A COMPARISON OF ANTENNULE STRUCTURE IN A SURFACE- AND A CAVE-DWELLING CRAYFISH, GENUS *ORCONECTES* (DECAPODA, ASTACIDAE)

BY

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ABSTRACT

Crayfish show many behavioral and morphological adaptations that serve to exploit chemical information in an aquatic environment. The primary chemosensory structure, the outer branch of the antennule, varies morphologically among species. A reasonable approach to the study of these variations is to compare morphological structures from crayfish living in different environments. The potential of this approach was evaluated by comparing the morphology and distribution of chemosensory sensilla (aesthetascs) along antennules of a cave- and a surface-dwelling crayfish. In comparison to the surface-dwelling species (*Orconectes cristavarius*), the cave-dwelling species (*O. australis packardi*) had longer antennules and longer individual aesthetasc sensilla. The surface species, however, had significantly more aesthetascs per annulus and a higher density of aesthetascs towards the distal end of the antennule. These data are discussed in terms of chemical sensitivity and chemoreception in turbulent environments.

RÉSUMÉ

Les écrevisses présentent des adaptations comportementales et morphologiques qui servent à exploiter l'information chimique dans un environnement aquatique. La structure chémosensible primaire, la rame externe de l'antennule, varie morphologiquement suivant les espèces. Une approche raisonnable de l'étude de ces variations est de comparer les structures morphologiques des écrevisses vivant dans différents environnements. Le potentiel de cette approche a été évalué en comparant la morphologie et la répartition des sensilles chémosensibles (aesthétasques) sur les antennules d'une écrevisse vivant en eaux de surface et d'une écrevisse vivant en eaux souterraines. Par rapport à l'espèce de surface (*Orconectes cristavarius*), l'espèce de grotte (*O. australis packardi*) avait de plus longues antennules et de plus longs aesthétasques. L'espèce de surface, cependant, avait significativement plus d'aesthétasques par anneau et une densité plus élevée d'aesthétasques vers l'extrémité distale de l'antennule. Ces données sont discutées en termes de sensibilité chimique et de chémoréception dans les environnements turbulents.

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INTRODUCTION

Chemical ecology of crayfish

Like many other decapod crustaceans, crayfish morphology appears to be well suited to exploit chemical information in the aquatic environment. Crayfish have been shown to use chemical signals to find prey (Keller et al., 2001), avoid predators (Hazlett & Schoolmaster, 1998), recognize mates (Hazlett, 1985), and assess social status (Zulandt-Schneider et al., 1999). Not only must crayfish detect and identify a diverse array of compounds, but the meaning and source of these signals must be decoded in an environment often characterized by complex mixing patterns and turbulence. It has been suggested that some aquatic animals, including crayfish, use information from the "turbulent odor plume" (Moore et al., 1991) to increase efficiency of finding odor sources (Moore & Grills, 1999). These tasks likely require complex physiological processing and recognition abilities in order to filter signal from noise. In addition, morphological structures most suited to detect chemical signals should vary depending on the distribution of the signals in the environment and their influence on behavior and fitness.

In general, the particular requirements of chemosensory structures should depend on (1) the importance of different chemical cues to the organism's behavior and ecology and (2) the hydrodynamic properties of the system. Therefore, we expect that species inhabiting different environments and facing diverse selection pressures may have chemoreceptive structures suited to their particular environment. In this paper we compare the primary chemoreceptive structure in the crayfish, the outer branch of the antennule, in two species of freshwater crayfish, *Orconectes cristavarius* Taylor, 2000, an epigean (surface-dwelling) species, and *Orconectes australis packardi* Rhoades, 1941, a troglobitic (obligate cave-dwelling) species.

Antennule structure and function

Although chemoreceptors are found on many parts of the crayfish exoskeleton, they are highly concentrated on the first pair of antennae, the antennules. The outer flagellum of each antennule contains sensilla (aesthetascs) that house chemoreceptive neurons (Tiernery et al., 1986; Sandeman & Sandeman, 1996). To facilitate diffusion of chemical signals, the cuticle in the distal lenticular region of each aesthetasc is thinner compared to the basal stem, dividing the aesthetasc into two regions. Aesthetascs are located along nearly the entire length of the antennule but are most numerous on the distal segments, or the annuli, of the antennule (Sandeman & Sandeman, 1996).

The morphology of antennules and individual aesthetascs varies among species and may reflect selection for particular mechanical or chemosensory requirements

(Tierney et al., 1986; Hallberg et al., 1997). Variation in the morphology or innervation patterns of aesthetasc sensilla may provide a means of modulating the sensitivity or specificity of the chemosensory response (Tierney et al., 1986). For example, fewer neurons in each aesthetasc might increase sensitivity by increasing the area of cuticle exposed per dendrite. On the other hand, more neurons could increase the range of detectable chemicals, at the expense of sensitivity, due to a greater diversity of cell types. Sensitivity could also be increased by the lengthening of the aesthetasc, as seen in copepods (Boxshall & Huys, 1998), or by altering the length or thickness of the cuticle in the distal lenticular region. It has been suggested that the arrangement of aesthetascs on the antennule is related to "flicking behavior" (Schmitt & Ache, 1979), and, in general, to the requirement to deliver odorants across the boundary layer surrounding the hair (Hallberg et al., 1997). Species may have different requirements for thinning this layer due to variation in the hydrodynamic regime or in the distribution of important chemical signals in the environment. Given the variety of habitats inhabited by crayfish, we expect that both the morphology of individual aesthetascs and their distribution along the antennule might vary across species due to divergent selection pressures.

Cave crayfish and surface crayfish comparisons

Freshwater crayfish are found in a variety of habitats under a variety of environmental conditions. One of the most dramatic examples of this variety is the contrast between subterranean and surface environments. Several species in the genus *Orconectes* are found in cave habitats. Like many cave-dwelling organisms, these species show conspicuous morphological features associated with the cave environment, including a slender body shape, elongate appendages, lack of pigmentation, and non-functional eyes. Along with these obvious external features, studies from other subterranean species suggest some cave organisms may have increased sensitivity to non-visual environmental cues (chemical, tactile) compared to surface-dwelling species (Poulson, 1963; Barr, 1967; Barr & Holsinger, 1985).

The ecological structure of the cave environment could lead to selection for higher chemical sensitivity. Cave production generally consists of allochthonous input of organic matter and limited microbial production (Barr, 1967). Floods and sinking streams provide pulses of organic material in the form of plant material and organisms suspended in the water column. In addition, surface organisms will occasionally enter the cave and excrete or die, providing a temporary food source for cave organisms. In general, high-quality food in the cave is in short supply and may be patchy. Under these circumstances we might expect strong selection for sensory structures that allow location of these sources, possibly over long distances. In contrast, surface streams are characterized by an abundance of allochthonous and autochthonous production. Although high-quality food "bonanzas" may

be sporadically available, more diffuse resources are present in the form of detritus and algal growth. Therefore, we might expect less selection in the surface environment for long-distance, high sensitivity chemoreceptive abilities.

Both cave and surface crayfish experience a diversity of hydrodynamic regimes throughout the year. In low-order streams in Kentucky, some surface stream pools may become isolated in the late summer, but in general there is some surface water flow within and between pools. Cave crayfish also experience a range of flow rates, but they can be found in isolated pools through much of the year. In many cases, these pools are characterized by little or no surface flow (Barr, 1967). Therefore, except during periodic flooding, cave crayfish experience a less turbulent environment than many surface crayfish. This difference in hydrodynamics could alter selection pressures on antennule morphology in several ways. First, lower turbulence might be associated with less physical disturbance on the antennular surface, allowing for more gossamer appendages and sensilla. Second, the distribution of chemical signals in caves should differ from that in surface environments due to differences in water velocity and substrate morphology (Atema, 1988; Moore et al., 1999). Different configurations of sensory structures could be most effective for extracting information from odor plumes in cave and surface habitats.

The purpose of this study is to compare antennule structure in a surface and a cave crayfish. Our intention is to use these data to generate hypotheses for the evolution of chemosensory structures in crayfish. Further morphological, behavioral, and physiological studies will be required to fully test these hypotheses.

METHODS

Orconectes cristavarius is a surface-dwelling crayfish found in head water streams in eastern Kentucky (Taylor, 2000). *Orconectes australis packardi* is a troglobitic crayfish found in subterranean streams in south-central Kentucky (Hobbs et al., 1977). We collected specimens of *O. cristavarius* from Indian Creek, Menifee County, KY and specimens of *O. a. packardi* from Sloans Valley Cave, Pulaski County, KY. All animals were kept in the lab and maintained on a diet of pellet fish food for at least one month before the study began. Several individuals molted during this period.

We removed the outer flagellum of the left antennule from 17 *O. cristavarius* and 20 *O. a. packardi* and mounted them on glass slides. These slides were observed under a Leica-MS5 dissecting microscope at 100 × magnification. Observations and photographs of individual aesthetascs required higher magnification and

were done using a Nikon, Optiphot-2 upright microscope using a $40 \times$ (0.55NA) objective with a $3.5 \times$ photographic magnification eyepiece.

We measured the relative length of each flagellum (flagellum length divided by the total body length from the tip of the rostrum to the posterior end of the telson). For each annulus of the flagellum, we counted the number of aesthetascs and acuminate setae (Tierney et al., 1986). Previous studies of *Orconectes* have shown that aesthetascs occur in distinct clusters on the distal and proximal end of each annulus (Tierney et al., 1986). For a subset of replicates in this study, we kept separate counts of aesthetascs occurring on each end (proximal and distal) of the annulus. To allow pooling of data and comparisons across species, we counted the number of aesthetascs in each of ten, equally spaced bins representing the percent of the total number of annuli along the antennule. For example, the first bin would represent all aesthetascs occurring in the first 10% of the annulus, starting at the proximal end. In order to assess differences between species in the morphology of individual aesthetascs, we measured the total length of the aesthetasc and the length of the distal lenticular region located on the tenth annulus from the distal end for individuals from each species.

RESULTS

Measurements of relative antennule length indicated that *Orconectes australis packardi* had significantly longer antennules relative to body length than *O. cristavarius* (table I, fig. 1). In both species, acuminate setae occurred within the first few proximal annuli (fig. 2) and steadily increased in number in more distal annuli. Approximately four times as many setae occurred within the most distal 10% of annuli (setae were usually most numerous on the last segment). *O. a. packardi* and *O. cristavarius* had overall similar numbers of setae per annulus (*O. a. p.*: 2.14 setae, *O. c.*: 2.11 setae; $t = 0.18$, $p = 0.85$). In *O. cristavarius*, a small number of aesthetascs were found towards the proximal end of the anntenule, starting within 30% of the total annuli (fig. 3). Aesthetascs became more numerous towards the distal end. We saw a similar pattern in *O. a. packardi*. However, there were overall fewer aesthetascs per annulus (*O. a. p.*: 2.10 aesthetascs, *O. c.*: 2.68 aesthetascs; $t = 3.90$, $p = 0.0005$). Proximal ends of each annulus had more aesthetascs than distal ends in both *O. cristavarius* ($t = 2.44$, $p = 0.03$) and *O. a. packardi* ($t = 10.46$, $p < 0.001$) (fig. 4). Total aesthetasc length ($t = 9.49$, $p < 0.001$) and the length of the distal lenticular region ($t = 7.97$, $p < 0.001$) were both significantly greater in *O. a. packardi* (fig. 5a). The relative length of the distal lenticular region, expressed as a percentage of the total aesthetasc length was also greater in *O. a. packardi* ($t = 3.51$, $p = 0.001$, fig. 5b).

Morphological measures of body and antennule length for *Orconectes australis packardi* Rhoades
and *Orconectes cristavarius* Taylor

Antennule length (mm)	Body length (mm)	Antennule length/body length
	O. a. packardi	
4.74	30	1.58
6.41	34	1.89
7.05	35	2.02
5.13	35	1.47
7.31	42	1.74
7.82	44	1.78
7.18	45	1.60
7.44	49	1.52
9.74	50	1.95
7.05	50	1.41
8.08	54	1.5
9.23	59	1.57
11.28	60	1.88
10.90	61	1.79
	O. cristavarius	
5.39	39	1.38
6.15	40	1.54
6.41	43	1.49
7.95	48	1.66
6.28	50	1.26
7.44	51	1.46
10.51	55	1.91
8.97	60	1.50
9.62	65	1.48
7.69	67	1.15
10.51	69	1.52

DISCUSSION

Our study showed that there is an elaboration of the chemosensory apparatus in
Orconectes australis packardi that is suggestive of increased chemical sensitivity.
Although already known from previous species descriptions (Fitzpatrick, 1987),
we confirmed that the relative length of the antennules is greater in *O. a. packardi*
than in *O. cristavarius*. We also found that the length of individual aesthetascs and
the relative length of the distal lenticular region were both greater in *O. a. packardi*.
These observations suggest that *O. a. packardi* neurons may be exposed to more
chemical cues which could lead to increased sensitivity.

It is interesting to note that there was no difference in the density or distrib-
ution of acuminate setae on the antennule. The clustering of these setae on the
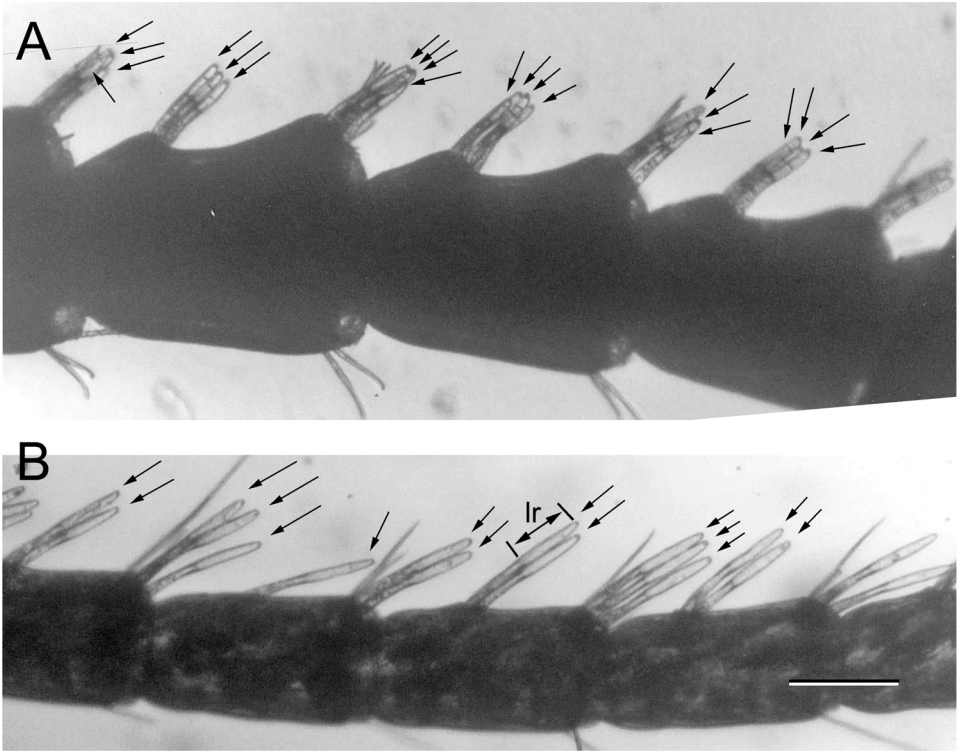distalmost annulus suggests a mechanoreceptive role. If the differences in anten-

Fig. 1. Photomicrograph of annuli of: A, *Orconectes cristavarius* Taylor, 2000; and, B, *Orconectes australis packardi* Rhoades, 1941. The number of setae (arrows) per aesthetasc is greater in the surface species then for the cave animal and the relative length of the lenticular region (lr) within each seta is greater for the cave species. In addition, there are more setae on average in the distal aesthetascs per annulus for both species. Distal is to the right for both figs. Scale bar equals 120 μm.

nule morphology were due to an overall increase in sensitivity to all environmental cues, we would expect more setae in *O. a. packardi*. This was not the case, leading us to conclude that the morphological differences we observed are probably associated with chemoreception.

Although the morphology of the individual aesthetascs is consistent with increased chemical sensitivity in *O. a. packardi*, the density of aesthetasc hairs suggests otherwise. In our study, *O. cristavarius* had significantly more aesthetascs per annulus compared to *O. a. packardi*. This was particularly true towards the distal end of the antennule. This morphological difference could potentially increase the exposure of receptor neurons to chemical signals in this species, leading to increased sensitivity. Alternatively, this difference could be a result of the differences in the hydrodynamic environment of these two species. *O. cristavarius* is likely to experience a greater range of water velocities in surface streams. If current think-
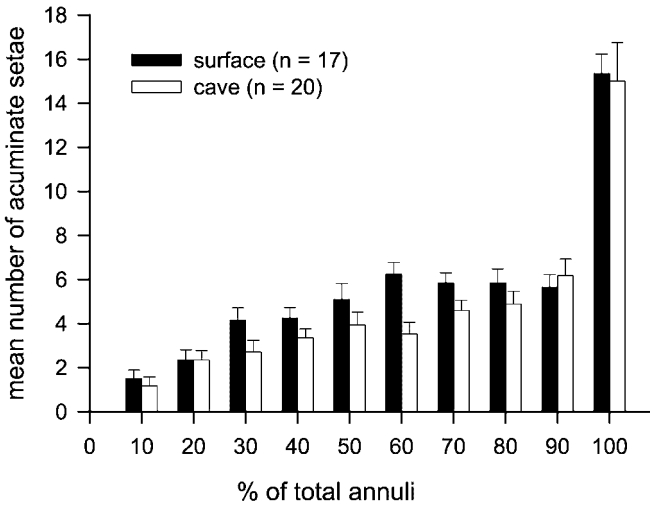
Fig. 2. Number of acuminate setae in each decile representing fractions of the total number of annuli. Values to the left are more proximal annuli. Means ±1 SEM are plotted for cave and surface crayfish.
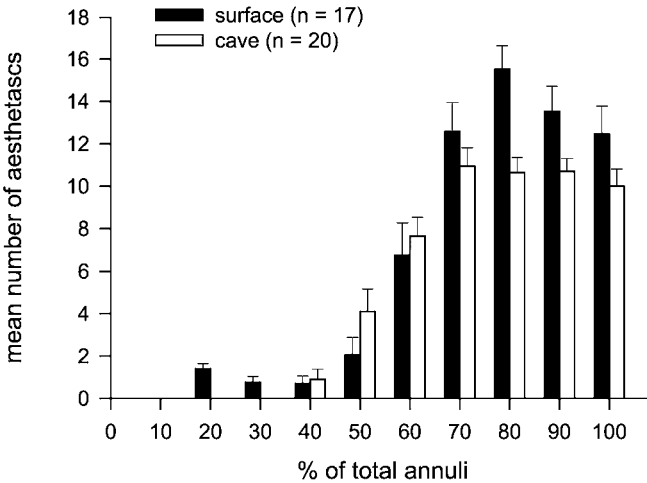

Fig. 3. Number of aesthetascs in each decile representing fractions of the total number of annuli. Values to the left are more proximal annuli. Means ±1 SEM are plotted for cave and surface crayfish.

ing concerning the extraction of information from turbulent odor plumes is correct (Atema, 1986; Moore et al., 1999), this would suggest the chemical reception structures of cave and surface crayfish would differ. Current theory does not provide clear predictions concerning how they should differ, but our data suggest an intriguing possibility. In turbulent environments, high energy mixing would lead to an odor plume (perhaps from a potential food item) with a structure skewed towards a smaller spatial scale. In contrast, odor plumes in low energy environments
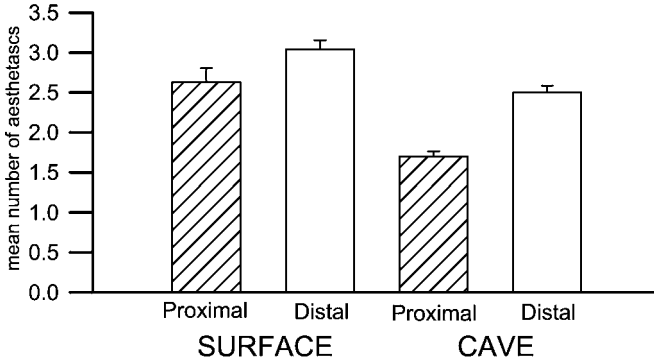
Fig. 4. The number of aesthetascs per annulus in each of two distinct clusters located on the proximal or distal end of each annulus. Means ±1 SEM are plotted for both surface and cave species.
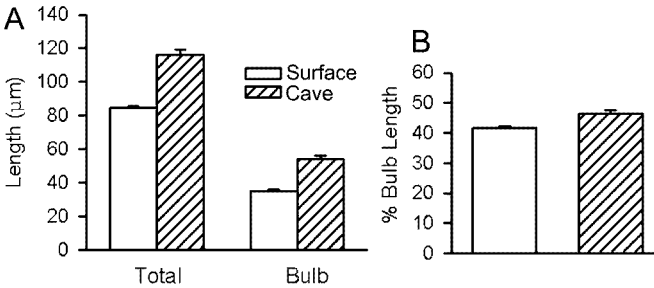

Fig. 5. A, The total aesthetasc length and the length of the distal lenticular region of the aesthetasc for surface and cave crayfish; B, percent of the total aesthetasc length attributable to the distal lenticular region. Means ±1 SEM are plotted on both graphs.

would be dominated by larger patches of odor. Therefore, we would predict that organisms in higher energy environments would benefit from an increased density of sensory hairs along the antennule. This would allow discrimination of fine scale patterns in the odor plume. In a less turbulent environment, the discrimination of these patterns may be less important, leading to a lower density of sensilla. An alternative hypothesis for this observed difference in morphology concerns the effect of turbulence on the mechanics of the sensory structures. Both species may have sensory structures that maximize sensitivity under the limitations imposed by physical damage caused by water flow. Surface crayfish antennule length or the distribution of aesthetascs could be an adaptation to avoid loss of chemosensory sensilla in a high-energy environment. The shorter, higher-density hairs might be more protected at the expense of greater exposure to chemical signals compared to longer, more isolated hairs in cave crayfish. This cost might be ameliorated in the surface streams by faster water flow, which would lead to thinner boundary layers surrounding the anntenules and increased exposure to chemicals during antennule flicking.

There appears to be a wide range of variation in the structure and distribution of chemosensory sensilla in crustaceans (Hallberg et al., 1997). There is also significant variation within crayfish species. For example, the number of aesthetasc hairs on each annulus varies among species: 1 row of 2-5 sensilla in *Cherax destructor* Clark, 1936 (cf. Sandeman & Sandeman, 1996), 2 rows of 5-10 sensilla in *Pacifastacus leniusculus* (Dana, 1852) (cf. Hallberg et al., 1997), 2 rows of 3-6 aesthetascs in *Orconectes propinquus* (Girard, 1852) (cf. Tierney et al., 1986), 2 rows of 1-4 sensilla in *Orconectes australis packardi* (this study), 2 rows of 1-7 sensilla in *Orconectes cristavarius* (this study). In addition, there is variation in the number of innervating olfactory receptor neurons and the length of individual sensilla (Sandeman & Sandeman, 1996; Tierney at al., 1996; Hallberg et al., 1997). The morphological variation documented in these studies suggests an opportunity to study the evolution of chemosensory structures in crayfish. Future research should involve correlating morphological structures with different environmental conditions. In addition, future work could include analysis of variation among life stages or sexes (Boxshall & Huys, 1998). Our study serves as a preliminary analysis of two species living under very different environmental circumstances. We hope that our analysis leads to further studies on the evolution of these structural variants in crayfish.

ACKNOWLEDGEMENTS

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