The Pheromone Receptor System of Male *Eulia ministrana* L., with Notes on Other Cnephasiini Moths

Ernst Priesner

Max-Planck-Institut für Verhaltensphysiologie,
D-8131 Seewiesen

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The antennal Sensilla trichodea of male *Eulia ministrana* (Tortricidae: Cnephasiini) contain specialist receptor cells for the sex-attractant components (*Z*)-9- and (*Z*)-11-tetradecenyl acetate and the attraction-inhibitors (*E*)-11-tetradecenyl acetate, (*Z*)-7-dodecenyl acetate, and (*Z*)-11-hexadecenyl acetate. The significance of the latter two compounds as potential sex pheromone components in Tortricidae is considered.

The subfamily Tortricinae (Lepidoptera: Tortricidae) is represented in the Palearctic region by more than 500 known species referred to three major tribes: the Tortricini, Archipini, and Cnephasiini. Razowski [1, 2] revised the Palearctic species of Cnephasiini and outlined phylogenetic relationships among genera based on morphological characters.

The chemical composition of the female sex pheromone has so far been reported for only one species of this moth tribe, the cereal tortrix *Cnephasia pumicana* Zeller. Virgin *C. pumicana* females produce a mixture of (*Z*)-9- and (*E*)-9-dodecenyl acetates and dodecyl acetate, in a ratio of *Z*9-12:Ac/*E*9-12:Ac/12:Ac of 3/5/2 [3]. The synthetic mixture selectively attracted conspecific males [3, 4] and has provided an effective trap bait used in current programs of population monitoring [5]. For a number of further Cnephasiini spp. the attraction of males to synthetic pheromone components of other Lepidoptera has been reported [5–17].

When studying olfactory receptor systems of male antennal *Sensilla trichodea* among selected Cnephasiini species I noticed a number of unusual features, not seen in other tortricid groups. For example, in some test species this receptor system was found to include specialist cells for (*Z*)-7-dodecenyl acetate and/or (*Z*)-11-hexadecenyl acetate; these two compounds (*Z*7-12:Ac, *Z*11-16:Ac) are common pheromone constituents in other Lepidoptera, *e.g.* Noctuidae, but were thus far unknown from Tortricidae pheromones (see [16]). On comparing different species groups of *Cnephasia*, the prevailing cell types were found to respond to 14-carbon acetates in some species but 12-carbon compounds in others, with the unusual combination of receptors for Δ8- and Δ9-dodecenyl acetates occurring even within the same species. Such findings prompted a more systematic analysis of pheromone receptor systems over different Cnephasiini taxa. For each species this included an electrophysiological survey of the different types of receptor cells present in the male olfactory hair sensilla. The "key compounds" of these cells were then field tested in varying mixture combinations, in order to derive behavioural functions for the different chemicals (and, hence, the corresponding receptor cells).

Results of these studies are presented here for the sole European representative of *Eulia, E. ministrana* L. This large leafroller moth, the first Cnephasiini species described taxonomically [18], is holarctic in distribution and common throughout Central Europe [19]. Recently Booij and Voerman [17] mentioned attraction of *E. ministrana* males to a binary combination of (*Z*)-9- and (*Z*)-11-tetradecenyl acetate (*Z*9-14:Ac, *Z*11-14:Ac) but did not report any capture details. In the present study, *E. ministrana* male attraction was noted in 1979 when field testing, at Seewiesen (Upper Bavaria), binary *Z*9-14:Ac/*Z*11-14:Ac mixtures against the noctuid moth *Polia pisi* L. In these tests the *P. pisi* males responded specifically to the 1/1 combination of the two chemicals [20] (subsequently established to be the natural female pheromone of this species [21]). Contrasting to this pattern, the *E. ministrana* captures occurred over a far broader range of mixture ratios, with a few males even responding to 100 µg of pure *Z*11-14:Ac alone (Table I). In a subsequent series of experiments the specificity of response was found to increase on lowering the overall attractant dose; at the level of 10 µg the *Z*11-14:Ac alone did not reveal significant captures, the preferred mixtures clearly being those containing 3–10% of *Z*9 isomer (Table II).

At this stage the hair sensilla on *E. ministrana* male antennae were analyzed electrophysiologically, using technical procedures and test chemicals as in previous work on other Tortricinae species (see

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Table I. Captures of *Eulia ministrana* males in tetratraps baited with varying combinations of *Z*11-14:Ac and *Z*9-14:Ac. Seewiesen, May 26 to June 12, 1979; six replicates.

Amount of chemical [µg/trap]		$\bar{X}$ males/trap
*Z*11-14:Ac	*Z*9-14:Ac	
100	0	6.2
100	1	25.0
100	3	38.2
100	10	40.8
100	30	26.7
100	100	15.3
30	100	5.0
10	100	0.8
0	100	0

Table II. Captures of *Eulia ministrana* males in tetratraps baited with varying combinations of *Z*11-14:Ac and *Z*9-14:Ac. Seewiesen, June 2 to 16, 1980; four replicates.

Amount of chemical [µg/trap]		$\bar{X}$ males/trap
*Z*11-14:Ac	*Z*9-14:Ac	
10	0	0
10	0.01	0
10	0.03	4.5
10	0.1	8.75
10	0.3	18.75
10	1	24.0
10	3	7.25
10	10	2.0
1	10	0
0	10	0

[22, 23]). The results definitely proved the presence in these sensilla of specialist cells for *Z*11-14:Ac and *Z*9-14:Ac, whereas a third type of cell responded highly-specifically to a stereoisomer, *E*11-14:Ac. Threshold responses to these "key" stimulants usually occurred at 10^{-3} µg or 10^{-2} µg (source load), and the response spectra to analogous chemicals closely conformed with those reported for these types of receptor cells from other tortricid species [23, 24]. All three compounds are common pheromone and attractant components identified from a number of Tortricinae moths [16].

Nerve impulse activity of two further cell types was regularly noted in these recordings. Systematic analysis of responses to test chemicals showed that these cells were specialist receptors for *Z*7-12:Ac and *Z*11-16:Ac, respectively. These cell types were not reported previously from Tortricidae but had already been studied for chemical response specificity in some other male Lepidoptera [24–27].

No evidence for any further cell type present in these sensilla was obtained. In particular, there were no receptor cells specific to further (monoenic or dienic) acetates known from tortricid pheromones (see [16]), or cells sensitive to alcohols or aldehydes. According to the present results the pheromone receptor system of male *E. ministrana* thus appears to be composed of five cell types, specific to *Z*7-12:Ac, *Z*9-14:Ac, *Z*11-14:Ac, *E*11-14:Ac, and *Z*11-16:Ac, respectively.

The three additional "key compounds" disclosed by these recordings were part of many synthetic lures operated against other lepidopteran spp. during the period of *E. ministrana* flight. No male *E. ministrana* ever responded to lures containing > 1% of any of these chemicals, *Z*7-12:Ac, *E*11-14:Ac, or *Z*11-16:Ac. For example, whereas small numbers of males were consistently captured by 100 µg or 1000 µg of pure *Z*11-14:Ac, captures disappeared by adding only 1% of the *E*11 isomer. This rendered attraction functions of these compounds unlikely, and led us to study their effects by third-component tests, using the combination of 10 µg *Z*11-14:Ac + 1 µg *Z*9-14:Ac as the basic lure. Trap types, pheromone dispensers and trap placement procedures were as in previous work on other moth species conducted in this test area (see [20]).

In tests using *E*11-14:Ac as a third component, a 0.3–1% admixture significantly reduced captures in comparison to the basic lure alone, with higher amounts abolishing any catch (Table III). Further test series (results not specified here) failed to show synergistic effects for trace (0.03%, 0.1%; [28]) admixtures of this compound. These data classified the *E*11-14:Ac as a strong sex attraction inhibitor for male *E. ministrana.*

Table III. Captures of *Eulia ministrana* males in tetratraps baited with 10 µg *Z*11-14:Ac + 1 µg *Z*9-14:Ac as the basic lure and *E*11-14:Ac as a third component. Seewiesen, May 25 to June 12, 1981; four replicates.

Amount [µg] of added *E*11-14:Ac	$\bar{X}$ males/trap
0	32.5
0.01	35.0
0.03	21.25
0.1	7.75
0.3	0.5
1	0
3	0
10	0

The *Z*7-12:Ac and *Z*11-16:Ac in analogous series did not show synergistic properties either. Admixtures of *Z*7-12:Ac to the *Z*11-/*Z*9-14:Ac lure proved strongly inhibitory (Tables IV, V), closely comparing to the effects found for the *E*11-14:Ac. With *Z*11-16:Ac, inhibitory effects occurred at approx. 30 fold higher relative amounts (Table IV).

Various further acetate analogues, and some alcohols and aldehydes also, were tested in an analogous manner, using mixture ratios ranging from 10/1/0.03 to 10/1/10. Compared to the 10/1 binary lure alone, none of these chemicals significantly enhanced or totally abolished captures.

Field trapping effects of the three C_{14} acetates as reported here for *E. ministrana* were already known from some other male Tortricinae moths. Thus, several *Pandemis* spp., including *P. pyrusana* Rob., *limitata* Rob. and *heparana* Den. et Schiff., and also the carnation tortrix *Epichoristodes acerbella* Walk., use pheromone blends based on specific *Z*11-/*Z*9-14:Ac mixtures, with the *E*11 isomer strongly inhibiting male attraction responses to these blends [29–34]. In the oak tortrix *Tortrix viridana* L., trap captures in response to *Z*11-14:Ac, the only chemical identified from the conspecific female pheromone secretion, strongly decreased on addition of small amounts of either *Z*9-14:Ac or *E*11-14:Ac [35, 36]. However, an even greater number of Tortricinae species is known to use sex-attractant pheromones based on blends of *Z*11-/*E*11-14:Ac [16]. The strong inhibitory effect of *E*11-14:Ac on *E. ministrana* male attraction may serve to maintain reproductive isolation against such species.

Table IV. Captures of *Eulia ministrana* males in tetratraps baited with 10 μg *Z*11-14:Ac + 1 μg *Z*9-14:Ac as the basic lure and *Z*7-12:Ac or *Z*11-16:Ac as a third component. Seewiesen, May 26 to June 12, 1981; four replicates.

Third chemical, amount [μg]		$\bar{X}$ males/trap
none		29.0
*Z*7-12:Ac,	0.1	4.25
	0.3	0.5
	1	0
	3	0
	10	0
*Z*11-16:Ac,	0.1	26.25
	0.3	31.75
	1	20.75
	3	5.0
	10	0

Table V. Captures of *Eulia ministrana* males in tetratraps baited with 10 μg *Z*11-14:Ac + 1 μg *Z*9-14:Ac as the basic lure and *Z*7-12:Ac as a third component. Seewiesen, May 30 to June 15, 1982; four replicates.

Amount [μg] of added *Z*7-12:Ac	$\bar{X}$ males/trap
0	27.25
0.003	24.0
0.01	28.75
0.03	9.0
0.1	2.25
0.3	0
1	0
3	0

The combination of specialist receptor cells for the three homologues, *Z*7-12:Ac, *Z*9-14:Ac and *Z*11-16:Ac, has been previously reported from some male Noctuidae spp. [20, 24–27, 37]. Male broom moths, *Polia pisi* L., and pine beauty moths, *Panolis flammea* Schiff., both use these three cell types along with specialist receptor cells for *Z*11-14:Ac; the only apparent difference to *E. ministrana* thus is the lack of *E*11-14:Ac receptor cells in these noctuids. Furthermore, in *P. pisi* males the field trapping response to a synergistic combination of *Z*9-/*Z*11-14:Ac decreased on addition of *Z*7-12:Ac or *Z*11-16:Ac as the third component [20], also closely resembling the pattern reported here for *E. ministrana.* This provides a striking example of evolutionary convergence in sex-attractant systems among taxonomically-distant species of moths.

The functional significance of the *Z*7-12:Ac and *Z*11-16:Ac receptor cells in *E. ministrana* is unclear but there is evidence that these compounds do have attractant functions in other Cnephasiini genera. For example, the sexual attractants established for Central European species of *Eana, Neosphaloptera,* and *Tortricodes* all included *Z*7-12:Ac as an essential constituent [38]. Male *Olindia schumacheriana* F., on the other hand, were highly attracted to pure *Z*11-16:Ac alone, with *Z*9-14:Ac inhibiting this response [38]. *Isotrias rectifasciana* Haw. and *I. hybridiana* Hbn., of the same tribe, both use sexual attractants consisting of a 1/1 combination of *Z*9-14:Ac + *Z*11-16:Ac, counteracted by *Z*7-12:Ac as an attraction-inhibitor [15, 18]. Electrophysiological analysis of male antennal hair sensilla in these various Cnephasiini species has established the presence of receptors for *Z*7-12:Ac, *Z*9-14:Ac and *Z*11-16:Ac, such as reported here for *E. ministrana.*

Studies of potential pheromone precursors located in the sex pheromone glands of female

Tortricidae [16, 39–41] have led to an outline of two major biosynthetic routes that could account for the majority of known sex pheromone components in this moth family. Each route starts from a particular saturated fatty acid and involves Δ11-desaturation followed by enzymatic chain shortening. According to this scheme, Δ9-14:Acyl intermediates should have derived from Δ11-16:Acyl precursors [16, 42], thus suggesting that the *Z*11-16:Ac represented a more ancient type of tortricid pheromone than the *Z*9-14:Ac. Whereas this latter compound is known from sex-attractant systems of various tortricid species [16], evidence for the use of *Z*11-16:Ac as a tortricid pheromone component is limited to the two genera mentioned above, *Olindia* and *Isotrias*. These two genera show a number of primitive morphological characters [1, 2] and may represent an old Cnephasiini stock. In light of these considerations, *Eulia* may represent an evolutionary stage that has abandoned the use of *Z*11-16:Ac as a sex-attractant component but retained the corresponding type of specialist receptor cell.

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