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Peripheral and behavioral plasticity of pheromone response and its hormonal control in a long-lived moth

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SUMMARY

Reproductive success in many animals depends on the efficient production of and response to sexual signals. In insects, plasticity in sexual communication is predicted in species that experience periods of reproductive inactivity when environmental conditions are unsuitable for reproduction. Here, we study a long-lived moth *Caloptilia fraxinella* (Ely) (Lepidoptera: Gracillariidae) that is reproductively inactive from eclosion in summer until the following spring. Male sex pheromone responsiveness is plastic and corresponds with female receptivity. Pheromone response plasticity has not been studied in a moth with an extended period of reproductive inactivity. In this study, we ask whether male antennal response and flight behavior are plastic during different stages of reproductive inactivity and whether these responses are regulated by juvenile hormone. Antennal response to the pheromone blend is significantly reduced in reproductively inactive males tested in the summer and autumn as compared with reproductively active males tested in the spring. Reproductively inactive autumn but not summer males show lower antennal responses to individual pheromone components compared with spring males. Treatment with methoprene enhances antennal response of autumn but not summer males to high doses of the pheromone blend. Behavioral response is induced by methoprene treatment in males treated in the autumn but not in the summer. Plasticity of pheromone response in *C. fraxinella* is regulated, at least in part, by the peripheral nervous system. Antennal and behavioral response to pheromone differed in reproductively active and inactive males and increased with methoprene treatment of inactive males.

Key words: juvenile hormone, pheromone response plasticity, *Caloptilia*, Lepidoptera, sex pheromone.

INTRODUCTION

Sexual behavior, including response to sexual signals, should be plastic in animals that have distinct reproductive cycles or undergo periods of reproductive inactivity, such as reproductive diapause in insects (Teal et al., 2000). Response to sexual signals should occur at reproductive maturity under suitable environmental conditions for mate finding, mating and production of offspring. Hormones act as mediators between the environment and animal nervous systems to ensure that mate-finding behavior is evoked when animals are physiologically able to produce offspring (Anton et al., 2007).

Response to sexual chemical signals or sex pheromones depends on the receiver's hormonal state in several taxa. In two fish species, hormonal status influences male sensitivity to female-produced sex pheromones. Adult male *Puntius schwanenfeldi* increase peripheral nervous system sensitivity to pheromone with increased androgen titer (Cardwell et al., 1995) whereas endocrine effects on the central nervous system mediate pheromone responsiveness in the goldfish *Carassius auratus* (Sorensen et al., 1987). Male hamsters respond to sex pheromone only if a threshold level of testosterone occurs in the brain (Wood and Swann, 2000). Young locusts treated with the gonadotropic hormone juvenile hormone (JH) respond to aggregation pheromone like sexually mature locusts whereas mature locusts deprived of JH have a reduced response to aggregation pheromone similar to that of young locusts (Ignell et al., 2001).

In moths (Lepidoptera), mate finding occurs *via* male response to female-produced, long-distance, sex pheromones. Pheromone response can increase predation risk and be energetically costly (Cardé and Haynes, 2004) and should be confined to a time when moths are sexually mature and the probability of locating a receptive female is high (Anton et al., 2007). Plasticity in pheromone response occurs in long-lived moth species in response to photoperiod and temperature (Dumont and McNeil, 1992), moth age (Turgeon et al., 1983; Dumont and McNeil, 1992; Gemeno and Haynes, 2000), mating status (Gadenne et al., 2001) and hormonal state (Gadenne et al., 1993).

JH is the major gonadotropic hormone in many Lepidoptera and regulates egg maturation in females and sex accessory gland development and mating behavior in both sexes (Denlinger, 2002; Ramaswamy et al., 1997). In migratory and long-lived Lepidoptera in which mating is delayed, JH controls sex accessory gland development and induction of pheromone response in adult males (Peter et al., 1981; Cusson et al., 1993; Duportets et al., 1996; Duportets et al., 1998; Anton et al., 2007). JH acid production in adult male *Pseudoletia unipuncta* correlates with responsiveness to female sex pheromone (Cusson et al., 1994). JH-deprived male *Agrotis ipsilon* show no response to female sex pheromone and response is restored with the implantation of the gland that produces JH, the corpora allata (CA) (Gadenne et al., 1993). Newly eclosed male *A. ipsilon* treated with a juvenile hormone analogue (JHA) are responsive to female sex pheromone whereas untreated males do not respond until three days post-eclosion (Gadenne et al., 1993).

In moth species studied to date, pheromone response plasticity associated with delayed reproduction is mediated in the central nervous system. The antennae of sexually immature male *A. ipsilon* are as responsive to female sex pheromone as antennae from reproductively active or JHA-treated males (Gadenne et al., 1993). Intracellular recordings show that JH affects the sensitivity of the

central olfactory neurons to pheromone (Anton and Gadenne, 1999; Gadenne and Anton, 2000). JH or JH acid mediates timing between reproductive development and pheromone responsiveness in long-lived male moths. However, it is not known if JH or JH acid play a role in pheromone response plasticity of male moths throughout a period of prolonged reproductive inactivity.

Caloptilia fraxinella (Ely) adults eclose in July when the majority of the population is in a reproductively immature state (Evenden et al., 2007), which is maintained until the following spring when adults emerge from overwintering sites to mate and lay eggs on newly flushed ash leaflets (Pohl et al., 2004). JH is important for the initiation of reproduction in female *C. fraxinella* in the spring (Evenden et al., 2007). Male *C. fraxinella* exhibit plasticity in response to sex pheromone that is dependent on physiological state, and their response is most acute in the spring when females are reproductively active (Evenden and Gries, 2008).

Here, we provide the first evidence that antennal response to pheromone is plastic throughout the adult stage of a long-lived moth. Furthermore, antennal and behavioral response to pheromone can be enhanced during a period of reproductive inactivity by treatment with a JHA, methoprene. However, the effect of methoprene on pheromone responsiveness varies with treatment time and pheromone stimulus during the prolonged period of reproductive inactivity.

MATERIALS AND METHODS

Moth collection

Early in the period of reproductive inactivity, summer moths were reared from pupae collected in leaf rolls from green ash *Fraxinus pennsylvanica* at various sites in Edmonton, Alberta, Canada (53 deg.34′N 113 deg.31′W) in June 2006 and 2007. Rolls were kept in individual 30 ml transparent plastic cups and ~70 cups were placed in transparent plastic bags with a damp paper towel to maintain humidity. Bags were held under summer conditions at 24°C with a 16 h:8 h light:dark cycle. Cups were checked twice weekly for adult eclosion. Moths were separated by sex, and males were provided with distilled water through a dental wick and transferred in individual 30 ml cups to a new growth chamber held under similar conditions until JHA treatment.

Later in the period of reproductive inactivity prior to overwintering, autumn moths were collected as adults from 16 August to 24 September 2006, from 24 August to 4 September 2007 and on 11 September 2008. Reproductively active moths were collected in the spring as adults from 14 April to 6 May 2007. Free-flying adult moths were collected at various sites in Edmonton, placed individually in glass vials and transported to the laboratory where they were separated by sex. Autumn and spring males were treated and housed in a similar manner as the summer males.

Methoprene treatment

A subset of reproductively inactive male moths collected in the summer and autumn were treated with 1 μg of methoprene (94.3% pure, Sigma-Aldrich, Oakville, ON, Canada) diluted in 1 μl of high-performance liquid chromatography (HPLC)-grade acetone (Fisher Scientific, Ottawa, ON, Canada) or 1 μl acetone alone as a control. Male moths were held using a gentle vacuum stream while treatments were applied exogenously to the ventral side of the abdomen. After treatment, males were provided with a 10% sugar solution and held in individual cups at 24°C under a reversed 16 h:8 h light:dark cycle for one week prior to the behavioral or electroantennography assay. Reproductively active males collected in the spring were not treated with methoprene but were used as a standard with which antennal response of untreated summer and autumn males was compared.

Electroantennogram experiments

Antennal response was measured to various doses of the full pheromone blend and the two individual pheromone components in the spring when males were reproductively active to establish a baseline of male response and again when males were in different phases of reproductive inactivity in the summer and autumn. Six additional electroantennogram (EAG) assays tested the hypothesis that methoprene treatment would alter the antennal response of males in different phases of reproductive inactivity to the pheromone blend and the individual components. EAG recordings were made using an IDAC-02 data acquisition controller system and EAG 2000 software (Syntech, Hilversum, The Netherlands). In preparation for antennal recordings, male moths were chilled at 4°C for at least 20 min before their antennae were excised and one antenna was attached to a stainless steel antenna holder using a small quantity of Spectra 360 conductive gel (Parker Laboratories, Orange, NJ, USA) and attached to a Syntech EAG probe (Type PRG-2, internal gain 10×). EAG recordings were conducted between the last 1–2 h of the photophase into the first 2 h of the scotophase. Single component pheromone loadings consisted of either (*Z*)-11-hexadecenal or (*Z*)-11-hexadecen-1-ol (Pherobank, Wageningen, The Netherlands) serially diluted in HPLC-grade hexane to obtain decade solutions between 0.0001 μg and 1 μg μl^{-1} hexane. Full blend pheromone loadings consisted of a 10:1 ratio of (*Z*)-11-hexadecenal and (*Z*)-11-hexadecen-1-ol (Pherobank) serially diluted in HPLC-grade hexane to obtain decade solutions between 0.0001 μg and 1 μg (*Z*)-11-hexadecenal μl^{-1} hexane. Fifty μl of each solution and 50 μl of a hexane control were pipetted individually onto 7×0.2 cm strips of folded Whatman no. 1 filter paper and allowed to evaporate in a fume hood. As a standard, 50 μl of the plant volatile (*E*)-2-hexenal (1 μg μl^{-1} hexane) was also pipetted onto filter paper and allowed to evaporate. Treated strips were inserted into disposable Pasteur pipettes. Stimulus puffs were generated with a Syntech CS-55 stimulus controller with a pulse duration of 0.2 s and flow of 10 ml s^{-1}. Antennal responses were measured as the maximum amplitude of depolarization elicited by the stimulus applied. Each antenna received a series of puffs delivered once every minute in the following order: hexane; 50 μg plant volatile; 0.005 μg pheromone; 50 μg plant volatile; 0.05 μg pheromone; 50 μg plant volatile; 0.5 μg pheromone; 50 μg plant volatile; 5 μg pheromone; 50 μg plant volatile; 50 μg pheromone; 50 μg plant volatile. We measured the response of at least 10 male antennae in each treatment group (methoprene, acetone and not treated) of each experiment (individual components, blend, spring, summer and autumn).

Behavioral experiments

The wind tunnel used in behavioral assays had a flight section 1.7 m long and 0.85 m high. Six 15 W bulbs diffused through white paper dimly illuminated the tunnel. Wind speed was 0.32–0.34 m s^{-1} and temperature was maintained at 25–26°C. Males were acclimatized to experimental conditions 30 min prior to initiation of the behavioral assay. Flights were conducted during the last hour of the photophase and the first two hours of the scotophase to a pheromone source consisting of 10 μg (*Z*)-11-hexadecenal and 1 μg (*Z*)-11-hexadecen-1-ol (Pherobank) in HPLC-grade hexane (Fisher Scientific, Ottawa, ON, Canada) released from a pre-extracted gray rubber septum (Phero Tech International, Delta, BC, Canada). Males were introduced individually into the wind tunnel in cylindrical wire cages (5 cm

diameter × 6 cm height) on a platform 20 cm from the downwind end. Once the moth was positioned in the pheromone plume, the lid of the cage was removed and males were allowed three minutes to respond to the pheromone source. Methoprene-treated and acetone-treated control males were flown alternately and each moth was flown only once. Untreated males were not flown in the wind tunnel assays due to a lack of moth availability. Behavioral responses to pheromone were recorded as: wing fanning, take-off from release device, lock-on to the pheromone plume, upwind-oriented flight and contact with the pheromone source.

Two separate wind tunnel experiments were conducted to test the hypothesis that methoprene treatment of males in different phases of reproductive inactivity (summer and autumn) would induce pheromone responsiveness as measured by upwind-oriented flight to a known attractive pheromone source (Evenden and Gries, 2008). Untreated, reproductively active males in the spring were flown in a previous experiment (Evenden and Gries, 2008). In the summer experiment, 1–17 treated and 5–17 control males were flown on each of the eight days for a total of 81 males from each treatment group over the course of the experiment. In the autumn experiment, 14–18 treated and control males were flown on each of the seven days for a total of 116 males from each treatment group over the course of the experiment.

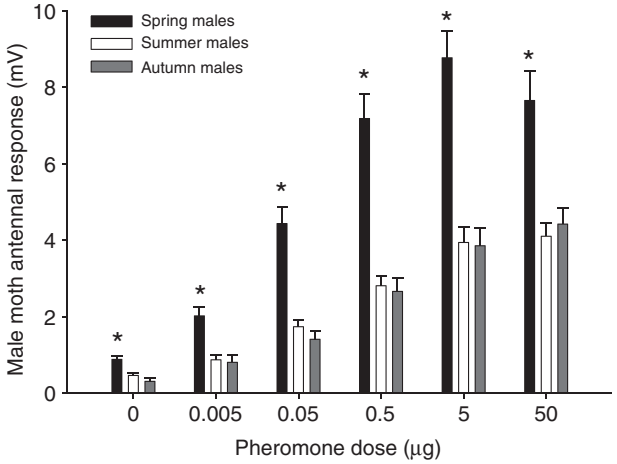

Fig. 1. Mean antennal response (+s.e.m.) from untreated male *Caloptilia fraxinella* that were reproductively active (spring, *N*=11) or in two different stages of reproductive inactivity (summer, *N*=11 and autumn, *N*=13) to increasing concentrations of sex pheromone. Asterisks represent significant differences in response between reproductively active males and males in both stages of reproductive inactivity (Tukey's test, $P \leq 0.002$).

Statistical analysis

Differences in mV antennal response among treatments at each stimulus dose in each electrophysiology experiment were compared using one-way analyses of variance (ANOVAs) followed by *post-hoc* Tukey's tests (v. 9, Systat Systems, Inc., Point Richmond, CA, USA) when necessary.

In the wind tunnel experiments, behavioral responses were recorded as + or – for each male so that the resulting data were binomially distributed. The proportion of methoprene-treated males *versus* control males that conducted each behavior was compared using 2×2 contingency tables (Systat Systems Inc., v. 9) in both experiments. The total proportion of responders pooled over time in each experiment was compared because a similar number of methoprene-treated and control males were flown on each day of the behavioral assay.

RESULTS

Electroantennogram experiments

Electrophysiological response of male antennae over the adult life stage varied depending on the physiological state of the moths. Antennae from reproductively active males collected in the spring responded in a dose-dependent manner to the full pheromone blend except that response peaked at the 5 μg dose and declined at the highest dose tested of 50 μg (Fig. 1). There was a significantly higher antennal response of reproductively active males to the pheromone blend at all doses compared with summer and autumn males collected in different stages of reproductive inactivity (Fig. 1). Response of antennae from both summer and autumn males did not show an upper threshold. When directly compared, there was no significant difference between the responses of antennae from summer and autumn males at any pheromone dose (Fig. 1). Reproductively active males showed a significantly higher antennal response than autumn males but not summer males to the four highest doses of the major component (*Z*)-11-hexadecenal (0.05 μg, 0.5 μg, 5 μg and 50 μg) tested alone (Fig. 2A). Antennal response by reproductively active males to the minor component (*Z*)-11-hexadecen-1-ol tested alone was greater than autumn male response at all doses and greater than summer male response at the highest dose tested (Fig. 2B). Unlike the response of reproductively active males to the full pheromone blend, there was no upper threshold of antennal response to either of the single components by males in any physiological state.

Plasticity in response to the full pheromone blend but not the individual components is influenced by methoprene treatment. Antennal response to the pheromone blend did not vary with methoprene treatment in the summer (Fig. 3A). By contrast, antennae from autumn-collected, methoprene-treated males responded significantly more to the two highest doses of the pheromone blend tested than antennae from acetone-treated and untreated autumn males (Fig. 3B). There was no effect of methoprene treatment on male EAG response to the full pheromone blend at the three lowest pheromone doses (0.005 μg, 0.05 μg and 0.5 μg) tested in either the summer or autumn experiments (Fig. 3A,B). There was also no significant effect of methoprene treatment on male EAG responses to the individual pheromone components at any of the doses tested in the summer or autumn (Fig. 4); however, in the autumn males there is a non-significant trend of increased response to both pheromone components by methoprene-treated males (Fig. 4C,D).

Behavioral experiments

Early in the period of reproductive inactivity none of the pheromone response behaviors normally elicited by reproductively active male moths to pheromone (Evenden and Gries, 2008) were enhanced by methoprene treatment (Fig. 5A). These data demonstrate that JHA treatment of summer males does not readily induce reproductive activity. By contrast, a greater proportion of males treated with methoprene in the autumn displayed wing fanning, lock-on to the pheromone plume, upwind-oriented flight and source contact behaviors than control moths treated with acetone alone (Fig. 5B). Although behavioral response to pheromone by summer and autumn males cannot be statistically compared because the experiments were necessarily conducted at different times, together the experiments show that the effect of methoprene treatment on the induction of pheromone responsiveness varies throughout the period of reproductive inactivity.

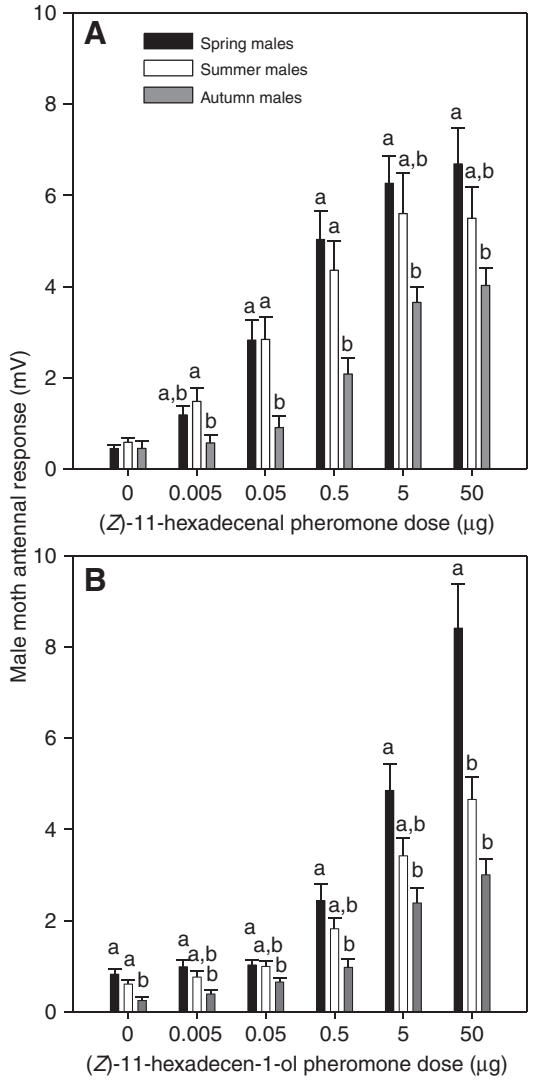

Fig. 2. (A) Mean antennal response (+s.e.m.) from untreated male *Caloptilia fraxinella* that were reproductively active (spring, *N*=13) or in two different stages of reproductive inactivity (summer, *N*=12 and autumn, *N*=11) to increasing concentrations of the major component (*Z*)-11-hexadecenal of the female sex pheromone. Different letters represent significant differences in response between reproductively active males and males in both stages of reproductive inactivity within one pheromone dose (Tukey's test, $P<0.05$). (B) Mean antennal response (+s.e.m.) from untreated male *C. fraxinella* that were reproductively active (spring, *N*=12) or in two different stages of reproductive inactivity (summer, *N*=12 and autumn, *N*=10) to increasing concentrations of the minor component (*Z*)-11-hexadecen-1-ol of the female sex pheromone. Different letters represent significant differences in response between reproductively active males and males in both stages of reproductive inactivity within one pheromone dose (Tukey's test, $P<0.05$).

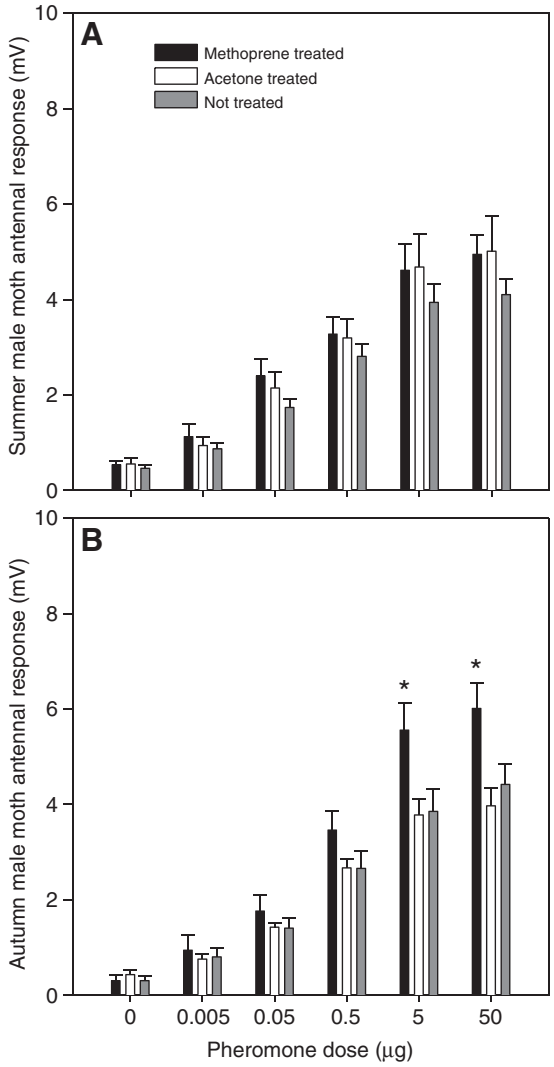

Fig. 3. (A) Mean antennal response (+s.e.m.) from reproductively inactive summer male *Caloptilia fraxinella* to increasing concentrations of sex pheromone. Males were either untreated (*N*=11) or treated with either methoprene in acetone (*N*=12) or acetone (*N*=12). Methoprene treatment did not impact male antennal response at any of the doses tested (ANOVA, $P>0.05$). (B) Mean antennal response (+s.e.m.) from reproductively inactive autumn male *C. fraxinella* to increasing concentrations of sex pheromone. Males were either untreated (*N*=13) or treated with either methoprene in acetone (*N*=11) or acetone (*N*=11). Asterisks represent a significant difference in antennal response between methoprene-treated and both acetone-treated and untreated males. Antennae from methoprene-treated males had a significantly greater response to the 5 μg (Tukey's test, $P<0.05$) and 50 μg (Tukey's test, $P<0.05$) pheromone stimuli than acetone-treated and untreated males.

DISCUSSION

Plasticity of pheromone responsiveness in moths appears to be tightly linked with reproductive maturity to ensure that mate finding occurs when conditions promote successful production of offspring (Turgeon et al., 1983; Dumont and McNeil, 1992; Gadenne et al., 1993). Pheromone response of male *C. fraxinella* is plastic throughout its prolonged adult life stage and response is most acute in the spring when males are reproductively active and should be pursuing females (Evenden and Gries, 2008). In the present study, we show that this plasticity exists in the peripheral nervous system, as antennal response of reproductively active spring males is higher than that of reproductively inactive males collected and tested in the summer and autumn. Peripheral chemoreceptor response changes with age in some adult moths (Seabrook et al., 1979; Domingue et al., 2006) and flies (Rees, 1970; Crnjar et al., 1990). The exact mechanism of peripheral nervous system plasticity in *C. fraxinella* males is not known but post-diapause female *Culex pipiens* mosquitoes possess a higher proportion of neurons that are

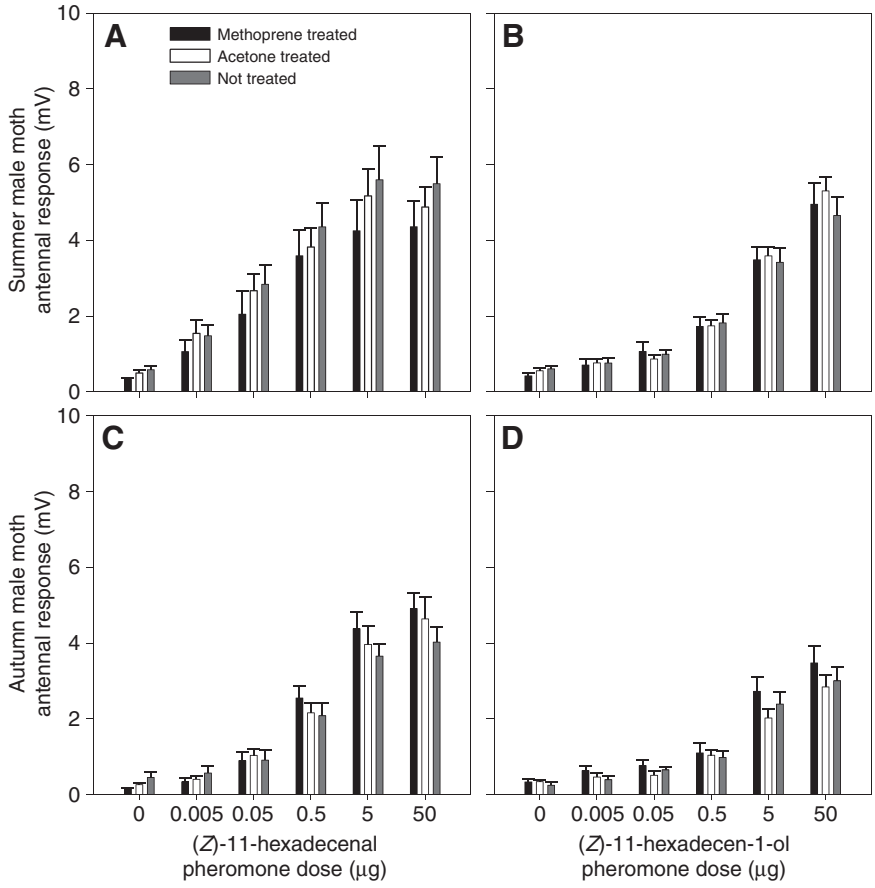

Fig. 4. Mean antennal response (+s.e.m.) from methoprene-treated, acetone-treated and untreated male *Caloptilia fraxinella* in two stages of reproductive inactivity. (A) Summer male response to the major female sex pheromone component, (*Z*)-11-hexadecenal ($P>0.05$). (B) Summer male response to the minor female sex pheromone component, (*Z*)-11-hexadecen-1-ol ($P>0.05$). (C) Autumn male response to the major female sex pheromone component, (*Z*)-11-hexadecenal ($P>0.05$). (D) Autumn male response to the minor female sex pheromone component, (*Z*)-11-hexadecen-1-ol ($P>0.05$). Methoprene did not impact male response to individual pheromone components in the summer or autumn.

highly sensitive to host cues than females in diapause (Bowen, 1990), which may result from upregulation of odorant receptors to host kairomones in active females. This type of plasticity occurs in another mosquito *Anopheles gambiae* in which odorant receptors for host cues are downregulated following a blood meal (Takken et al., 2001). It is likely that plasticity of pheromone response in *C. fraxinella* is regulated by allelic polymorphism of genes that control a more general reproductive diapause phenomenon in this species, and on-going research in our laboratory is examining variation in male accessory gland morphology and protein expression and spermiogenesis throughout the extended adult life stage. Allelic variation of the *Drosophila melanogaster* foraging gene (Shaver et al., 1998) and associated expression of a protein kinase influences behavioral response to food odors in adult flies (Shaver et al., 1998). EAG responses by *D. melanogaster* antennae to host volatiles also vary with expression of circadian clock genes (Krishnan et al., 1999).

The plasticity of antennal response to individual pheromone components in *C. fraxinella* followed a different pattern than to the full blend. Antennae from males collected and tested in the summer at the beginning of the period of reproductive inactivity responded to the main aldehyde component intermediately between reproductively active spring males and males collected in the autumn prior to overwintering. This may be because a small proportion of the population that eclose in the summer are not in a state of reproductive inactivity (Evenden et al., 2007). Alternatively, the antennal response to the blend may be driven in part by greater plasticity in response to the minor alcohol component. This would corroborate our previous findings from field behavioral studies in which summer males were attracted to lures releasing off ratio pheromone blends with high levels of the alcohol component (Evenden and Gries, 2008).

Long-lived moths show plasticity in pheromone response with age that is under endocrine control and correlated with a period of delayed reproductive maturity (Dumont and McNeil, 1992; Turgeon et al., 1983; Gadenne et al., 1993). However, in species studied to date, sexual receptivity occurs over a period of days after eclosion rather than after months, as is the case in *C. fraxinella* (Evenden et al., 2007). Pheromone response of male *P. unipuncta* increases with age and reproductive maturity over 5–7 days post-eclosion (Turgeon et al., 1983; Dumont and McNeil, 1992) and is correlated with JH acid biosynthesis (McNeil et al., 1994). JHA treatment of sexually immature, newly eclosed, male *A. ipsilon* promotes behavioral response to pheromone in a wind tunnel, similar to that typically seen in mature, three-day-old males (Gadenne et al., 1993). In the current study, we have evidence from both EAG and behavioral experiments that JH is at least partially involved in the control of pheromone response induction in male *C. fraxinella*. However, the effect of exogenous treatment with a JHA varied depending on the stage during the period of reproductive inactivity in which treatment occurred and whether the stimulus consisted of the complete pheromone blend or individual components. These data suggest that other environmental cues are necessary to fully induce reproductive behaviors. Methoprene treatment increased both antennal and behavioral response to the pheromone blend in autumn but not summer males. This response can be attributed to methoprene treatment and not the solvent in which it was applied (Critchley and Almeida, 1973) as antennal response of acetone-treated and untreated male antennae were statistically similar in all experiments. Insect physiological state changes gradually throughout the period of reproductive inactivity (Koštál, 2006), and

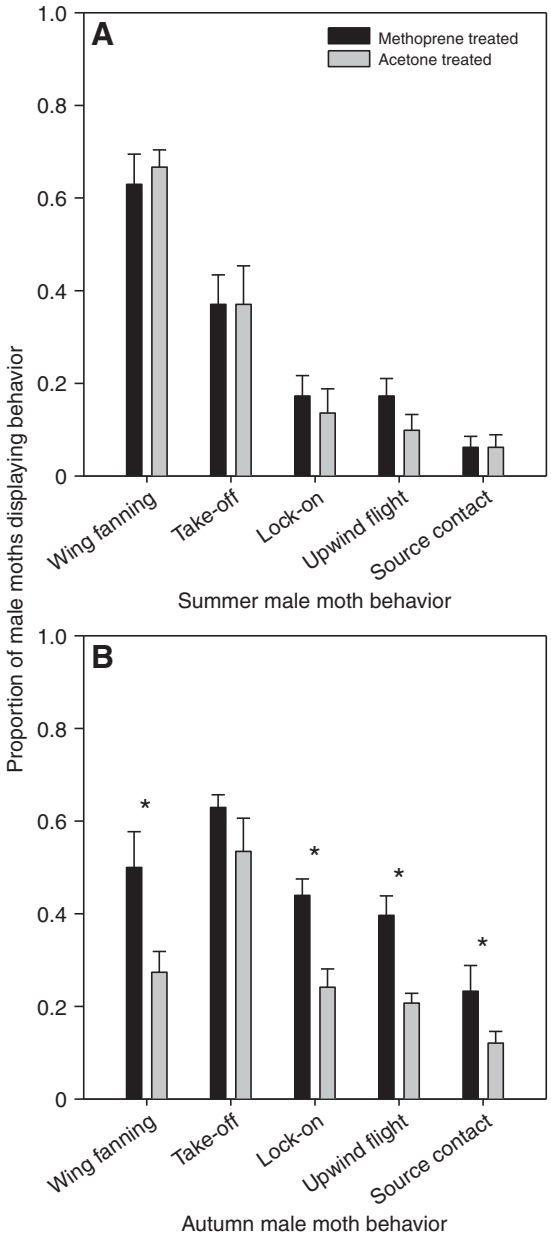

Fig. 5. (A) Mean proportion (+s.e.m.) response of male *Caloptilia fraxinella* collected in the summer in an early state of reproductive inactivity to sex pheromone in a wind tunnel. Males were treated with either methoprene in acetone or acetone alone. There was no effect of methoprene treatment on subsequent moth behavior (χ^2, $P>0.05$ for all behaviors). (B) Mean proportion (+s.e.m.) response of male *C. fraxinella* collected in the autumn in a late state of reproductive inactivity to sex pheromone in a wind tunnel. Males were treated with either methoprene in acetone or acetone alone. Asterisks represent a significant difference in each marked behavioral response between methoprene-treated and control males. Methoprene treatment significantly increased the proportion of males wing fanning (χ^2, $P<0.001$), locking-on to the pheromone source (χ^2, $P=0.001$), flying upwind toward the pheromone source (χ^2, $P=0.002$) and contacting the pheromone source (χ^2, $P=0.025$). Take-off from the release device was not impacted by methoprene treatment (χ^2, $P>0.05$).

the effect of JH or JHA treatment on the termination of reproductive diapause will depend on when hormone treatment is applied. This is probably because gene expression varies during diapause initiation, maintenance and termination, and upregulation of genes involved in diapause termination as a result of JH treatment will vary depending on time of treatment (Tauber and Tauber, 1976; Denlinger, 2002). A greater proportion of female *C. fraxinella* collected in autumn and treated with methoprene mate and produce eggs than females collected and treated in summer early in the period of reproductive inactivity (Evenden et al., 2007). Similarly, in the lady beetle *Coccinella septempunctata bruckii* summer aestivation is successfully terminated by JHA treatment, which induces increased respiration and initiation of oogenesis in females (Sakurai et al., 1986). However, JHA treatment only slightly impacted respiration and oogenesis of overwintering beetles (Sakurai et al., 1986).

Endocrine regulation of pheromone response by JH in other insects occurs at the level of central nervous system processing and not at the peripheral nervous system level (Payne et al., 1970; Anton and Gadenne, 1999; Ignell et al., 2001; Anton et al., 2007). Results from the current study show that there is some effect of methoprene treatment on response to pheromone in the peripheral nervous system, as antennae from autumn males treated with methoprene and stimulated by the pheromone blend were more responsive than control antennae. Female *Culex* mosquitoes exhibit a shift between diapause and non-diapause physiological states in their ability to detect host cues (lactic acid) at the peripheral nervous system level (Bowen, 1990) that is regulated in part by JH (Hancock and Foster, 2000). Female spruce budworm *Choristoneura fumiferana* detect sex pheromone as measured by electroantennography and this response is reduced after JHA treatment (Palaniswamy et al., 1979). Methoprene treatment alone was not enough to enhance autumn male *C. fraxinella* antennal response to the same level as in reproductively active males, so other factors are involved in this response plasticity. Furthermore, EAG response to the individual components did not differ with methoprene treatment, suggesting that JH may differentially affect the response of olfactory response neurons presented with the blend. Although interactions between pheromone components at the level of the peripheral nervous system processing in moths are thought to be minimal, there is some evidence for modulation of response when two or more pheromone components are presented simultaneously (O'Connell at al., 1986) or in the presence of host plant volatiles (Ochieng et al., 2002). Interestingly, JH treatment of *A. ipsilon* differentially affected the response of pheromone-sensitive antennal lobe interneurons; JH-induced the response of interneurons to the pheromone blend but not to individual components (Gadenne and Anton, 2000).

In animals that undergo a period of reproductive inactivity, plasticity in response to sexual signals would be adaptive and promote mate finding at times when animals are reproductively mature. Antennal and behavioral response to sex pheromone signals by male *C. fraxinella* is plastic during the adult lifespan (Evenden and Gries, 2008), and male antennal response to female sex pheromone is highest in the spring, when males are reproductively active. This plasticity appears to be regulated, at least in part, by JH, which also terminates reproductive diapause in female *C. fraxinella* (Evenden et al., 2007). Therefore, JH is the major gonadotropic hormone in *C. fraxinella* and also acts to appropriately time male and female encounters in this long-lived moth.

LIST OF ABBREVIATIONS

CA	corpora allata
EAG	electroantennogram
HPLC	high performance liquid chromatography
JH	juvenile hormone
JHA	juvenile hormone analogue

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REFERENCES

Anton, S. and Gadenne, C. (1999). Effect of juvenile hormone on the central nervous processing of sex pheromone in an insect. *Proc. Natl. Acad. Sci. USA* **96**, 5764-5767.

Anton, S., Dufour, M. C. and Gadenne, C. (2007). Plasticity of olfactory-guided behavior and its neurobiological basis: lessons from moths and locusts. *Entomol. Exp. Appl.* **123**, 1-11.

Bowen, M. F. (1990). Post-diapause sensory responsiveness in *Culex pipiens*. *J. Insect Physiol.* **36**, 923-929.

Cardé, R. T. and Haynes, K. F. (2004). Structure of the pheromone communication channel in moths. In *Advances in Insect Chemical Ecology* (ed. R. T. Carde and J. G. Millar), pp. 283-332. Cambridge: Cambridge University Press.

Cardwell, J. R., Stacey, N. E. and Tan, E. S. P. (1995). Androgen increases olfactory receptor response to a vertebrate sex pheromone. *J. Comp. Physiol. A* **176**, 55-61.

Critchley, B. R. and Almeida, A. A. (1973). Side effects of solvents, especially acetone, used for the application of juvenile hormone mimics and chemosterilants. *Bull. Entomol. Res.* **63**, 1-6.

Crnjar, R., Yin, C. M., Stoffolano, Jr, J. G., Barbarossa, I. T., Liscia, A. and Angioy, A. M. (1990). Influence of age on the electroantennogram response of the female blowfly (*Phormia regina*) (Diptera: Calliphoridae). *J. Insect Physiol.* **36**, 917-921.

Cusson, M., Yagi, K. J., Tobe, S. S. and McNeil, J. N. (1993). Identification of release products of corpora allata of male and female armyworm moths, *Pseudaletia unipuncta*. *J. Insect Physiol.* **39**, 775-783.

Cusson, M., Tobe, S. S. and McNeil, J. N. (1994). Juvenile hormones: their role in the regulation of the pheromonal communication system of the armyworm moth, *Pseudaletia unipuncta*. *Arch. Insect Biochem. Physiol.* **25**, 329-345.

Denlinger, D. L. (2002). Regulation of diapause. *Annu. Rev. Entomol.* **47**, 93-122.

Domingue, M. J., Roelofs, W. L., Linn, C. E., Jr and Baker, T. C. (2006). Effects of egg-to-adult development time and adult age on olfactory neuron response to semiochemicals in European corn borers. *J. Insect Physiol.* **52**, 975-983.

Dumont, S. and McNeil, J. N. (1992). Responsiveness of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) males, maintained as adults under different temperature and photoperiodic conditions, to female sex pheromone. *J. Chem. Ecol.* **18**, 1797-1807.

Duportets, L., Dufour, M. C., Becard, J. M., Gadenne, C. and Couillaud, F. (1996). Inhibition of male corpora allata activity and sexual pheromone responsiveness of the black cutworm, *Agrotis ipsilon*, by the hypocholesterolemic agent, fluvastatin. *Arch. Insect Biochem. Physiol.* **32**, 601-611.

Duportets, L., Dufour, M. C., Couillaud, F. and Gadenne, C. (1998). Biosynthetic activity of corpora allata, growth of sex accessory glands and mating in the male moth *Agrotis ipsilon* (Hufnagel). *J. Exp. Biol.* **201**, 2425-2432.

Evenden, M. L. and Gries, R. (2008). Plasticity of male response to sex pheromone depends on physiological state in a long-lived moth. *Anim. Behav.* **75**, 663-672.

Evenden, M. L., Armitage, G. and Lau, R. (2007). Effects of nutrition and methoprene treatment upon reproductive diapause in *Caloptilia fraxinella* (Lepidoptera: Gracillariidae). *Physiol. Entomol.* **32**, 275-282.

Gadenne, C. and Anton, S. (2000). Central processing of sex pheromone stimuli is differentially regulated by juvenile hormone in a male moth. *J. Insect Physiol.* **46**, 1195-1206.

Gadenne, C., Renou, M. and Sreng, L. (1993). Hormonal control of pheromone responsiveness in the male back cutworm *Agrotis ipsilon*. *Experientia* **49**, 721-724.

Gadenne, C., Dufour, M. C. and Anton, S. (2001). Transient post-mating inhibition of behavioural and central nervous responses to sex pheromone in an insect. *Proc. Biol. Sci.* **268**, 1631-1635.

Gemeno, C. and Haynes, K. F. (2000). Periodical and age-related variation in chemical communication system of black cutworm moth, *Agrotis ipsilon*. *J. Chem. Ecol.* **26**, 329-342.

Hancock, R. G. and Foster, W. A. (2000). Exogenous juvenile hormone and methoprene, but not male accessory gland substances or ovariectomy, affect the blood/nectar choice of female *Culex nigripalpus* mosquitoes. *Med. Vet. Entomol.* **14**, 376-382.

Ignell, R., Couillaud, F. and Anton, S. (2001). Juvenile-hormone-mediated plasticity of aggregation behavior and olfactory processing in adult desert locusts. *J. Exp. Biol.* **204**, 249-259.

Koštál, V. (2006). Eco-physiological phases of insect diapause. *J. Insect Physiol.* **52**, 113-127.

Krishnan, B., Dryer, S. E. and Hardin, P. E. (1999). Circadian rhythms in olfactory responses of *Drosophila melanogaster*. *Nature* **400**, 375-378.

McNeil, J. N., Cusson, M., Delisle, J. and Tobe, S. S. (1994). Hormonal control of sexual behavior in moths that migrate in response to predictable or unpredictable deterioration. In *Perspectives in Comparative Endocrinology* (ed. K. G. Davey, R. E. Peter and S. S. Tobe), pp. 464-468. Ottawa: National Research Council of Canada.

Ochieng, S. A., Park, K. C. and Baker, T. C. (2002). Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*. *J. Comp. Physiol. A* **188**, 325-333.

O'Connell, R. J., Beauchamp, J. T. and Grant, A. J. (1986). Insect olfactory responses to components of pheromone blends. *J. Chem. Ecol.* **12**, 451-467.

Palaniswamy, P., Sivasubramanian, P. and Seabrook, W. D. (1979). Modulation of sex pheromone perception in female moths of the eastern spruce budworm, *Choristoneura fumiferana* by altosid. *J. Insect Physiol.* **25**, 571-574.

Payne, T. L., Shorey, H. H. and Gaston, L. K. (1970). Sex pheromones of noctuid moths: factors influencing antennal responsiveness in males of *Trichoplusia ni*. *J. Insect Physiol.* **16**, 1043-1055.

Peter, M. G., Shirk, P. D., Dahm, K. H. and Roller, H. (1981). On the specificity of juvenile hormone biosynthesis in the male *Cecropia* moth. *Z. Naturforsch.* **36**, 579-585.

Pohl, G., Saunders, C. and Barr, W. B. (2004). *Caloptilia fraxinella* (Lepidoptera: Gracillariidae), a new pest of ash (Oleaceae: Fraxinus spp.) on the Canadian prairies. *Can. Entomol.* **136**, 733-736.

Ramaswamy, S. B., Shu, S., Park, Y. I. and F., Zeng. (1997). Dynamics of juvenile hormone-mediated gonadotropism in the Lepidoptera. *Arch. Insect Biochem. Physiol.* **35**, 539-558.

Rees, C. J. C. (1970). Age dependency of response in an insect chemoreceptor sensillum. *Nature* **227**, 740-742.

Sakurai, H., Hirano, T. and Takeda, S. (1986). Physiological distinction between aestivation and hibernation in the lady beetle, *Coccinella septempunctata bruckii* (Coleoptera: Coccinellidae). *Appl. Entomol. Zool.* **21**, 424-429.

Seabrook, W. D., Hiral, K., Shorey, H. H. and Gaston, L. K. (1979). Maturation and senescence of an insect chemosensory response. *J. Chem. Ecol.* **5**, 587-594.

Shaver, S. A., Varnam, C. J., Hilliker, A. J. and Sokolowski, M. B. (1998). The foraging gene affects adult but not larval olfactory-related behavior in *Drosophila melanogaster*. *Behav. Brain Res.* **95**, 23-29.

Sorensen, P. W., Hara, T. J. and Stacey, N. E. (1987). Extreme olfactory sensitivity of mature and gonadally-regressed goldfish to a potent steroidal pheromone, 17*a*,20β-dihydroxy-4-pregnen-3-one. *J. Comp. Physiol. A* **160**, 305-313.

Takken, W., van Loon, J. J. A. and Adam, W. (2001). Inhibition of host-seeking response and olfactory responsiveness in *Anopheles gambiae* following blood feeding. *J. Insect Physiol.* **47**, 303-310.

Tauber, M. J. and Tauber, C. A. (1976). Insect seasonality: diapause maintenance, termination, and postdiapause development. *Annu. Rev. Entomol.* **21**, 81-107.

Teal, P. E., Gomez-Simuta, Y. and Proveaux, A. T. (2000). Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. *Proc. Natl. Acad. Sci. USA* **97**, 3708-3712.

Turgeon, J. J., McNeil, J. N. and Roelofs, W. L. (1983). Responsiveness of *Pseudaletia unipuncta* males to the female sex pheromone. *Physiol. Entomol.* **8**, 339-344.

Wood, R. I. and Swann, J. M. (2000). Neuronal integration of chemosensory and hormonal signals in the control of male sexual behavior. In *Reproduction in Context* (ed. K. Wallen and J. E. Schneider), pp. 423-444. Cambridge, MA: MIT Press.