Origin, transfer to female and identification of a likely male anti-male pheromone in the silver-washed fritillary, *Argynnis paphia* (Lep.: Nymphalidae)

Peter Ockenfels [1], Michael Boppré [1], Ottmar W. Fischer [1] & Stefan Schulz [2]

[1] Forstzoologisches Institut, Albert-Ludwigs-Universität, D-79085 Freiburg i.Br., Germany

[2] Institut für Organische Chemie, Technische Universität Braunschweig, Hagenring 30, D-38106 Braunschweig, Germany

boppre@fzi.uni-freiburg.de

Summary

In the course of studies on pheromone communication in the silver-washed fritillary, we found that the male valves are glandular and that the female 'dorsal sac', described by Urbahn (1913) and Treusch (1971), contains a glutinous liquid after mating. Morphological and GLC/MS studies have revealed that during copulation males transfer □-farnesene and 1-dodecyl-acetate from the valves into the female's dorsal sac where they likely serve as repellents for conspecific males.

Introduction

The silver-washed fritillary, *Argynnis paphia* L., is a classical example in invertebrate ethological studies (e.g., Magnus 1950, 1954). With respect to chemical communication it is one of the most interesting butterflies (Boppré 1984). Although male courtship is initiated exclusively by visual cues, at close range pheromones come into play. Not only do males have alar androconial organs (cf. Fig. 1; Barth 1944) which are brought into contact with female antennae in a late stage of the complex courtship sequence, virgin females expose and direct a pair of lateral clubs (cf. Fig. 2A) to stimulate an approaching male chemically (Treusch 1967).

Interested in the linkage of visual and chemical communication, we are studying structures, chemicals and behaviour of *Argynnis* and related taxa.

Already 85 years ago, a peculiar cavity (the "dorsal sac") in the female abdomen of A. paphia was described by Urbahn (1913), and Clark (1926) reported females of other Argynninae to have a characteristic post-mating smell to the human nose. For Heliconius, Gilbert (1976) provided evidence for the existence of an "anti-aphrodisiac", obviously of male origin, in mated females.

Here wereport on a re-investigation of the histology of the female dorsal sac, the fine-structure of the male valves, and results of chemical analyses as well as behavioural tests in A. paphia in order to elucidate post-mating chemical com-munication in this species.

Fig. 1 Male of *Argynnis paphia* L. (Note the androconial patches on forewings; arrows). Wingspan: 50 mm

Morphology and histology

The "dorsal sac" of the female

The dorsal sac consists of a pair of cavities situated beneath the 7th tergite with a joint orifice located medially between tergites 7 and 8 (Fig. 2). It has been described in detail by Urbahn (1913) who believed it to be extrudable, glandular and its secretion to stimulate males. Treusch (1971) found the dorsal sac to contain material in mated females only and suggested it to be spermatophoric. According to our histological studies, however, it is non-glandular and not connected to any internal structure. The inner walls of the sacs are lined by a fine cuticular layer showing irregularly distributed spines. The epidermal cells are small and do not show any signs of a secretory function.

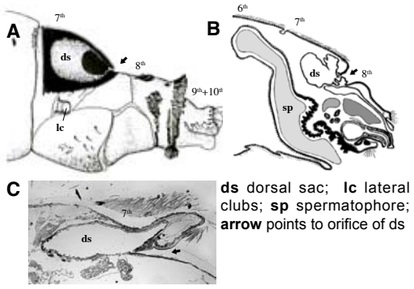


Fig. 2 **A** Location of the dorsal sac at the female abdominal tip (from Treusch 1971); **B** longitudinal section through abdominal tip (after Urbahn 1913); **C** histology of dorsal sac

ds dorsal sac; **lc** lateral clubs; **sp** spermatophore; **arrow** points to orifice of ds

The material in the dorsal sac is colloidal and pale white in colour in older females, while it appears as a viscose clear liquid in freshly mated females.

The glandular valves of the male

The valves (Fig. 3A) posses a voluminous cavity (Fig. 3B) into which glandular cells (arranged in a single layer (Fig. 3C)) secrete material. This can be released through a narrow opening which forms a kind of crease, running along almost the entire length of the valve.

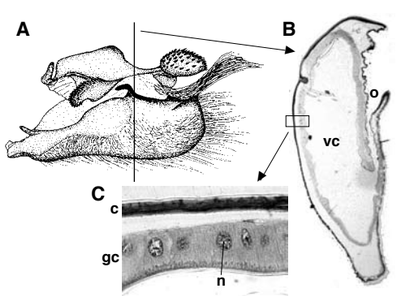


Fig. 3 Lateral view (**A**) and cross sections of male valve (**B, C**); **gc** glandular cells beneath cuticle (**c**); **n** nucleus; **vc** valval cavity; **o** opening

Transfer of secretion

During copulation the male valves with their apical hair bundle (cf. Fig. 3A) form a ridge to transfer the valves' secretion into the dorsal sac (Fig. 4).

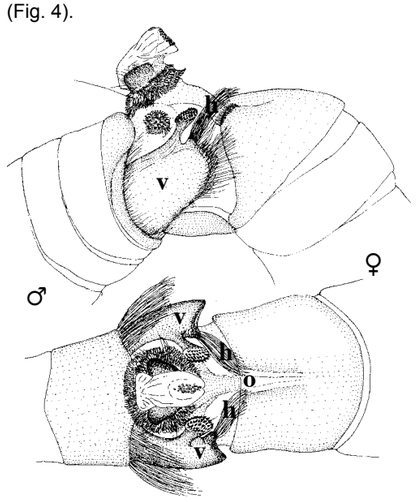


Fig. 4 In coitu position of *Argynnis paphia* viewed laterally (above) and dorsally (below). **v** valve; **h** bundle of hairs; **o** orifice of dorsal sac

Chemistry of secretion

Methods

Entire valves or filter paper tips (usually used in tooth surgery) were employed to collect secretion from the cavities of valves, these were then extracted with $CHCl_2$. Material from dorsal sacs of mated females was obtained by gently pressing the abdomen and removing the content with forceps or with the filter paper tips already mentioned (Fig. 5). The crude extracts were stored at -18°C until subjected to capillary gas chromatography/mass spectrometry.

A Hewlett-Packhard 5890 instrument with a fused-silica-column (30 m x 0.25 mm i.d.) with Rtx 5 as stationary phase was employed. Temperature increased from 60 to 280°C at 3°C/min. The HP 5890 GC was coupled to a HP 5972 MSD. Pure synthetic samples were used as reference compounds.

Fig. 5 Collection of material from dorsal sac with a filter paper tip

Results

Extracts of male valves contain numerous volatile compounds (Fig. 6). Quantitatively, the secretion is dominated by □-farnese (**1**) and 1-dodecyl-acetate (**2**), which occur in amounts of up to 100 µg in unmated males. **1** has previously not been reported for lepidopteran glands while **2** is a common constituent of female pheromones in a large number of moths (cf. Arn et al. 1998).

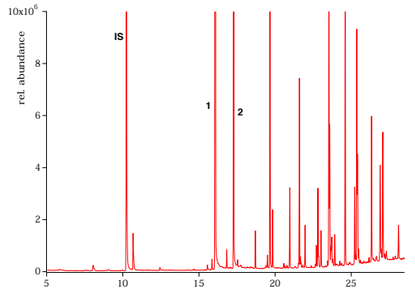
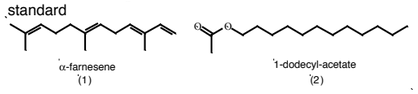


Fig. 6 Chromatogram of extract of male valves. **IS** internal standard



The content of the female dorsal sac by **1** and **2** as major compounds (Fig. 7). This clearly shows that **1**+**2** are transferred from the valves into the female dorsal sac.

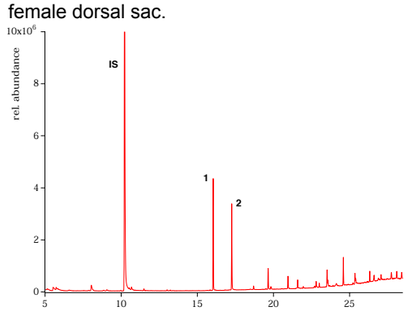


Fig. 7 Chromatogram of extract of material from female dorsal sac of a mated female. **IS** internal standard

The change in solidity of the material (liquid in valval cavity and freshly mated females vs colloidal in females several days after mating) has not yet been explained. Analyses of valval secretion left under ambient laboratory conditions for 48h prior to extraction and analysis revealed chromatograms almost identical to those of dorsal sac contents (cf. Figs 7 and 8). This finding contradicts the idea that the change might be due to any substance contributed by the female and it indicates that non-volatile components are transferred as well.

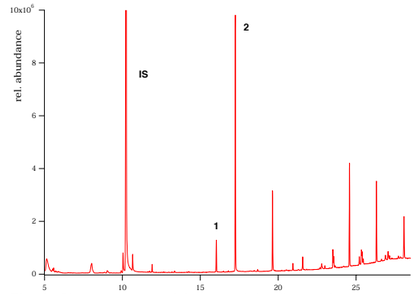


Fig. 8 Chromatogram of the material collected from a valval cavity and stored for 48 h under ambient laboratory conditions prior to extraction. **IS** internal standard

Analyses of valval secretion on suc-cessive days after mating revealed that the amount of **1**+**2** is reduced to 40% in a male 1 day after mating compared to a virgin male; 10 days after mating males contain 90% of the original amount. This demonstrates that there is continuous production of secretion.

Bioassays

It is most likely that **1**+**2** act as repellents to con-specific males, however, it is most difficult to bioassay these presumed pheromone components although both are available in synthetic form: Male courtship can easily be initiated with dummie butterflies made of coloured paper (cf. Magnus 1950, 1954). At such a visual dummie a male remains for less than 2 sec without close inspection. However, if it has been impregnated with an extract of a virgin female, males court such a dummy for at least 30 sec and up to 20 min (Boppré et al. unpubl. data). There is great individual variation in the length of stays due to male motivation as well as to fast evaporation of the stimulating odour(s).

The addition of extracts of female dorsal sacs to visual dummies impregnated with extracts of virgin females caused many males to stay shorter than without the male pheromone. However, we do not yet have sufficient data for statistical treatment.

Conclusive bioassays of compounds **1** and **2** must await identification of the female phe-ro-mone and its availability in synthetic form. Extensive studies with extracts of virgin females cannot be done because in the field unmated females are hardly found and the species is difficult to rear; also, it is protected by German law although it is common in our area.

Discussion

The 'impregnation' of a female by a male with a chemical 'chestity belt' has been suggested for *Heliconius* (Gilbert 1976) and could be substanciated now for *Argynnis paphia*. Preliminary studies with related species (*A. aglaja* and *A. adippe*) indicate that a secretory function of the valves and the transfer of its products to the female is common in this genus.

Our findings also provide further evidence for the close relation of *Heliconius* and *Argynnis* (cf. Harvey 1991). Our histological studies with *A. paphia*, *H. melpomene* and *Dryas julia* supplement Eltringham's (1925) reports on glands in valves of several heliconiine and argynnine butterflies.

Our chemical studies, also as yet unpublished, indicate that there is no species-specificity in the likely male anti-male pheromones, neither in *Argynnis* nor in *Heliconius*.

Prior to 'final' discussions, we will con-tinue to pursue our effords to elucidate chemical communication in *Argynnis* / *Heliconius*.

Acknowledgements

We thank Anita Kiesel for skillful assistance in histology and rearing *Argynnis* as well as G. Gföll for drawing Figs 3A and 4.

Selected references

Arn H, Tóth M, Priesner E (1998) Pherolist. http://www-pherolist.slu.se

Barth R (1944) Die männlichen Duftorgane einiger *Argynnis*-Arten. Zool Jb Anat 68: 331-362

Boppré M (1984) Chemically mediated interactions between butterflies. Symp R ent Soc 11: 259-275. (= The Biology of Butterflies, Vane-Wright RI, Ackery PR (eds), London: Academic Press). Reprinted 1989 by Princeton University Press)

Clark AH (1926) Notes on the odors of some New England butterflies. Psyche 33: 1-5

Eltringham H (1925) On the abdominal glands in Heliconius (Lepidoptera). Trans R ent Soc Lond 64: 269-276

Gilbert L (1976) Postmating female odor in *Heliconius* butterflies: a male-contributed antiaphrodisiac? Science 193: 419-420

Harvey DJ (1991) Higher classification of the Nymphalidae. Pp 255-273 in Nijhout F The Development and Evolution of Butterfly Wing Patterns. Washington, London: Smithosonian Institution Press

Magnus DBE (1950) Beobachtungen zur Balz und Eiablage des Kaisermantels *Argynnis paphia* L. (Lep., Nymphalidae). Z Tierpsychol 7: 435-449

Magnus DBE (1954) Experimentelle Untersuchungen am Kaisermantel zur Analyse optischer Auslösungsreize. Deutscher Entomologentag (Hamburg 1953): 58-75

Treusch HW (1967) Bisher unbekanntes gezieltes Duftanbieten paarungsbereiter *Argynnis paphia*-Weibchen. Naturwissenschaften 54: 592

Treusch HW (1971) Zur Kenntnis der weiblichen Duftorgane bei Inachis io, *Agrynnis paphia* und *Gonepteryx rhamni* (Lepidoptera, Nymphalidae und Pieridae). Dissertation, TH Darmstadt

Urbahn E (1913) Abdominale Duftorgane bei weiblichen Schmetterlingen. Jenaische Z Naturwiss 50: 277-358

Comments

v/98