

Odour-mediated sexual attraction in nabids (Heteroptera: Nabidae)

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Abstract. In many insects, mate finding is mediated by volatile sex pheromones, but evidence in nabids is still fragmentary. The role of odour-mediated sexual attraction in two nabid species, *Nabis pseudoferus* and *N. rugosus*, was studied in a Y-tube olfactometer. Females of the two species were significantly attracted by odours of conspecific males, and males of *N. rugosus*, but not of *N. pseudoferus*, were attracted by odours of conspecific females. Odours of conspecifics of the same gender were unattractive. These results suggest the existence of male pheromones in both species and a different female pheromone in *N. rugosus*.

INTRODUCTION

In arthropods, mates use visual, chemical and/or acoustic communication to find each other (Lewis, 1984), but volatile sex pheromones are the most widespread means of sexual attraction (Cardé & Minks, 1997). Although communication between genders is well-studied in some heteropterans (e.g. Aldrich, 1988, 1995; Landolt & Philips, 1997), there is little information on pheromones in the Nabidae, a predatory heteropteran family, (Aldrich, 1988; Lattin, 1989), which is remarkable in view of their importance for pest suppression in agroecosystems (Coll & Ruberson, 1998; Braman, 2000). Moreover, nabids reveal unusual phenomena in their mating behaviour. Males of several nabid species have retained a bilaterally symmetric structure of their genitalia including their parameres, but only one paramere is used for copulation (Remane & Thielen, 1973; Roth & Remane, 1993). In order to understand the evolution of this phenomenon more background information about sexual communication and mating behaviour are needed.

Sound measurements revealed no evidence that nabids use acoustic signals (Leston, 1957), and the extent to which they use visual cues to find mates is unknown (Freund & Olmstead, 2000; Roth & Remane, 2003). A role for chemical cues in sexual attraction has been suggested for nabids (Harris, 1928; Söcknick, 1974; Carayon, 1977; Pericart, 1987; Hartwig, 1990; Roth & Remane, 2003), but the evidence so far concerns the existence of male pheromones in the nabid *Prostemma guttula* (Kott, 2000) only (see Aldrich, 1988, 1995).

In this article we present results on odour-mediated sexual attraction in two European nabid species, *Nabis pseudoferus* and *Nabis rugosus*. These species are polyphagous predators in natural and semi-natural habitats with dense vegetation, including grasslands (Pericart, 1987), where they occur in densities of about 2–4 individuals per m² (Roth, 1999). Hence, the mean distance between the genders is in the order of 1 m. They have similar life cycles, are usually univoltine and overwinter as dormant adults. *Nabis pseudoferus* is macropterous and can readily fly (Southwood, 1960), both genders of *N. rugosus* are brachypterous and only the hindwings are developed. These differences in dispersal capacity may affect mate finding in the field. Fertilisation and oviposition occurs in spring and early summer, but insemination occurs both in autumn and spring

(Pericart, 1987; Kott et al., 2000). The reasons for mating both in autumn and spring are unknown. Possibly, mating before winter dormancy serves as a kind of fertility insurance because mortality of nabid males during winter is high (see Kott et al., 2000).

We tested for attraction of males and females of the two nabid species to odours of conspecifics of both genders in a bioassay using a Y-tube olfactometer.

MATERIAL AND METHODS

Due to difficulties with rearing of European nabids (Roth, 1999), we used field collected specimens of *Nabis rugosus* L. and *N. pseudoferus* Remane. In total about 400 individuals were caught with a sweep net in grasslands in Thuringia (Germany) in late summer and autumn. In the laboratory, nabids were kept individually in petri dishes (t = 18–20°C, r.h. = 60–65%, 17L : 7D) and were supplied with wet tissue paper as a source of water and *Drosophila* spp. as prey. Owing to this relatively laborious method, the number of individuals available for experiments was rather limited.

Bioassay

Olfactometer experiments were carried out using a Y-shaped glass tube with a white Y-shaped metal wire runway in the centre (Sabelis & van de Baan, 1983). The Y-tube was positioned at an angle of 30° with the arms higher than the base, because preliminary experiments showed that the bugs were more active in this set-up. The base of the Y-tube was connected to a vacuum pump, causing air to flow from the arms to the base. The wind speed was ca. 0.5 m/s in each arm of the tube and was measured with a hot-wire anemometer. With equal wind speeds in both arms, the odours form two neatly separated fields in the base of the Y-tube with the interface coinciding with the metal wire (Sabelis & van de Baan, 1983). The odour source (i.e. one individual male or female) was kept in a glass tube with mesh at both ends and wet tissue paper for moisture supply. This tube was connected to one of the arms of the olfactometer. As alternative odour source, a similar tube without heteropterans but with wet tissue was connected to the other arm. Thus, each tested individual always had the choice between an odour of a conspecific (odour source) and a control odour (fresh air).

TABLE 1. Response of male and female *Nabis rugosus* to odours of the opposite gender (I; II) and to odours of the same gender (III; IV) when offered against clean air. Significance of the G-Tests is indicated by * ($p < 0.05$) and ** ($p < 0.01$).

Test	No. of individuals		No. of indiv. responding to		G-Test		Statistical summary		
	tested	responding	odour	control	df	G-value	Test	df	G-value
I Male response to females odours									
1	13	13	8	5	1	0.70			
2	10	8	7	1	1	5.06*	Pooled	1	5.61*
3	11	10	6	4	1	0.40	Heterogeneity	3	2.19
4	10	10	7	3	1	1.64	Total	4	7.81
II Female response to males odours									
1	11	11	9	2	1	4.82*	Pooled	1	10.74**
2	10	10	8	2	1	3.85*	Heterogeneity	2	0.29
3	11	11	8	3	1	2.35	Total	3	11.03*
III Male response to males odours									
1	11	11	7	4	1	0.83	Pooled	1	1.06
2	11	10	6	4	1	0.40	Heterogeneity	2	0.24
3	13	13	7	6	1	0.08	Total	3	1.31
IV Female response to females odours									
1	12	12	6	6	1	0.00	Pooled	1	0.13
2	10	10	6	4	1	0.40	Heterogeneity	2	1.92
3	10	10	3	7	1	1.65	Total	3	2.05

Individual nabids were released, one at a time, by putting them on the downwind end of the wire after disconnecting the pump from the base tube. A test trial started with reconnecting the pump to the olfactometer and ended with removing the bug when it had reached the end of one of the arms or after it had spent 5 min in the olfactometer. Per replicate 10–13 individuals were tested. The two tubes were interchanged to correct unforeseen asymmetries in the set-up after each of the five nabids had reached the end of one of the arms. Preliminary experiments showed that the individuals tested were not significantly attracted or repelled by tubes in which a conspecific had walked before. Two to 4 replicates were conducted, each with a new odour source. Because of the restricted numbers of individuals available, some individuals were tested in more than one olfactometer experiment. However, all individuals were used for a choice-experiment involving intraspecific attraction only once. Hence, they were always naive with regard to the odour sources offered, but they differed with regard to experience of the set-up. This experience proved not to affect choice (S. Roth, pers. obs.).

The following combinations of responder and odour source were tested: (1) male to female, (2) female to male, (3) male to male, (4) female to female.

Because there is no evidence that copulations are restricted to a specific period during the day in either nabid species studied; the olfactometer experiments were mostly conducted during 10.00 am – 05.00 pm. In general, nabids mate and remate easily under laboratory conditions (see Roth & Remane, 2003) and, therefore we did not rigorously control lighting and temperature for the olfactometer experiments.

Statistical analysis

To enable testing for heterogeneity among replicates in each experiment, we applied a replicated goodness-of-fit test (G-statistic) against a 1 : 1 null hypothesis (Sokal & Rohlf, 1995). Differences between treatments were tested in pairwise comparisons between results using 2×2 contingency tables and G-tests of independence.

RESULTS

Nabis rugosus

In *N. rugosus*, about 94% of 188 individuals made a choice for one of the odour sources within 5 min. Males were significantly attracted to females in one out of four replicates and the other 3 replicates showed the same trend, resulting in non-significant heterogeneity among replicates and a significant overall response (Table 1, Experiment I). Females were significantly attracted to males in 2 out of 3 replicates and the third replicate showed the same trend, resulting in non-significant heterogeneity and a significant pooled response (Table 1, Experiment II). Neither gender was significantly attracted or repelled by odours of the same gender (Table 1, experiment III and IV).

Nabis pseudoferus

In total, 85% of the 136 *N. pseudoferus* tested chose one of the odour sources within 5 min. Females of *N. pseudoferus* were attracted to male odours (Table 2, Experiment II), but this attraction was not significant in any of the separate replicates. This is likely to be due to the low numbers of animals tested in each replicate. However, the heterogeneity among replicates was small ($G_H = 0.48$), indicating that all replicates show the same trend, and the pooled results show a significant attraction of females to male odours.

In contrast, males were not significantly attracted to female odours (Table 2, Experiment I). However, heterogeneity among replicates (G_H) was high and males were significantly attracted to odours of females in one out of three tests. With an exception of one test in the male to male experiment, neither gender was significantly attracted or repelled by odours of the same gender (Table 2, Experiment III and IV).

DISCUSSION

We have shown that female nabids of both species are attracted to conspecific males. This is not the first inference on the existence of male pheromones in heteropterans in general (Aldrich, 1995) and in nabids in particular (Kott, 2000). How-

TABLE 2. Response of male and female *Nabis pseudoferus* to odours of the opposite gender (I; II) and to odours of the same gender (III; IV) when offered against clean air. Significance of the G-Tests is indicated by * ($p < 0.05$).

Test	No. of individuals		No. of indiv. responding to		G-Test		Statistical summary			
	tested	responding	odour	control	df	G-value	Test	df	G-value	
I Male response to females odours										
1	10	10	4	6	1	0.40	Pooled	1	0.15	
2	10	8	7	1	1	5.06*	Heterogeneity	2	5.82	
3	10	8	3	5	1	0.51	Total	3	5.97	
II Female response to males odours										
1	10	9	7	2	1	2.94				
2	11	8	5	3	1	0.51	Pooled	1	5.94**	
3	11	7	5	2	1	1.33	Heterogeneity	3	0.48	
4	11	10	7	3	1	1.64	Total	4	6.42	
III Male response to males odours										
1	11	11	6	5	1	0.09	Pooled	1	0.33	
2	11	9	5	4	1	0.11	Heterogeneity	2	3.83	
3	10	7	1	6	1	3.96*	Total	3	4.16	
IV Female response to females odours										
1	10	10	3	7	1	1.65	Pooled	1	0.57	
2	11	10	7	3	1	1.65	Heterogeneity	2	4.81	
3	10	8	2	6	1	2.10	Total	3	5.38	

ever, males attract females in most heteropteran families (Aldrich, 1988, 1995), with exceptions for Miridae (e.g. King, 1973; Blommers et al., 1988; McBrien & Millar, 1999) and Lygaeidae (Marques et al., 2000). The presence of female pheromones or attraction to female odours, as shown only for *N. rugosus*, is novel for the Nabidae. Neither species showed significant attraction to odours of conspecifics of the same gender. This shows that the attraction was caused by gender-specific odours, suggesting the presence of gender-specific pheromones. Although we found significant attraction to members of the opposite gender, 30–40% of the individuals were not attracted. One reason for this may be that the nabids were given only 5 min for a choice and this may be too short a time interval for some individuals. Secondly, there may be individual differences in sexual activity and/or physiological state. These differences may lead to (a) differences in the response of the insects tested, and (b) differences in the attractiveness of the animals used as odour source. Individual variation in sexual behaviour has been reported for several insects (McNeil, 1991; Raina et al., 1992; Landolt & Phillips, 1997). For example, the mating activity in some species of nabids depends on the age of the individual (Koschel, 1971a; Perkins & Watson, 1972). Thirdly, not all conditions relevant for a response to sex pheromones can be provided in olfactometer experiments. For example, host plant cues stimulate and regulate pheromone production in several insects (Raina et al., 1992; Landolt & Phillips, 1997). Fourthly, the release of pheromones was not controlled in our experiments. In fact, we tested attraction to odours of conspecifics, regardless of the individual nabids used as odour source producing pheromones. It has been suggested that the release of at least some of the male sex pheromones of several species of nabids is associated with specific behaviour of the males, in which males stroke with either of two hind legs (their tibia bears a row of long spines) against the corresponding “comb” of an abdominal organ (Ekblom, 1926; Harris, 1928; Leston, 1957; Kott, 1995). It may serve to enhance diffusion of pheromones and/or may provide additional cues to females, either through the air or via the substrate (Kott, 2000; Roth & Remane, 2003). However, males attractive to females were not observed to show this spe-

cific behaviour in our experiments. Possibly, the olfactometer set-up did not trigger the males that were used as odour source to produce pheromones, and this may be another reason for low responsiveness of the females in the olfactometer. Moreover, the set-up might cause tested individuals to release defensive secretions (including alarm pheromones) (see Aldrich, 1985, 1995). This might explain the occasional repellent behaviour to conspecifics in male *N. pseudoferus* (Table 2).

Nevertheless, our results show that the experimental design can be used to study odour-mediated long range attraction in nabids. Moreover, they are the first experimental evidence for odour-mediated sexual attraction of species of the subfamily Nabinae.

Future studies should focus on chemical identification of the sex pheromones of nabids. Such studies have been carried out for phytophagous heteropterans (Groot et al., 1998; McBrien & Millar, 1999), showing that sex pheromone compounds may be obscured by products from glands that produce defensive secretions (Aldrich, 1988, 1995). Elucidation of the chemical structure of the sex pheromone will provide new perspectives for analysing the sexual behaviour of nabids and contribute to the study of the ecology of this important group of predators (Koschel, 1971b; Remane & Thielen, 1973; Söcknick, 1974; Carayon, 1977; Richards & Harper, 1978; Kott, 2000; Roth & Reinhardt, 2003; Roth & Remane, 2003).

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REFERENCES

- ALDRICH J.R. 1988: Chemical ecology of the Heteroptera. *Annu. Rev. Entomol.* **33**: 211–238.
 ALDRICH J.R. 1995: Chemical communication in the true bugs and parasitoid exploitation. In Cardé R.T. & Bell W.J. (eds): *Chemical Ecology of Insects 2*. Chapman and Hall, New York, pp. 318–363.

- BRAMAN S.K. 2000: Damsel bugs (Nabidae). In Schaefer C.W. & Panizzi A.R. (eds): *Heteroptera of Economic Importance*. CRC Press, Boca Raton, pp. 639–656.
- BLOMMERS L., BUS V., DE JONGH E. & LENTJES G. 1988: Attraction of males by virgin females of the green capsid bug *Lygocoris pabulinus* (Heteroptera: Miridae). *Entomol. Ber. Amst.* **48**: 175–179.
- CARDÉ R.T. & MINKS A.K. (eds) 1997: *Insect Pheromone Research: New Directions*. Chapman and Hall, New York, 734 pp.
- CARAYON J.P. 1977: Insémination extra-génitale traumatique. In Grassé P.P. (ed.): *Traité de Zoologie. Vol. 8(5A)*. Masson, Paris, pp. 351–390.
- COLL M. & RUBERSON J.R. (eds) 1998: *Predatory Heteroptera: Their Ecology and Use in Biological Control*. Entomological Society of America, Lanham, MD, 233 pp.
- EKBLOM T. 1926: Morphological and biological studies of the Swedish families of Hemiptera – Heteroptera. *Zool. Bidrag Uppsala* **10**: 31–180.
- FREUND R.L. & OLMSTEAD K.L. 2000: The roles of vision and antennal olfaction in enemy avoidance by three predatory heteropterans. *Environ. Entomol.* **29**: 733–742.
- GROOT A.T., VAN DER WAL E., SCHUURMAN A., VISSER J.H., BLOMMERS L.H.M. & VAN BEEK T.A. 1998: Copulation behaviour of *Lygocoris papulinus* under laboratory conditions. *Entomol. Exp. Appl.* **88**: 219–228.
- HARRIS H.M. 1928: A monographic study of the Hemipterous family Nabidae as it occurs in North America. *Entomol. Am.* **9**: 1–97.
- HARRIS V.E. & TODD J.W. 1980: Temporal and numerical patterns of reproductive behaviour in the southern green stinkbug, *Nezara viridula* (Hemiptera: Pentatomidae). *Entomol. Exp. Appl.* **27**: 105–116.
- HARTWIG O. 1990: *Untersuchungen zu Wanzen der Gattung Nabis als Element der Insektenfauna in Winterweizen*. Master Thesis, University of Halle/Saale, Germany.
- KING A.B.S. 1973: Studies of sex attraction in the cocoa capsid, *Distantiella theobroma* (Heteroptera: Miridae). *Entomol. Exp. Appl.* **16**: 243–254.
- KOSCHEL H. 1971a: Zur Kenntnis der Raubwanze *Himacerus apterus* F. (Heteroptera, Nabidae). Teil 1. *Z. Angew. Entomol.* **68**: 1–24.
- KOSCHEL H. 1971b: Zur Kenntnis der Raubwanze *Himacerus apterus* F. (Heteroptera, Nabidae). Teil 2. *Z. Angew. Entomol.* **68**: 113–137.
- KOTT P. 1995: Zur Biologie von *Prostemma guttula* F. (Heteroptera: Nabidae). *Mitt. Intern. Entomol. Ver.* **20**: 31–49.
- KOTT P. 2000: Zur Biologie von *Prostemma guttula* F. (Heteroptera: Nabidae). Teil II. *Mitt. Intern. Entomol. Ver.* **25**: 41–74.
- KOTT P., ROTH S. & REINHARDT K. 2000: Hibernation mortality and sperm survival during dormancy in female Nabidae (Heteroptera: Nabidae). *Opusc. Zool. Flumin.* **182**: 1–6.
- LANDOLT P.J. & PHILIPS T.W. 1997: Host plant influences on sex pheromone behaviour of phytophagous insects. *Annu. Rev. Entomol.* **42**: 371–391.
- LATTIN J.D. 1989: Bionomics of the Nabidae. *Annu. Rev. Entomol.* **34**: 383–400.
- LESTON D. 1957: The stridulatory mechanisms in terrestrial species of Hemiptera – Heteroptera (Geocorisae). *Proc. Zool. Soc. Lond.* **128**: 369–386.
- LEWIS T. (ed.) 1984: *Insect Communication*. Academic Press, Orlando, FL, 414 pp.
- MARQUES F.A., McELFRESH J.S. & MILLAR J.G. 2000: Female-produced sex pheromone of the predatory bug *Geocoris puncticeps*. *J. Chem. Ecol.* **26**: 2843–2855.
- McBRIEN H.L. & MILLAR J.C. 1999: Phytophagous bugs. In Hardie J. & Minks A.K. (eds): *Pheromones of Non-Lepidopterous Insects Associated with Agricultural Plants*. CABI Publishing, Oxon, pp. 277–304.
- McNEIL J.N. 1991: Behavioural ecology of pheromone-mediated communication in moths and its importance in the use of pheromone traps. *Annu. Rev. Entomol.* **36**: 407–430.
- MILLAR J.G., RICE R.E. & WANG Q. 1997: Sex pheromone of the mirid bug *Phytocoris relativus*. *J. Chem. Ecol.* **23**: 1743–1754.
- PÉRICART J. 1987: Hemiptères Nabidae d'Europe occidentale et du Maghreb. *Faune de France* **71**: 1–169.
- PERKINS V.P. & WATSON T.F. 1972: Biology of *Nabis alternatus* (Hemiptera: Nabidae). *Ann. Entom. Soc. Am.* **65**: 54–57.
- RAINA A.K., KINGAN T.G. & MATTOO A.K. 1992: Chemical signals from host plant and sexual behaviour in a moth. *Science* **255**: 592–594.
- REMANE R. & THIELEN U. 1973: Steuerungsmechanismen beim Kopulationsverhalten von Nabiden-Arten (Hemiptera – Heteroptera) – ein Beitrag zur Frage der Beziehung zwischen Organstruktur, Funktion und Evolution. *Z. Wiss. Zool.* **186**: 89–197.
- RICHARDS L.A. & HARPER A.M. 1978: Oviposition by *Nabis alternatus* (Hemiptera: Nabidae) in alfalfa. *Can. Entomol.* **110**: 1359–1362.
- ROTH S. 1999: *Zur räumlichen und zeitlichen Habitatnutzung von Nabidenarten (Nabidae, Heteroptera) in Rasenökosystemen unter besonderer Berücksichtigung von Nabis brevis und Nabis rugosus*. PhD Thesis, University of Jena, Germany. Cuvillier, Göttingen.
- ROTH S. & REINHARDT K. 2003: Facultative sperm storage in response to nutritional status in a female insect. *Biology Letters – Proc. Zool. Soc. London (Suppl.)* **270**: S54–S56.
- ROTH S. & REMANE R. 2003: Zur Reproduktionsbiologie der Nabinae (Insecta: Heteroptera: Nabidae). *Entomol. Abh. Staatl. Museum Tierkunde Dresden* **60**: 3–22.
- SABELIS M.W. & VAN DE BAAN H.E. 1983: Location of distant spider mite colonies by phytoseiid predators: demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. *Entomol. Exp. Appl.* **33**: 303–314.
- SOKAL R.R. & ROHLF F.J. 1995: *Biometry*. Freeman, New York, 887 pp.
- SÖKNICK R. 1974: *Beiträge zur Biologie von Dolchonabis limbatus (Dhlb.) (Hemiptera, Heteroptera, Nabidae)*. Master Thesis, University of Marburg, Germany.
- SOUTHWOOD T.R.E. 1960: The flight activity of Heteroptera. *Trans. R. Entomol. Soc. Lond.* **8**: 112–220.

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