

Sex Pheromone Communication in the Family Zygaenidae (Insecta: Lepidoptera): a Review

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Abstract: Recently about sex pheromone communication in the family Zygaenidae a significant information is available for quite a lot of species of the subfamilies Zygaeninae and Procridinae and a very scarce information for only one species of the subfamily Chalcosiinae while nothing is known in this respect about the members of the fourth zygaenid subfamily, Callizygaeninae. Besides historical remarks the review covers the main aspects of the sex pheromone communication in each Zygaenidae subfamily separately which includes: sex pheromone glands, female reproductive behaviour: calling behaviour, chemistry of the sex pheromones and sex attractants known, male reproductive behaviour: searching behaviour, diurnal rhythms of pheromone communication. In addition, examples of use of synthetic pheromones for the control of zygaenid pests and use of sex pheromone traps for faunistic investigations of zygaenids are also presented.

The only zygaenid species mentioned in the comprehensive review book “Insect sex pheromones” by MARTIN JACOBSON (JACOBSON, 1972) is *Zygaena filipendulae* (LINNAEUS, 1758), but this was only to cite two old papers reporting the attraction of males of this species by virgin females of *Lasiocampa quercus* (LINNAEUS, 1758). Up to now, of the four Zygaenidae subfamilies currently recognized (TARMANN, 2004), significant information about sex pheromone communication is available for the subfamilies Zygaeninae and Procridinae; evidence for such communication is available for only one member of the subfamily Chalcosiinae, and nothing is known in this respect about Callizygaeninae species.

The first report on sex pheromone communication of a member of the subfamily Zygaeninae was that of ZAGATTI, RENOU (1984) who found that caged *Z. filipendulae* females strongly attracted conspecific males in the field. Subsequently, such attraction was demonstrated for many other *Zygaena* species (Tremewan, 2006; HOFMANN, KIA-HOFMANN, 2010). The first report of attraction of Procridinae species males to conspecific females was that of DOLIDZE et al. (1980) who used caged *Theresimima ampelophaga* (BAYLE-BARELLE, 1808) females to catch

males of the same species in the field. Further experimental evidence about using pheromone signals of the females of this species for attracting conspecific males was provided by SUBCHEV, HARIZANOV (1990). Subsequently, attraction of males to conspecific calling females indicating sex pheromone communication was also observed in some other Procridinae species (TANAKA, KOSHIO, 2002; EFETOV, 2001). The only Chalcosiinae species for which such sex pheromone communication was proven is *Elcysma westwoodii* (SNELLEN VAN VOLLENHOVEN, 1863) – females of this species placed in Petri dishes with holes attracted conspecific males in the field, while females in Petri dishes without holes did not (KOSHIO, HIDAKA, 1995).

The simplest scheme of any communication comprises sender – signal – channel – received signal – receiver in which the “received signal” differs from the “signal” sent by the sender by a possible noise that has been added (BAKER, 1993). In sex pheromone communication in insects, and in the zygaenid moths in particular, the most common sender is the female and the receiver is the male. In this case the main components of the sender for sending signals are the sex pheromone glands which produce

and release the pheromone and the specific so-called “calling behaviour”, which is characterized by a specific calling posture. The organs that percept the pheromone signal in the males are their antennal sensilla trichoidea with the so-called “odour specialists” receptor cells. Perception of the signal evoked a specific behaviour in males, starting with a directed flight and ending with copulation.

Sex pheromone glands

According to PERCY-CUNNINGHAM, MACDONALD (1987), up to the time of publishing their review on the sex pheromone producing glands in insects, female moths of 16 families of Lepidoptera had been examined, the sex pheromone glands identified, and the structural characteristics of the gland cells at least partly identified, but the family Zygaenidae was not among them.

Subfamily Zygaeninae

To the best of my knowledge no special morphological and histological investigations on the sex pheromone glands in Zygaeninae are available. ZAGATTI, RENOU (1984) stated that the sex pheromone gland in *Z. filipendulae* is a yellow “pocket” located dorsally on the intersegmental membrane between the 8th and 9th segments. This is supported by a picture of a calling female but not by any morphological and histological evidence. There is also a statement by Clas Naumann, cited as “unpublished”, in BODE, NAUMANN (1988) saying that: “In *Zygaena* the actual sex pheromone gland is located in the dorsal intersegmental fold between the 8th and 9th abdominal segment” but again this was not supported by published results, neither in the cited paper nor anywhere else. Actually, this is the common location of female sex pheromone glands in almost all Lepidoptera (PERCY-CUNNINGHAM, MACDONALD, 1987) and thus there is no reason to hesitate that this is the case also in the members of Zygaeninae. This was supported by observations by HOFMANN, KIA-HOFMANN (2010) on the calling behaviour of other *Zygaena* species in which females extrude their abdominal tips at the time of calling and attract conspecific females, as in the case of *Z. filipendulae* (see below). The different calling postures in females belonging to species of the genus *Neurosymphoca* described by the same authors (see below) could indicate a different distribution of the gland cells not forming a dorsal pocket-like gland but, for example, a ventrally located gland or ring-like pheromone gland.

Subfamily Procridinae

In order to find the position of the glands secreting

the sex pheromone in *T. ampellophaga*, a member of the subfamily Procridinae, extracts of different parts of the abdomen of females of this species were tested for biological activity and, as a result, it was found that among the extracts of abdominal tips, dorsal abdominal integument and ventral abdominal integument, only extracts of the dorsal abdominal integument clearly excited conspecific males (SUBCHEV, HARIZANOV, 1990). This location was confirmed by further investigations by electron microscopy which showed that the gland cells forming the sex pheromone gland in *T. ampellophaga* females are situated on the anterior part of the 3rd-5th abdominal tergites and the glandular epithelium of the gland consists of two cell types: gland cells with a central microvillined cavity and wrapping cells (HALLBERG, SUBCHEV, 1997). This location coincides well with the specific calling behaviour in the females of this species (see below). The sex pheromone secreting section was also described in the females of another Procridinae species, viz. *Illiberis pruni* DYAR, 1905 (LI *et al.*, 1996a), but unfortunately the paper is in Chinese and no details are provided in the short English summary. Bearing in mind that the specific location of the pheromone glands in Lepidoptera females correlates with the specific calling posture, and available information on the calling posture of females of many other Procridinae species (see below), we could predict that the location of the sex pheromone gland in *T. ampellophaga* is common for all members of the tribe Procridini of the zygaenid subfamily Procridinae and most likely also for the members of the second Procridinae tribe, the Artonini (see below). This specific location of the pheromone gland, which is unique within the Lepidoptera (a similar location is known only for the family Psychidae (SUBCHEV, 2003)), coincides well with the specific structure of the sex pheromone compounds known for the Procridini members (see below).

Subfamily Chalcosiinae

There is no information about any sex pheromone glands in members of this subfamily.

Female reproductive behaviour: calling behaviour

Subfamily Zygaeninae

The first special observations on the calling behaviour of *Zygaena* species was that of ZAGATTI, RENOU (1984) on *Z. filipendulae*. The authors established that at the time of attraction of conspecific males in nature, the females of this species are motion-

less with the extruded tip of the abdomen exposing a pocket-like yellow pheromone gland located dorsally on the intersegmental membrane between the 8th and 9th abdominal segments. TOSHOVA *et al.* (2008) have observed that the females of *Z. niphona* BUTLER, 1877, also extrude their abdominal tips when calling. Such calling behaviour was observed in females of some other *Zygaena* species: *Z. tamarra* CHRISTOPH, 1889; *Z. hindukuschi* KOCH, 1937; *Z. favonia* FREYER, 1844; *Z. loyselii* OBERTHÜR, 1876; *Z. algira* BOISDUVAL, 1834; *Z. carniolica* (SCOPOLI, 1763); *Z. truchmena* EVERSMAAN, 1854; *Z. dorycnii* OCHSENHEIMER, 1808; *Z. armena* EVERSMAAN, 1851; *Z. nevadensis* RAMBUR, 1858; *Z. aurata* BLACHIER, 1905; *Z. youngi* ROTHSCHILD, 1926; *Z. alluaudi* OBERTHÜR, 1922; *Z. fausta* (LINNAEUS, 1767); *Z. marcuna* OBERTHÜR, 1888; *Z. maroccana* ROTHSCHILD, 1917; *Z. transalpina* (ESPER, 1780); *Z. angelicae* OCHSENHEIMER, 1808); *Z. seitzi tenhageni* HOFMANN & TREMEWAN, 2003; *Z. haematina* KOLLAR, 1849; *Z. felix* OBERTHÜR, 1876; *Neurosymploca* sp. (HOFMANN, KIA-HOFMANN, 2010). The same authors noted that *Zygaena* females extrude the abdominal tip with the pheromone gland in a prolonged form, while in the calling females belonging to species of the genus *Neurosymploca* the last abdominal segments and the extruded abdominal tip with the pheromone gland are curved upward, which suggests a different location of the pheromone gland (see above).

Subfamily Procridinae

For the first time an unusual female calling behaviour for a member of the subfamily Procridinae and for Lepidoptera was reported by SUBCHEV, HARIZANOV (1990). The observations made by one of the latter authors (M. SUBCHEV) have revealed that *T. ampellophaga* females, at the time of active attraction of the conspecific males, instead of protruding the abdominal tip with the pheromone gland on it, as is the case with almost all Lepidoptera families with well-developed sex pheromone communication, they curve their abdomens dorsally and keep this posture for hours. Further detailed investigations have shown also that in the calling females the antennae are directed forward and the wings are spread and directed downward; thus the proximal parts of the first dorsal tergites that are usually covered by the preceding ones become exposed (TOSHOVA, SUBCHEV, 2003). This unusual calling behaviour completely coincides with the position of the pheromone gland in this species (see above). A similar calling posture was subsequently observed in *I. pruni* (LI *et al.*, 1996b; NISHIHARA, WIPKING, 2003), *I. rotundata* (TANAKA, KOSHIO, 2002; TOSHOVA, SUBCHEV, 2005),

and also in members of three other Procridinae genera: *Rhagades*: *R. pruni* ([DENIS & SCHIFFERMÜLLER], 1775) (EFETOV, 2001; TOSHOVA, SUBCHEV, 2005); *Zygaenoprocris*: *Z. taftana* (ALBERTI, 1939); and *Jordanita*: *J. budensis* (ALBERTI, 1939), *J. subsolana* (STAUDINGER, 1862) and *J. chloros* (HÜBNER, 1813) (EFETOV, 2001). EFETOV (2001) also mentioned the genus *Adscita*, but provided no illustration, as in the case with the species of the above-mentioned genera, nor was any other evidence provided to support this. Recently a calling behaviour similar to that of *T. ampellophaga* females was observed also in females of the North American Procridini species *A. rectorius* and *N. constans* (TARMANN, personal communication). New data (TARMANN, personal communication) have shown also that not only the females of the representatives of the tribe Procridini, to which belong all Palaearctic and Nearctic Procridinae, but also females of the Australian genus *Pollanisus*, which belongs in the tribe Artonini, display a calling behaviour different from that in Zygaeninae and in most Lepidoptera families: *Pollanisus* females do it in a similar but slightly different way from that observed in Procridini females – they spread their wings like *T. ampellophaga* females and expose the dorsal part of the abdomen, but the latter is not bent downward but is almost straight; in addition, they vibrate the abdomen at a high frequency, which most likely helps to diffuse the pheromone from the glands.

Subfamily Chalcosiinae

KOSHIO, HIDAKA (1995) showed experimentally that *E. westwoodii* females attracted conspecific males by using pheromones, but they failed to describe a clear calling posture for this species.

Sex pheromones and sex attractants

In this paper the generally accepted definitions of sex pheromones and sex attractants in insects and in Lepidoptera in particular are used: sex pheromones are chemical(s) identified in the pheromone gland of one of the sexes (the common case in lepidopterans is in the females) of a certain species which, after synthesis, show biological activity, i.e. they attract and/or excite the individuals of the opposite sex, while sex attractants are synthetic pheromone compounds which were found just to attract and/or excite one of the sexes in a certain species. In the latter case there is no information on whether these attractant chemical(s) are present in the pheromone glands of the sex which attracts, i.e. we have no information on whether these attractants are natu-

ral products. Thus we can call the sex pheromone compound(s) of a certain species also sex attractants (after proving their biological activity) but cannot call each a synthetic sex attractant sex pheromone before these have been proved, which means before finding this attractant compound(s) as a natural product in the sex pheromone gland of the corresponding females.

Subfamily Zygaeninae

According to EL-SAYED (2012), sex pheromones are known for five *Zygaena* species: *Z. carniolica*, *Z. filipendulae*, *Z. purpuralis* (BRUNNICH, 1763), *Z. trifolii* (ESPER, 1783) and *Z. viciae* ([DENIS & SCHIFFERMÜLLER], 1775). Actually, in the cited reference (PRIESNER et al., 1984), none of these species' sex pheromone identification results was presented and thus we should accept the compounds given for the species only as their sex attractants (see above). Thus, surprisingly, no sex pheromone for any Zygaeninae species is known (published) so far.

The known sex attractants for *Zygaena* species are presented in Table 1.

Investigations on the sex pheromone communication of zygaenids, and their sex attractants in particular, were organized by E. PRIESNER in collaboration with C. M. NAUMANN in the early 1980s. Unfortunately these investigations were not completed and most likely many results remain unpublished because of the unexpected disappearance of the organizer of the project, E. PRIESNER. In the only paper to be published (PRIESNER et al., 1984), the results of electrophysiological investigations on antennal receptor cells of 12 *Zygaena* species and on *Reissita simonyi* (REBEL, 1899) and *Epizygaenella caschmirensis* (KOLLAR, 1844) responding specifically to certain insect pheromone compounds were

presented. In the same paper, preliminary (no details about methods used and experimental results obtained were given) information about sex attractants for six *Zygaena* species was also reported. A ternary combination of (*Z*)-7-dodecenyl acetate (Z7-12Ac) + (*Z*)-9-tetradecenyl acetate (Z9-14Ac) + (*Z*)-11-hexadecenyl acetate (Z11-16Ac) or (*Z*)-5-dodecenyl acetate (Z5-12Ac) + Z7-12Ac + Z9-14Ac or Z7-12Ac + (*Z*)-7-tetradecenyl acetate (Z7-14Ac) + Z9-14Ac was found to be attractive to males of these six species, but the optimal ratio of the compounds for attraction of only five species (*Z. carniolica*, *Z. filipendulae*, *Z. purpuralis*, *Z. trifolii* and *Z. viciae* ([DENIS & SCHIFFERMÜLLER], 1775)) was specified, while for the sixth one, *Z. lonicerae* (SCHEVEN, 1777), this ratio was not defined (Table 1). In another *Zygaena* species, *Z. niphona*, Z7-12Ac and Z9-14Ac elicited the strongest EAG response from the males, so it is supposed that these compounds were included in the pheromone system of this species (TOSHOVA et al., 2008). Preliminary field tests support this statement (HORIE, TOSHOVA, SUBCHEV, unpublished) but further tests are needed to confirm it. Sex attractants for another four *Zygaena* species (*Z. ephialtes* (LINNAEUS, 1767), *Z. hippocrepidis* (HÜBNER, 1799), *Z. scabiosae* (SCHEVEN, 1777), *Z. transalpina* (ESPER, [1780])) have been established by field screening in different countries in Europe (Table 1). Single specimens (1-2) of *Z. fausta*, *Z. nevadensis gallica* OBERTHÜR, 1898, and *Z. hilaris* OCHSENHEIMER, 1808, were caught in traps baited with synthetic pheromones (MAYER, McLAUGHLIN, 1991; EL-SAYED, A. M. 2012) but these were most probably occasional catches. GRICHANOV et al. (1995) reported on catches of *Z. filipendulae* in traps containing (*Z*)-11-tetradecenyl acetate (Z11-14Ac) and (*E*)-11-tetradecenyl acetate (E-11-14Ac); however, because of missing details in the methods

Table 1. Sex attractants for members of subfamily Zygaeninae. Z8-12Ac = (*Z*)-8-dodecenyl acetate, E8-12Ac = (*Z*)-8-dodecenyl acetate, Z11-14OH = (*Z*)-11-tetradecenol; for the full names of the other compounds – see the text

Species	Sex attractant	References
<i>Z. carniolica</i>	Z7-12Ac+Z9-14Ac+Z11-16Ac (100:30:3)	PRIESNER et al., 1984
<i>Z. ephialtes</i>	Z11-14Ac	DECAMPS et al., 1981
<i>Z. filipendulae</i>	Z5-12Ac+Z7-12Ac+Z9-14Ac (3:100:10)	PRIESNER et al., 1984
<i>Z. hippocrepidis</i>	Z11-14Ac	DECAMPS et al., 1981
<i>Z. lonicerae</i>	Z5-12Ac+Z7-12Ac+Z9-14Ac (?:?:?)	PRIESNER et al., 1984
<i>Z. purpuralis</i>	Z7-12Ac+Z7-14Ac+Z9-14Ac (100:10:100)	PRIESNER et al., 1984
<i>Z. osterodensis (scabiosae)</i>	Z8-12Ac+E8-12Ac (24:1)	HRDY et al., 1989
<i>Z. transalpina</i>	Z11-14Ac+Z11-14OH (?:?)	BENZ VON SALIS, 1973
<i>Z. trifolii</i>	Z7-12Ac+Z9-14Ac+Z11-16Ac (100:100:30/10)	PRIESNER et al., 1984
<i>Z. viciae</i>	Z7-12Ac+Z9-14Ac+Z11-16Ac (10:100:10)	PRIESNER et al., 1984

used (purity of the compounds used, number of traps used, statistics etc.) and relatively low catches – mean of 11.3 per trap for more than 70 days, these results are not reliable.

An interesting phenomenon in the sex pheromone communication is cross attraction, i.e. attraction of males of one species by females of another, which indicates similarities in the sex pheromone of these two species, e.g. common main pheromone component or components. Usually in such cases the species isolation is based on different isolating mechanisms that also include a different ratio of the sex pheromone components in cases where species use multiple compounds in their specific pheromone. HOFMANN, KIA-HOFMANN, (2010) reported on cross attraction in the following *Zygaena* species: *Z. storaiae flaugeri* REISS & REISS, 1974, males to females of *Z. transpamirina andarabensis* KOCH, 1938, and *Z. youngi* ROTHSCHILD, 1926, and *Z. alluaudi* OBERTÜR, 1922, males to females of *Z. algira* BOISDUVAL, 1834. In the same paper further examples of cross attraction were reported but these concern the attraction of males to females of more than one species caged together and thus it is not clear which females of a species were attracted to the males of a certain species.

Subfamily Procridinae.

The first successful identification of a sex pheromone for a member of the subfamily Procridinae (although incomplete – see below) was that of *Harrisina brillians* BARNES & MCDUNNOUGH, 1910 (this nominal taxon is a synonym of *Harrisina metallica* STRETCH, 1885) by MYERSON *et al.* (1982) who isolated 2-butyl (7Z)-tetradecenoate (7-14) from the volatiles emitted from females of this species and found that the synthetic racemic mixture of this compound was biologically active towards conspecific males in the laboratory. Subsequently, in field tests, the active enantiomer was found to be (2S)-butyl (7Z)-tetradecenoate (S-7-14) (SODERSTROM *et al.*, 1985). For the first time the full stereochemistry was unambiguously defined for the sex pheromone of a member of the Zygaenidae, and the subfamily Procridinae in particular, by SUBCHEV *et al.* (1998, erratum – 1999) – the sex pheromone of *T. ampellophaga* was identified by couples GC-EAG, GC-MS and synthesis as (2R)-butyl (7Z)-tetradecenoate (R-7-14); a small amount of the S enantiomer (2S)-butyl (7Z)-tetradecenoate (S-7-14) was also found in female pheromone gland extracts. Subsequently, two sex pheromone compounds, 2-butyl (7Z)-dodecenoate (7-12) and 2-butyl (9Z)-tetradecenoate (9-14), were identified for another Procridinae species, *Illiberis rotundata* JORDAN, 1907. By bioassay

the active enantiomers for this species proved to be (2R)-butyl (7Z)-dodecenoate (R-7-12) and (2R)-butyl (9Z)-tetradecenoate (R-9-14) (SUBCHEV *et al.*, 2009). The most active ratio of these two compounds for *I. rotundata* males was found to be 30 : 100–50 : 100 (SUBCHEV *et al.*, 2011). As early as the 1990s, LI *et al.* (1997a) had tried to determine the sex pheromone of another *Illiberis* species, *I. pruni*, but the identification was only partial – they found that extracts of females contain unsaturated butyl dodecenoate with unknown stereochemistry and an unknown number and position of the double bond(s). Recently, preliminary field tests conducted in Japan have shown that the males of this species are attracted to R-7-12 (SUBCHEV *et al.*, 2011) and further experiments revealed an optimal attractant mixture of R-7-12 and R9-14 in a ratio of 100 : 10 (SUBCHEV *et al.*, 2013). On the other hand, when comparing the published mass spectrum of the natural butyl dodecenoate (LI *et al.* 1997a) and the mass spectrum of synthetic R-7-12, a full coincidence was found (SUBCHEV *et al.* 2009). The latter fact and the activity of R-7-12 as an attractant for *I. pruni* males reported in the present paper strongly suggest that this compound is the main sex pheromone component in this species (SUBCHEV *et al.*, 2013).

Cross attraction was observed for the following Procridini species: *T. ampellophaga* males to *R. pruni* females and *R. pruni* males to *T. ampellophaga* female; *Rhagades amasina* males to *R. pruni* females and *Jordanita horni* males to *J. subsolana* females (EFETOV, 2001).

It is worth mentioning that there are indications that although the sex pheromone system in Artonini seems to be similar to that of Procridini, at least as regards female calling behaviour (see above), the pheromone compounds used by the members of the former tribe are most likely different from those known as sex pheromones for Procridini species – none of the latter showed any attraction to Australian Artonini in a large field screening trial in that country (TARMANN, TOSHOVA, unpublished). Of course, this fact is not enough to exclude that some compounds could be common as a sex pheromone component to these two Procridinae tribes.

Actually, our knowledge about the chemistry of Procridinae species is still very poor. However, analogous to the case with the common sex pheromone compounds of most Lepidoptera, which are, in most cases, acetates, alcohols and aldehydes with 10-18 C chain and one or two double bonds, we can expect the existence of Procridinae sex pheromones with a longer and shorter C chain than the presently known 12 and 14 ones, with more than

Table 2. Sex attractants for members of the subfamily Procridinae

Species	Sex attractant	References
<i>Acoloitus falsarius</i>	(2R)-butyl (9Z)-tetradecenoate	LANDOLT, P., R. HEATH (1991)
<i>A. novaricus</i>	(2S)-butyl (9Z)-tetradecenoate	LANDOLT, P., R. HEATH (1991)
<i>A. rectarius</i>	(2R)-butyl (9Z)-tetradecenoate	TARMANN, G., M. SUBCHEV, unpublished
<i>Adscita geryon</i>	(2S)-butyl (7Z)-dodecenoate	SUBCHEV <i>et al.</i> , 2010 EFETOV <i>et al.</i> , 2014
<i>A. manni</i>	(2S)-butyl (7Z)-dodecenoate	SUBCHEV <i>et al.</i> , 2010 EFETOV <i>et al.</i> , 2014
<i>Harrisina americana</i>	(2R)-butyl (9Z)-tetradecenoate	LANDOLT, P., R. HEATH, 1991)
<i>H. coracina</i>	(2S)-butyl (9Z)-tetradecenoate	LANDOLT, P., R. HEATH, 1991
<i>H. guatemalena</i>	(2S)-butyl (9Z)-tetradecenoate	LANDOLT, P., R. HEATH, 1991
<i>Illiberis pruni</i>	(2R)-butyl (7Z)-dodecenoate (2R)-butyl (9Z)-tetradecenoate	SUBCHEV <i>et al.</i> , 2013
<i>Jordanita anatolica</i>	(2R)-butyl (7Z)-dodecenoate (2S)-butyl (7Z)-dodecenoate	EFETOV <i>et al.</i> , 2010
<i>J. horni</i>	(2R)-butyl (7Z)-dodecenoate (2S)-butyl (7Z)-dodecenoate	EFETOV <i>et al.</i> , 2011
<i>J. notata</i>	(2R)-butyl (7Z)-dodecenoate	SUBCHEV <i>et al.</i> , 2010 EFETOV <i>et al.</i> , 2014
<i>Neoalbertia constans</i>	(2S)-butyl (9Z)-tetradecenoate	TARMANN, G., M. SUBCHEV, unpublished
<i>Neoilliberis fusca</i>	(2R)-butyl (9Z)-tetradecenoate	TARMANN, G., M. SUBCHEV, unpublished
<i>Neoprocris aversa</i>	(2R)-butyl (9Z)-tetradecenoate	TARMANN, G., M. SUBCHEV, unpublished
<i>Pyromorpha dyari</i>	(2S)-butyl (7Z)-dodecenoate	TARMANN, G., M. SUBCHEV, unpublished
<i>Rhagades pruni</i>	(2R)-butyl (7Z)-dodecenoate	SUBCHEV <i>et al.</i> , 2010
<i>Tripocris cyanea</i>	(2S)-butyl (9Z)-tetradecenoate	TARMANN, G., M. SUBCHEV, unpublished
<i>Zygaenoprocis chalcoclora</i>	(2R)-butyl (7Z)-dodecenoate + (2R)-butyl (9Z)-tetradecenoate OR (2R)-butyl (7Z)- dodecenoate	HOFMANN, A., M. SUBCHEV unpublished
<i>Z. eberti</i>	(2R)-butyl (7Z)-dodecenoate + (2R)-butyl (9Z)-tetradecenoate OR (2R)-butyl (7Z)- dodecenoate	HOFMANN, A., M. SUBCHEV unpublished
<i>Z. taftana</i>	(2R)-butyl (7Z)-dodecenoate	EFETOV <i>et al.</i> , 2011

one double bond in the chain and with a site for the double bond/s different to that of the ones known so far. Evidence to support this is the fact that preliminary results on the identification of the *Jordanita subsolana* (STAUDINGER, 1862) sex pheromone have shown the presence of two compounds, 2-butyl dodecenoate and a 2-butyl dodecadienoate, the latter being the first doubly unsaturated Procridinae pheromone (W. FRANCKE, personal communication).

After successful identification and synthesis of pheromone compounds in *H. metallica*, *T. ampellophaga* and *I. rotundata*, by field screening these compounds, sex attractants for a total of 19 Procridinae species were recognised (Table 2).

Screening of the different enantiomers of 7-14 resulted in revealing sex attractants for five north American Procridinae species, including

two *Acoloitus* species and three *Harrisina* species (LANDOLT *et al.*, 1986; LANDOLT, HEATHY, 1991). After identification of the sex pheromone of *T. ampellophaga*, R-7-14, its four synthetic enantiomers were screened in field trials in several countries in Europe, but only examples of the target species were caught (SUBCHEV *et al.*, 2003; 2006; 2008a; 2008b, RAZOV *et al.*, 2014): the same results were obtained also in the Asian part of Turkey (EFETOV *et al.*, 2001). Much more successful was the field screening of the synthetic pheromone compounds identified in *I. rotundata* and their enantiomers in both Europe and Asia. By large-scale field screening of the latter compounds using sticky traps in Europe, sex attractants were found for *Adscita geryon*, *A. manni*, *Jordanita notata* and *Rhagades pruni* (SUBCHEV *et al.*, 2010). The sex attractants of *A. manni* and *J.*

notata were further confirmed by visual observations of male flight responses to different lures in the field (EFETOV *et al.*, 2014). Another field experiment in Turkey revealed that *Jordanita anatolica* (NAUFOCK, 1929) males were very strongly attracted to a 1 : 1 mixture of R-7-12 and (2*S*)-butyl (7*Z*)-dodecenoate (S-7-12). By using visual observations, sex attractants were found also for *Zygaenoprocris taftana* and *Jordanita horni* in Armenia (EFETOV *et al.* (2011). Single males of *Adscita albanica* were caught in sticky traps baited with pheromone compounds containing S-7-12 in 2006 and 2007 respectively in Ukraine (Crimea) (SUBCHEV *et al.*, 2010), but these catches are not enough for any conclusions. Field investigations originally organized for *I. rotundata* in Naruto, Tokushima, Japan, in 2008-2010 also showed an attraction of males of *I. pruni* to R-7-12 alone, and to a mixture containing this compound and R-9-14. Moreover, a more precise test organized in 2011 clearly showed that for *I. pruni* males the most active attractant was the mixture containing R-7-12 and R-9-14 in a ratio of 100 : 10. (SUBCHEV *et al.*, 2013).

A field screening test organized in the U.S.A. resulted in establishing the sex attractants of six Procridinae species belonging to four genera: R-9-14 attracted males of *Acoloitus rectarius* DYAR, 1898, *Neoilliberis fusca* (EDWARDS, 1884) and *Neoprocris aversa* (EDWARDS, 1884). S-9-14 attracted males of *Nealbertia constans* (EDWARDS, 1881) and *Triplocris cyanea* BARNES & McDUNNOUGH, 1910; *Pyromorpha dyari* (JORDAN, 1913) males were attracted to S-7-12 (TARMANN, SUBCHEV, unpublished). It is worth mentioning that no attraction of any Zygaenidae species was observed when screening the available Procridinae synthetic sex pheromones in Australia (TARMANN, SUBCHEV, unpublished).

Summarizing the data presented in Table 2, one can conclude that on the whole the species from the Palaearctic ecozone (genera *Adscita*, *Illiberis*, *Jordanita*, *Rhagades* and *Zygaenoprocris*) use 2-butyl-(7*Z*)-dodecenoate for sex attraction, while those from the Nearctic ecozone (genera *Acoloitus*, *Harrisina*, *Nealbertia*, *Neoilliberis*, *Neoprocris* and *Triplocris*) use 2-butyl-(9*Z*)-tetradecenoate. The only exceptions are the Palaearctic *T. ampellophaga* with sex pheromone components R-7-14 and S-7-14, and the North American *Pyromorpha dyari* the males of which were almost equally well attracted by S-7-12 alone and by a mixture of the latter compound and S-9-14. The Asian species *I. rotundata* and *I. pruni* also use a mixed sex attractant – R-7-12 and R-9-14 (SUBCHEV *et al.*, 2009, 2011, 2013).

Male reproductive behaviour: searching behaviour

On the whole the sequences in the behavioural response of the males to natural (conspecific calling females) or artificial (synthetic compound(s)) pheromone sources are common for all lepidopterans, including zygaenids which use sex pheromone communication. These include three main steps: oriented flight to the source, natural (calling female) or artificial (synthetic pheromone), hovering around the source when found and attempts to copulate with the source.

Subfamily Zygaeninae.

For some *Zygaena* species, e.g. *Z. filipendulae* and *Z. trifolii*, two mate-locating strategies for locating females have been claimed: use of optical cues in the morning and sex pheromone signals from calling females in the afternoon (NAUMANN *et al.*, 1999). According to HOFMANN, KIA-HOFMANN (2010) the two mate-locating strategies described for the two above-mentioned species have been observed also for some other European *Zygaena* species (not specified) but its application to the whole genus and especially its validity to the species of the other two subgenera (*Mesembrynus* HÜBNER, [1819], and *Agrumenia* HÜBNER, [1819]) as well as to the Oriental and Afrotropical Zygaeninae needs further investigations. The authors stated also that the optical cue system used by males of *Z. trifolii*, which takes place during the early morning and occasionally leads to ‘morning copulae’, cannot be confirmed as a general strategy within the genus and may vary from species to species, depending on the ecological circumstances (e.g. altitude, semi-desert, woodland).

In *Z. filipendulae* the long-range attraction of males is mediated by calling females emitting pheromones; then visual cues affect the guidance of the flying male in the 50 cm range (ZAGATTI, RENOU, 1984). Similar male behaviour was observed in *Z. niphona* – the male is attracted by a calling female and when the latter is approached the male becomes excited and starts hovering around her and, with hair-pencils opened, tries to copulate (KOSHIO, 2003).

Subfamily Procridinae

TOSHOVA *et al.* (2007) have investigated experimentally the precopulatory phase of the male mating behaviour of *T. ampellophaga* in the field using a female model and a synthetic sex pheromone. They found that oriented flight of wild *T. ampellophaga* males could be observed at a distance of more than 10 m from the experimental area and at a height of

more than 3 m. After approaching the experimental platform *ca* 90% of the males landed, walked up-wind, fluttered their wings and moved their antennae. Then they touched the stimulus (chemical or chemical+visual) with antennae or tarsi, curved the abdomen towards the stimulus and exposing their genitalia they tried to copulate. The authors also found that a female model (visual stimulus) attached to a pheromone source (chemical stimulus) significantly increased the number of males contacting and showing copulatory responses compared to their reaction to a pheromone stimulus alone.

TANAKA, KOSHIO (2002) described the sequence in male sexual behaviour in *I. rotundata* as follows: searching and approaching calling females with fluttering wings and moving antennae; courting the female with fluttering wings and trying to grasp her abdomen with his valvae; successful copulation after which the male folds his wings and turns around so that the two partners are back to back.

Diurnal rhythms of pheromone communication

As a rule the time of the day when females release pheromones corresponds with the time when the conspecific males react to the sex pheromone – this ensures gathering of the mating partners. Thus the diurnal rhythms could be investigated by observation on calling females, the reaction of males to a pheromone source (excitement or attraction) or, indirectly, time of mating.

Subfamily Zygaeninae

The usual time of mating activity in *Zygaena* species (information on other genera is not available) is afternoon. Laboratory observations on *Z. niphona* females (L : D 15 : 9; 27°C) have shown that calling occurs from the third period of the photophase till its end, with the maximum between the 10th-13th hour of the photophase that coincides with the period of the copulation of the moths of this species in the field (TOSHOVA *et al.*, 2008). According to HOFMANN, KIA-HOFMANN (2010), in almost all arboreal and eremic *Zygaena* species in Europe, North Africa and the Middle East, the pheromone-induced mating takes place during the late afternoon. These authors stated also that this is valid and is supported by observations in Morocco (*Z. aurata* BLACHIER, 1905; *Z. favonia*; *Z. youngi* ROTHSCHILD, 1926; *Z. alluaudi* OBERTHÜR, 1922; *Z. algira*, *Z. fausta* (LINNAEUS, 1767); *Z. marcuna* OBERTHÜR, 1888; *Z. maroccana* ROTHSCHILD, 1917) and in Germany (*Z. transalpina* (ESPER, 1780); *Z. angelicae* OCHSENHEIMER, 1808). Calling females

and the attraction of males at that time of the day were observed by one of the authors (AH) in Iran (*Z. seitzii tenhageni* HOFMANN & TREMEWAN, 2003; *Z. haematina* KOLLAR, 1849) and Morocco (*Z. felix* OBERTHÜR, 1876; *Z. fausta*).

Subfamily Procridinae

Observations on diel patterns of activity connected with pheromone communications were undertaken for four Procridinae species (CARR *et al.*, 1992). For all these species, *H. brillians*, *H. americana*, *A. falsarius* and *T. ampellophaga*, observations were done in the field using traps baited with synthetic pheromones, and for the last-mentioned species also by observation on calling behaviour in the laboratory. *H. brillians* males are attracted to pheromone traps in the field from 6.00 h until 14.00 h, with a peak between 7.00 h and 9.00 h. Similar patterns of catches were observed also in traps baited with virgin females. *H. americana* males were attracted to pheromone traps in the morning between 5.30 h and 11.30 h while *A. falsarius* was most active during the heat of mid-afternoon with catches being recorded from 12.30 h to 18.30 h (LANDOLT, HEATH, 1987). In contrast to these three American species, the European *T. ampellophaga* has a much longer period of pheromone-induced activity. DOLIDZE *et al.* (1980) recorded two periods of catches of *T. ampellophaga* males to traps baited with conspecific virgin females – during the first half of the day and in the evening. Similar results were reported by SUBCHEV *et al.* (2004). In addition, laboratory observations have shown that females of this species start calling just before the start of photophase and lasted till the end of the photophase (TOSHOVA, SUBCHEV, 2003).

Direct observations on the diurnal rhythm of calling were organized also for two more Procridinae species, *R. pruni* and *I. rotundata*: similarly to *T. ampellophaga* and *R. pruni*, the females start calling at the beginning of the photophase and continue to do so almost until the end of photophase, while *I. rotundata* females call from the sixth hour of the photophase until its end (TOSHOVA, SUBCHEV, 2003). Similar results for the latter species were reported earlier by TANAKA, KOSHIO (2002). In *Illiberis consumata* the peak of the courtship flight occurs between 14.00 h and 17.00 h; no details on female behaviour were provided (NISHIHARA 2003).

Subfamily Chalcosiinae

According to KOSHIO (1996) the mating time of *E. westwoodii*, the only Chalcosiinae species investigated in this respect, is early morning.

Use of synthetic pheromones for the control of zygaenid pests

Synthetic pheromones could be used for the control of insect pests, for example in the detection, seasonal monitoring and direct control (male confusion technique and mass trapping). The following Zygaenidae species for which sex attractants are known so far are included in TARMANN'S (2003) list of Zygaenidae pests: *Acoloitus falsarius*, *A. novaricus*, *Harrisina americana*, *H. coracina*, *H. metallica* (= *H. brillians*), *I. pruni*, *R. pruni* and *T. ampellophaga*. To this list should be added *I. rotundata* (see SUBCHEV, 2009).

After the identification of the sex pheromone of *T. ampellophaga*, pheromone traps were used for detecting this pest in some European countries and in Asiatic Turkey. As a consequence, and in some cases after a long period of time when data had been lacking, the presence of the pest was confirmed again in Bulgaria (SUBCHEV *et al.*, 2008), Croatia (RAZOV *et al.*, 2014), France (RYMARCZYK, DROUET, 2006), Greece (SUBCHEV *et al.*, 2006), Hungary (VOIGT *et al.*, 2000; SUBCHEV *et al.*, 2003), Romania (SUBCHEV *et al.*, 2008), Italy (SUBCHEV *et al.*, 2008), Ukraine (SUBCHEV *et al.*, 2008) and Turkey (CAN *et al.*, 2010, CAN *et al.*, 2014)

After identification of sex pheromones and the finding of sex attractants for the Procrinae species mentioned above, sex pheromone traps have been successfully used for seasonal monitoring of the North American pests *H. americana* and *A. falsarius*, the Asian pests *I. rotundata* and *I. pruni* as well as the vine bud moth *T. ampellophaga* occurring mainly in Europe but also in the Middle East and North Africa. By means of sex pheromone traps, three generations of *H. americana* and two generations of *A. falsarius* were ascertained in Florida, U.S.A. (LANDOLT, HEATH, 1987). Again, by using sex pheromone traps the only generation of *I. rotundata* was found to occur during the period from the end of May to the end of June (SUBCHEV *et al.*, 2011) and that of *I. pruni* in June (SUBCHEV *et al.*, 2013). Seasonal monitoring using sex pheromone traps in Bulgaria (TOSHOVA, SUBCHEV, 2002) and Hungary (VOIGT *et al.*, 2000; SUBCHEV *et al.*, 2003; SUBCHEV *et al.*, 2004) showed the presence of only one generation of the vine bud moth, *T. ampellophaga*, in these two countries, which began to

emerge at the end of May and continued to do so until the beginning of August. In Greece observations of this pest using pheromone traps have revealed one generation in the central part of the country and on the island of Lefkada, with a flight period occurring from the end of May to the end of July; on Rhodos there are two generations, the first occurring in June, the second from the end of July to the end of August (SUBCHEV *et al.*, 2006). Two-year investigations using pheromone traps for the vine bud moth in the eastern Mediterranean region of Turkey have shown that the pest produces one or two generations per year, depending on local conditions and unknown factors: the moths of the first generation fly in June while those of the second generation occur in August (CAN *et al.*, 2010). Sex pheromone traps were also used to study the seasonal flight of *T. ampellophaga* in the Aegean region of Turkey where two generations were found – the first in June and the second in August (CAN *et al.*, 2014).

Use of sex pheromone traps for faunistic investigations

As with other kinds of traps for insects, with or without baits, sex pheromone traps or just sex pheromone baits could also be used successfully for faunistic investigations. The weak point of the pheromone traps is their selectivity, resulting in a fragmented picture of the species diversity within a certain insect group – only the species using a certain sex attractant(s) as used in the baits will be detected. However, there are also advantages – because of their high sensitivity even rare species and/or species at a very low population density at a certain site could be found. Good examples for Zygaenidae, and Procrinae in particular are the investigations with synthetic pheromone compounds organized originally for searching sex attractants for new species in Europe (e.g. EFETOV *et al.*, 2011; EFETOV *et al.* 2014; RAZOV *et al.*, 2014; SUBCHEV *et al.*, 2010) and the U.S.A. (TARMANN, unpublished).

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