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Surface glycoproteins in copepods: potential signals for mate recognition

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Abstract

The mechanism male copepods use to recognize mates is not well understood. Both chemical and mechanical cues have been implicated, but the relative importance of these is not known. This lack of knowledge is despite the belief that mate recognition has a critical role in maintaining reproductive isolation of many species and influences the direction of evolution. Glycoproteins are used as mating signals by a number of aquatic organisms including rotifers, ciliates, and algae. We have developed techniques for selectively probing surface glycoproteins in zooplankton using fluorescently labeled lectins. We examined surface glycoproteins on the urosomes of several species of marine and freshwater copepods to develop insight into their role in mate recognition. Calanoids *Labidocera aestiva*, *Centropages hamatus*, and *Acartia tonsa* were collected in the northern Gulf of Mexico and screened with 12 lectins representing a wide variety of carbohydrate affinities. The harpacticoid *Coulana* sp. originally collected from Florida was screened for the same 12 lectins. The freshwater calanoid *Skistodiaptomus pygmaeus* and the cyclopoid *Mesocyclops edax* also were investigated and compared to the marine species. The sites of lectin binding were consistent across species. Most fluorescence was observed in the urosome at the caudal rami, gonopore, margin of the genital segment, and urosome segment junctions. The signal contrast (signal/background ratio) along the urosome ranged from 3–51 which seems ample for males to discriminate a glycoprotein signal from noise. Our observations clearly demonstrate that glycoproteins on the urosome of females from all six species are present at sites expected to be important in mate recognition.

Introduction

Discriminating potential mates among individuals from different populations and species is one of the major challenges facing males in sexually reproducing populations. This discrimination is accomplished by communication between female and male about a female's receptivity and species identity. Sexual communication has been thoroughly studied in several insects and the molecular nature of sexual signals identified (Stadler, 1984; Carde & Baker, 1984). In most insects, pheromones control reproductive behavior and elucidation of their modes of action has been central to understanding insect population dynamics and speciation.

Chemical communication in aquatic animals also is thought to be well developed, but as yet no molecule with pheromonal activity has been isolated and characterized. This is despite the widespread belief that chemoreception is probably as important in crustaceans as it is in insects (Dunham, 1978, 1988). One group where progress in identifying mating pheromones recently has been made is brachionid rotifers. In the marine rotifer *Brachionus plicatilis*, a mating pheromone is detected through contact chemoreception by males who respond with characteristic mating behavior (Snell & Hawkinson, 1983). The pheromone is a surface glycoprotein (Snell *et al.*, 1988; Snell, 1989; Snell & Nacionales, 1990; Snell & Morris, 1993), glycosylated with N-linked oligosaccharides composed of galactose, mannose, N-acetylglucosamine, and fucose subunits. Lectins with affinity for this oligosaccharide have been used to block male mate recognition and to purify the glycoprotein from rotifer homogenates. Lectins also have been useful in localizing the glycoprotein on the body surface of rotifer females (Snell *et al.*, 1993).

Mating pheromones long have been hypothesized to play an important role in copepod mating (*e.g.* Parker, 1902; Gauld, 1957; Jacobs, 1961; Fleminger,

1967), but identification of chemicals with demonstrable pheromonal activity has proven elusive. Behavioral evidence suggests that males are attracted to females by waterborne pheromones in *Eurytemora affinis* (Katona, 1973), *Calanus pacificus* and *Pseudocalanus* sp. (Griffiths & Frost, 1976), *Centropages typicus* (Blades, 1977), *Labidocera aestiva* (Blades & Youngbluth, 1979, 1980), *Diaptomus* (2 species) (Chow-Fraser & Maly, 1988; Watras, 1983), *Pseudodiatomus* (3 species) (Jacobs, 1961; Jacoby & Youngbluth, 1983), *Oithona davisae* (Uchima & Murano, 1988), *Euterpina acutifrons* (Haq, 1972), and *Tigriopus californicus* (Burton, 1985; Lazzaretto *et al.*, 1990). Although soluble pheromones appear to play an important role in mate location, mate recognition probably occurs upon male contact (Katona, 1973). Calanoid males initially contact prospective mates by grasping their caudal rami with their right geniculate antennule (A1) (Blades, 1977; Blades & Youngbluth, 1979; Jacoby & Youngbluth, 1983; Watras, 1983). Presumably there is a chemosensory stimulus at this site since males grasping females at other sites immediately release them. As mating proceeds, males grasp the female's urosome with their right chelate 5^{th} leg (P5) and use the left 5^{th}leg to attach their spermatophore (Blades & Youngbluth, 1979, 1980). In some species, males 'stroke' the female's urosome in the vicinity of the gonopore prior to spermatophore attachment (Blades-Eckelbarger, 1991). This behavior has been interpreted as males searching for a chemosensory stimulus.

Mating behavior of male calanoid copepods, therefore, suggests the presence of chemical signals on the body surface of females that might be important in mate recognition. We were interested in examining the molecular characteristics of these signals, their localization on the body surface of females, and their intensity relative to background. Our hypothesis is that chemical signals present on the surface of female copepods serve as markers of regions important in copulation and spermatophore placement. Using fluorescently labeled lectins, we have been able to identify surface glycoproteins that could be important in these reproductive activities.

Materials and methods

Copepods used in these experiments were obtained from several locations. The calanoids *Centropages hamatus*, *Labidocera aestiva*, and *Acartia tonsa* were collected in the northern Gulf of Mexico 26 April 1992 about 1 km offshore at Turkey Point, Florida near the Florida State University Marine Laboratory. These samples were collected with a plankton net in 5–15 m of water and kindly supplied by Robert Lutz. The cyclopoid *Mesocyclops edax* and the calanoid *Skistodiaptomus pygmaeus* were collected 8 August 1992 in Berry Reservoir in Rome, Georgia by Dr. Steve Swartz and identified by Dr. Mark Boileau. The harpacticoid *Coulana* sp. (formerly *Scottolana canadensis*) was collected in Sebastian River, Florida by Dr. Darcy Lonsdale and maintained in her lab since 1984. This Florida population is reproductively isolated from other *C. canadensis* populations collected along the coast in the northeast USA (Lonsdale *et al.*, 1988). Most of our analyses were on frozen samples which were shipped to Georgia Tech where all experiments were conducted. Comparison of lectin binding in live and frozen females of these species revealed no differences in the pattern or intensity of lectin binding.

Lectin binding experiments were performed with two replicate males and females of *Coulana* sp. and three replicate *Centropages hamatus* females for each lectin. For *Labidocera aestiva*, *Acartia tonsa*, *Mesocyclops edax*, and *Skistodiaptomus pygmaeus* 2–3 females were examined for each of the 12 lectins tested. Marine copepods were placed in a 24-well plate in 1 ml of 15 ppt synthetic seawater prepared from Instant Ocean salts. Freshwater copepods were placed in synthetic freshwater containing 96 mg $NaHCO_3$, 60 mg $CaSO_4 \cdot 2H_2O$, 60 mg $MgSO_4 \cdot 7H_2O$, and 4 mg KCl in one liter of deionized water. This medium has a pH of 7.4–7.7, a hardness of 80–100 mg $CaCO_3$ per liter and an alkalinity of 60–70 mg per liter. The seawater contained 0.1 mg $\cdot$ ml^{-1} of the lectin to be tested and exposures lasted for 5 minutes. Preliminary experiments indicated that this exposure period with relatively low lectin concentrations was sufficient to get labeling without non-specific binding. All lectins were conjugated to fluoroisothiocyanate (FITC) which allowed lectin binding sites to be visualized with epifluorescent microscopy. After lectin exposure, copepods were transferred with a micropipet to a wash well containing 1 ml of clean seawater to remove unbound lectin. Animals then were transferred to a microscope slide and examined for fluorescence at 50× magnification. An Olympus BH2 microscope had an excitation filter at 450–490 nm and an emission filter at 515 nm. A Javelin Newvichip CCD camera mounted on the microscope recorded the images which were digitized with a Data Translation QuickCapture card on a Mac-

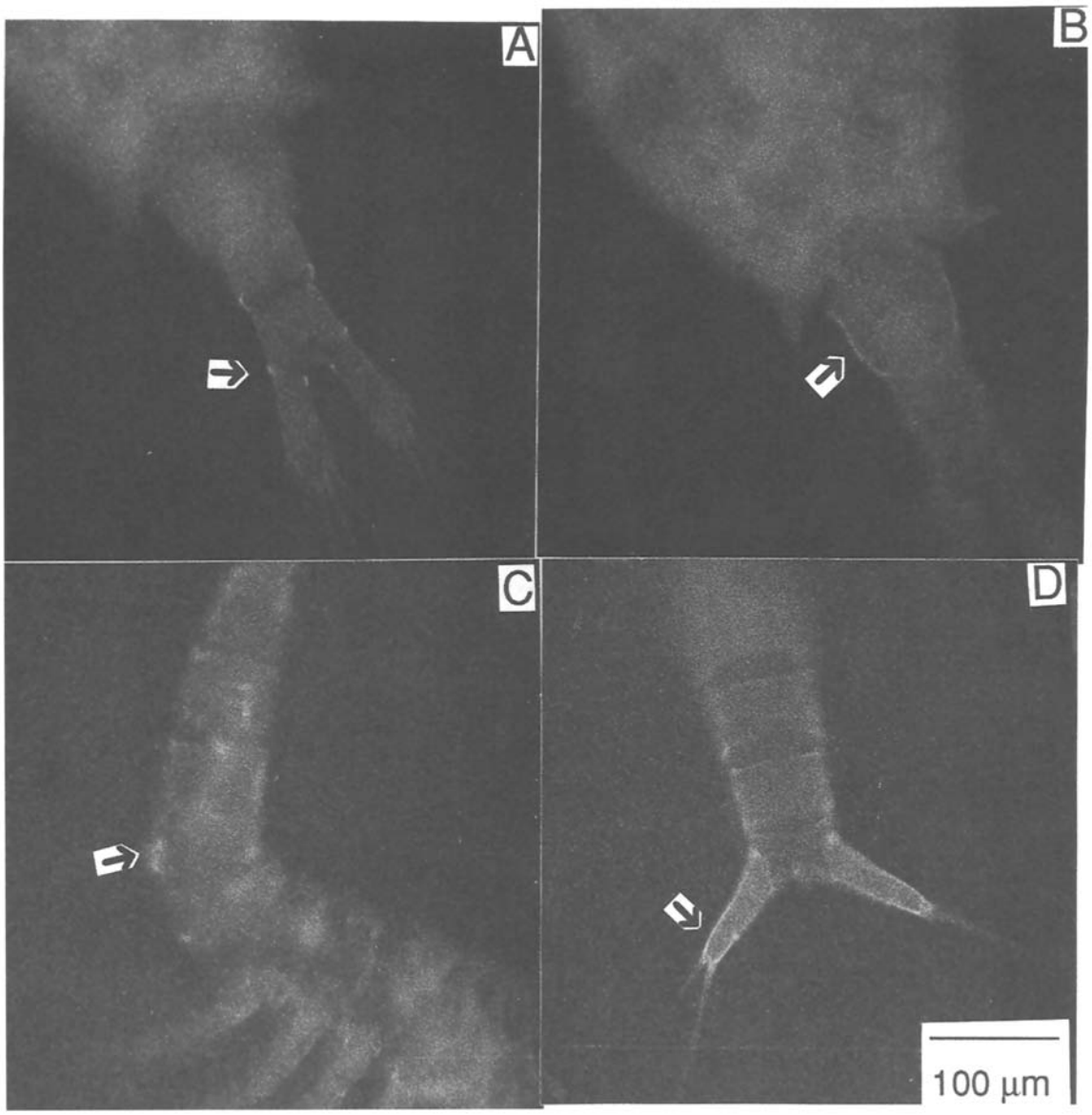

Fig. 1. Photomicrographs of lectin binding sites. A – *Centropages hamatus* female, *Vicia* lectin, urosome segment junctions; B – *Centropages hamatus* female, *Vicia* lectin, margin of genital segment; C – *Centropages hamatus* female, *Pisum* lectin, gonopore; D – *Coulana* sp., female, *Pisum* lectin, margin of caudal rami.

intosh IIci computer. NIH Image software (available from the U.S. National Institutes of Health, Bethesda, Maryland) was used to analyze the intensity of fluorescence localized at various sites on the body surface.

Lectins tested were selected to represent four major classes of oligosaccharide affinities (Table 1). Lectins are proteins or glycoproteins of non-immune origin that bind carbohydrates specifically and non-covalently (Sharon & Lis, 1989). The carbohydrate binding specificities of many lectins are well known (Goldstein & Poretz, 1986), including those used in our experiments. The pattern of lectin binding can be used to assess the molecular structure of a glycoprotein (Osawa & Tsuji, 1987). Lectins were obtained from Sigma Chemical Company, which isolated them from plants, naming them for their plant sources: *Pisum sativum* (pea), *Vicia faba* (fava bean), *Lens culinaris* (lentil), *Canavalia ensiformis* (Concanavalin

A, jack bean), *Lycopersicon esculentum* (tomato), *Triticum vulgaris* (wheat germ), *Glycine max* (soybean), *Erythrina corallodendron* (coral tree), *Bauhinia purpurea* (camel's foot tree), *Ricinus communis* (castor bean), *Tetragonolobus purpurea* (winged pea), *Ulex europaeus* (gorse).

Results

The binding of 12 lectins to the body surface of *Centropages hamatus* females was investigated (Table 2). Little fluorescence was observed on the prosome suggesting that glycoproteins with affinity for the lectin probes are in low abundance at these sites. When prosome fluorescence was observed, it was associated with the margin of the cephalothorax and the junctions between segments. In contrast, several sites on the urosome consistently bound lectins. Five major binding sites were identified including the lateral margin of the genital segment, urosome segment junctions, gonopore, fork of the caudal rami, and tip of the caudal rami (Fig. 1A-D).

All 12 lectins bound to the margin of the genital segment with fluorescence intensities of 4–13× background. Only 6 of the 12 lectins bound at the junctions of urosome segments. All four lectin classes were represented, however, and they yielded fluorescence intensities 6–11× background. Ten of 12 lectins bound to the gonopore with fluorescence intensities ranging from 5–13× background. The lectin *Pisum* produced especially strong fluorescence around the gonopore which was 41× background. Fluorescence in the fork of the caudal rami was observed for only *Pisum* of the 12 lectins screened, which yielded a fluorescence 9× background. Similarly, only *Bauhinia* lectin bound to the tip of the caudal rami, producing fluorescence 7× background. The variation among replicate measures was low, with coefficients of variation averaging 38% for all lectins.

The degree of lectin binding can be used to estimate the relative abundance of glycoproteins along the surface of the urosome. Examples of this kind of data are shown for selected lectins binding to female *C. hamatus* (Fig. 2). Fluorescence localized at the urosome segment junctions was observed for *Pisum*, *Erythrina*, and *Ulex*. This suggests that surface glycoproteins with affinities for these lectins are at 10, 12 and 9× higher densities, respectively, than in the surrounding area. A similar pattern can be observed for the gonopore. A sharp peak of fluorescence about 37× background

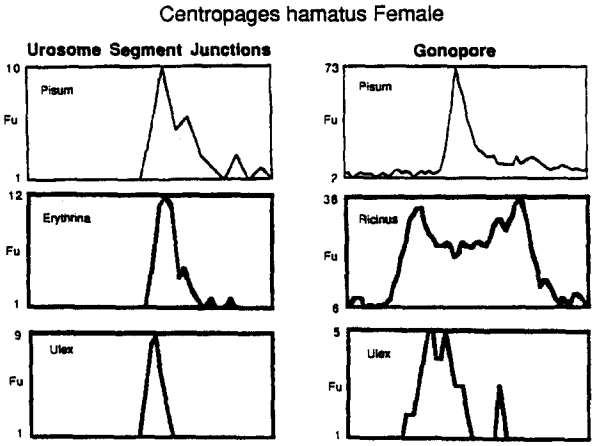

Fig. 2. Fluorescence intensities associated with urosome segment junctions and gonopores of *Centropages hamatus* females. Fu is arbitrary fluorescence units, the lowest value on each graph representing background and the highest value the maximum fluorescence detected. The × axis is linear distance of a transect through the urosome junctions or across the gonopore. The lectin probes used in each experiment are indicated on the graphs.

was detected with *Pisum* lectin, clearly signaling the location of the gonopore on the urosome. Likewise, *Ricinus* and *Ulex* lectins revealed glycoproteins around the gonopore 6 and 5× background levels.

None of the lectins had localized binding in the prosome of *Coulana* sp. females, but binding was intense and localized at several urosome sites (Table 3, Fig. 1D). Nine of 10 lectins screened bound to urosome segment junctions with intensities of 16–46× background. Similarly, 9 of 11 lectins bound to the gonopore with intensities of 18–37× background. Ten of 10 lectins screened bound to the lateral margin of the genital segment and the tip of both caudal rami, producing a range of fluorescence intensities from 17–57× background. None of the lectins bound to the fork of the caudal rami. Replicate measures for all 12 lectins yielded coefficients of variation that averaged 21%.

Variation in relative fluorescence intensity along the caudal rami and across the gonopore is illustrated in Fig. 3. A transect across the caudal rami demonstrates the higher fluorescence intensities at the lateral margins, with values ranging from 12–42× background. The gonopore was also a region of strong fluorescence with the *Vicia* lectin producing a signal 41× background and the *Ulex* and *Ricinus* lectins were 20 and 18× background, respectively.

Lectin binding to *Coulana* sp. males was similar to females except that strong binding was observed in the antennules (Table 4). All 12 lectins tested bound to

Table 1. Lectin binding specifities*. Man - mannose, glc - glucose, glcNAc - N-acetylglucosamine, neu5Ac - N-acetylneuraminic acid, gal - galactose, galNAc - N-acetylgalactosamine, fuc - fucose.

Lectin	Nominal specifity
Glucose/Mannose group	
Pisum sativum	αMan> αGlc = GlcNAc
Vicia faba	αMan> αGlc = GlcNAc
Lens culinaris	αMan> αGlc = GlcNAc
Canavalia ensiformis	αMan> αGlc = GlcNAc
N-Acetlglucosamine group	
Lycopersicon esculentum	GlcNAc (β 1,4 GlcNAc) 1–3
Triticum vulgaris	GlcNAc (β 1,4 GlcNAc) 1–2 βGlcNAc>Neu5Ac
N-Acetylgalactosamine/Galactose group	
Glycine max	α and βGalNAc>α and βGal
Erythrina corallodendron	Galβ1, 4GlcNAc>αGalNAc
Bauhinia purpurea	α and βGalNAc>α and βGal
Ricinus communis	β and αGal>GalNAc
L-Fucose group	
Tetragnolobus purpureas	αL-Fuc>LFucα1,2Galβ1, 4GlcNAc>> L-Fucα1,2Galβ1,3GlcNAc
Ulex europaeus I	αL-Fuc

* From Goldstein & Poretz, 1986.

Table 2. Lectin binding in *Centropages hamatus* females. Table entries are ratios of background fluorescence to structure of interest. Numbers in parentheses are standard errors. A value of 0 means no fluorescence was detected.

Lectin	Binding Sites				
	Margin genital segment	Urosome segment junctions	Gonopore	Fork caudal rami	Tip caudal rami
Lyco	7.0	8.0 (2.0)	0	0	0
Pisum	8.2 (1.9)	10.0 (0.0)	41.3 (4.8)	9.2 (1.6)	0
Tetra	10.0 (1.0)	6.0	0	0	0
Vicia	8.0 (1.0)	11.0	13.0 (0.6)	0	0
Lens	8.0 (1.7)	0	7.5 (1.5)	0	0
Ulex	6.0 (2.1)	9.0	5.5 (0.5)	0	0
Glyc	4.0 (2.8)	0	4.5 (3.2)	0	0
Eryth	6.0 (0.0)	8.5 (2.9)	6.5 (0.5)	0	0
ConA	3.7 (0.9)	0	6.5 (0.5)	0	0
Bauh	4.5 (0.5)	0	8.7 (0.9)	0	7.0
Trit	13.2 (2.5)	0	6.5 (1.5)	0	0
Ricin	10.8 (2.5)	0	6.6 (0.6)	0	0

Table 3. Lectin binding to *Coulana* sp. females. Table entries are ratios of background fluorescence to structure of interest. Numbers in parentheses are standard errors. A value of 0 means no fluorescence was detected and nm means not measured.

Lectin	Binding sites				
	Urosome segment junctions	Gonopore	Margin caudal rami	Fork caudal rami	Tip caudal rami
Lyco	nm	23.0	nm	0	40.0
Pisum	37.0 (1.0)	25.0	25.5 (3.5)	0	34.5 (8.5)
Tetra	25.0 (7.0)	0	26.0 (9.0)	0	19.0 (6.0)
Vicia	46.0 (2.0)	36.5	34.5 (7.5)	0	45.5 (5.5)
Lens	nm	26.0	17.0	0	34.5 (2.5)
Ulex	32.5	20.0 (0.5)	27.0	0	57.0
Glyc	0	0	nm	0	19.0
Eryth	16.0 (3.0)	nm	43.0	0	20.0
ConA	36.5 (0.5)	32.0	43.0 (1.0)	0	44.0
Bauh	16.0 (0)	17.5 (0.5)	21.0	0	28.0 (1.0)
Trit	30.0	23.0	25.5	0	nm
Ricin	21.0	18.0	18.5 (7.5)	0	nm

Table 4. Lectin binding to *Coulana* sp. males. Table entries are ratios of background fluorescence to structure of interest. Numbers in parentheses are standard errors. A value of 0 means no fluorescence was detected and nm means not measured.

Lectin	Binding sites				
	Antennules	Gonopore	Margin caudal rami	Fork caudal rami	Tip caudal rami
Lyco	26.0 (5.0)	25.0 (4.0)	nm	0	22.0 (0)
Pisum	13.0 (6.0)	23.0	36.5 (5.5)	30	22.0 (0)
Tetra	20.0	26.0	27.5 (1.5)	0	19.5 (1.5)
Vicia	25.5 (6.5)	31.0	45.0	0	20.0
Lens	21.5 (3.5)	30.0	nm	0	21.5 (3.5)
Ulex	10.9 (0.6)	51.0 (11.0)	21.3 (9.6)	0	nm
Glyc	22.0 (1.0)	36.5 (1.5)	33.5 (2.5)	0	21.0 (3.0)
Eryth	36.0 (1.0)	0	16.5 (2.5)	0	nm
ConA	21.0	28.0	44.5 (2.5)	0	nm
Bauh	33.5 (0.5)	29.5 (4.5)	41.0 (4.0)	0	25.0
Trit	48.0 (4.0)	0	35.5 (0.5)	0	nm
Ricin	11.0	37.0	12.3	0	nm

both left and right antennules, producing fluorescence intensities 11–48× background. Ten of 12 lectins tested bound to the gonopore and 10 of 10 bound to the margin of the caudal rami, with fluorescence intensities ranging from 12–51× background. Seven of 7 lectins screened bound to the tip of the caudal rami in

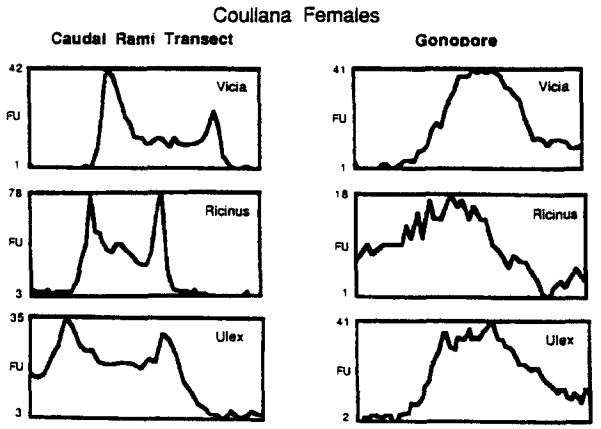

Fig. 3. Fluorescence intensities associated with the caudal rami and gonopore of *Coulana* sp. females. Fu is arbitrary fluorescence units, the lowest value on each graph representing background and the highest value the maximum fluorescence detected. The X axis is linear distance of a transect across the caudal rami or the gonopore. The lectin probes used in each experiment are indicated on the graphs.

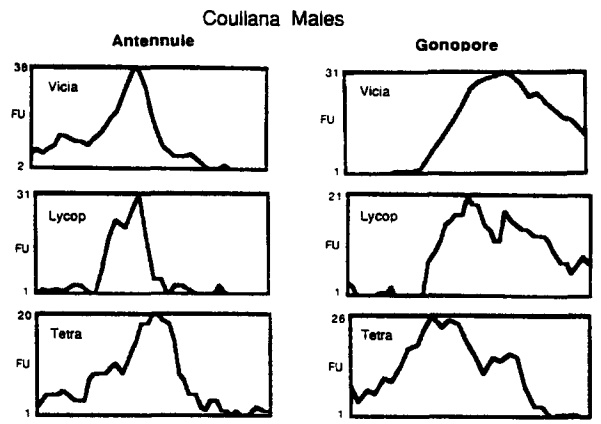

Fig. 4. Fluorescence intensities associated with the antennules and gonopore of *Coulana* sp. males. Fu is arbitrary fluorescence units, the lowest value on each graph representing background and the highest value the maximum fluorescence detected. The X axis is linear distance of a transect across the antennules or gonopore. The lectin probes used in each experiment are indicated on the graphs.

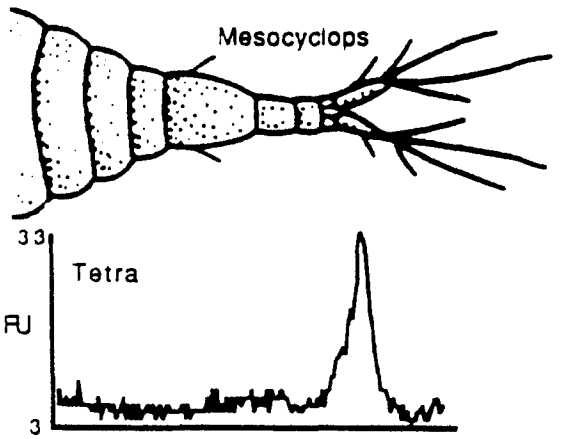

Fig. 5A. Fluorescence intensities along a transect down the urosome of 4 copepod species: a – *Mesocyclops edax*, b – *Skistodiaptomus pygmaeus*, c – *Labidocera aestiva*, d – *Acartia tonsa*. FU is arbitrary fluorescence units, the lowest value on each graph representing background and the highest value the maximum fluorescence detected. The X axis is linear distance of a transect down the urosome. The lectin probes used in each experiment are indicated on the graphs.

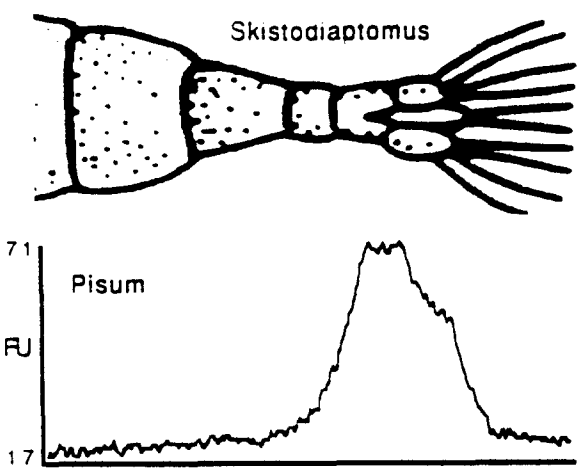

Fig. 5 B.

males, but only *Pisum* out of 12 lectins tested bound to the fork of the caudal rami. Fluorescence intensities at these sites were consistently 20–30× background. Coefficients of variation for all 12 lectins screened averaged 20%.

Variation in relative fluorescence intensities across the *Coulana* sp. male antennules and the gonopore can be seen in Fig. 4. The lectins *Lycopersicon*, *Tetragonolobus*, and *Vicia* produced fluorescence intensities of 31, 20, and 19× background, respectively. The gonopore was clearly distinguishable from its surroundings by the binding of the fluorescently labeled lectins *Vicia*, *Lycopersicon*, and *Tetragonolobus*. These lectins produced fluorescence intensities that were 21–31× background.

Observation of lectin binding in *Centropages hamatus* and *Coulana* sp. indicated that the most binding to surface glycoproteins occurred in the urosome. To determine the generality of this result we examined four additional copepod species from different genera. We screened *Mesocyclops edax*, *Skistodiaptomus pygmaeus*, *Labidocera aestiva*, and *Acartia tonsa* using

Table 5. Comparison of lectin binding among four copepod species.

Species	Lectins screened	Lectins binding	Major binding site
Mesocyclops edax	12	6	tip caudal rami
Skistodiaptomus pygmaeus	12	5	fork & tip caudal rami
Labidocera aestiva	12	4	fork caudal rami
Acartia tonsa	12	1	2 & 3 urosome junction

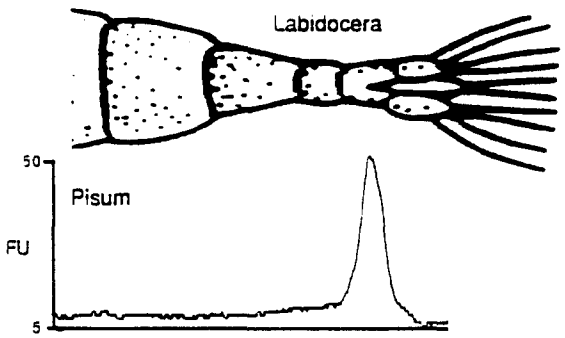

Fig. 5 C.

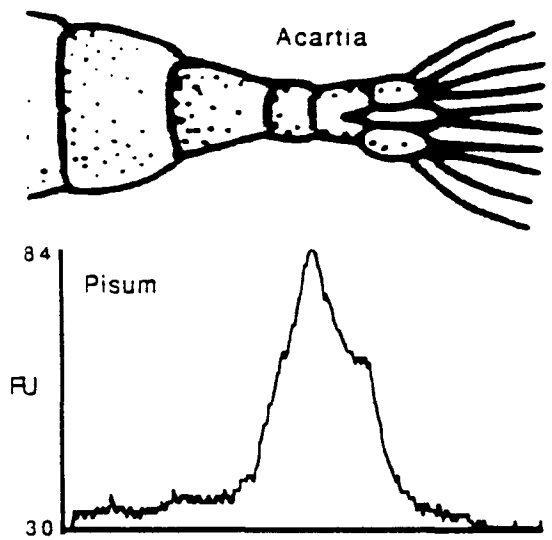

Fig. 5 D.

12 lectins and found significant binding of 6, 5, 4, and 1 lectins, respectively (Table 5). Transects of fluorescence intensity along the urosome illustrate signal contrast against background (Fig. 5A–D). For *M. edax* exposed to the *Tetragonolobus* lectin, the strongest fluorescence was found at the tip of the caudal rami. A sharp peak of fluorescence 11× background allowed the tip to be discriminated from other parts of the urosome. A broader peak encompassing the fork and tip of the caudal rami was observed with *Pisum* binding to *S. pygmaeus*. This fluorescence was 4× background levels. In *L. aestiva*, the major site of fluorescence was the fork of the caudal rami which was 10× background with *Pisum* labeling. In *A. tonsa*, fluorescence was most intense at the junction between the 2^{nd} and 3^{rd} urosome segments where *Pisum* labeling produced fluorescence 3× background.

Discussion

Several aspects of calanoid mating behavior suggest the involvement of contact pheromones. One of the best studied species is *Labidocera aestiva* (Blades & Youngbluth, 1979; Blades-Eckelbarger, 1991). After contact, males use their right geniculate antennule to grasp females by their caudal rami or setae. If a male grasps a female at any other site, he immediately releases her (Blades, 1977). This behavior suggests that a specific signal is received through the male's antennule from the female's caudal rami, but whether the signal is chemical or mechanical is unknown. Our data on lectin binding demonstrated the presence of glycoproteins in this region which could act as mating pheromones. Males could use these signals, whose intensities were several fold background, for recognition of conspecifics.

After initial contact, specific sensory hairs on the antennule stimulate males to move into copulatory position, grasping the female's urosome with their right chelate 5^{th} leg (P5) (Blades-Eckelbarger, 1991). The urosome grip must be firm and precise because it influences the placement of the spermatophore. The male's left 5^{th} leg is used to 'stroke' the genital region exploring for a site to attach the spermatophore (Blades & Youngbluth, 1979). Stroking behavior may pro-

vide males with important chemical cues necessary for accurate spermatophore placement. The site of placement is critical since copepod sperm are aflagellate and non-motile and males lack a copulatory organ (Blades-Eckelbarger, 1986). Slightly misplaced spermatophores may significantly reduce the number of sperm transferred. The area around the gonopore has glandular pores in some species (Blades & Youngbluth, 1979; Blades-Eckelbarger, 1991), some of which might secrete chemical signals useful in copulation. Our observations of dense glycoproteins in the genital region indicate that males have an opportunity to use these as signals to guide spermatophore attachment. An hypothesis emerging from our data is these surface glycoproteins are critical to mate recognition and sperm transfer. Inactivation of these glycoproteins or blocking access to them could interfere with copulation. Katona (1973) showed that heat-killing *Eurytemora affinis* females by exposure to 65 °C for 15–30 s greatly diminished their attractiveness to males. This observation suggests that a heat-labile compound is necessary for mate recognition and that female movement is not required for mating in this species.

Less is known about the mating behavior of harpacticoids than calanoids, but several aspects have been described (Haq, 1972; Burton, 1985). Male *Tigriopus californicus* use their geniculate antennules to grasp young females in the CII-CV copepodid stages. Males maintain their grasp until the females complete their terminal molt when fertilization occurs (Boxshall, 1990). Males prefer CV's over younger copepodid stages and rarely attempt to grasp adult females. These precopulatory mate guarding behaviors suggest that receptors in male antennules have the ability to discriminate among developmental stages, but the role of chemoreception is unknown. Knowledge of mating behavior in cyclopoids is even more limited with a few observations reported by Gophen (1979) and Uchima & Murano (1988).

The fact that several types of lectins bound to most species suggests that more than one type of glycoprotein is present. For *Centropages hamatus* and *Coulana* sp. females and *Coulana* sp. males, lectins representing all four classes bound. At the other extreme, *Acartia tonsa* bound only *Pisum* lectin to surface glycoproteins on its urosome. The surface of some copepod species may be more densely populated with glycoproteins than others and some may rely more heavily on chemical signals in mating. The fact that occurrence of lectin binding to the urosomes of species representing calanoid, cyclopoid, and harpacticoid orders suggests that the presence of surface glycoproteins is a widespread and general characteristic.

The sites of lectin binding were consistent across species. Most fluorescence was observed in the urosome at the caudal rami, gonopore, margin of genital segment, and urosome segment junctions. Behavioral observations suggest that signals on the caudal rami may be used for species recognition, those on the gonopore for spermatophore placement, and the remaining sites for general male orientation during copulation. The signal contrast (signal/background ratio) along the urosome ranged from 3–51 which seems ample for males to discriminate a glycoprotein signal from noise. Lectin binding was observed on the antennules of *Coulana* sp. males, but not *Coulana* females or females of the 5 other species examined. All 12 lectins surveyed bound to male antennules which behavioral evidence suggests have an important sensory role in mating. In contrast, no lectin binding was seen on the left 5^{th} leg, which in calanoids is used in stroking behavior prior to spermatophore attachment (Blades & Youngbluth, 1979).

Investigation of mating pheromones in copepods may have other benefits. The existing taxonomy of genera and species is inadequate, largely due to the near complete dependence on morphology for discriminating boundaries. If mating pheromones are molecules that male copepods use to discriminate conspecifics, they might prove especially useful to taxonomists. Isolation, purification and the production of antibodies to copepod mating pheromones could provide useful new tools to simplify and speed species identification.

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