Specificity of Synthetic Sex-Attractants in *Zygaena* Moths

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Species-specific sex-attractants are reported for western Palearctic *Zygaena* moths of the *carniolica, viciae, filipendulae*, and *purpuralis* species groups. The attractants were established by combined electrophysiological and field behavioural studies and consist of ternary combinations of (*Z*)-7-dodecenyl/(*Z*)-9-tetradecenyl acetates with a particular further (*Z*)-alkenyl acetate. Data on other Zygaenini taxa are included.

The distribution of the approx. 100 valid species of the genus *Zygaena* F. ("burnet moths") is exclusively limited to the Palearctic faunal region [1–3]. Related genera are found in the Oriental and Ethiopian faunal regions; all of these form the monophyletic tribe Zygaenini [3, 4]. Usually *Zygaena* species are day-flying moths, with the period of flight limited to 3–4 weeks in the summer months. The adult moths have an aposematic pattern which makes field observations on population density and flight behaviour rather easy [5, 6].

The chemical composition of the female sex pheromone is unknown for any species of the Zygaenini tribe. However, several authors have reported captures of *Zygaena* males in sticky traps baited with synthetic pheromones of other Lepidoptera. Thus, (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac), alone or in combination with the corresponding alcohol or (*E*)-11 isomer, was found to attract males of the four closely-related species *Z. transalpina* Esp., *hippocrepidis* Hbn., *angelicae* Ochs. and *ephialtes* L. [7–9]. Renou and Decamps [10] provided electroantennogram (EAG) data that confirmed the specific effect of *Z*11-14:Ac on *Z. hippocrepidis* males. These authors also demonstrated high EAG responses of male *Z. filipendulae* L. to the lepidopteran pheromone (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac) but failed to attract males of this species to synthetic test chemicals in olfactometer and field tests [10].

We have studied sex-attractant systems throughout western Palearctic *Zygaena* moths. In each species, the male antennal *Sensilla trichodea* (generally known to contain pheromone-sensitive receptor cells in the Lepidoptera) were analyzed electrophysiologically with respect to the different cell types present, using test chemicals and experimental procedures as in similar work on other moths [11]. The "key compounds" of the different cell types found were subsequently exposed in the field in varying mixture combinations; those mixtures that showed attractancy were further modified, until response maximum was reached.

In some species the type A cell [12] of the receptor system responded specifically to *Z*11-14:Ac. This pattern applied to *Z. transalpina, hippocrepidis, angelicae* and *ephialtes* (mentioned above), and also to *Z. dorycnii* Ochs., *loti* Den. et Schiff., *armena* Ev., and *laeta* Hbn. In some other species, including *Z. hilaris* Ochs. and *fausta* L., receptor systems with the type A cell specific to the (*E*)-11 isomer, *E*11-14:Ac, were found. The single cell and field trapping data obtained on these species will be considered elsewhere.

Here we report on a group of *Zygaena* moths in which the type A cell of the pheromone receptor system responded specifically to either *Z*7-12:Ac or the longer-chain homologue, *Z*9-14:Ac. The species studied included: *Z. filipendulae* L., *trifolii* Esp., *lonicerae* Schev., *viciae* Den. et Schiff., *lavandulae* Esp., *anthyllidis* Bsd., *exulans* Hoch., *romeo* Dup., *osterodensis* Reiss, *rhadamanthus* Esp., *sarpedon* Hbn., *thevestis* Stgr., *tamara* Chr., *minos* Den. et Schiff., *purpuralis* Brünn. and *carniolica* Scop. To the best of our knowledge, males of these species have not previously been attracted to synthetic compounds in laboratory or field tests.

For 12 *Zygaena* species and two members of the closely related genera *Reissita* and *Epizygaenella* the specific cell type combinations found are listed in Table I. Each of the seven cell types involved responded specifically to a particular (*Z*)-alkenyl acetate, *viz.* (*Z*)-5-, (*Z*)-7- and (*Z*)-9-dodecenyl

* 35th contribution to the knowledge of the genus *Zygaena* Fabricius 1775 and related genera (Insecta, Lepidoptera, Zygaenidae).

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Table I. Cell type combinations in male olfactory hair sensilla [a], and composition of optimized synthetic sex-attractant, for selected species of *Zygaena*, *Reissita* and *Epizygaenella*.

Test species	Specialist receptor cell for [b]							Synthetic attractant
	Z5-12: Ac	Z7-12: Ac	Z9-12: Ac	Z7-14: Ac	Z9-14: Ac	Z11-14: Ac	Z11-16: Ac	
Z. filipendulae	x	xA			x			Z5-12:Ac/Z7-12:Ac, Z9-14:Ac, ratio 3/100/10
Z. trifolii	x	xA		?	x			same compounds, ratio 100/100/30 (100/100/10)
Z. lonicerae	x	xA			x			
Z. viciae	x	xA			x		?	same compounds, ratio 10/100/10
Z. anthyllidis	?	xA	?		x			
Z. exulans		x	x		xA			
Z. sarpedon		x	x		xA			
Z. thevestis		x			xA	x		
Z. tamara		xA	x		x	x		
Z. minos		x	?		xA	x		
Z. purpuralis		xA	?	x	x			Z7-12:Ac/Z7-14:Ac/Z9-14:Ac, ratio 100/10/100
Z. carniolica		xA	x		x		x	Z7-12:Ac/Z9-14:Ac/Z11-16:Ac, ratio 100/30/3
R. simonyi		x	?		xA		x	
E. caschmirensis			?		xA	x	x	

[a] x, presence of cell type fully established (A, type A cell of receptor system); ?, questionable evidence; empty space, cell type not found in indicated species.
[b] The seven (*Z*)-alkenyl acetates are listed with first the position of the olefinic double bond and secondly the number (−12 till −16) of C atoms in the alcohol moiety.

acetate (*Z*5-12:Ac, *Z*7-12:Ac, *Z*9-12:Ac); (*Z*)-7-, (*Z*)-9- and (*Z*)-11-tetradecenyl acetate (*Z*7-14:Ac, *Z*9-14:Ac, *Z*11-14:Ac); and (*Z*)-11-hexadecenyl acetate (*Z*11-16:Ac). There was no evidence for any additional cell type (responsive to a further, monoenic or dienic acetate, or an alcohol or aldehyde analogue) present in the 12 *Zygaena* species listed. The above (*Z*)-alkenyl acetates elicited nerve impulse responses from the respective cell type at the low stimulus amount of 10^{-2} µg, the usual threshold value obtained with lepidopterous pheromone receptor cells under the chosen experimental setup. In addition, the graded responses of these cells towards a broader set of analogous test chemicals (not listed here) closely conformed with the response spectra reported for (*Z*)-alkenyl acetate receptor cells in other moth species [11, 14–17], including species known to use these compounds as pheromone components. This is further evidence that the above alkenyl acetates should be the true "key compounds" of these male *Zygaena* receptor cells and, thus, the candidate structures for the as yet unidentified female pheromones of these species. All 16 species listed had receptor cells for both *Z*7-12:Ac and *Z*9-14:Ac, combined with 1 or 2 further cell types in each species (Table I).

The above (*Z*)-alkenyl acetates were tested for attractancy towards wild *Zygaena* males in approx. 100 mixture combinations. Most experiments were carried out on the basis of visual observations of male flight responses, while in the beginning we used sticky traps. The compound mixtures (major component(s), 100 µg) were applied to red serum bottle caps (∅ 1.8 cm) which were fixed to strips of cardboard (8 × 2 cm). These "bait-strips" were suspended from a horizontally-stretched string at a height of 0.7 m and at distances of 2 m from each other. The relative positions of the "bait-strips" were altered during the course of the experiments and the numbers of *Zygaena* males attracted, and eventual landing and close-range responses [18] were noted continuously. Given a responsive male population and appropriate external test conditions [18], the order of preference among 20 different

lures could thus be established within approx. 2 hours.

For the *Zygaena* species treated here, the optimized sexual attractants all consisted of combinations of Z7-12:Ac/Z9-14:Ac with a particular third compound, in a specific mixture ratio. Ternary combinations with Z5-12:Ac attracted *Z. filipendulae, viciae* and *trifolii* (for optimum ratio, see Table I), and also *Z. lonicerae* (optimum ratio yet to be determined). Males of *Z. purpuralis* and *Z. carniolica* did not respond to these mixtures but were highly attracted to ternary combinations with Z7-14:Ac and Z11-16:Ac, respectively (Table I). In *Zygaena* species where the type A cell responded specifically to Z9-14:Ac (such as the high-alpine *Z. exulans*), only mixtures based on this compound as the major constituent were attractive.

The specificity of response was pronounced with all test species. Male *Z. filipendulae*, for example, were maximally attracted to the 3/100/10 combination of Z5-12:Ac/Z7-12:Ac/Z9-14:Ac; these same compounds in the ratios 10/100/10, 10/100/3, 100/100/30 or 3/100/30 attracted much less males. With *Z. trifolii*, mixtures of these compounds of 100/100/30 and 100/100/10 were highly attractive, whereas the 100/100/100, 100/100/3 or 30/100/30 mixtures elicited only one-tenth as many flight approaches. Analogously in *Z. purpuralis*, males responded maximally to the combination of Z7-12:Ac/Z7-14:Ac/Z9-14:Ac of 100/10/100, but only weakly to ratios such as 100/10/10 or 100/100/100. Binary combinations of Z7-12:Ac/Z9-14:Ac elicited occasional flight attempts in *Z. filipendulae* and *Z. purpuralis* but were totally unattractive for *Z. viciae* or *Z. trifolii* males.

The mixtures ratios indicated in Table I applied to Central European test populations. With some species we have obtained evidence for geographic variation of the optimally attractive compound combination. For example, in field tests conducted in Spain (Prov. Gerona) in June 1981, males of a five-spotted population of *Z. filipendulae* were strongly attracted to Z5-12:Ac/Z7-12:Ac/Z9-14:Ac mixtures with a high Z5-12:Ac portion, such as the 100/10/10 ratio, and even responded to the binary combination of Z5-12:Ac/Z7-12:Ac of 100/10, but showed only weak responses to ternary mixture ratios such as preferred by Central European *filipendulae* populations. *Z. carniolica* males in Central Europe responded strongly to ternary mixtures of Z7-12:Ac/Z9-14:Ac/Z11-16:Ac (see Table I) whereas in some test populations from eastern Turkey the males were more responsive to combinations of Z7-12:Ac/Z9-14:Ac with another "key compound", Z9-12:Ac. Such data indicate the need of systematically testing the synthetic attractant mixtures over the geographic range of distribution in some *Zygaena* species.

Included in Table I were *Reissita simonyi* Rebel and *Epizygaenella caschmirensis* Koll. These two genera exhibit a number of morphological characters that are considered to link *Zygaena* with the phylogenetically more ancient Ethiopic Zygaenini [3, 4]. *R. simonyi* (a species endemic to the Arabian Peninsula) appears to use the same four cell types as found in *Z. carniolica*, whereas in *E. caschmirensis* (from the Southern Himalaya) the receptor system showed a unique combination of cell types for Z9-14:Ac, Z11-14:Ac and Z11-16:Ac. It is noteworthy that the combination of the former two cell types also occurred in South African species of *Orna* (not listed in Table I), which are presumed to have preserved a great number of primitive characters among recent Zygaenini [3, 4]. In *Zygaena*, we have found the combination of receptor cells for Z9-14:Ac and Z11-14:Ac in *Z. thevestis, Z. minos* and *Z. tamara*. They belong to the subgenus *Mesembrynus* which is believed to have retained a number of plesiomorphic characters with reference to the ground plane of the genus [4].

The optimized (ternary) *Zygaena* attractants elicited a sequence of male behavioural actions [18] identical to those observed in response to conspecific, calling females. The outstanding attractive potency of these synthetic lures was particularly evident when relating the numbers of responding males to the size of local, isolated populations. For example in *Z. trifolii*, a local population studied near Diepholz (Oldenburg, northern Germany), restricted to an area of approx. 100×200 m, was estimated to consist of 200–250 males at the time of testing. From this population the synthetic lure elicited 130 attraction responses (approx. 100 individuals) on a single day. Similarly in *Z. purpuralis*, in an isolated population near Höxter (Weserbergland, northern Germany), 30 males responded to the synthetic attractant within a few minutes after exposure; the total population in this area (approx. 50×300 m) was estimated to be not more than 100 male moths. Throughout such tests, less than

0.5% of other *Zygaena* species were attracted, even when present in larger numbers within the test area.

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