



Communication via sex pheromones within and among *Arrenurus* spp. mites (Acari: Hydrachnida; Arrenuridae)

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Abstract. We present direct experimental evidence of pheromone use in six species of *Arrenurus* and indirect evidence for four species, including members of the subgenera *Megaluracarus*, *Truncaturus*, and *Arrenurus*. Water in which females were housed elicited arrestant behaviour in males, males oriented to the source, and at least some individuals in each species assumed the male readiness posture, a precursor to coupling. Most species responded to water treated with conspecific females, but there was also interspecific sex pheromone responsiveness. *Arrenurus manubriator* and *A. megalurus* demonstrated reciprocal pheromone cross-attractancy. Males of *A. major*, *A. marshallae*, and *A. birgei* responded to water from females of related species from within their subgenera. *Arrenurus apetirolatus* males failed to respond to conspecific female-treated water, but the same water elicited arrestant behaviour and orientation in *A. manubriator*. Heterospecific reactions to female-conditioned water were limited to cases involving members of the same species group and were not seen between species representing different species groups or different subgenera. The species for which cross-attractancy has been demonstrated commonly co-occur in nature, so apparently these pheromones are of limited value for species recognition. Shared reaction to sex pheromones provides additional evidence for inferring close phylogenetic relationship among species, and thus far, corresponds with morphological evidence based on adult males and larvae.

Introduction

Recently, Smith and Hagman (2002) demonstrated that in at least one species of *Arrenurus* water mite, females produce a male-attractant stimulus that can be extracted from water – presumably a pheromone. When exposed to this stimulus, males stop swimming (arrestant behaviour) and either remain motionless or crawl slowly towards the source, and if strongly stimulated, assume a characteristic readiness posture (Proctor and Smith 1994) with the fourth legs held against the dorsum and bent at the genuotibial joint. Males of various other *Arrenurus* spp. have been reported to exhibit this posture (e.g., Böttger 1962; Proctor and Wilkinson 2001). Given that various species share the male readiness position, it is quite possible that those species also use sex pheromones.

Sonenshine (1985) reviewed chemical communication in Acari, and noted that many species use the same or similar chemicals, which is in strong contrast

to the species-specificity seen in many insects, especially Lepidoptera (e.g., Löfstedt 1993). Much of the specificity in insects is based upon species responding only to relatively precise blends of isomers, sometimes requiring small quantities of secondary components (e.g., Silk and Kuenen 1988; Löfstedt 1993; Roelofs 1995; Mori and Kuwahara 2000). Our impression from Sonenshine (1985) and subsequent papers is that sex pheromones for many species of Acari are single compounds or simple mixtures.

The genus *Arrenurus* is species-rich: there are over 900 described species (Viets 1987) and it is not difficult to discover undescribed species in most parts of the world. Given the species diversity and the relative simplicity of many acarine sex pheromones, it is quite probable that there is substantial interspecific sex pheromone responsiveness. We have two aims in our study: (1) to investigate whether female-emitted male-attractant sex pheromones are widespread among *Arrenurus* species, and (2) to explore species-specificity of the pheromones used.

Materials and methods

Collection and maintenance of mites

Most of the mites used in this study were collected from three field sites in Southern Ontario, Canada (Table 1). The Black River and Salmon River were sampled at their junctions with Highway 7 (ca. 10 km east of Madoc, and ca. 20 km east of Kaladar, respectively). Telephone Bay of Lake Opinicon, located approximately 6 km south-west of Chaffeys Lock, ca. 50 km north-east of Kingston, was also a source of experimental animals. Collections were made following the methods of Smith et al. (2001) using D-frame nets in waters of 0.25–1.5 m depth. Adult *Arrenurus* (*Megaluracarus*) *manubriator* Marshall were available from a sixth generation laboratory colony established from inseminated females previously collected from Telephone Bay of Lake Opinicon. A fifth generation colony of *Arrenurus* (*Truncaturus*) *rufopyriformis* Habeeb was another source of experimental animals, having originated from females collected from Mèr Bleue Bog (located on the south-eastern edge of Ottawa, Ontario, by Blackburn Hamlet). Both of these species are atypical of parasitengonine mites in that they forego larval feeding and any association with insect hosts (Smith 1998) which makes it feasible to maintain reproducing colonies in the laboratory. Adults of an undescribed species (*Arrenurus* (*Arrenurus*) new species, near *reflexus*) were raised from engorged larvae removed from parasitized adult *Leucorrhinia frigida* Hagen dragonflies netted at Hebert Bog (located ca. 14 km south-west of Chaffeys Lock on the Opinicon Road) in early July, 1995.

Field-collected mites were stored unfed and in darkness at 15 °C until about 1 week before being used in experiments. They were then warmed to and kept at room temperature (range 21–26 °C) under ambient fluorescent lighting

Table 1. Localities and dates for field-collected *Arrenurus* spp. mites used in this study. Subgenera '(A)': *Arrenurus*, '(M)': *Megaluracarus*.

Lake Opinicon, September 2, 1995

- A. (A.) americanus* Marshall
- A. (A.) falcicornis* Marshall
- A. (A.) fissicorniformis* Cook
- A. (A.) magnicaudatus* Marshall
- A. (M.) apetiolutus* Piersig
- A. (M.) birgei* Marshall
- A. (M.) intermedius* Marshall
- A. (M.) megalurus* Marshall

Salmon River, September 3, 1995

- A. (A.) americanus* Marshall
- A. (A.) fissicornis* Marshall
- A. (A.) flabellifer* Marshall
- A. (A.) gennadus* Cook
- A. (A.) major* Marshall
- A. (A.) pseudosuperior* Cook
- A. (A.) superior* Marshall
- A. (M.) apetiolutus* Piersig
- A. (M.) birgei* Marshall
- A. (M.) megalurus* Marshall

Black River, September 3, 1995

- A. (A.) flabellifer* Marshall
 - A. (A.) magnicaudatus* Marshall
 - A. (M.) marshallae* Piersig
 - A. (M.) megalurus* Marshall
 - A. (M.)* new species, near *unisinuatus*
-

(ca. 6–10 h lighting per day) and fed. Mites of most species were provided an excess of living ostracods from laboratory colonies. Adult *Arrenurus (Arrenurus) pseudosuperior* Cook and *Arrenurus (Arrenurus) superior* Marshall were maintained on small immatures of *Daphnia magna* Straus. Colonies of *A. manubriator* and *A. rufopyriformis* were kept in polypropylene containers, continually in room conditions, with an abundance of ostracods. Tap water aged for at least 24 h was used for housing mites and for experiments.

Experimental methods

Female-conditioned water was produced by storing female mites of a given species in a glass beaker (1 ml of water per mite) for 24 h at room temperature. Food was withheld during this period to avoid potential kairomones from prey. Water was conditioned using as many females as were available up to a maximum of 250. A comparable volume of water destined for use as a control was also stored in a glass beaker at room temperature for 24 h. Control and

female-conditioned water was used in experiments immediately after the 24 h period. In both cases, beakers were kept covered with parafilm.

The design was to simultaneously test a group of males of a given species, using two sources of water: female-conditioned and an untreated control. The test arena was a glass petri dish (9 cm in diameter, 1.5 cm deep) containing 30 ml of 24-h aged tap water. The dish had a line drawn down the middle of the underside, thus dividing it into two regions. Two syringes were suspended opposite each other equidistant from the midline of the test arena and each about 2.5 cm from the dish's edge, with the needle tips just below the water's surface. One syringe contained control water, the other had female-conditioned water. During a test, the plungers of the two syringes were removed simultaneously so that the water in the two syringe barrels would drain by gravity. Responses by males were scored immediately after the syringes had finished draining. Only males exhibiting arrestant behaviour were counted, and we specifically tested whether male arrestant behaviour was oriented towards the putative pheromone (i.e., number of males on the side with the female-treated water vs. the number on the control side of the arena). We compared counts of arrested males from the two sides using a one-tailed sign test of association: the null hypothesis was that there would be no apparent orientation of stationary mites (non-significant, or negative result), the alternative hypothesis was that arrestant males would cluster on the side corresponding to the female-conditioned water (a positive result, being statistically significant). This criterion was used because males could stop swimming for other reasons besides an arrestant response to a pheromone source. Counting males that assumed the readiness posture would be a more definitive test, but it was impossible to accurately score groups of males tested simultaneously because individuals frequently hold the posture for only a fraction of a second to a couple of seconds, and it was too difficult to reliably recognize the posture without magnification. We found that males tested as groups were no more likely to show arrestant behaviour than males tested individually, and testing in groups allowed for larger sample sizes with less time and effort. However, we also noted that arrested males are more likely to exhibit the readiness posture after a collision with another mite, as also reported by Böttger (1962).

Given that the only way to demonstrate the presence of pheromone was to see a response by males, it was necessary to elicit an arrestant behaviour in males oriented to the source of female-conditioned water as a positive control. Pheromone production drops off dramatically when females are not well-fed or are in poor condition, and similarly, males will only exhibit an arrestant response when they are well-fed and in good condition. A positive result from testing males with conspecific female-conditioned water was an optimal control for the males' responsiveness to stimuli and for the females' production of pheromone. A test in which males responded to water from heterospecific females was still informative even with negative results from the controls, but negative results from heterospecific tests without evidence for male

receptiveness and the presence of pheromone were ambiguous. Species without some positive evidence for pheromone use were dropped from the study.

A series of tests were run in each comparison, with female-conditioned water first being presented to conspecific males to test whether this species used female-emitted sex pheromones (and whether the males and females were in adequate condition). After a test, males were placed into a new petri dish with fresh water and allowed to sit for 15 min before the next test was conducted. After males had first been tested with conspecific female-conditioned water, the same males were tested with a sequence of water conditioned by females of other *Arrenurus* species. As many males as available up to a maximum of 40 per species were used in each comparison. Seven sets of comparisons were conducted in total, given that it was not possible to conduct tests with a wide range of species combinations within a reasonable period of time. All tests within a set were conducted within a two day period, including repeats of conspecific tests. Consequently, results of sets of comparisons are presented separately, and certain reference species are included in several different sets. The first set was repeated as confirmation, given that it was the first evidence for heterospecific responsiveness to pheromones among *Arrenurus* species; we decided to conduct other sets of tests to further explore the scope of cross-species reactivity to pheromones after having made this discovery. In all but one series of tests, 3 ml syringes were used each with 3 ml of solution, and it took approximately 75 s for the syringes to drain; in the fifth set of comparisons, 1 ml syringes and 1 ml of each solution were used and draining took about 25 s. Syringes used for female-treated water from a given species of mite were used for that species throughout a set of comparisons, and new syringes were used for each set of comparisons.

Results

There was no evidence of pheromone communication in nine species and they were omitted from further consideration. Male *Arrenurus* (*Megaluracarus*) *wardi* Marshall and *Arrenurus* (*Megaluracarus*) new species, near *unisinuatus* were tested with water from their own females and with water from the other species, but none showed a response. Females could not be reliably identified or were too few for *Arrenurus* (*Megaluracarus*) *intermedius* Marshall, *Arrenurus* (*Arrenurus*) *fissicornis* Marshall, *Arrenurus* (*Arrenurus*) *falcicornis* Marshall, *Arrenurus* (*Arrenurus*) *magnicaudatus* Marshall, *Arrenurus* (*Arrenurus*) *flabelifer* Marshall, *Arrenurus* (*Arrenurus*) *fissicorniformis* Cook, and *Arrenurus* (*Arrenurus*) *gennadus* Cook. Males of these species failed to respond to heterospecific female-conditioned water and were dropped from the study. We only had one male of *A. superior* and no females, but we nevertheless tested it with water conditioned by female *A. pseudosuperior*: the male arrested and assumed the readiness posture.

In the first set of comparisons (Table 2) we concentrated on species of the subgenus *Megaluracarus*, namely various combinations of *A. manubriator*, *Arrenurus megalurus* Marshall, *Arrenurus birgei* Marshall, and *Arrenurus marshallae* Piersig. Females of *A. marshallae* could not be reliably identified and only males were used in the study. This comparison was repeated, forming two trials. Male *A. manubriator* and *A. megalurus* responded and oriented to water from their own and each other's females in both trials (Table 2). Almost all arrested males would cluster on the side of the arena near the test syringe, and more distant males would crawl slowly towards the source. Water conditioned with female *A. manubriator* did not elicit a response from *A. marshallae* males, while water from *A. megalurus* females did cause *A. marshallae* males to arrest and orient (statistical significance in trial 2, $p = 0.059$ in trial 1; Table 2). Male *A. birgei* did not respond to conspecific stimuli, but responded to *A. manubriator* and *A. megalurus* female water: few oriented in trial 1 and neither case was statistically significant, but there were significant responses by *A. birgei* males to water from females of both species in trial 2 (Table 2).

Table 2. Tests for pheromone response as indicated by male arrestant behaviour among species of the subgenus *Megaluracarus*.

No. of males	Species of males	Female water	No. of males at test	No. of males at control	Sign test
Trial 1					
40	<i>A. manubriator</i>	<i>A. manubriator</i>	23	0	$p < 0.001$
40	<i>A. megalurus</i>	<i>A. megalurus</i>	26	2	$p < 0.001$
40	<i>A. megalurus</i>	<i>A. manubriator</i>	18	3	$p < 0.001$
40	<i>A. manubriator</i>	<i>A. megalurus</i>	13	2	$p < 0.005$
40	<i>A. marshallae</i>	<i>A. manubriator</i>	3	1	$p = 0.313$
40	<i>A. marshallae</i>	<i>A. megalurus</i>	11	4	$p = 0.059$
40	<i>A. birgei</i>	<i>A. birgei</i>	1	0	$p = 0.500$
40	<i>A. birgei</i>	<i>A. manubriator</i>	4	0	$p = 0.063$
40	<i>A. birgei</i>	<i>A. megalurus</i>	1	0	$p = 0.500$
Trial 2					
40	<i>A. manubriator</i>	<i>A. manubriator</i>	32	0	$p < 0.001$
16	<i>A. megalurus</i>	<i>A. megalurus</i>	12	1	$p < 0.005$
16	<i>A. megalurus</i>	<i>A. manubriator</i>	9	0	$p < 0.005$
40	<i>A. manubriator</i>	<i>A. megalurus</i>	29	0	$p < 0.001$
38	<i>A. marshallae</i>	<i>A. manubriator</i>	1	0	$p = 0.500$
38	<i>A. marshallae</i>	<i>A. megalurus</i>	7	0	$p < 0.008$
40	<i>A. birgei</i>	<i>A. birgei</i>	0	0	$p = 1.000$
40	<i>A. birgei</i>	<i>A. manubriator</i>	10	0	$p < 0.001$
40	<i>A. birgei</i>	<i>A. megalurus</i>	5	0	$p < 0.032$

A group of males of a given species were given simultaneous presentation of female-conditioned water of either the same or a different species and of control water. The number showing arrestant behaviour in the half of the test arena by the source of female-treated water and control water were recorded. Data were tested using a one-tailed sign test. The two trials were conducted in different weeks and hence are kept separate.

The second set of comparisons centered on testing whether *Arrenurus* (*Megaluracarus*) *apetiolutus* Piersig would respond to *A. manubriator* and *A. megalurus*. Males of *A. manubriator* and *A. megalurus* responded to water conditioned with conspecific females, but *A. apetiolutus* males did not (Table 3). We continued testing *A. apetiolutus* males with heterospecific-conditioned water and there was a significant arrestant and orientation response in two trials with water from *A. manubriator* females but no response with that from *A. megalurus* (Table 3).

Table 3. Tests for pheromone response as indicated by male arrestant behaviour among species of the subgenus *Megaluracarus* (see Table 2).

No. of males	Species of males	Female water	No. of males at test	No. of males at control	Sign test
40	<i>A. manubriator</i>	<i>A. manubriator</i>	27	0	$p < 0.001$
40	<i>A. megalurus</i>	<i>A. megalurus</i>	31	0	$p < 0.001$
38	<i>A. apetiolutus</i>	<i>A. apetiolutus</i>	1	1	$p = 0.500$
40	<i>A. manubriator</i>	<i>A. apetiolutus</i>	10	1	$p < 0.006$
40	<i>A. manubriator</i>	<i>A. apetiolutus</i>	15	0	$p < 0.001$
40	<i>A. megalurus</i>	<i>A. apetiolutus</i>	2	1	$p = 0.500$

Table 4. Tests for pheromone response as indicated by male arrestant behaviour among species of the subgenus *Arrenurus* (see Table 2).

No. of males	Species of males	Female water	No. of males at test	No. of males at control	Sign test
40	<i>A. americanus</i>	<i>A. americanus</i>	27	2	$p < 0.001$
19	<i>A. n.sp. nr. reflexus</i>	<i>A. n.sp. nr. reflexus</i>	10	1	$p < 0.006$
14	<i>A. pseudosuperior</i>	<i>A. pseudosuperior</i>	13	1	$p < 0.001$
40	<i>A. americanus</i>	<i>A. n.sp. nr. reflexus</i>	0	0	$p = 1.000$
14	<i>A. pseudosuperior</i>	<i>A. n.sp. nr. reflexus</i>	0	0	$p = 1.000$
19	<i>A. n.sp. nr. reflexus</i>	<i>A. pseudosuperior</i>	0	0	$p = 1.000$
40	<i>A. americanus</i>	<i>A. pseudosuperior</i>	0	0	$p = 1.000$
14	<i>A. pseudosuperior</i>	<i>A. americanus</i>	3	2	$p = 0.500$
19	<i>A. n.sp. nr. reflexus</i>	<i>A. americanus</i>	1	0	$p = 0.500$

Table 5. Tests for pheromone response as indicated by male arrestant behaviour among species of the subgenus *Arrenurus* (see Table 2).

No. of males	Species of males	Female water	No. of males at test	No. of males at control	Sign test
40	<i>A. americanus</i>	<i>A. americanus</i>	25	2	$p < 0.001$
24	<i>A. major</i>	<i>A. americanus</i>	18	0	$p < 0.001$
12	<i>A. pseudosuperior</i>	<i>A. pseudosuperior</i>	11	1	$p < 0.004$
24	<i>A. major</i>	<i>A. pseudosuperior</i>	4	3	$p = 0.706$
17	<i>A. n.sp. nr. reflexus</i>	<i>A. n.sp. nr. reflexus</i>	12	1	$p < 0.003$
23	<i>A. major</i>	<i>A. n.sp. nr. reflexus</i>	4	3	$p = 0.706$

We then shifted focus to species of the subgenus *Arrenurus*, testing four species: *Arrenurus americanus* Marshall, *Arrenurus major* Marshall, *A. pseudosuperior*, and *Arrenurus* n. sp., near *reflexus*. Males of *Arrenurus americanus* Marshall, *A. pseudosuperior*, and *Arrenurus* n. sp., near *reflexus* all yielded positive responses to water from females of their own species (Tables 4 and 5) but not to heterospecific sources (Table 4). Female *A. major* could not be reliably identified and were excluded, but males were tested with female-conditioned water from the other three species, arresting and orienting only to water from *A. americanus* females (Table 5).

The remainder of the study was concerned with testing whether pheromone responses occurred between species of different subgenera. Three species representing different subgenera were used in the fifth set of comparisons, namely

Table 6. Tests for pheromone response as indicated by male arrestant behaviour among species from the subgenera *Truncaturus*, *Megaluracarus*, and *Arrenurus* (see Table 2).

No. of males	Species of males	Female water	No. of males at test	No. of males at control	Sign test
40	<i>A. manubriator</i>	<i>A. manubriator</i>	30	0	$p < 0.001$
30	<i>A. rufopyriformis</i>	<i>A. rufopyriformis</i>	23	0	$p < 0.001$
19	<i>A. n.sp. nr. reflexus</i>	<i>A. n.sp. nr. reflexus</i>	10	1	$p < 0.006$
19	<i>A. n.sp. nr. reflexus</i>	<i>A. manubriator</i>	2	0	$p = 0.250$
40	<i>A. manubriator</i>	<i>A. n.sp. nr. reflexus</i>	1	0	$p = 0.500$
30	<i>A. rufopyriformis</i>	<i>A. n.sp. nr. reflexus</i>	0	0	$p = 1.000$
19	<i>A. n.sp. nr. reflexus</i>	<i>A. rufopyriformis</i>	1	0	$p = 0.500$

Table 7. Tests for pheromone response as indicated by male arrestant behaviour among species of the subgenus *Megaluracarus* and *Arrenurus* (see Table 2).

No. of males	Species of males	Female water	No. of males at test	No. of males at control	Sign test
40	<i>A. manubriator</i>	<i>A. manubriator</i>	25	0	$p < 0.001$
40	<i>A. americanus</i>	<i>A. americanus</i>	28	2	$p < 0.001$
40	<i>A. americanus</i>	<i>A. manubriator</i>	3	0	$p = 0.125$
40	<i>A. manubriator</i>	<i>A. americanus</i>	0	0	$p = 1.000$

Table 8. Tests for pheromone response as indicated by male arrestant behaviour among species of the subgenus *Megaluracarus* and *Arrenurus* (see Table 2).

No. of males	Species of males	Female water	No. of males at test	No. of males at control	Sign test
40	<i>A. manubriator</i>	<i>A. manubriator</i>	27	0	$p < 0.001$
10	<i>A. pseudosuperior</i>	<i>A. pseudosuperior</i>	7	0	$p < 0.008$
40	<i>A. manubriator</i>	<i>A. pseudosuperior</i>	0	0	$p = 1.000$
10	<i>A. pseudosuperior</i>	<i>A. manubriator</i>	0	0	$p = 1.000$

A. manubriator, *A. rufopyriformis*, and *A. n. sp.*, near *reflexus*. In all three, female-conditioned water elicited a response by conspecific males but not by heterospecific males (Table 6). The same result occurred when *A. manubriator* was tested against *A. americanus* (Table 7) and *A. pseudosuperior* (Table 8).

Discussion

Female-emitted male-attractant sex pheromones appear to be widespread: we present evidence of chemical communication involving at least 10 species representing three subgenera of *Arrenurus*. In most cases, males reacted to conspecific female-conditioned water, but in *A. apetirolatus* we could only demonstrate that female-conditioned water elicited an arrestant response in male *A. manubriator*. All species demonstrating arrestant behaviour also exhibited the male readiness posture. Previous work by Proctor and Wilkinson (2001) showed that courtship and mating behaviour is similar in *A. manubriator*, *A. rufopyriformis*, and *A. n. sp.*, near *reflexus*, three species also used in our study and representing three different subgenera. We suggest that female-emitted sex pheromones, the male arrestant response, male readiness posture, and the male's use of the fourth legs to grab the female are elements of a generalized courtship behaviour that is presumably plesiotypic for this genus. It is certainly not a universal pattern: for example, there are notable (and presumably apotypic) exceptions (e.g., *Arrenurus (Arrenurus) planus* Marshall; Proctor and Wilkinson 2001).

Unfortunately, it is difficult to interpret negative data: males typically will not arrest or exhibit a readiness posture and females will produce little if any pheromone unless they are well-fed and in good condition. When males of one species fail to respond to pheromones of another species, it may be because the pheromone truly fails to release arrestant behaviour or it could reflect poor condition of either the emitters or receivers. Presumably, this is why *A. birgei* males would cluster and arrest near the source of female-conditioned water from *A. manubriator* but not to water from females of its own species, and conversely, male *A. manubriator* would respond to female-conditioned water from *A. apetirolatus* yet conspecific males were unresponsive. Subsequent to this study, we have found that laboratory-raised mites that have been segregated as males or females at adult transformation (and therefore, remain virgin) are the most responsive and reliable test organisms. Inconsistent results or lack of a pheromone response in the present study could be because of poor condition of the mites, or that in some species, previously mated females having reduced or curtailed their pheromone production. It is likely that most females used in our study were not virgins.

Apparently, it is quite common for members of the same species-group to arrest and orient to heterospecific pheromone sources. *Arrenurus manubriator*, *A. megalurus*, *A. marshallae*, *A. apetirolatus*, and *A. birgei* showed some degree of cross-species reactions, although not in all combinations. Male

A. manubriator and *A. megalurus* respond to female-conditioned water from each other's females, yet male *A. manubriator* but not *A. megalurus* responded to water from *A. apetirolatus* females. Several possible explanations exist: (1) species may use the same chemical or compound as a pheromone but have different thresholds (e.g., Kaae et al. 1973); (2) species may use different but related chemicals (in this case, *A. megalurus* would presumably have a pheromone intermediate in structure to chemicals used by the other two species); (3) pheromones consist of several chemicals in a blend, different species share some or all components, and the likelihood of interspecific responses depends on similarity of blends in constituent chemicals and ratios among those chemicals (typical with many insects, especially Lepidoptera, e.g., Löfstedt 1993).

Whereas there was cross-attractancy among many species within a subgenus, it did not extend across subgenera or throughout all members of a subgenus. Subgenera in the genus *Arrenurus* are largely considered to be artificial constructs, based upon superficial shape similarities of the adult males (Cook 1974). However, there are recognizable species groups (based on male morphology) within the subgenera that presumably reflect real phylogenetic affinities. Interspecific sex pheromone responsiveness provides additional insight, reinforcing concepts of species groups and implying relationships among these groups. Adult male *Arrenurus* (*Megaluracarus*) *megalurus*, *A. marshallae*, and *A. intermedius* are so similar morphologically that they intergrade into each other (*marshallae* group; Cook 1954b, 1976). Larvae of these species share a number of characters with *A. birgei* (*birgei* group; Cook 1954a, 1976) and *A. apetirolatus*, with a conspicuous synapomorphy being that seta Lh1 is located on coxal plate III rather than on the membranous idiosomal integument (Smith 1990). Larvae of *A. manubriator* are quite similar to those of *A. rotundus*; both have setae Lh1 in the plesiomorphic location on the idiosomal integument, but share with all of the forementioned species a broad differentiated marginal band on the dorsal plate and anterolateral corner of coxal plate III (Smith 1990), which again appear to be synapomorphies. These species appear to form a cohesive group within the subgenus *Megaluracarus*, shown above to have the same or similar sex pheromones. In contrast, *A. wardi* and *A. n. sp.*, near *unisinuatus* do not show affinities with these species but have larvae very similar to those of a cluster of species characterized by a heart-shaped hump on the distal end of the male cauda (Cook 1954a); we call the *cardiacus* group. We did not see any evidence for pheromone communication within or between these two species, nor did they react to *A. manubriator* female-conditioned water. It is not clear whether *A. wardi* and *A. n. sp.*, near *unisinuatus* belong to a clade of species that do not use female-emitted sex pheromones, or whether they were not producing and responding to pheromones because of suboptimal conditions in our experiments.

The subgenus *Arrenurus* includes a series of conspicuous species groups based upon male morphology (Cook 1974) but these are also reflected by similarities in larval form. The *americanus* group (Cook 1954b, 1976) includes *A. americanus* and *A. major*, the latter of which responded to

female-conditioned water of the former but *A. americanus* did not respond to pheromone sources from other species of the subgenus. *Arrenurus pseudosuperior* belongs to the *superior* species group (Cook 1954b, 1976) and the one available male of *A. superior* responded to water from female *A. pseudosuperior* but *A. pseudosuperior* did not react to water conditioned by females of other species. *Arrenurus* n. sp., near *reflexus* was the only species in our study belonging to a third species group (we would cluster it with the *fissicornis* group; Cook 1954b) and only responded to water from its own females. Again, negative data are hard to interpret: there was no evidence of pheromone activity involving *A. magnicaudatus* (of the *superior* group) and both *A. fissicornis* and *A. fissicorniformis* (*fissicornis* group). Subsequent casual observations using laboratory-reared mites confirmed that *A. fissicornis* and *Arrenurus* (*Arrenurus*) *bleptopetiolatus* Cook (*fissicornis* group) males will respond to water conditioned by their own and each other's females.

The lack of species-specificity in chemical communication among many mites is a striking contrast to studies involving insects, as has previously been noted by Sonenshine (1985). This is not universal: Mori and Kuwahara (2000) found that various species of *Caloglyphus* spp. (Astigmata: Acaridae) did have distinctly different sex pheromones. It is controversial as to why there is species-specificity in sex pheromones for many insects, especially Lepidoptera. The classical paradigm is that it developed as a premating isolating mechanism to prevent hybridization, whereas another interpretation is that species-specific pheromones represent character displacement developed between already reproductively isolated species (Löfstedt, 1993). Regardless of why species-specificity is so widespread with insect sex pheromones, the question is why it is much less pronounced with mite sex pheromones.

There is strong potential for *Arrenurus* spp. males to be attracted to heterospecific females in their natural habitats. It is common to collect large numbers of *Arrenurus* spp. from one water body, and we have collected over 30 *Arrenurus* spp. from Telephone Bay of Lake Opinicon. It is not uncommon to get ten species from one net-full of substrate, and the species for which we demonstrated cross-attractancy commonly co-occur on this scale in various water bodies of Ontario. Heterospecific couplings between *Arrenurus* spp. regularly occur in containers when researchers are collecting and sorting living material; presumably this is an artifact of disturbance and artificially high densities, although perhaps such pairings occur naturally. One possibility is that sex pheromones in *Arrenurus* spp. are a long-range attractant, but that individuals can then use short-range species-specific cues or courtship behaviour to discriminate among potential mates. Another possibility is that stimulated males produce their own sex pheromone, which are species-specific. *Arrenurus* species with interspecific sex pheromone responsiveness usually have males that are unambiguously different in physical form, while congeneric species of insect that do not exhibit cross-attractancy are often difficult to identify using morphological characters (e.g., *Choristoneura* spp., Silk and Kuenen 1988; *Yponomeuta* spp., Löfstedt 1993). Males of many *Arrenurus* spp.

assume the readiness position and use their fourth legs to grab and grapple the female into position (Proctor and Wilkinson 2001), but the extravagance of male form and diversity among species (while females are cryptically similar; Cook 1976) strongly implies sexual selection and it is probable that female choice still operates to some degree despite male adaptations to circumvent it.

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