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Effects of egg-to-adult development time and adult age on olfactory neuron response to semiochemicals in European corn borers

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Abstract

We used the cut-sensillum technique to assess the effect of both adult age and egg-to-adult development time on olfactory neuron responses of Z strain moths of the European corn borer, *Ostrinia nubilalis*. Compounds tested included the pheromone components, (*Z*)-11-tetradecenyl acetate and (*E*)-11-tetradecenyl acetate, the behavioral antagonist, (*Z*)-9-tetradecenyl acetate, and components of the *O. furnicalis* (Asian corn borer) sex pheromone, (*Z*)-12-tetradecenyl acetate and (*E*)-12-tetradecenyl acetate. The proportion of moths having neurons responding to the two *O. nubilalis* sex pheromone components and antagonist increased with longer development time and age. The spike frequency of neurons responding to (*E*)-11-tetradecenyl acetate and the antagonist increased with longer development time. Fourteen of 45 moths with neurons sensitive to either of the *O. nubilalis* pheromone components responded to (*Z*)-12-tetradecenyl acetate or (*E*)-12-tetradecenyl acetate. The likelihood of (*Z*)-12-tetradecenyl acetate stimulating a neuron similar in spike shape and waveform to that responding to (*E*)-11-tetradecenyl acetate increased with development time.

Keywords: Development time; Olfactory receptor neurons; *Ostrinia nubilalis*; *Ostrinia furnicalis*; Sex pheromone

1. Introduction

It has been a common observation that in moth pheromone communication systems males often do not respond optimally to female sex pheromones until 2–6 days after emergence (Shorey and Gaston, 1964; Shorey et al., 1968; Werner, 1977; Szöcs and Tóth, 1979; Tóth, 1979; Turgeon et al., 1983; Gemeno and Haynes, 2000). In many such cases male response begins within a day of the age at which females begin calling (Werner, 1977; Szöcs and Tóth, 1979; Tóth, 1979; Gemeno and Haynes, 2000).

The effects of age have also been examined at various levels of the neurophysiological pathway for pheromone olfaction in moths. The responsiveness of antennal lobe interneurons increased in adult *Agrotis ipsilon* males from 1 to 5 days old (Greiner et al., 2002). Male moths tend to exhibit maximum amplitude electroantennogram (EAG) response a few days into adulthood, followed by a gradual decline in responsiveness with age (Rees, 1970; Schweitzer et al., 1976; Seabrook et al., 1979). However, in one case this peak occurred in the pharate adult stage (Payne et al., 1970). Furthermore, in *Argyrotaenia velutinana* there is no effect of adult age on EAG response (Roelofs and Comeau, 1971). In the silk moth *Antheraea pernyi*, ageing causes a decline in olfactory receptor neuron (ORN) response to pheromones, which is linked to the death of dendrites (Kumar et al., 1998). Because ORN responsiveness for *Antheraea pernyi* was measured using 2–3-day age cohorts, the possibility that ORN response peaked a day or two after emergence cannot be excluded.

The European corn borer, *Ostrinia nubilalis* has a well-researched sex pheromone system that utilizes two components, (*Z*)-11-tetradecenyl acetate (Z11-14:OAc) and (*E*)-11-tetradecenyl acetate (E11-14:OAc). Populations of *O. nubilalis*, are marked by geographic differences in voltinism and pheromone blend (Klun, 1975; Kochansky et al., 1975; Roelofs et al., 1985). There is a bivoltine race in

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which females produce a 99:1 ratio of E11/Z11-14:OAc, as well as univoltine and bivoltine races that utilize a 3:97 ratio of E11/Z11-14:OAc. The behavioral preferences of males for particular isomeric ratios match the pheromone component blend ratios produced by females of the same strain (Roelofs et al., 1987). Regardless of such preferences, male antennae of all strains of the European corn borer have long trichoid sensilla with two separate but co-compartmentalized ORNs that are exclusively responsive to one of the two isomers (Hansson et al., 1987, 1994; Hallberg et al., 1994; Cossé et al., 1995). There is also a third co-compartmentalized ORN sensitive to (*Z*)-9-tetradecenyl acetate (Z9-14:OAc). The latter compound is a behavioral antagonist for this species (Klun and Robinson, 1971; Struble et al., 1987; Glover et al., 1989). Shorter trichoid sensilla located more distally on the antennae might have only one or two neurons that are responsive to Z11-14:OAc, E11-14:OAc, or Z9-14:OAc (Hallberg et al., 1994).

Recently, Roelofs et al. (2002) discovered so-called rare *O. nubilalis* males in their laboratory colony that responded in a flight tunnel by flying upwind to both the Asian corn borer (*Ostrinia furnicalis*) and *O. nubilalis* pheromone (Linn et al., 2003). The *O. furnicalis* sex pheromone is comprised of an approximately 40:60 blend of (*E*)-12-tetradecenyl acetate (E12-14:OAc) to (*Z*)-12-tetradecenyl acetate (Z12-14:OAc) (Klun et al., 1980, Ishikawa et al., 1999).

We were interested in the responsiveness of normal and rare European corn borer male ORNs to the Asian corn borer pheromone components relative to the *O. nubilalis* components. To begin this assessment and ensure that we would be measuring relative ORN responsiveness using physiologically optimal males, we needed to determine whether the proportion of males having ORNs responsive to the 11- and 12-tetradecenyl acetates, as well as the spike frequencies of these ORNs, varied with age or egg-to-adult development time. Here we examine the effects of adult age and development time on *O. nubilalis* ORN responsiveness using Z-strain univoltine males.

2. Material and methods

2.1. Insects

A colony of the univoltine Z race of European corn borer was maintained in the laboratory of Roelofs as previously described (Roelofs et al., 1985). All eggs laid on egg sheets over the course of a single day from a colony of approximately 240 moths were reared to pupal stage in isolation. Surviving male pupae were mailed overnight via courier to Penn State University 27 days after the eggs were laid. Pupae and emerging males were maintained at 25 °C on a 16:8 L:D photoperiod, 40–50% RH. As adults began emerging at day 30, they were separated from pupae to isolate each daily cohort in 3L cages. By day 38, emergence ceased and 58 males had been collected for analysis. Electrophysiological recordings of each cohort were initiated so as to distribute them from 0 to 6 days after emergence. Depending on the size of the cohort, one to three moths were tested from a cohort on each day.

2.2. Electrophysiological recordings

For all adult males, we recorded from the ORNs within individual antennal sensilla using the cut-sensillum technique (Kaissling, 1974; van der Pers and denOtter, 1978). Each antenna was excised from the head of the moth, with the antenna placed in a saline-filled Ag/AgCl recording electrode. We used a micromanipulator to maneuver the antenna such that a single trichoid sensillum rested on the tip of a vertically positioned tungsten knife. The tip of the sensillum was left hanging over the edge of the vertical knife. The sensillum tip was then cut using a horizontally oriented glass knife that was maneuverable with another micromanipulator. The cut sensillum was then contacted with a saline-filled glass micropipette containing an Ag/AgCl recording electrode.

The AC signal from the recording electrode passed through the built-in amplifier (DAM50, World Precision Instruments, Sarasota, FL, USA) of the portable recording unit into a computer. To monitor neural activity we used an external loudspeaker in conjunction with computer software (Syntech Autospike v.32; Syntech, Hilversum, The Netherlands) for observing action potentials.

A stream of purified, humidified air blew continuously over the antenna (10 ml/s) through a 25 cm-long glass tube (8 mm ID), the end of which was placed 2 cm from the antenna. Using a stimulus flow-controller device (SFC-2, Syntech), a 50-ms air pulse at 40 ml/s flow rate was injected through the odor cartridge and into the airstream. Linear flow through the airstream was ~0.3 m/s. At least 30 s were allowed to elapse between stimulations.

Syntech software was also used for later analyzing data by counting the number of spikes within 300 ms from the initiation of neuronal activity. As previously reported, there was little spontaneous background activity (Cossé et al., 1995) and initiation of response was easily discerned.

2.3. Odor cartridges

We created serial dilutions (1, 10, and 100 μg/μl) of Z11-14:OAc, E11-14:OAc, Z9-14:OAc, Z12-14:OAc, E12-14:OAc (Pherobank, The Netherlands) in 1 ml HPLC-grade hexane. We confirmed a >98% purity of the compounds, as well as equivalencies of the concentrations of each compound via gas chromatography. For each concentration, 10 μl was pipetted onto a $0.5 \times 2.0\,cm^2$ filter paper strip held in a 15 cm-long Pasteur pipette odor cartridge. The filter paper loadings thus were 10 μg, 100 μg, and 1 mg for all five compounds.

2.4. Sampling protocol

The protocol was developed with the primary purpose of detecting responsiveness to Z12-14:OAc and E12-14:OAc.

All recordings were performed using the long trichoid sensilla near the base of the antenna, which tend to have three co-compartmentalized neurons responding to Z11-14:OAc, E11-14:OAc, and Z9-14:OAc (Hallberg et al., 1994). We first tested for responses to Z12-14:OAc and E12-14:OAc (in either order) with the 10 μg pipette loading in the first sensillum contacted. We followed this by testing with the respective 100 μg cartridges in the same order on the same sensillum. If there was a response to either compound, which was always at 100 μg rather than 10 μg, we then continued to test for responses to Z9-14:OAc, Z11-14:OAc, and E11-14:OAc at 100 μg. If the ORN did not respond initially to Z12-14:OAc or E12-14:OAc, we attempted stimulations with only these compounds (100 μg before 1 mg) with two more sensilla. Finally, whether or not an ORN responded to Z12-14:OAc or E12-14:OAc, we continued to test up to 10 sensilla with all five compounds at 100 μg and then 1 mg, until at least three were successfully stimulated by Z9-14:OAc, Z11-14:OAc, and E11-14:OAc. The high dosage of 1 mg was used primarily to double check that there was not any responsiveness at all to a given compound in moths that had not responded to lower doses. Only three of 146 total positive responses to the five compounds among 58 moths were obtained exclusively at the 1 mg dose.

We attempted to examine the pair of antennae from each moth in the manner described above. However, for 17 of the 58 moths sampled, only one antenna was used. Such instances arose when obvious deformation of one antenna was observed, or one antenna was lost or damaged in the experimental preparation.

2.5. Statistical analyses

Two different approaches were undertaken to explore the effects of development time and adult age on responsiveness to pheromone components. All spike analyses were performed using Autospike 32. All statistical analyses were performed using SAS 9.1.

Because we allocated much more effort to unresponsive than responsive antennae, analyzing variation at the sensillum level is problematic. We also found that in many individuals, none of their ORNs responded to the pheromone or antagonist at any dosage. This lack of response occurred despite the antennae appearing to be in good condition. Also in such cases, ORNs exhibited occasional spontaneous spike activity characteristic of this species' background firing rate, showing that the electrode had established a good connection with the sensillum. Thus, we first determined whether there were positive responses in any sensillum from an individual moth at any concentration for each of the five test compounds. This approach allowed us to consider each moth as a responder or non-responder for each compound. With this data set, we performed a stepwise logistic regression for the binary response to each compound versus the independent variables, development time, adult age, and their interaction.

Next we considered only sensilla in which successful responses were recorded. Here we performed a stepwise least-squares regression of number of spikes per successful response versus development time, adult age, and left versus right antennal location of sensillum. We counted the number of spikes within 300 ms of the onset of electrophysiological activity. Generally, only responses to 100 μg of each test compound were considered. If no response occurred at 100 μg, but did occur at 1 mg, we assigned a value of 1 for the spike count.

For the spike-count regression, we confirmed the statistical significance of the results by using a maximum-likelihood model. Because of the unbalanced design with respect to multiple sensillar recordings per individual, moth identity could not be added as a factor to the least-squares model. In the maximum-likelihood model we added the identity of the moth sampled as a random effect, in addition to the fixed effects that were used previously for the least-squares model. A Newton–Rapson algorithm was used to calculate maximum-likelihood (Milliken and Johnson, 1984). In this algorithm the significance of each effect is evaluated by removing it from the model and evaluating the change in maximum likelihood.

3. Results

Spike trains in response to Z11-14:OAc, E11-14:OAc, and Z9-14:OAc (Fig. 1) were similar to those found in previous studies, coming from three co-compartmentalized ORNs in each sensillum (Hansson et al., 1987; Roelofs et al., 1987). The response to Z11-14:OAc was characterized by spike activity from a large-spiking ORN (Fig. 1), whereas the response to E11-14:OAc came from a smaller-spiking neuron. Another small-spiking neuron, usually the smallest of the three, responded to Z9-14:OAc (Fig. 1). Of the 58 males tested, 45 had sensilla in which we were able to record responses to Z11-14:OAc, 35 to E11-14:OAc, and 45 to Z9-14:OAc.

Throughout our experiments, responses to Z12-14:OAc or E12-14:OAc were rare, occurring for only 14 of the 58 moths tested (Tables 1 and 2), respectively. From these individuals with unusual responses, 16 sensilla were found with ORNs responding to Z12-14:OAc, and nine sensilla had ORNs responding to E12-14:OAc. For every ORN in which Z12-14:OAc or E12-14:OAc elicited responses, there were also responses with a similar spike size to Z11-14:OAc or E11-14:OAc, if the contact was able to be maintained. Eleven ORNs responding to Z12-14:OAc exhibited spike sizes and waveforms indistinguishable from those of the ORN responding to Z11-14:OAc (Fig. 1), with the other five ORNs responding to Z12-14:OAc having spike sizes more like those of the ORN sensitive to E11-14:OAc. All nine ORNs responding to E12-14:OAc were similar in size and waveform to the ORN responding to E11-14:OAc (Fig. 1). For several sensilla sampled from *O. nubilalis* not included in this experiment, we have observed both large

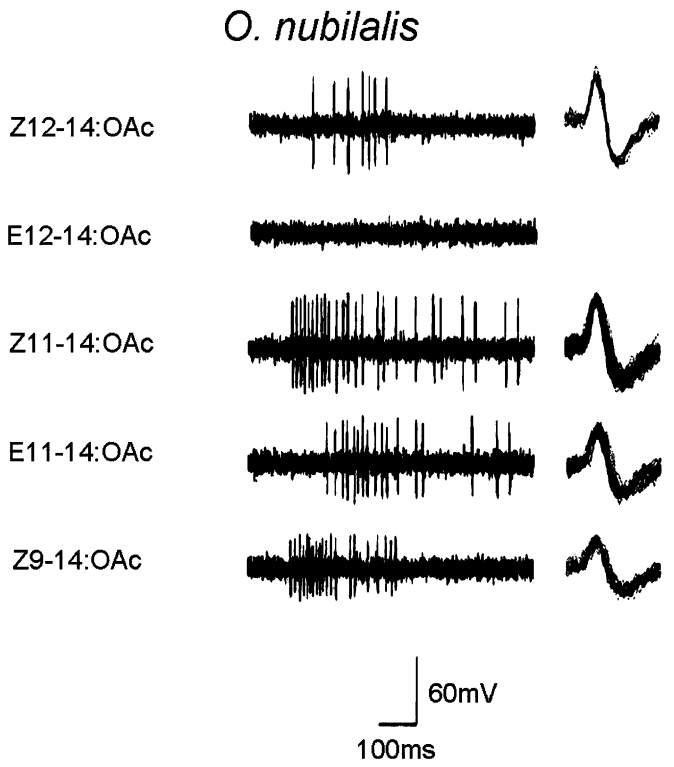

Fig. 1. Sample ORN responses and cumulative waveforms in male *O. nubilalis* from a 2-day-old moth with 37-day egg–adult development to 100 μg of each of the five test compounds. The large-spike-size ORN responded to Z12-14:OAc as well as to Z11-14:OAc.

Table 1
Response classes of individual moths over the observed range of egg-to-adult development times

Development time	30	31	32	⩾33	All
No response	7	3	0	0	10
Z9-14:OAC	1	0	1	1	3
Z11-14:OAC	1	1	1	0	3
Z11 & Z9-14:OAC	3	3(1)[a]	0	1	7(1)
Z11, E11 & Z9-14:OAC	6(3)	9(3)	8(2)	12(5)	35(13)
Sum	18(3)	16(4)	10(2)	14(5)	58(14)

Numbers in parenthesis represent number of moths in each category with responses to Z12-14:OAc or E12-14:OAc. Further detail about all such unusual responses provided in text on a per sensillum basis.

[a]In this case the response to Z12-14:OAc exhibited similar spike size to that also observed in response to Z11-14:OAc.

and intermediate-sized spiking neurons responding to Z12-14:OAc or E12-14:OAc.

The probabilities of an individual responding to the two *O. nubilalis* pheromone components Z11-14:OAc and E11-14:OAc, and the antagonist Z9-14:OAc increased with both egg-to-adult development time (Fig. 2; Table 1) and adult age (Fig. 3; Table 2). Longer development time and age significantly increased the likelihood of response to all three compounds. There were never significant interaction effects between development time and age added in the stepwise regression analyses. There was also a significant increase in the probability of individuals having an ORN response to Z12-14:OAc that was indiscernible from the E11-14:OAc with development time (Fig. 2; Table 3). While there was a visible increase in the probability of Z12-14:OAc stimulating an ORN appearing similar to that excited by Z11-14:OAc in the five individuals of last age class (Fig. 3), there was no statistically significant effect for this response with respect to adult age or development time (Table 3).

For least-squares stepwise regression of possible age, development, and antenna effects on spike frequency (Table 4), no factors were included for Z11-14:OAc, (Table 4) despite a large sample size. However, development time had a highly significant effect on responsiveness to E11-14:OAc (Fig. 4) and Z9-14:OAc (Fig. 5). These effects were significant despite large variation within all the cohorts that sometimes included very high responsiveness to pheromone for the earlier developers. No factors were included for regressions modeling responses to Z12-14:OAc and E12-14:OAc, but the sample sizes were small for such analyses. The maximum-likelihood models for development time as a factor for E11-14:OAc and Z9-14:OAc responses (Table 4) showed similar significance levels to the least-squares models.

4. Discussion

The increased likelihood of males having ORNs responsive to the pheromone components of the European corn borer with longer development times suggests there might be a trade-off between potential mate-finding benefits of early emergence and the full development of the peripheral pheromone olfaction system that reports presence and relative abundance of compounds involved in mate-finding. Constraints on sexual competition have been studied in many other contexts (c.f. review by Andersson, 1994). Later-developing *O. nubilalis* males have the strongest ORN responsiveness to pheromone components, but may risk emerging after most females are ready to mate. On the other hand, if the lower responsiveness of ORNs in earlier-emerging males results in reduced interneuron activity through the CNS, then earlier-emerging males may be less likely to detect the presence of calling, unmated females and be less likely to locate them. As a result there may be strong selection on males to emerge at an intermediate time when the peripheral nervous system will be receptive, yet females will still be available.

Although studies directly assessing the effect of age on behavioral response to sex pheromones in *O. nubilalis* males do not appear in the literature, there have been no indications of age effects in flight tunnel experiments. Males have been used for behavioral assays when they are 1 day old (Cossé et al., 1995). Likewise, behavioral activity has always been very high in this strain of European corn borer. While 13 of 58 moths did not respond to either pheromone component in any sensilla (Table 1), flight tunnel experiments using this colony recently showed that

Table 2
Response classes of individual moths over the experimental range of adult age classes

Adult age	0	1	2	3	4	5	6	All
No response	2	3	3	0	0	2	0	10
Z9-14:OAC	2	0	0	0	1	0	0	3
Z11-14:OAC	1	0	1	1	0	0	0	3
Z11 & Z9-14:OAC	1	2	0	1	2	0	1(1)[a]	7(1)
Z11, E11 & Z9-14:OAC	3(2)	5(1)	5(2)	6(3)	5(2)	7(1)	4(2)	35(13)
Sum	9(2)	10(1)	9(2)	8(3)	8(2)	9(1)	5(3)	58(14)

Numbers in parenthesis represent number of moths in each category with responses to Z12-14:OAc or E12-14:OAc. Further detail about all such unusual responses provided in text on a per sensillum basis.

[a]In this case the response to Z12-14:OAc exhibited similar spike size to that also observed in response to Z11-14:OAc.

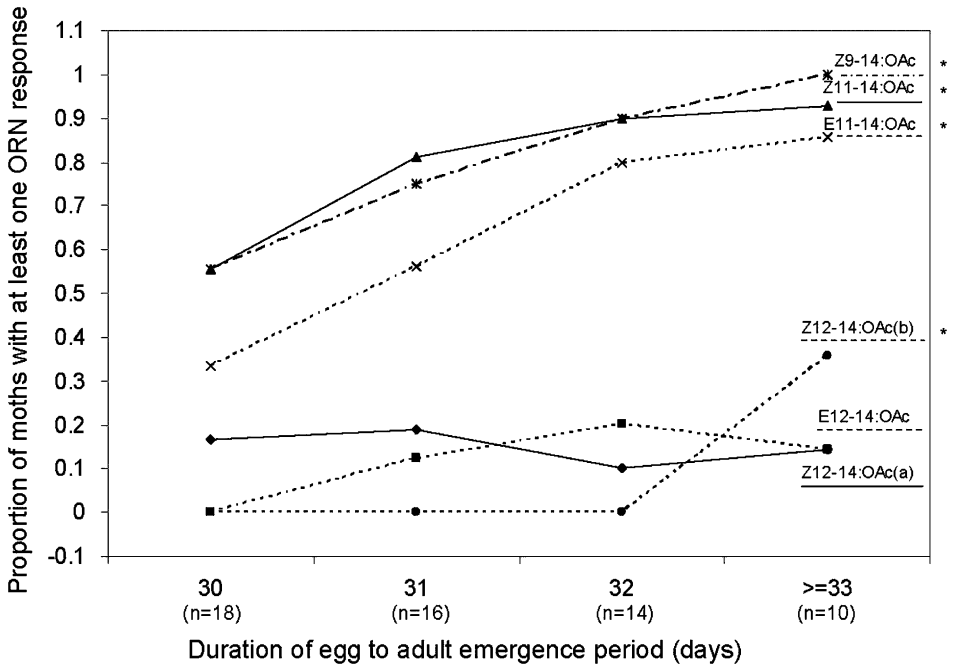

Fig. 2. Proportion of males having ORNs responding at any dosage to tetradecenyl acetates for adults emerging at different days. Different adult ages are represented for each day. All males emerging after day 33 were further combined for display. Number of individuals (n) contributing to frequency given for each emergence time. Solid lines represent stimulation of the large-spiking ORN. Ordinary dashed lines indicate excitation of the intermediate ORN. Patterned dashed lines show stimulation of the smallest ORN. *Significant regression (Table 3).

98% of the tested males ($n = 213$) exhibited complete flights to the source (Linn, unpublished results), a result consistent with other published results (Linn et al., 1997). Furthermore, although our study demonstrated an effect of increased ORN responsiveness with increased age, many young males' ORNs were responsive (Table 2, Fig. 3). It is thus difficult to reconcile the apparent differences between the ages of maximum physiological responsiveness that we found and the maximum behavioral responsiveness found previously. However, it is well known that CNS sensitivity is more than 100 times greater than peripheral ORNs due to the convergence of tens of thousands of pheromone-component-sensitive ORNs onto perhaps only 100 or so projection interneurons (Boeckh and Boeckh, 1979). Behavioral thresholds should be similarly lowered as a result of such convergence-related amplification of the signal.

There are multiple potential mechanisms that may explain the development time and age-specific patterns described in this study. For all analyses involving detection of ORN responsiveness to Z11-14:OAc, E11-14:OAc, and Z9-14:OAc, increased age and development time had positive effects. Developmentally regulated processes that affect all three ORNs within each sensillum may be responsible for such a pattern. For example there is only one pheromone binding protein of conserved structure in *O. nubilalis* and *O. furnicalis*, which presumably would transport any of the tested compounds through the sensillar lymph to the ORNs (Willett and Harrison, 1999). Endocrine regulation of such an element

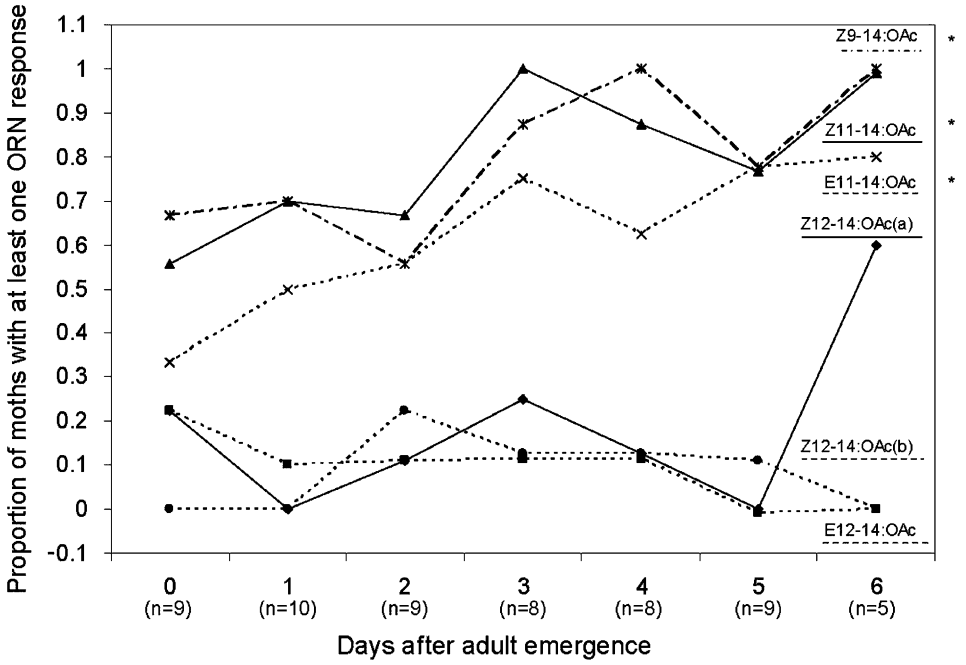

Fig. 3. Proportion of males having ORNs responding at any dosage to tetradecenyl acetates by adults at different ages after emergence, combining classes of different emergence times. Number of individuals (n) contributing to frequency given for each adult age. Solid lines represent stimulation of the large-spiking ORN. Ordinary dashed lines indicate excitation of the intermediate ORN. Patterned dashed lines show stimulation of the smallest ORN. *Significant regression (Table 3).

Table 3
Summary of stepwise regression using a logit model for response to five tetradecenyl acetates in 58 moths

Compound	Intercept[a]	Development time	Adult age
Z12-14:OAc(a)[b]	−1.69 ($p<0.001$)	Not included	Not included
Z12-14:OAc(b)	−40.7 ($p=0.007$)	1.15 ($p=0.008$)	Not included
E12-14:OAc	−2.16 ($p<0.001$)	Not included	Not included
Z11-14:OAc	−21.5 ($p=0.029$)	0.691 ($p=0.028$)	0.452 ($p=0.019$)
E11-14:OAc	−27.6 ($p=0.002$)	0.848 ($p=0.003$)	0.517 ($p=0.005$)
Z9-14:OAc	−39.9 ($p=0.008$)	1.29 ($p=0.009$)	0.494 ($p=0.017$)

[a]Parameter significance tests have 1 d.f (intercept, development time, and adult age). The interaction development time × adult age was also considered for each model but never included. Entries into the development time or adult age columns indicate that these effects are statistically significant.
[b]Responses to Z12-14:OAc are classified as (a) and (b) for the respective large- and small-spiking ORNs.

Table 4
Summary of stepwise regression for spike frequency response (spikes/0.3 s at 100 μg pipette loading) to five tetradecenyl acetates

Compound	Stepwise least squares regression[a]			Maximum-likelihood model[b]
	N	Intercept	Development time	Δ log likelihood
Z12-14:OAc(a)[c]	11	6.36 ($p<0.001$)	Not included	—
Z12-14:OAc(b)	5	5.40 ($p<0.108$)	Not included	—
E12-14:OAc	9	7.11 ($p=0.004$)	Not included	—
Z11-14:OAc[d]	84	13.2 ($p<0.001$)	Not included	—
E11-14:OAc	55	−31.1 ($p=0.011$)	1.27 ($p=0.001$)	7.8 ($p=0.005$)
Z9-14:OAc	96	−24.8 ($p=0.189$)	1.42 ($p=0.016$)	4.9 ($p=0.027$)

[a]Stepwise regression model selected used development time, adult age, and development time × adult age as possible independent variables. Entries into the development time column indicate that these effects are statistically significant.
[b]Maximum likelihood used to test for significance of development time in ANOVA model with development time as a fixed effect and Individual as a random effect.
[c]Responses to Z12-14:OAc are classified as (a) and (b) for the respective large and small-spiking ORNs.
[d]Analyses repeated using square root transformation to ensure normality. Results are not affected.

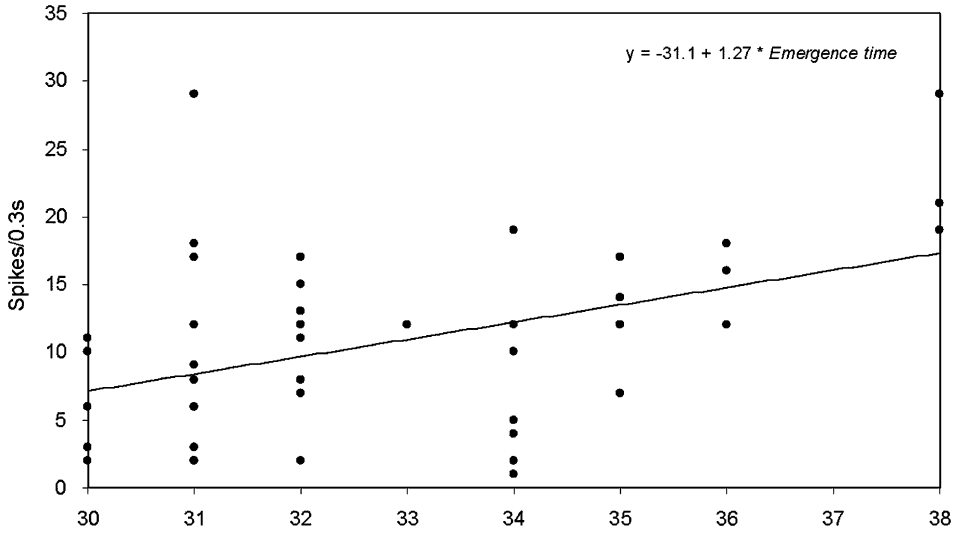

Fig. 4. Spike frequency versus emergence time for all sensilla having ORNs responding to E11-14:OAc at a 100 μg pipette loading. Significant regression equation included (Table 4).

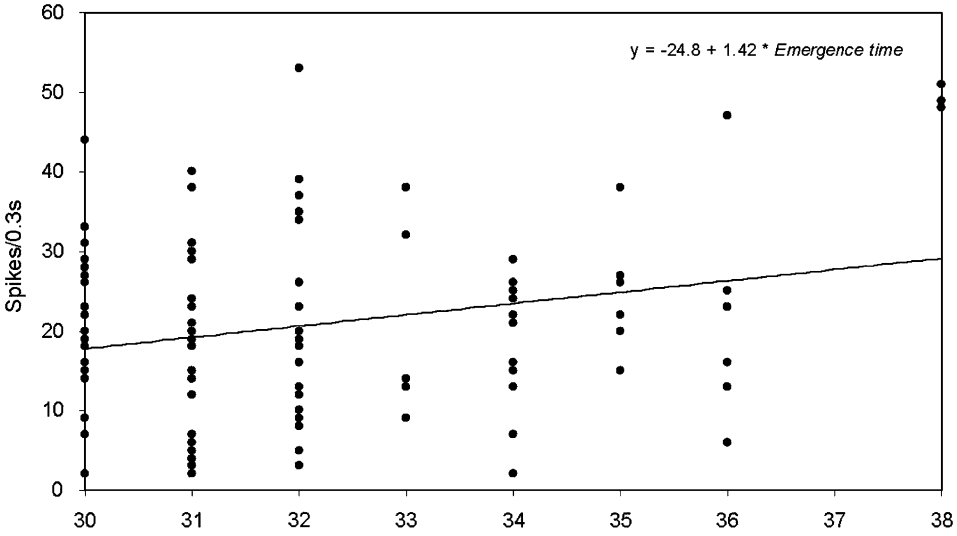

Fig. 5. Spike frequency versus emergence time for all sensilla having ORNs responding to Z9-14:OAc at a 100 μg pipette loading. Significant regression equation included (Table 4).

could account for the observed chronological effects on responsiveness.

However, significant effects upon spike frequency occurred with respect to development time for E11-14:OAc and Z9-14:OAc, but not for Z11-14:OAc. This result suggests developmentally regulated factors unique to each ORN. Perhaps expression of receptor proteins is differentially regulated such that ORNs for the main pheromone component Z11-14:OAc have a full compliment of receptors earlier than those responsive to E11-14:OAc or Z9-14:OAc. Expression of bombykol olfactory receptors has been shown to be developmentally regulated in silkworms (Sakurai et al., 2004), but no link between physiological sensitivity and receptor expression has been established.

Responses to E12-14:OAc and Z12-14:OAc were relatively rare in our study, generally occurring in less than 20% of males (Figs. 2 and 3). The ORNs responsive to E12-14:OAc or Z12-14:OAc were always equally or more highly responsive to E11-14:OAc and Z11-14:OAc. Less than 5% of *O. nubilalis* males from this colony respond to the *O. furnicalis* pheromone blend behaviorally (Roelofs et al., 2002; Linn et al., 2003). These rare individuals, that also respond to their own *O. nubilalis* pheromone blend, likely arise from such portions of the population that have a broader ORN-tuning profile that includes heightened responsiveness to Z12- and E12-14:OAc.

Although ORN responsiveness to the 12-tetradecenyl acetate isomers is undoubtedly required for rare *O. nubilalis* males to fly to the Asian corn borer

pheromone, the central nervous system interpretation of such ORN stimulation will be of similar importance. The amount of pheromone-component-specific input to each glomerulus in the MGC depends upon both the numbers of ORNs responding to a component at a given dose and the firing frequencies of these ORNs. We observed that the effect of longer egg-to-adult development was an increase in the proportion of individuals in which there was an 'E11-14:OAc-like' ORN responding to Z12-14:OAc. There was not a corresponding increase in the proportion of 'Z11-14:OAc-like' ORNs responding to Z12-14:OAc and so in these longer-developing individuals the Z12-/E12-14:OAc blend ratio that is reported to the central nervous system might be different from that in faster-developing animals.

Our findings should be taken into account for conducting, and interpreting results from, further studies of ORN-response profiles in rare and normal *O. nubilalis* individuals. Successful ORN recordings can be made more frequently with older than with younger males, and so behavioral testing to classify males as normal or rare can be done early in adulthood without hampering subsequent electrophysiological testing of these males. It must be realized that there might be an increase in responsiveness of the E11-14:OAc-tuned ORN to the *O. furnicalis* pheromone component Z12-14:OAc in longer-development-time males. There is no indication as yet that development time affects behavioral responsiveness to *O. nubilalis* pheromone components or those of *O. furnicalis*. However, our results emphasize the importance of correlating electrophysiological recordings with particular individuals whose behavioral tendencies have been clearly identified in wind tunnel flight tests, which is the protocol that we have been following in our subsequent experiments.

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