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Ecological, Evolutionary, and Functional Correlates of Sensilla Number and Glomerular Density in the Olfactory System of Decapod Crustaceans

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ABSTRACT

One of the features common among olfactory systems for vertebrate and invertebrate species is the division of the primary processing area into distinct clumps of synaptic neuropil, called *glomeruli*. The olfactory glomeruli appear to serve as functional units of olfaction and are the location of the primary processing between chemosensory afferents and second-order neurons. Although glomeruli are found across all phyla, their numbers and size appear to be characteristic for each species, giving rise to the speculation that there is a relationship between glomerular number and function. It has been hypothesized, for example, that animals with more glomeruli may be able to resolve a wider range of odors. Crustacean species are distributed among freshwater, marine, and terrestrial habitats in arctic, temperate, and tropical climates. They also exhibit a variety of lifestyles and behaviors in which olfaction may play a dominant role. Feeding, for example, ranges from carnivorous, through subaquatic and terrestrial omnivorous scavenging, to filter feeding. Mating and territorial behaviors also are known to involve chemical signals. The current study examines glomerular numbers in the olfactory lobes of 17 crustacean species from six of the seven taxa now included in the reptantian decapods. Estimates of the glomerular numbers were obtained from the analysis of sectioned material treated immunocytochemically with an antibody against synapsin that labels proteins contained in neuronal terminals. The numbers of glomeruli found in the different species were then compared with the volume of the glomerular neuropil, numbers of olfactory sensilla, life styles, habitat, and phylogenetic affinities. The picture that emerges from these correlations is that the decapod crustaceans have exploited various strategies in the construction of their olfactory systems in which the problems of size, sensitivity, and selectivity have all interacted. We find a continuum across the groups ranging from those that favor a high convergence of receptor neurons onto a few glomeruli to those that share a small number of receptor neurons among many glomeruli. The potential functional consequences of these differences are discussed. J. Comp. Neurol. 455: 260–269, 2003.

Indexing terms: phylogeny; eureptantia; aesthetasc; olfactory receptor neuron; glomeruli; chemosensation

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The most obvious and frequently cited similarity between the invertebrate and vertebrate olfactory systems is the projection of the olfactory receptor neurons to a single area of the brain, where they end in concentrations of neuropil called *olfactory glomeruli*. These glomeruli are typically arranged around the periphery of a lobe, and the afferent fibers from the sensory neurons project to and penetrate the lobe. All glomeruli in any one animal, with the exception of the macroglomerular complex of insects, are generally about the same size and appear to have principally the same neuronal circuitry (Hildebrand and Shepherd, 1997). Hence there appears to be something in the first stage of olfactory processing in all animals that requires the initial distribution of the primary afferent inputs into an array of complex but similar subunits of neuropil, arranged in parallel, before the information is passed to higher order systems in the central nervous system. Although there are many similarities in the functional organization of olfactory systems, these parallels are more likely to be convergent than inherited from a last common ancestor (Strausfeld and Hildebrand, 1999).

In the vertebrates and invertebrates, odor selectivity begins with the olfactory receptor neurons (ORNs), where receptor molecules in the membranes of the receptor neurons bind to odor molecules or parts of them (Buck and Axel, 1991; Clyne et al., 1999; Vosshall et al., 1999). In most species, each receptor cell expresses very few, perhaps only one, odor receptor molecule on its surface. One exception to this trend, however, is the chemosensory neurons in *Caenorhabditis elegans*, each of which expresses multiple receptor molecules (Sengupta et al., 1993; Wes and Bargmann, 2001). In either case, different parts of a large and complex odor molecule may bind to and activate several different olfactory receptor neurons and be represented in a unique set of active channels (Dryer, 2000). For both vertebrate and invertebrate olfactory systems, accumulating evidence suggests that the receptor neurons project in an orderly way to the glomeruli so that those receptor neurons expressing similar classes of odorant receptor molecule on their membranes will converge onto common glomeruli (Kauer, 1991; Ressler et al., 1994; Galizia et al., 1998; Gao et al., 2000; King et al., 2000).

Olfaction, however, is much more than simply sorting complex molecules into channels, and the situation is complicated because natural odors seldom consist of a single molecule in a pure form but are most often mixtures of molecules. After detection, animals have to code the identified odors so that they can be stored and compared with others or with the same odor at a later time (Laurent, 1996, 1997; Gelperin, 1999). The many examples of both vertebrate and invertebrate animals relying primarily on olfaction to find food and for inter- and intraspecific communication testify to the importance of this sensory modality.

Although it is not certain how much olfactory processing is completed within the glomeruli, it has been suggested, at least for mammals, that the repertoire of odors that can be detected and discriminated is correlated with the number of glomeruli that are contained in the olfactory bulb (Kauer and Cinelli, 1993; Hildebrand and Shepherd, 1997). This concept of odotopic compartmentalization in the glomeruli of the primary olfactory processing areas is less certain for invertebrates. However, the Decapod crustaceans have anatomically well-defined olfactory centers in their brains and provide excellent material for a comparative study of the numbers of olfactory glomeruli in a phylogenetically closely related group of animals that are nevertheless highly diverse in size and life style. Decapods have radiated into habitats ranging from close to the vents of the deepest ocean trenches through the antarctic oceans and up into the trees of the tropics. Few live for only a single season, and in fact the general rule is a life of many years coupled with continual growth in the size of the animal and its brain. The number of glomeruli in the crustacean brain, however, as with those of the vertebrates and insects (LaMantia et al., 1992; Rospars and Hildebrand, 1992; Baier and Korsching, 1994), reaches a fixed level early in the animal's life and then does not change, although the size of the individual glomeruli does increase as the animal grows (Helluy et al., 1996).

The first synaptic area of the olfactory system in the decapod crustaceans is located in the olfactory lobes. These are two spherical neuropils, one on each side of the brain and forming part of the deutocerebrum. Each olfactory lobe in adult crustaceans is the dominant, and perhaps only, target neuropil for axons from an array of special chemoreceptor sensilla (aesthetascs) on the antennule (Sandeman and Denburg, 1976; Mellon et al., 1989; Mellon and Munger, 1990). Recently, interneuronal projections to the olfactory lobes from the mandibular and maxillary neuromeres in lobster embryos have been discovered (Sullivan and Beltz, 2001) and are the first indication that chemoreceptors on other parts of the body may also be involved in olfaction, although the nature of the input to these interneurons has not yet been determined. The aesthetasc sensilla on the antennules are characterized by being smooth walled and containing the dendrites of olfactory receptor neurons (ORNs) that lie in a cluster at their bases (Tierney et al., 1986; Spencer and Linberg 1986; Grünert and Ache, 1988; Gleeson et al., 1996). Each olfactory lobe receives inputs from the receptors on the antennule ipsilateral to it, and, unlike the antennal lobes of insects, the inputs to the olfactory lobes are predominantly, and perhaps even purely, chemoreceptive. The olfactory neuropil in the decapod crustacean is organized into roughly conical or cylindrical columns (glomeruli) arranged radially around the lobe (Fig. 1). The glomeruli can be resolved into three subsections, the cap, subcap, and base (Sandeman and Luff, 1973; Schmidt and Ache 1992; Helluy et al., 1996). While the organization of the olfactory system as a whole appears to be conserved among the decapods, some differences exist in the anatomy of the lobes of the species we examined in this study. Although all have their output fibers in the large olfactory globular tract that leaves the center of the lobe to project medially and anteriorly toward the protocerebrum (Mellon et al., 1992), the Brachyuran olfactory lobes are characterized by having a second large tract of fibers extending from the center of the lobe and spreading out over the surface of the lobe itself. There are also differences in the dimensions of the columnar glomeruli throughout the decapods: The glomeruli of some species are tall and narrow, whereas those of others are short and broad (Fig. 1).

For this study, we collected data from 70 individual animals from the eureptantian decapods, with 21 species drawn from the Achelata, Homarida, Astacida, Thalassinida, Anomala, and Brachyura (Scholtz and Richter, 1995). We had enough material from 17 of these species to take detailed measurements of the olfactory organ

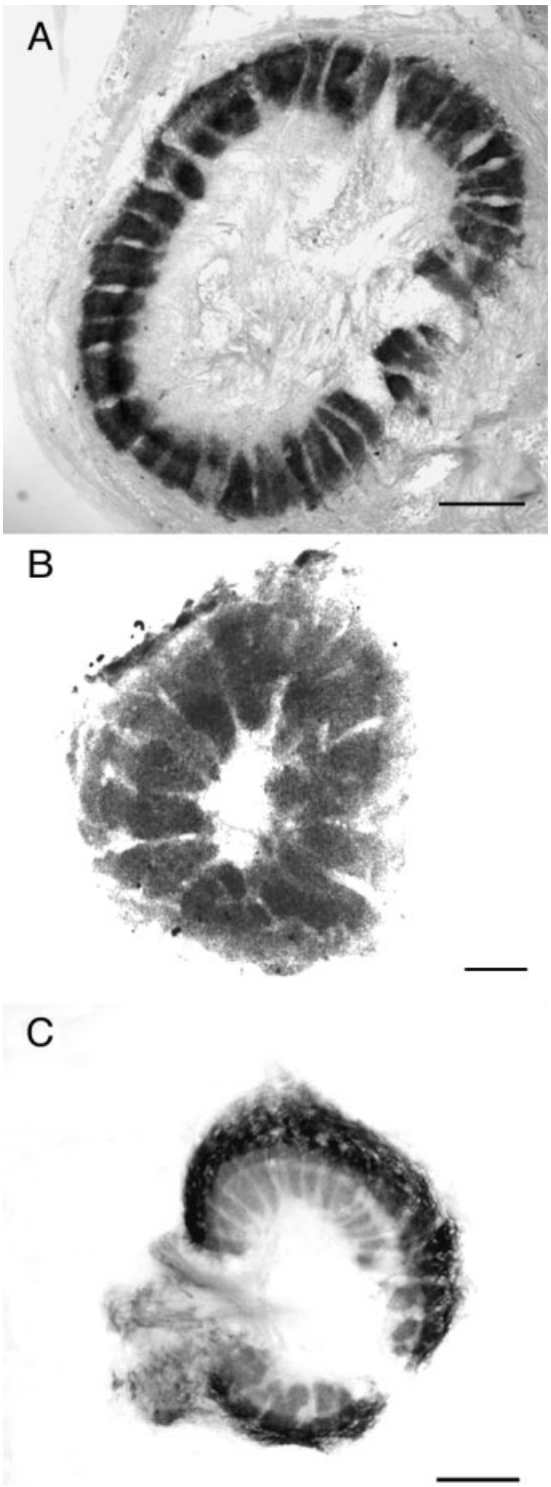

Fig. 1. The olfactory lobes of the decapod species examined are made up of columnar glomeruli. Examples used to illustrate this anatomy are sections through the olfactory lobe of *Homarus americanus* (**A**), *Cherax destructor* (**B**), and *Libinia dubia* (**C**) that have been labeled with antisynapsin antibody. Scale bars = 200 μm in A,C, 100 μm in B.

and the olfactory lobes in the brain, and these are presented in this report.

We have not set out to test the hypothesis that more glomeruli mean a richer olfactory experience. Instead, we have sought only to document the range of olfactory glomerular numbers in the decapods, the range that occurs within the separate orders, and whether there is any correlation between glomerular numbers and life style, habitat, phylogenetic group, or number of olfactory sensilla. There are indications from our study that more glomeruli in the olfactory lobe may be associated with a particular life style. In general, the picture that has emerged is that the decapod crustaceans have exploited various strategies in the construction of their olfactory systems in which the problems of size, sensitivity, and selectivity have all interacted. We find a continuum across the species ranging from those that favor a high convergence of receptors onto a few glomeruli to those that share a small number of receptors among many glomeruli.

MATERIALS AND METHODS

Animals

Live specimens were collected from the field or obtained from marine resource centers and biological supply houses. The size of the animals presents a problem peculiar to the crustaceans, insofar as it is known that they continue to grow throughout their lives, resulting in an increase in the number of peripheral receptor sensilla (including those on the olfactory organs) and in the brain volume (Helluy et al., 1996; Sandeman et al., 1998). The numbers of olfactory glomeruli are characteristic for each species, and these numbers are established early in life (Helluy et al., 1996). Therefore, glomerular numbers are thought to be invariant in decapods, as in insects (Rospars and Hildebrand, 1992), and this feature is insensitive to the specific size or life stage of the animal. However, because we also wanted to compare the numbers of olfactory sensilla (aesthetascs) and the size of the olfactory centers both within and between species, specimens for each species were chosen that were as close as possible in size and that represented midlife stages. For comparisons between species, we took into account the nature of decapod growth, which is rapid in the juvenile stages but which, under natural conditions, usually slows, until eventually molting occurs infrequently, and may even cease. In selecting animals, we avoided both juveniles and the largest reported size for any species, choosing instead animals that represented intermediate-sized, sexually mature life stages.

Dissection and preservation

Prior to removal of the olfactory organs (antennules) and brains, the animals were packed in ice for 0.5–1 hour, after which they were immobile. The head cavity was opened, and the brain was removed under cold saline and fixed in a 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.2) for approximately 24 hours, after which they were moved into 0.1 M phosphate buffer. The antennules, cut off at their bases, were processed in the same way. The brains were infiltrated with increasing concentrations of sucrose (10%, 20%, 30% in ddH_2O) until tissues were saturated. Brains were left in 30% sucrose with 0.15% sodium azide overnight to ensure complete infiltration.

Sectioning

Brains were attached to the chilled chuck of a cryostat with Miles Tissue-Tek OCT compound and sectioned at 50 μm from the dorsal to the ventral surface. The sections were mounted in serial order on cold Fisherbrand Colorfrost/Plus microscope slides and then warmed and dried before immunocytochemical processing.

Immunocytochemistry

Olfactory glomeruli are the sites for the synaptic contact between the olfactory receptor cells and the first-order interneurons in the brain. A label specific for synapses would therefore be expected to stain the glomeruli and allow them to be seen against the less heavily labeled surrounding tissue. We found this to be the case when we applied a primary antisynapsin antibody (SYNORF-I) that had been raised against *Drosophila* synapsin to the sectioned brains of the crustaceans.

Mounted sections were washed three times over the course of 1 hour in 0.1 M Sorenson's buffered saline (PBS; pH 7.2) to remove any remaining fixative and then incubated in a solution of 3% normal goat serum (NGS) to block nonspecific antibody binding and 0.3% Triton X-100 (TX-100) in PBS for 1 hour. Sections were then exposed overnight at 4°C to a 1:40 dilution of SYNORF-I antibody (mouse monoclonal antisynapsin; courtesy of Dr. Erich Buchner, Germany) in PBS with 0.3% TX-100 and 3% NGS. The sections were then washed six times in PBS over 2 hours and incubated at room temperature for 2 hours in sheep anti-mouse alkaline phosphatase (SAM-AP) diluted 1:200 in PBS with 0.3% TX-100 and 3% NGS. The sections were then washed six times over 2 hours in PBS and reacted with a Sigma Fast insoluble alkaline phosphatase 5-bromo-4-chloro-3-indolyl phosphate nitroblue tetrazolium (BCIP/NBT) for 10–40 minutes until synaptic regions in the brain began to turn dark purple. The sections were then rinsed in ddH_2O for 30 minutes and mounted in GelMount (Biomedia, Foster City, CA). Edges of the coverslips were lined with fingernail enamel to seal the sections for permanent storage.

Measurements

For each individual, we recorded the weight, the volume of the olfactory neuropils on both sides of the brain, the dimensions of the olfactory glomeruli, and the number of aesthetasc sensilla on the antennules. To determine the volume of the olfactory neuropil from the sections, each section was viewed with a microscope connected to a Sony video system and analyzed with a Compix digital morphometric computer system using C Imaging software (Compix Inc.). Initially, all darkly labeled regions of the olfactory neuropil were selected, and the areas were calculated and recorded by the computer system. The areas of the serial sections were then added and multiplied by the section thickness (50 μm) to provide a total volume of the glomerular neuropil.

Next, a mean value was obtained for the volume of a single glomerulus. Because the glomeruli are neither conical nor cylindrical but a combination of these, the volume was derived from calculating the average of the cylindrical volume and the conical volume. The length and cross-sectional areas of the glomeruli used in these calculations were measured directly from the labeled tissue. Finally, an estimate of the glomerular number for each olfactory lobe could be made by dividing the total glomerular volume by the volume of a single glomerulus.

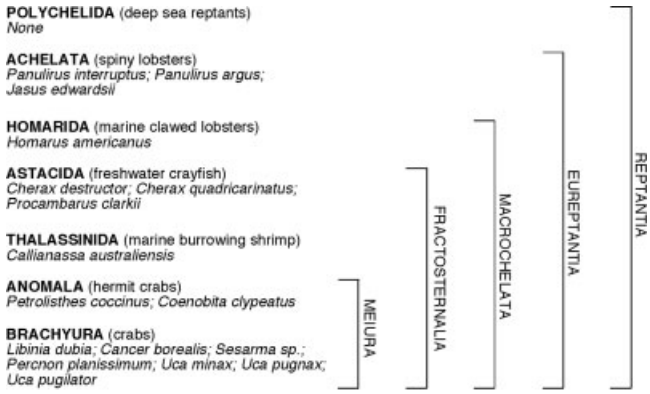

Fig. 2. The subdivision of the crustacean decapod Reptantia according to Scholtz and Richter (1995). The Reptantia contain all seven groups, the Eureptantia six of these, the Macrochelata five, the Fractosternalia four, and the Meiura two. The species that we used in this study are listed beneath each of the six eureptantian taxa.

Aesthetasc counts were made with the light microscope. The aesthetascs were identified from other sensilla on the antennules by their smooth profiles and their location on the annuli of the lateral flagella of the antennules. Various methods were employed to ensure that the aesthetascs were separated from one another so that each could be counted. Counts were made from whole mounts of the antennular flagella, sectioning flagella or trimming the aesthetascs, and counting the cut bases.

Statistical analysis

Scatterplots were made and statistical analyses conducted with SSPS (version 10.1) software (SSPS, Inc., Chicago IL). A Pearson's correlation test and regression were conducted to determine the relationships between the various measured and calculated parameters, such as glomerular number, aesthetasc counts, OL neuropil volume, and glomerular volume.

Photomicrographs

Sections were examined with a Nikon photomicroscope, and representative sections were photographed with a Spot digital camera (Diagnostic Instruments, Inc.). Images were adjusted for brightness and contrast using Adobe Photoshop 5.5.

RESULTS

Scholtz and Richter (1995) have provided a phylogenetic revision of the Reptantia in which ranks have been abandoned, and we have adopted their scheme in this study. Briefly, the Reptantia have been arranged into seven taxa (Fig. 2). One of these, the Polychelida, contains only deep sea animals, and we were not able to obtain any species belonging to this taxon. The remaining taxa are within the Eureptantia. We were able to obtain species from all six taxa in this group, as shown in Figure 2. Additional material was available to us, but we did not include these animals in the study because we were in possession of only a single specimen or were unable to obtain a full set of

TABLE 1. Means of Olfactory Neuropil Volume, Glomerular Volume, Calculated Glomerular Number, Aesthetasc Count, and the Convergence Ratio for the 17 Species Examined in This Study

Taxon	Species	n	OL neuropil volume (μm^3)	Glomerular volume (μm^3)	Glomerular number	Aesthetasc count	Convergence ratio (aesthetasc/glomeruli)
Achelata	*Panulirus interruptus*	2	344,922,004	287,884	1,202	1,786	1.486
	Panulirus argus	2	154,068,687	117,862	1,332	1,255	0.942
	Jasus edwardsii	3	591,956,438	616,475	961	1,537	1.599
Homarida	*Homarus americanus*	2	141,159,589	591,583	249	1,262	5.068
Astacida	*Cherax destructor*	3	24,187,019	110,975	230	130	0.565
	Cherax quadricarinatus	3	24,735,814	74,298	334	237	0.710
	Procambarus Clarkii	3	9,790,377	19,585	503	133	0.264
Thalassinida	*Callianassa australiensis*	3	6,588,788	28,041	235	22	0.094
Anomala	*Coenobita clypeatus*	3	120,352,292	153,833	799	519	0.650
	Petrolisthes coccnicus	3	12,359,013	18,947	655	328	0.501
Brachyura	*Cancer borealis*	2	165,730,818	229,666	733	540	0.737
	Libinia dubia	3	20,327,317	39,338	454	319	0.703
	Percnon planissimum	3	28,765,244	58,705	495	555	1.121
	Sesarma sp.	3	6,617,077	14,887	446	33	0.074
	Uca minax	3	4,558,497	17,779	284	39	0.137
	Uca pugilator	3	3,114,604	13,140	234	28	0.120
	Uca pugnax	3	3,012,080	8,034	374	26	0.070

measurements on the olfactory neuropils and sensilla from the same individuals.

The means of measurements from two or three individuals for each species are presented in Table 1. These data represent the olfactory neuropil volume, the volume for single glomeruli, the aesthetasc sensilla count, and the calculated glomerular count. Table 1 also shows the calculated ratio of aesthetasc sensilla per glomerulus, which we refer to as the *convergence ratio*. In the following analyses, we explore whether there is some common principle in the organization of the olfactory system in the decapod crustacea and whether there is a correlation between the structure of the systems and the life style, habitat, or phylogeny of the individual species.

Aesthetasc count and olfactory neuropil volume

A common feature of crustacean olfactory systems is the narrow diameter of the axons of olfactory receptor neurons (between 0.1 and 0.2 μm) and the high density of the neuropil in the olfactory glomeruli where the endings of the ORNs synapse with the even smaller diameter processes of local and projection neurons (Sandeman and Luff, 1973; Spencer and Linberg, 1986; Mellon and Alones, 1993; Sandeman et al., 1995). If we assume a similar packing density of the glomerular neuropil across all taxa, a simple relationship may be found between the sensory input and the volume of olfactory neuropil that it subtends.

For the purpose of our analysis, the sensory input is defined as the numbers of aesthetascs on the antennules and includes the assumption that, among the decapods (but not all crustaceans, see Hallberg et al., 1992), the aesthetasc sensillum takes on a relatively constant form with, it is estimated, between 100 and 300 olfactory receptor neurons at its base (Ghiradella et al., 1968; Laverack, 1988; Mellon et al., 1989; Gleeson et al., 1996). The dendrites of the ORNs extend up into the shaft of the aesthetasc sensillum (Tierney et al., 1986; Grünert and Ache, 1988). The volume of the olfactory neuropil is the summed volume of the glomeruli that can be detected with the synapsin antibody. The glomeruli are separated by areas of many fine fibers, but both the synapsin label and the ultrastructural analyses indicate that the areas between the glomeruli contain few, if any, synapses (Sandeman and Luff, 1973).

Quantification of the sensory input (i.e., numbers of aesthetascs) and olfactory neuropil volume has the advantage that these are measurements that can be taken directly and quite accurately from each individual animal. Our data reveal a positive relationship between the amount of sensory input and the volume of the synapsin-labeled neuropil contained in the olfactory lobes ($r = 0.912$, $P < 0.0001$), indicating that 83% ($r^2 = 0.83$) of the variance in olfactory neuropil volume is explained by the aesthetasc count. This relationship is observed when all species are examined, regardless of major differences in the individual numbers of aesthetascs or volumes of the lobes between species.

This trend is maintained in all the species we examined, suggesting that they share the common morphological feature of larger numbers of sensory inputs requiring more central neuropil to accommodate them. This provides us with a common point of reference that is not sensitive to the large diversity we find across species in body size, neuropil volume, glomerular number, and aesthetasc number.

Aesthetasc count and glomerular number

The question addressed by comparing the aesthetasc count with the number of glomeruli is whether the olfactory neuropil in the different species is divided into relatively equal-sized portions, each representing a common integrative neural unit that receives a standard quantum of receptor neurons. In other words, do more afferents simply mean more glomeruli? If this were the case, then one would expect a positive correlation between the numbers of aesthetascs and glomeruli.

The data in Table 1 confirm that there is only a weak correlation between the numbers of aesthetascs and the numbers of glomeruli into which the olfactory lobe is subdivided ($r = 0.674$, $P < 0.0001$). This indicates that only 45% ($r^2 = 0.45$) of the variance in glomerular numbers is explained by aesthetasc counts. Small numbers of aesthetascs can be associated with relatively high glomerular numbers (e.g., *Sesarma* sp. 33 aesthetascs, 446 glomeruli) or large numbers of aesthetascs with smaller numbers of glomeruli (e.g. *Homarus americanus* 1,262 aesthetascs, 249 glomeruli). This is an interesting result in that it suggests that the olfactory neuropil is not parcelled out into equal integrative units receiving standard afferent inputs. Instead, it indicates that the receptor input is

divided differently among the glomeruli in different species, perhaps reflecting some functional adaptation of the particular species in terms of the numbers of afferents that project to a particular glomerulus.

Olfactory neuropil volume and glomerular number

Studies on lobsters from embryonic through adult stages (Helluy et al., 1996) and measurements on crayfish of different sizes (D.C. Sandeman, unpublished results) indicate that glomeruli in the olfactory neuropils of any one species enlarge in size, but not number, as the animal grows. In *H. americanus*, the fixed number of glomeruli characteristic of this species is attained by the end of larval life, prior to the settling of the juvenile lobsters to the benthos.

Comparing the volume of the olfactory neuropil of each species with the number of glomeruli that are contained within it, we find that this relationship is not consistent across the species from which data were gathered, resulting in a weak correlation ($r = 0.672$, $P < 0.0001$, $r^2 = 0.45$). The consequences of this are that a large volume of olfactory neuropil does not necessarily mean that it will contain a large number of glomeruli, nor will a small volume of neuropil mean that it will contain a small number of glomeruli. Olfactory neuropil volume is clearly not a predictor of the size or the number of glomeruli into which the neuropil is partitioned.

Glomerular numbers are also not related to differences in the volume of a single glomerulus ($r = 0.394$, $P = 0.006$); only 16% ($r^2 = 0.16$) of the variance in glomerular numbers is explained by the values for glomerular volume. Neither are glomerular numbers correlated with differences in body size between species. Compare, for example. *H. americanus* with *Panulirus argus*, which have similar body sizes and olfactory neuropil volumes but dramatically different glomerular numbers (249 vs. 1,332, respectively).

Glomerular number, taxon, and habitat

The suggestion of relative constancy of glomerular number within phylogenetic taxa is supported by data in Table 1 showing that glomerular numbers within some taxa fall into quite a narrow range. It could be argued that this narrow range reflects the fact that the animals chosen for the study also covered a narrow range of body size for each species. On the other hand, the variation of glomerular number between species (from <250 to >1,300) is much larger than within the taxa, suggesting that specific glomerular numbers are indeed characteristic for each species. This becomes evident when glomerular numbers of the different species are plotted in ascending order (Fig. 3). Species included in the Achelata taxon are grouped at the upper end of this series, but members of the Astacida and Brachyura, for example, are distributed broadly along the gradient of glomerular number.

In terms of habitat, the 17 species sampled represent crustaceans occupying marine (nine species), freshwater/estuarine (seven species), and terrestrial (one species) environments. Table 1 illustrates that the marine organisms have a much broader range of glomerular numbers (~230 to ~1,330) than the freshwater/estuarine species (~230 to ~500), but there appears to be no obvious and general association between glomerular number and either habitat or taxon. Indeed, occupation of a particular habitat would not necessarily dictate the olfactory preferences for any of the animals living there. A parallel here is the relatively small numbers of glomeruli in the olfactory lobes of some insects (e.g., honeybees) that live in tropical habitats, which are rich in odor varieties; nevertheless, these insects are specialists that are able to detect only a small number of these odors.

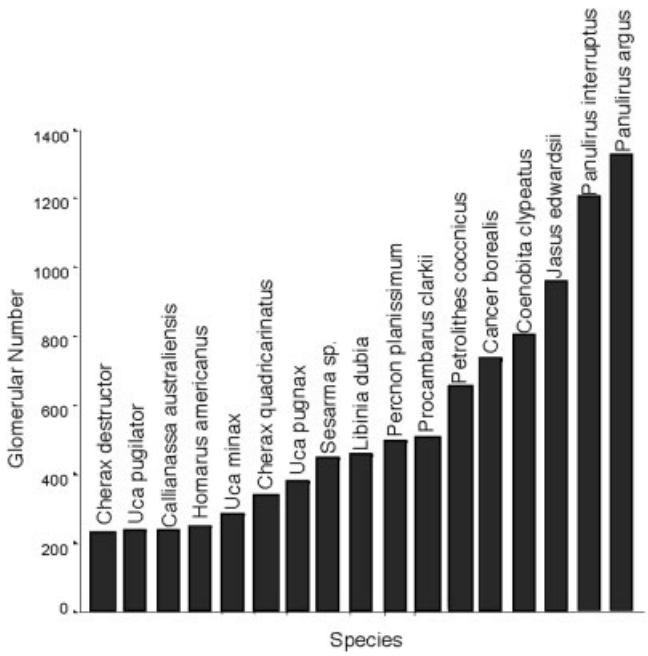

Fig. 3. Comparison of the numbers of glomeruli in the 17 species used in this study by arranging them in an ascending series. The number of glomeruli in the olfactory lobes is graded from 230 to 1,332 across the species, and the species do not group into their taxa in such a series.

Aesthetascs per glomerulus: convergence ratios

From the above discussion it is apparent that parameters such as body size, volume of the olfactory neuropil, and aesthetasc numbers are not good predictors of the numbers of glomeruli that are contained in the olfactory lobe of any one species of decapod. Because the number of glomeruli is characteristic of each species, there must be some combination of factors that determines this. The numbers of glomeruli are not likely to be sensitive to environmental stimuli, insofar as they are defined early in life. In the American lobster, at least, the numbers of olfactory glomeruli are fixed by the end of larval life, prior to the time when the organism settles to the benthos, which will be its habitat thoughout juvenile and adult life (Helluy et al., 1996).

We have entertained the idea that features of a species' "olfactory life style" may provide clues to why glomerular numbers vary so widely across the decapod Crustacea. For example, foraging and social behaviors that are guided by olfactory cues depend on the sensitivity and selectivity of the olfactory system. In any sensory system, high convergence of afferents onto second-order neurons in the brain would result in an amplification of the stimulus signals by

simple summation with an accompanying loss of the resolution of any small differences between the incoming channels. A high sensitivity may then be coupled with a low discriminative ability. A broad divergence of the same quantity of sensory input may indicate a separation of the sensory signals across a number of central channels, thus preserving small differences between the incoming lines but at the expense of sensitivity that would have been achieved by the summation of the inputs. The two situations are not alternatives but more likely represent the ends of a gradient between the two extremes.

Translated into olfactory systems, a high convergence of ORNs onto a few glomeruli would imply a high sensitivity to the signals carried in those fibers. With the assumption that crustacean olfactory systems are odotopically arranged and that fibers converging on one glomerulus carry similar information, those animals with many ORNs (aesthetascs) converging onto a small number of glomeruli would be able to detect relatively few odors but at low concentrations. Those species in which small numbers of ORNs (aesthetascs) project to many glomeruli could be expected to be sensitive to a wide range of odors but only at higher concentrations. Clearly, there will be animals that fall between the two extremes.

A "convergence ratio" for the crustaceans can be calculated by dividing the total number of aesthetascs (which reflects the numbers of sensory neurons) by the number of glomeruli to which they project. When these ratios are plotted as an ascending series, we find that there is a large range of values across the species we examined (Fig. 4A). *H. americanus*, for example, has the highest convergence ratio of 5.06 sensilla per glomerulus, whereas *Uca pugnax* has a ratio as low as 0.07 aesthetascs per glomerulus. However, the order in which the species appear along the abscissa is interesting in terms of the habitats the animals occupy. The lowest ratios are found in species that are estuarine (1–5), and most are exposed to air at low tide. The next in the series are the freshwater crayfish (6,8,10) and several marine crabs (7,11,12). The animals with the highest convergence ratio are the larger marine lobsters (13,15–17). Expressing the data as scatterplots in which the convergence ratios are expressed in relation to either the habitat or the taxon shows that some animals cluster with habitat (Fig. 4B) and others with taxon (Fig. 5).

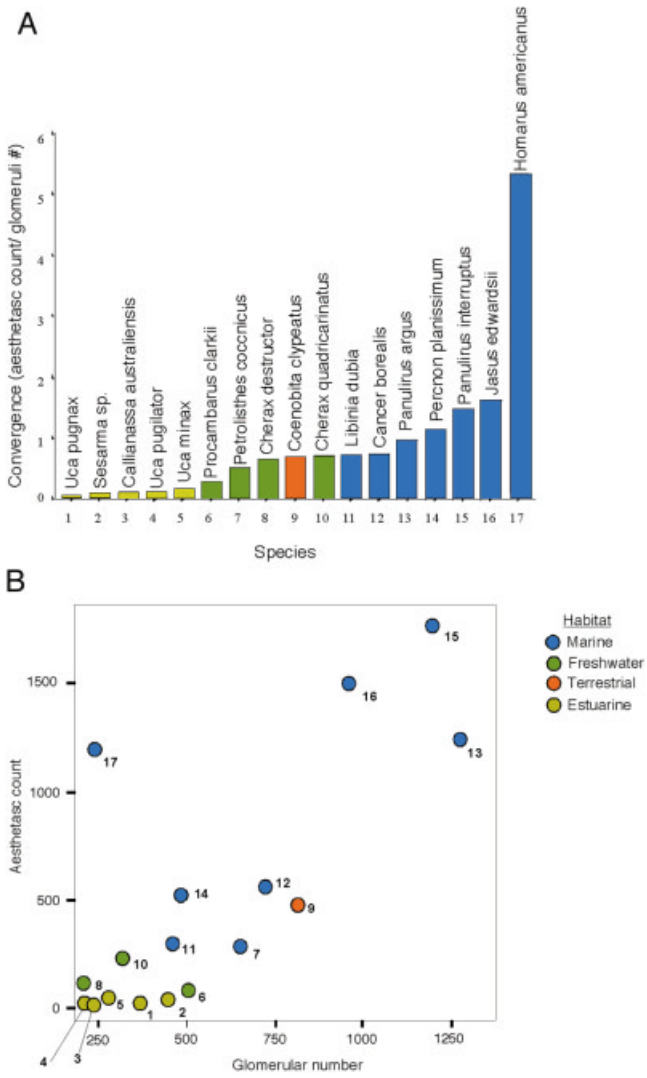

Fig. 4. **A:** The convergence ratio (aesthetasc sensilla per glomerulus) of the species we examined arranged into an ascending series. The species in the histogram are numbered consecutively and colored according to their habitat (see key in B). These numbers and colors identify the points in the scatterplot in **B**. Both the histogram and the scatterplot indicate that animals living in different habitats cluster into groups, with the lowest convergence ratio found in the estuarine environment and the highest in the deep marine environment.

DISCUSSION

Olfaction is an important sensory modality for many crustaceans, but our grasp of its significance for the behaviour of most species is tenuous, particularly insofar as few animals rely on a single sensory modality. Also, given the extreme size range covered by these animals (the smallest juvenile stages in *Homarus* weigh about 0.15 g and the largest adults up to 15 kg, an increase of 10,000 times), it is likely that the olfactory needs of the animal will change over its life. Indeed, this could lie behind the increase in olfactory inputs and the presumed greater sensitivity with size; however, the numbers of glomeruli, and presumably the numbers of odors that can be discriminated, remain the same. In addition, the relative volume of the brain dedicated to olfaction is variable among the different decapod species, a feature that was recognized by Hanstroem (1926), who noted inversely proportional sizes of visual and olfactory neuropils in crustaceans, so that large primary visual centers were associated with small primary olfactory centers and vice versa. It is doubtful, then, that any single factor can be found that will be a completely reliable predictor of glomerular numbers or size. Nevertheless, we do find that, although the glomerular number is not determined by simple anatomical parameters or even taxon and habitat, the *functional architecture* of the olfactory system revealed by the convergence ratio is indeed associated with phylogenetic affinities and habitat.

There are assumptions made in the calculation of the convergence ratios that have to be considered. First, we make the assumption that the numbers of ORNs within the aesthetasc sensilla of the 17 species we examined vary only by a factor of 3 or less. For the species in which direct

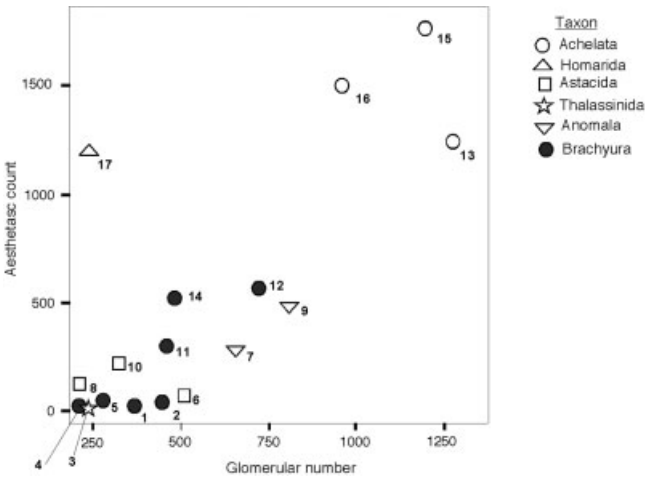

Fig. 5. Scatterplot in which the clustering of convergence ratios (aesthetasc number per glomerulus) of the different taxa are expressed. This indicates separate clustering of the Achelata, Astacida, and Anomala. The Homarida do not cluster with any other group. The Thalassinida are found with the Brachyurans. The least clustered are the Brachyurans, which range from the lowest covergence ratios to bordering on the Achelata with the highest convergence ratios.

counts of sensory neurons have been made (*Callinectes sapidus*, *Coenobita clypeadus*, *H. americanus*, *Panulirus argus*, *Procambarus clarkii*), there is consistency in numbers of sensory neurons per aesthetasc (Ghiradella et al., 1968; Laverack, 1988; Mellon et al., 1989; Gleeson et al., 1996). Also, we do find a good correlation between the numbers of aesthetasc sensilla and the size of the olfactory neuropil across all species, suggesting that the numbers of inputs associated with the sensilla are within a fairly narrow range.

Second, aesthetasc number increases with growth, whereas the numbers of glomeruli remain the same throughout life; hence the convergence ratio will also change throughout the life of the animal. This adds yet another complexity to a comparison across species. We have been able to go some way toward addressing this difficulty by using animals within species of about the same size and that represent sexually mature adults in the middle of the normal size range for the species. It is also possible in some species to estimate the magnitude of the change in the convergence ratio with growth. For example, data on the body size and aesthetasc numbers are available for *Cherax destructor* (Sandeman et al., 1998) and for *Homarus* (B.S.B. and D.C.S., unpublished observations). In both animals, the relationship between the aesthetasc number and body size is remarkably linear, allowing one to predict the numbers of aesthetascs on the antennules from the length of the carapace. In *C. destructor*, for example, the carapace length in millimeters will, if multiplied by a factor of 4, provide the numbers of aesthetascs on the antennule. In *Homarus*, the factor is 13.2. These factors allow us to estimate the differences in the convergence ratios that we could expect to see over a range of sizes. For *C. destructor* the mean convergence ratio in Table 1 is 0.56, representing an animal with a carapace length of about 32.2 mm. Animals with a carapace length of 42.5 mm would have about 170 aesthetascs and a convergence ratio of 0.73, a value that would not move *C. destructor* out of the cluster of species with which it is associated in the scatterplot in Figure 5. Likewise, the mean convergence ratio for *Homarus* in Table 1 represents an animal with a carapace length of about 95.5 mm. An animal with a carapace length of 85 mm would have 1,122 aesthetasc sensilla and a convergence ratio of 4.5, which is still the highest among all the species considered. Therefore, although convergence increases as the animal grows, the position of the convergence value for a single species in relation to others does not appear to change. The difference in convergence ratios in the very small and large individuals of the same species is in itself of considerable interest, perhaps reflecting the increased olfactory sensitivity necessary to support the expanding requirements for nourishment that accompany a significant size change. As noted above, in the American lobster, the largest adults can weigh up to 10,000 times the weight of the juveniles.

Despite the constraints of the convergence ratios, where they have been compared with phylogeny and with habitat, we find that different species are clustered together in ways that may be meaningful in terms of their olfactory abilities. Considering habitat first, we find that five species, *Uca pugnax*, *Sesarma* sp., *Callianassa australiensis*, *Uca pugilator*, and *Uca minax* (1–5 in Fig. 4A), cluster together in the scatterplot in Figure 4B with the lowest projection ratios of all the species. All of these animals, with the exception of *Callianassa*, live in estuarine mudflats or mangroves, are exposed at high tide, and spend a good proportion of their lives foraging and interacting in air. The freshwater crayfish, *P. clarkii*, *C. destructor*, and *Cherax quadricarinatus* constitute a second cluster of animals with convergence ratios at the lower end of an intermediate group of animals (6–12 in Fig. 4A). The other members of the intermediate group are the marine crabs (*Libinia dubia*, *Cancer borealis*) and the hermit and porcelain crabs (*Coenobita clypeatus*, *Petrolisthes coccnicus*). The highest convergence ratios are found in a cluster of large, deep marine lobsters and crayfish and a marine crab (*Panulirus argus*, *Percnon planissimum*, *Panulirus interruptus*, *Jasus edwardsii*, *H. americanus*; 13–17 in Fig. 4A). *Homarus* is unusual in having a convergence ratio that is much higher than that of all the others.

If each glomerulus represents an olfactory processing unit, then we could conclude that the olfactory system of *H. americanus* functions at high sensitivity relative to that of *U. pugilator*, which, because it has about the same number of glomeruli, may able to detect the same range of odors (not necessarily the same odors) but at low sensitivity. The low convergence ratios of *U. pugnax*, *Callianassa*, and *Sesarma* do not mean that there are too few afferents to share among the glomeruli, because each aesthetasc sensillum has a large number of ORN at its base, and these most likely will, as with those of freshwater crayfish, be distributed to all glomeruli (Mellon and Munger 1990). Several studies of crayfish support the proposal that the animal's entire chemoreceptive range is represented by the sensory neurons housed in each aesthetasc sensillum and that the multiplicity of sensilla functions to raise only the sensitivity and does not increase the diversity of the odors that can be detected (Mellon and Munger 1990; Mellon and Alones, 1993; Sandeman and Sandeman, 1996; Steullet et al., 2000; Harrison et al., 2001).

Plotting the convergence ratio in relation to the taxa results in clustering that has a phylogenetic basis. Members of the Achelata, the evolutionarily oldest group examined in this study (see Fig. 2), are restricted to one cluster with the highest convergence ratios. The Astacida and Anomala are grouped together in a cluster with intermediate convergence ratios. The Thalassinid and Homarid representatives are restricted, respectively, to clusters with the lowest and highest convergence ratios. Brachyurans, the evolutionarily youngest taxon among those we considered, have members in all clusters, a feature that we suggest reflects the broad radiation of the members of this taxon into a wide range of habitats.

Our original focus in this study was the subdivision of olfactory neuropil into glomerular subunits. The induction of glomeruli during development by incoming afferents in insects and crustaceans (Tolbert and Siriani, 1990; Helluy et al., 1996; Sandeman 2000) is relevant in that the induced glomeruli may reflect the type of odor receptor molecules of the afferents that project to them. The induction of a macroglomerulus in the antennal lobe of gynandromorphic moths is a particularly good example in this context (Schneiderman and Hildebrand, 1986). Therefore, the developmental relationship between the ingrowing afferents and the induced glomeruli suggests that the division of the olfactory lobe neuropil into glomeruli may reflect the level of diversity of the afferents growing in from the periphery in the various species of decapod crustaceans. A very diverse set of sensory neuron types could lead to the neuropil being divided into many glomeruli, such as in the Achelata and Anomala. A small range of sensory neuron types would induce fewer separate glomeruli, such as in *H. americanus*, but, because there are a large number of sensory neurons, the glomeruli will be large. The sensitivity of the systems would depend entirely on how many sensory neurons were present. Accordingly, we predict that a high diversity of sensory neuron types will indeed be found in the Panulirids and a narrower range in *H. americanus*. The test of this hypothesis must wait until the molecular nature of the odorant receptor molecules in the antennules of crustaceans has been defined.

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