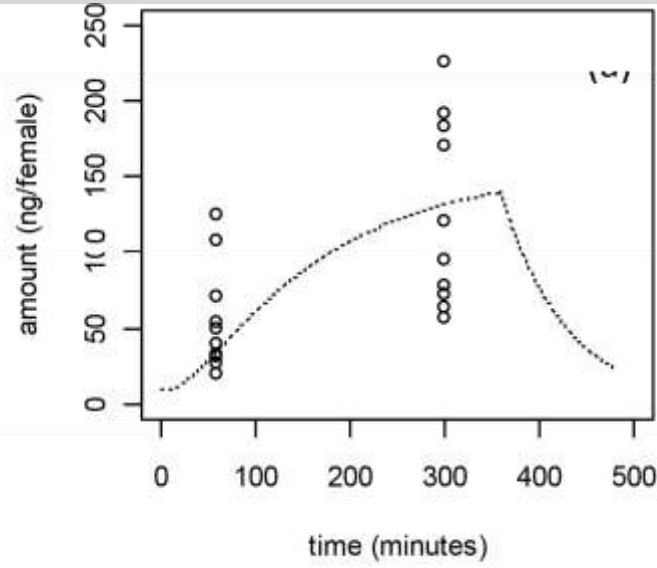


Figure 3

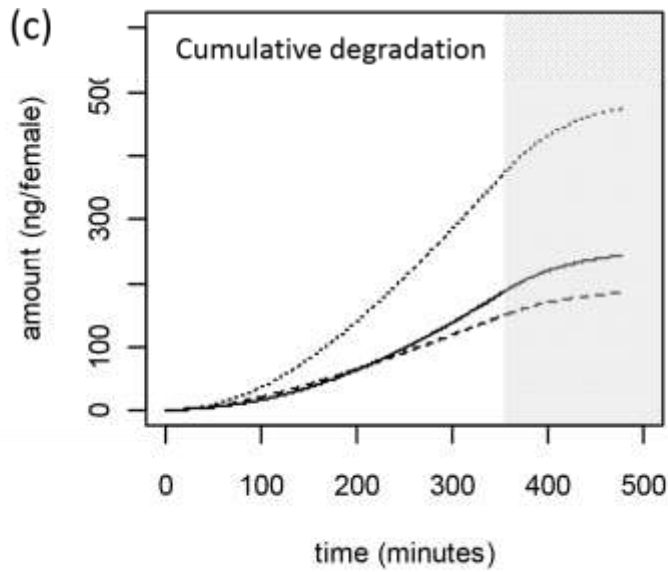
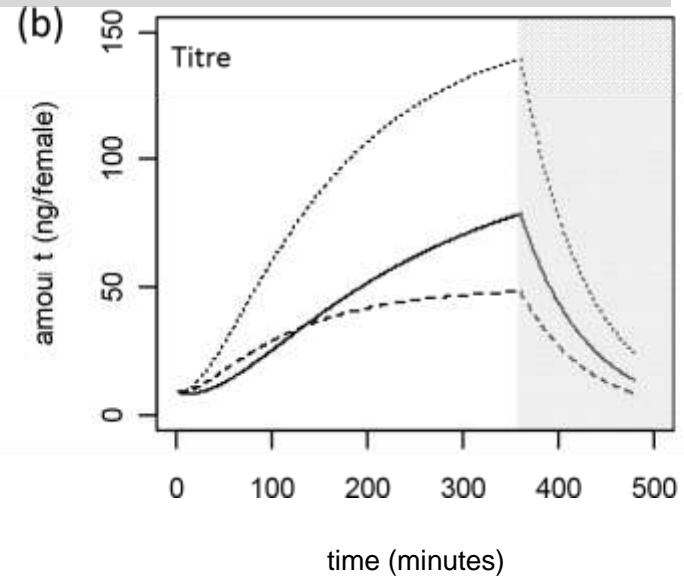
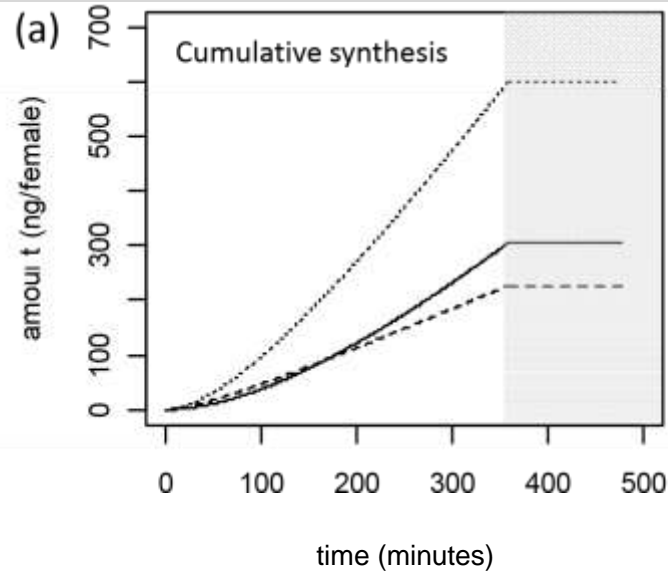


Figure 4

