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Mating attempts between the Scarlet Tiger Moth, *Callimorpha dominula* L., and the Cinnabar Moth, *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), involve a common sex pheromone composition

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Abstract. It has been suggested that a common sex pheromone composition may account for interspecific sexual interactions observed with certain moths in the Arctiidae. In this study, it is demonstrated that the sex pheromones released by females of the Scarlet Tiger Moth, *Callimorpha dominula* L., and the Cinnabar Moth, *Tyria jacobaeae* L., have similar activities and elute at the same retention time on analysis by coupled gas chromatography (GC)-electrophysiology with males from each species. Peak enhancement on GC, chiral GC and coupled GC-mass spectrometry using authentic compounds show that the sex pheromone for both *C. dominula* and *T. jacobaeae* is (3*Z*,6*Z*,9*S*,10*R*)-9,10-epoxyheneicosa-3,6-diene.
Key words. Mating; pheromone; epoxyheneicosadiene; enantiomer; Arctiidae; *Callimorpha*; *Tyria*; Lepidoptera.

The sex pheromones have been identified for many Lepidoptera, including the Arctiidae (Tiger and Footman Moths)[1], but none have been accurately defined for pairs from separate species that show coupling behaviour. The present study was stimulated by one of the authors (A. C.) who observed matings between the Cinnabar Moth, *Tyria jacobaeae*, and the Scarlet Tiger Moth, *Callimorpha dominula*. The objective was to investigate the possibility, originally suggested by Kettlewell[2,3], that a sex pheromone composition common to the participating species was responsible for this behaviour.

Moths, and to some extent butterflies, from different species, but generally within the same family, are often observed *in copula* or in other sexual interactions (see ref. 4 and refs therein). Such behaviour has been recorded most frequently in the Arctiidae, possibly because many members of this family are active during the day and have brilliant coloration and are thereby more readily observed. Attempted copulation was noticed between *Phragmatobia fuliginosa* (Ruby Tiger) and *T. jacobaeae*, although the sexes involved were unspecified[5], and males of *P. fuliginosa* were assembled by *C. dominula* females[6]. Mutual assembly was found for *Arctia villica* (Cream-spot Tiger) and *Parasemia plantaginis* (Wood Tiger)[7]. A *Spilosoma luteum* (Buff Ermine) female attracted *Arctia caja* (Garden Tiger) males[8] and *A. caja* females were reported to assemble *P. fuliginosa* males[9]. Examples of cross-species interactions within other taxonomic groupings of the Lepidoptera include the observation that *Xanthorhoe montanata* (Silver-ground Carpet) and *X. spadicearia* (Red Twin-spot Carpet) (Geometridae) males assembled to females of *Biston betularia* (Peppered Moth) (Geometridae)[3], and a complex of assembling observations was compiled on *Orgyia thyellina*, *O. recens* and *O. antiqua* (Lymantriidae)[10]. A number of inter-specific and inter-generic pairings in butterflies were recently reported by Tennent[4]. Couplings between butterflies and moths have also been observed, for example with *Erynnis tages* (Dingy Skipper) (Hesperiidae) and *Euclidia glyphica* (Burnet Companion) (Noctuidae)[11,12]. For the day-flying species, it is likely that vision plays a part in such interactions, but a common sex pheromone composition may also have a role.

Materials and methods

Insects and extracts. *C. dominula* and *T. jacobaeae* larvae were collected on the Wirral Way, West Kirby, from comfrey (*Symphytum officinale*, Boraginaceae) and ragwort (*Senecio jacobaea*, Asteraceae), respectively. Pupae were later sexed and transferred to a regime of 16-h day length at 25 °C. Two days after eclosion, virgin females were observed for 'calling': both species were found to evert the abdominal sex pheromone gland in a pulsed manner[13]. Glands from insects showing this behaviour were removed by dissection into redistilled pentane, with

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5 glands in 100 µl for *C. dominula* and 4 glands in 100 µl for *T. jacobaeae*. Portions of these solutions were either stored in glass vials at −20 °C for immediate electrophysiological analysis or sealed under N_2 in glass ampoules for longer storage prior to further analyses.

Electrophysiology. Electroantennographs (EAG) were recorded using Ag-AgCl glass electrodes filled with a saline solution (as in ref. 14, but without glucose). Antennae from unmated male moths (1–2 days after eclosion) were excised and suspended between the two electrodes. Signals generated by the antenna were passed through a high impedance amplifier (Syntech UN-03b) and monitored on an oscilloscope and a chart recorder. The stimulus was delivered into a purified airstream (1 l/min) flowing continuously over the preparation.

Coupled gas chromatography-electroantennography (GC-EAG). The GC-EAG system, in which the antennal preparation is directly coupled to the capillary column of the gas chromatograph, has been described previously[15]. Separation of the female gland extracts was achieved on an AI 93 gas chromatograph equipped with a cold on-column injector and a flame ionisation detector (FID). The column (50 m × 0.3 mm i.d. HP-1) was maintained at 40 °C for 1 min and then programmed at 5°/min to 100 °C and then at 10°/min to 250 °C. The carrier gas was hydrogen.

Coupled gas chromatography-mass spectrometry (GC-MS). GC-MS employed an identical column to that used for GC-EAG, directly coupled to the MS (70-250 VG Analytical). Ionisation was by electron impact at 70 eV, 230 °C. The GC was maintained at 30 °C for 5 min and then programmed at 5°/min to 180 °C. Tentative identifications by GC-MS were confirmed by comparison with authentic samples and then by peak enhancement on GC[16].

Optical isomer determination. For chiral GC, the column (25 m × 0.25 mm i.d. fused silica capillary coated with hepta-kis-2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin) was programmed from 60–165 °C at a rate of 2°/min, with hydrogen as the carrier gas.

Chemicals. Authentic samples of (3*Z*,6*Z*,9*S*,10*R*)-9,10-epoxyheneicosa-3,6-diene and the (9*R*,10*S*)-enantiomer were synthesised according to published methods[17]. Samples were diluted in purified hexane for GC and GC-MS studies.

Results

Extracts of glands from female *C. dominula* and *T. jacobaeae* showed significant EAG activity with males of both species. The extracts were analysed by coupled GC-EAG and, for each sample, a single major peak at the same retention time in the FID trace elicited a strong response from males of both species (e.g. fig. 1). No other regions of EAG activity were discernible in either sample. These results suggested that the sex pheromones for *C. dominula* and *T. jacobaeae* comprised a single common component. As the principal objective of the study was to characterise this component fully, detailed quantitative analyses were not undertaken. Nonetheless, a small difference between the species was observed, with the pheromone titre for *C. dominula* approximately two-fold higher and apparently associated with a lower EAG sensitivity in males of this species.

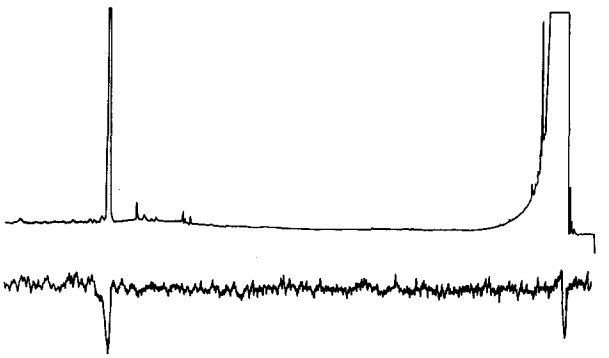

Figure 1. Coupled GC-EAG on *Tyria jacobaeae* male. Upper trace: GC of female *Callimorpha dominula* sex pheromone gland extract. Lower trace: simultaneous EAG response of male *T. jacobaeae*.

With *T. jacobaeae*, GC-MS analysis and peak enhancement on GC confirmed the earlier report that the major sex pheromone component for this species is (3*Z*,6*Z*)-9,10-epoxyheneicosa-3,6-diene[18]. These authors suggested that the specific optical isomer of the natural pheromone was 9*S*,10*R*-, but this was inferred from EAG studies only. Traces of related compounds, probably the corresponding triene and the C_{20} analogue found earlier[18,19], were detected by GC-MS but, in the present study, showed no EAG activity.

GC-MS of the *C. dominula* extract suggested that the main component was again the (3*Z*,6*Z*)-9,10-epoxyheneicosa-3,6-diene, since its mass spectrum was almost identical to that published previously[18], showing the principal fragmentation pattern of a 9,10-epoxyalka-3,6-diene[20]. Peak enhancement on GC confirmed this assumption. Traces of other compounds similar to those in the *T. jacobaeae* extract were detected, but again without associated EAG activity.

The absolute stereochemistry for both pheromone samples was established by GC on the chiral column. The (3*Z*,6*Z*,9*S*,10*R*)- and the (9*R*,10*S*)- enantiomers were well separated on GC by using the cyclodextrin derivative as the stationary phase and an α-value, i.e. rt (9*S*,10*R*): rt (9*R*,10*S*) = 1.002, was obtained. Thus, at a retention time of ca. 80 min, the enantiomers were separated by a difference of more than 10 s, with the (9*S*,10*R*)- enantiomer representing the later eluting peak. Investigation of the synthetic samples showed an enantiomeric excess (ee) of ca. 97% for both compounds. Co-injection of the synthetic compounds and

extracts of pheromone glands proved that, in both species, the natural epoxyheneicosadiene shows predominantly the (9*S*,10*R*)- configuration. With respect to enantiomeric composition, a slight difference was found insofar as *C. dominula* showed a particularly pure compound with an ee of more than 99%, while *T. jacobaeae* contained 2–3% of the enantiomer. The main pheromonal component of the two species is therefore the (3*Z*,6*Z*,9*S*,10*R*)-9,10-epoxyheneicosa-3,6-diene. This report is the first identification of the sex pheromone for *C. dominula* and also confirms chemically the inferred stereochemistry[18] for *T. jacobaeae*.

Discussion

It is shown that both *C. dominula* and *T. jacobaeae* produce sex pheromones comprising one neurophysiologically active component, which is identified unequivocally as (3*Z*,6*Z*,9*S*,10*R*)-9,10-epoxyheneicosa-3,6-diene. This common pheromone composition would be expected to have a role in the observed interspecific coupling behaviour.

T. jacobaeae and *C. dominula* are well known in ecological research, the former because of its role in the population dynamics of the noxious weed ragwort, *Senecio jacobaea*[21], and the latter because of its unique importance in the early development of ecological genetics[22]. Although the respective host plants for the two species may have related defence chemistry with which the insects can interact, the pheromonal epoxyheneicosadiene will undoubtedly be synthesised de novo by the insects from a fatty acid precursor[23]. Given that many Arctiidae have some similarity in coloration, it is only possible for species with the same sex pheromones to co-exist if there is some spacial or temporal separation in their mating behaviour. The two species described here normally frequent different habitats, with *T. jacobaeae* occurring as localised meadow or steppe biotypes and *C. dominula* preferring damp forests and woodland clearings or lush valleys[24]. In addition, *T. jacobaeae* usually mates around sunrise[21], whereas the calling and mating of *C. dominula* females takes place in the early afternoon[7] (V. Philpott, pers. comm.). However, *T. jacobaeae* males are also active during the day and this, in addition to the common pheromone composition, may account for female *C. dominula* occasionally being found *in copula* with male *T. jacobaeae* in the wild. Another effect of the common pheromone component may be that any resultant mating disruption could cause the exclusion of *C. dominula* from areas where *T. jacobaeae* is particularly abundant.

A number of other species in the Arctiidae are known to employ the epoxyheneicosadiene as a sex pheromone component. Although the absolute stereochemistry was not established, the presence of this compound in the sex pheromone of *P. fuliginosa*[1] may account for the sexual interactions with *T. jacobaeae* and *C. dominula* observed previously[5,6]. The compound has also been identified for *Estigmene acrea*[25], *Hyphantria cunea*[26], *Cymbalophora pudica*[27], *Halisodota leda*[28] and *Creatonotos gangis* and *C. transiens*[29]. It would be of interest to determine any cross-species sexual interactions involving these species. Also, the pheromonal basis for the other reported couplings could be investigated by the approaches described here.

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