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PRE- AND POSTEMBRYONIC DEVELOPMENT, GROWTH AND TURNOVER OF OLFACTORY RECEPTOR NEURONES IN CRAYFISH ANTENNULES

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Summary

The antennules of the crayfish *Cherax destructor* can first be observed as antero-laterally located lobes in embryos that have reached the 50 % stage of development. Clusters of cells that are probably the olfactory receptor neurones (ORNs) appear at the distal end of these lobes, which later differentiate into the lateral flagella of the antennules. New clusters of ORNs and segments are added at the proximal end of the lateral flagellum throughout the postembryonic stages and well into the juvenile adult stage. From a comparison of the exuvia and the newly emerged flagella in animals over a wide range of sizes, we conclude that, once the animals reach a certain size (approximately 7 mm carapace length), the most distal, and oldest, segments of the antennule are shed. Growth occurs from the proximal end of the flagellum, and the addition of new ORNs is the result of a delayed differentiation of the flagellar segments that takes place at the proximal end of the chemoreceptor array, about halfway along the flagellum.

Key words: development, crustacean, olfaction, receptors, *Cherax destructor*.

Introduction

An olfactory facility for the detection and processing of molecular signals in their environment is present in both vertebrate and invertebrate animals. In all animals, such systems follow a remarkably similar anatomical and functional plan in which chemical signals bind to receptor sites on the membranes of olfactory receptor neurones that, in turn, project exclusively to brain areas with glomerular-structured neuropile (see reviews by Ache, 1991; Hildebrand, 1995). Crustacean olfactory systems conform to this overall plan. Their olfactory receptor neurones (ORNs) are located in clusters of approximately 100 cells beneath unique hair-like sensilla called aesthetascs, found only on the distal segments of the outer flagella of the antennules (Tierney *et al.* 1984, 1986).

A significant body of information exists on the ORNs, on their central projections and on the anatomy and physiology of some central neurones in the adults of several decapods (Schmidt and Ache, 1992; Mellon and Alones, 1993, Sandeman and Sandeman, 1994; Wachowiak and Ache, 1994; Sandeman *et al.* 1995). Much less is known about the time of appearance of ORNs and aesthetascs in the embryos and their subsequent development and growth in the adults.

Crustaceans continue to increase in size during their lifetimes and therefore continually add ORNs to the enlarging antennules at each moult (Mellon and Alones, 1993). It is not clear whether this process also involves the loss of ORNs, resulting in the receptor turnover characteristic of vertebrate and some invertebrate olfactory systems (Moulton, 1974; Chase and Rieling, 1986; Chase and Tolloczko, 1993).

In this paper, we describe the first appearance of ORNs and aesthetasc sensilla in the antennules of crayfish embryos. We have followed the embryonic and postembryonic development and the addition of aesthetasc hairs during the animal's lifetime by comparing freshly moulted exuvia with their newly emerged antennules. We conclude that, in both juveniles and adults, ORNs and aesthetasc sensilla are added at the proximal end of the receptor array. In the adult, older distal ORNs are shed at each moult, resulting in a turnover of the olfactory receptors.

Materials and methods

Cherax destructor Clark, an Australian freshwater crayfish, was used for this study. Animals were obtained from ponds near Sydney and maintained in aquaria in the laboratory. Male and female animals of approximately the same size were paired to mate. After egg laying, the females were separated from the males and allowed to raise their brood in isolation. Embryos could be harvested from the females as required, and the developmental stage of the embryos was determined by previously established criteria (see below).

The antennular cuticle of the embryo and early postembryonic stages is transparent, and the development of

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the ORNs and aesthetasc sensilla is visible in unfixed and unstained whole mounts using differential contrast microscopy. The development of the ORNs was followed in individual pre- and post-moult animals in this study. Silver impregnation (Blest and Davie, 1980) of sectioned embryonic and postembryonic antennules aided the identification of developing ORNs.

The comparison between the exuvia and newly moulted antennules provided some of the most useful information for this study. The intermoult period of the postembryonic stages POI and POII is well known (Sandeman and Sandeman, 1991) so that they could be examined very shortly before they moulted. In this condition, the exuvia has often already parted from the antennule and a direct comparison can be made between the segments and sensilla of the antennule and those of the exuvia. Moulting in the older animals often occurs during the daylight hours, and we were able to retrieve the exuvia before the newly moulted animal consumed it. The antennules of such animals were removed and fixed with their matching exuvia in 70 % ethanol. Antennules, matching exuvia and all sensilla were then drawn using a stereomicroscope *camera lucida* and compared with one another, segment by segment. A minimum of 10 animals from each stage was examined. Scanning electron microscope (SEM) photographs were used to confirm the results obtained from the light microscope. Material for the SEM was fixed in 70 % alcohol, dehydrated in a graded alcohol series, cleared in xylene and placed in a dust-free container to allow the xylene to evaporate. Dry antennules were mounted on stubs covered with double-sided black carbon tape (Alltech adhesive carbon tape G 3939), sputter-coated with gold and viewed with a Leica Cambridge SEM at 20 kV.

The embryonic development of *Cherax destructor* has been staged as percentages using anatomical criteria (Sandeman and Sandeman, 1991). Egg deposition is defined as 0 % and hatching as 100 % development. At 20 °C, this takes 40 days. The hatchling is the first postembryonic stage (POI) and is attached to the mother's swimmerets for 5–7 days until the next moult. The second postembryonic stage (POII) clings to the swimmerets of the mother for another 14 days before moulting again to produce the first adult stage, ADI. ADI animals move about over the mother's body and will leave her for short periods. Approximately 14 days later, the ADIs moult into the second and independent adult stage ADII. From then on, animals grow in size by alternating moult and intermoult periods throughout their lives.

Results

The first antennae, or antennules, in freshwater crayfish each consist of the coxopodite, basipodite and ischiopodite which bears two flagella, one lateral and one medial. The three basal joints contain muscles, and those in the ischiopodite move the flagella. The flagella themselves consist of a large number of annular segments connected by arthrodial membranes and are free of muscle tissue. In the adults, both flagella bear mechanoreceptive hairs, most of which fall into three categories: feathered hairs that lie flat against the shaft of the antennule; and small and large smooth-shafted guard hairs that project at right angles to the long axis of the antennule. At higher magnification, the shafts of the guard hairs can be seen to be sculpted into many small protuberances. The large guard hairs are segmented (Fig. 1A,B,C). The distal segments of the lateral flagellum bear the characteristic thin-walled chemoreceptive aesthetasc sensilla. The aesthetasc sensilla occur in transverse rows containing between two and five sensilla. No segment of the *Cherax destructor* flagellum ever has more than a single row of sensilla, in contrast to the situation in *Orconectes propinquus* or *Procambarus clarkii* which have two rows of sensilla per segment (Tierney *et al.* 1986; Mellon and Alones, 1993). Dendrites of the ORNs extend into the shafts of the aesthetascs, which have a thin cuticular wall permeable to dyes (Tierney *et al.* 1986) and to radioactive leucine (Sandeman and Denburg, 1976; Mellon *et al.* 1989).

Development of the olfactory receptor neurones and aesthetasc sensilla

The antennules, second antennae and small mandibles can be identified as single lobe-like appendages in the head region of embryos that have reached 40–50 % development. The antennules are unsegmented and are nearly equal in length to the unsegmented second antennae (Fig. 2A). The first indication of the biramous adult form of the antennules appears at 55 % development with small protuberances visible about halfway along the medio-caudal edges of the antennule, indicating that the lateral antennular flagellum is the first to differentiate. There is no segmentation at this stage (Fig. 2B).

At 60 % development, the lateral flagellum of the antennule has lengthened to keep pace with the second antenna, but the bud of the medial flagellum remains relatively small. Oval aggregations of cells at the most distal end of the lateral flagellum signal the beginning of the first olfactory receptor neurone clusters and confirm that differentiation of the antennule begins at the distal end of the lateral flagellum. The first signs of segmentation at the base of the antennule appear as small indentations proximal to the lateral and medial flagellar bifurcation.

By 85 % development, antennular development has proceeded to the point where five clusters of ORNs can be identified in the lateral flagellum, the most distal being the oldest and most advanced, the others being added proximally (Fig. 2C). The three basal joints are now more clearly indicated by indentations of the cuticle. The medial flagellum is approximately half the length of the lateral flagellum and their proximal bases rest on the most distal of the common segments, the ischiopodite.

At hatching, the POI caryfish is virtually bare of external receptor hairs (Fig. 3A). The antennules are clearly differentiated into three basal segments and two flagella, the lateral still larger than the medial. The lateral flagellum has five segments; a distal segment and four shorter more proximal segments (Fig. 3B).

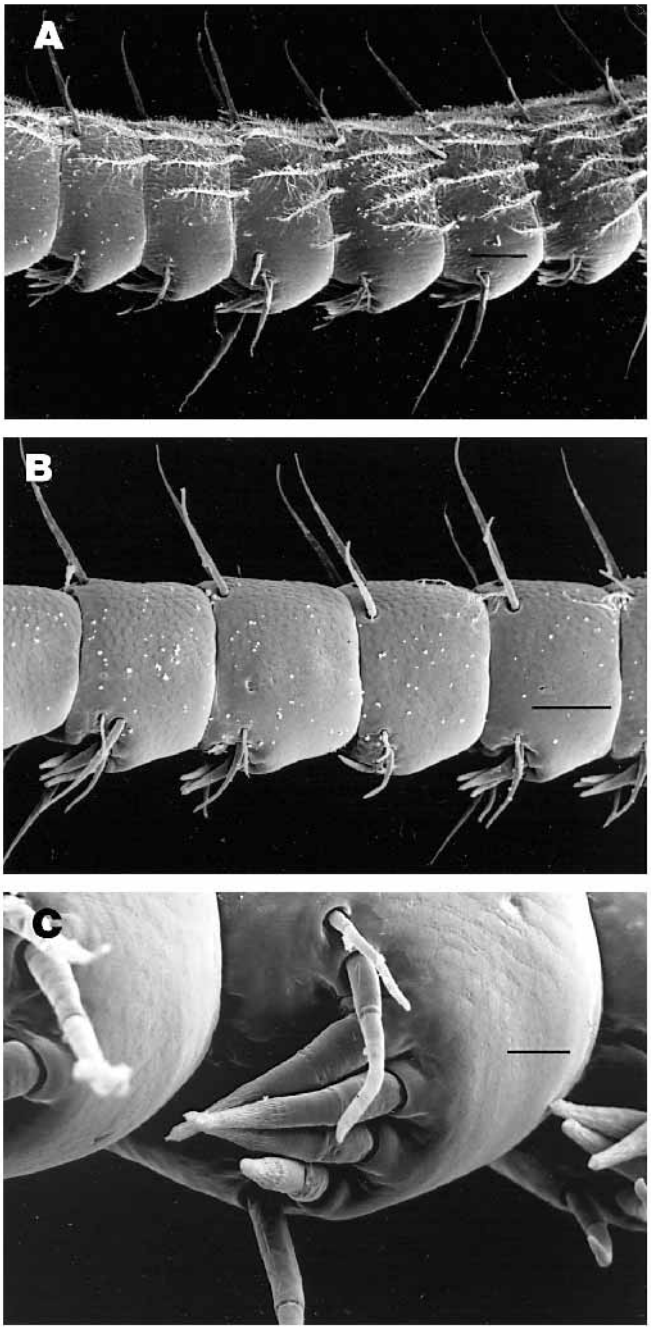

Fig. 1. Scanning electron micrographs of the receptors on the lateral flagellum of the antennule of *Cherax destructor*. (A) Part of the flagellum near the proximal end of the aesthetasc array. Distal is to the left. The aesthetascs project distally and ventrally from the distal edges of each segment. Feathered hairs, lying flat against the flagellum, are distributed here on the lateral and dorsal sides of the flagellum. (B) The distal part of the antennule bears no feathered hairs. Small and large guard hairs project out from the dorsal and ventral surfaces of the flagellum but are not necessarily present on each segment. (C) Aesthetasc sensilla occur in single rows on the ventral surface of the segments and are flanked by small and/or large guard hairs. Scale bars, A,B, 100 μm; C, 25 μm.

Clusters of ORNs lie beneath the transparent cuticle of the lateral flagella. The distal segment of each flagellum contains

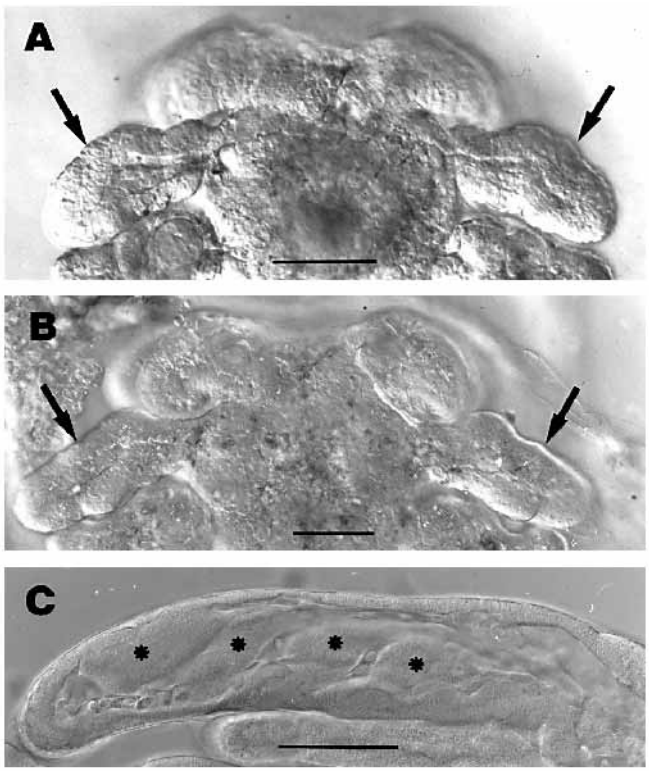

Fig. 2. Differential interference contrast micrographs of the anterior of crayfish embryos to show the development of the antennule. (A) At 45 % development, arrows show the lobed antennules. (B) By 55 % development, the antennules (arrows) have lengthened and show the first signs of becoming biramous. (C) At 85 % development, five clusters of olfactory receptor neurones (ORNs), of which four can be seen here (asterisks), are arranged along the lateral flagellum, the most distal being the oldest. Scale bars, 100 μm.

three such clusters. Given that differentiation of the flagellum proceeds from the distal end and that the distal segment is the oldest, we refer to this as the first flagellar segment and number the flagellar segments in ascending order from distal to proximal.

Aesthetasc sensilla materialize beneath the cuticle between day 0 and day 5 of the POI stage and can be clearly discerned in the three most distal segments, just prior to moulting (Fig. 4A). The distal segment contains three aesthetascs, the next proximal segment one aesthetasc and the third segment from the tip, one or two aesthetascs. Outlines of the aesthetascs in the most proximal segments of the lateral flagellum are visible but appear not to be as advanced as in the more distal segments. The segments of the lateral flagellum lengthen beneath the cuticle during the 5–7 day intermoult period of the POI animals. Three new segments (6, 7 and 8) appear immediately distal to the ischiopodite and proximal to the five existing segments.

On day 6 at 20 °C, the POI moults to emerge as a POII animal with external receptor hairs and aesthetasc sensilla on the antennules. The lateral flagellum of the POII stage contains 7–8 segments. Heralded by their subcuticular appearance in the POI stage, segment 1 of the POII lateral flagellum bears three

Fig. 3. (A) The absence of external receptor hairs on the body of a whole post-hatching stage I (POI) animal can be seen in this scanning electron micrograph of a specimen that was snap-frozen in liquid nitrogen before gold-coating. The segmented lateral flagella of the antennules are longer and thicker than the medial branches. (B) The naked, long lateral and shorter medial flagella of the antennule of a POI animal. The antennule was dehydrated and air-dried. Scale bars, 100 μm.

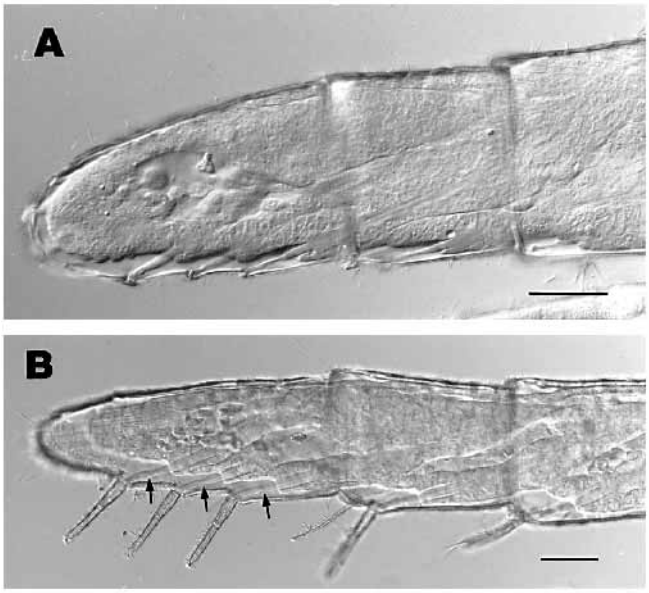

Fig. 4. (A) Differential contrast micrograph of a post-hatching stage POI antennule showing the emerging aesthetasc sensilla lying beneath the exuvium shortly before the animal moulted. The three aesthetasc sensilla that will occupy the first (most distal) segment in the POII antennule can be clearly seen. The single sensillum that will appear on the second segment and one of the two sensilla that will appear on the third segment of the POII antennule are also visible. (B) The 3, 1, 1 pattern of aesthetasc sensilla on the three most distal segments of the POII animal. This animal is about to moult into adult stage I (ADI), and the aesthetasc sensilla in the three most distal segments can be seen retracting from the exuvium (arrows). No new distal sensilla or segments are visible beneath the old exuvium. Scale bars, 100 μm.

external aesthetasc sensilla. Segment 2 carries one sensillum, and segment 3 has one or two sensilla (Fig. 4B). Aesthetascs on segments 4 and 5 are clearly established beneath the transparent cuticle and less clearly defined in segments 6, 7 and 8.

The POII moults into the ADI after 14 days and the aesthetascs on segments 4 and 5 emerge as free sensilla. Another two segments are added immediately distal to the ischiopodite, the aesthetascs beneath the cuticle of segments 6, 7 and 8 mature, and the ADI crayfish typically possesses a lateral antennular flagellum with 8–9 segments. The ADI animals show the typical ‘flicking’ behaviour of the antennules and, for the first time, search for food and eat it. Five receptor hairs, possibly mechanoreceptors, emerge at the very tip of segment 1 of the lateral flagellum. Similar receptor hairs appear lateral to the aesthetascs on segments 2, 3 and 4.

The development of the aesthetasc hairs was followed over the next three stages, i.e. ADII, ADIII and ADIV, and was seen to conform to the above plan, new segments always appearing at the base of the flagellum (Fig. 5). The intermoult period increases beyond the ADIV stage to a point at which it becomes impractical to follow individual animals.

Nevertheless, counts of the aesthetasc sensilla in animals with carapace lengths ranging from 7 to 40 mm show that the

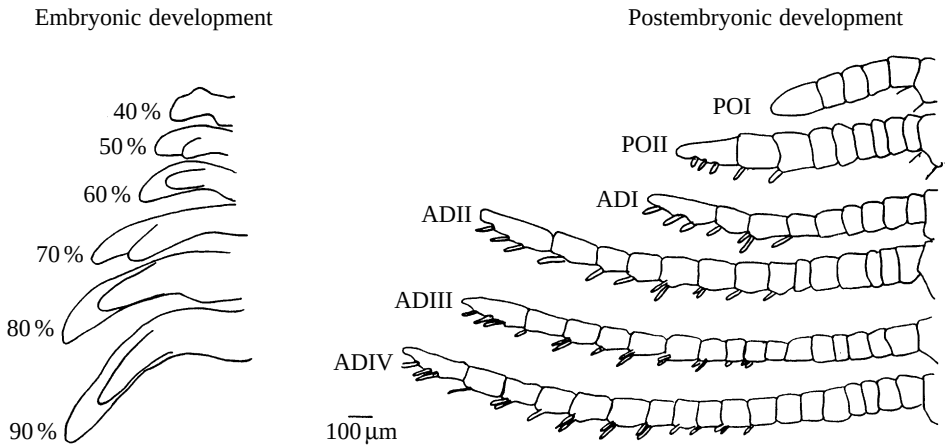

Fig. 5. *Camera lucida* tracings of developing antennules in embryonic and postembryonic crayfish, compiled from different animals. The 3, 1, 2 or 3, 1, 1 patterns of aesthetasc sensilla first appearing in the POII persist through to adult stage IV (ADIV).

number of segments carrying aesthetasc sensilla increases continually, as does the average number of aesthetascs on each segment (Table 1).

The numbers of aesthetascs per segment also varies in relation to the position of the segment along the flagellum. In ADI to ADIV crayfish, the distal segments are characterised by the 3, 1, 2, or 3, 1, 1 pattern carried over from the postembryonic stages. As the animals grow, this is replaced by a pattern in which the distal segments generally carry fewer sensilla than the more proximal segments. In all cases, however, the most proximal aesthetasc-bearing segment has fewer sensilla than those immediately distal to it. The gradual increase in the numbers of aesthetascs per segment during growth of the animal could be the result of the lateral addition of the aesthetascs to the segments at each moult or of the shedding of the older more distal segments and the replacement of these at the proximal end by segments bearing more sensilla, or both. The loss of the 3, 1, 2 pattern of the juveniles at an early stage would support the idea of distal shedding, as would the presence in the larger animals of a greater number of aesthetascs on the distal segments.

Growth and turnover in the adult

The distribution of the guard and feather hairs along the lateral flagellum of the antennule in *Cherax destructor* is of considerable importance for this study because it provides a way to identify individually each segment along the flagellum. At the proximal end of the flagellum, very few guard hairs are present and the feathered hairs surround each annulus. More distally, and several segments before the proximal end of the aesthetasc array, we find a greater proportion of guard hairs and the absence of the feathered hairs on the ventral surface of the segments. Some segments here have one or two aesthetasc sensilla and small swellings across the segment in line with any aesthetascs present.

The presence of the small or large guard hairs on segments bearing aesthetasc sensilla is variable. Some aesthetasc-bearing segments have no guard hairs, some have only small or only large guard hairs and some have both. SEM photographs show that segments without guard hairs do not have scars from the possible loss of such hairs. The particular complement of guard hairs on any segment does not follow any repeated or predictable pattern along the length of the flagellum so that, as a sequence, each flagellum is unique.

Three possibilities follow from this observation in relation to the addition of ORN aesthetasc sensilla at moulting in adults. (1) If ORNs and aesthetasc sensilla are added only at the proximal end of the receptor array, as in the juveniles, then particular segments of the distal ends of the exuvium and flagellum will match if they are placed side by side. In addition, if some distal segments are shed at the distal end, the exuvium should protrude beyond the new flagellum when the two are aligned at their matching point. (2) If ORNs and aesthetascs are added distally, the proximal segments of exuvium and flagellum will match and, at the matchpoint, the new flagellum should protrude beyond the exuvium. (3) If new aesthetascs and guard hairs are added along the entire receptor array, it will not be possible to match the segments of the exuvium and the new flagellum.

The comparisons were carried out by first recording, in horizontal columns, the numbers of receptors on aesthetasc-bearing segments of both exuvia and flagellum. The columns were then placed alongside one another with the most proximal aesthetasc-bearing ‘segments’ in register (Fig. 6). ‘Segments’ of the exuvium that carried the same number of a particular receptor type (i.e. small guard hairs, large guard hairs or aesthetascs) as their corresponding flagellar segments were totalled and expressed as a percentage of all the segments compared. The exuvial column was then shifted by one segment past the flagellar column and all perfectly matching segments were tallied as before. The exuvial column was first moved ‘distally’ past the antennule and then ‘proximally’ from the starting point.

Exuvia of 10 animals ranging from 35 to 55 mm in carapace length were compared with their newly moulted flagella. As a control, flagella from the left side were compared with those from the right side on the same animals.

The comparison using the numbers of large guard hairs produced the clearest result. Here, the possibilities are limited to the presence of no hairs, one hair or two hairs. To test the method, two columns of numbers were chosen at random between 0 and 2 and compared as described above.

Table 1. *The numbers of aesthetasc sensilla on each segment of the lateral flagellum of individual animals, ranging from stage ADI to a carapace length of 40 mm*

	ADI	7 mm	10 mm	15 mm	20 mm	30 mm	40 mm
Distal	3	3	1	1	3	3	3
	1	1	2	1	3	3	4
	2	2	2	2	3	3	4
	2	4	2	2	3	2	2
	2	2	2	3	2	3	3
	(2)	2	2	2	3	3	4
		2	3	2	3	2	4
		2	3	2	3	3	4
		3	3	1	3	3	3
		3	3	3	3	3	3
		2	3	3	3	4	3
		1	3	3	3	4	3
		1	3	3	3	3	3
		(2.2)	3	3	4	3	3
			3	3	3	3	4
			3	3	3	3	4
			3	2	3	2	3
			3	2	3	3	4
			3	3	3	3	3
			3	3	3	3	4
			2	4	4	3	5
			1	4	4	4	3
			(2.5)	4	4	4	4
				4	3	4	4
				1	3	2	4
				(2.5)	3	3	3
					3	3	2
					3	3	4
					3	2	4
					3	(3)	3
					3		4
					1		3
					(3)		4
							4
							2
							4
							3
							4
							4
							2
							4
							2
Proximal							(3.4)

The first number recorded in the columns is the most distal segment of the flagellum. The postembryonic pattern of 3, 1, 2 sensilla on the distal and next most proximal segments persists until the animals have reached approximately 7 mm in length. The average number of aesthetasc sensilla per segment along the receptor array of each animal (numbers in parentheses) increases as the animals become larger, reaching about 3.4 in an animal of 40 mm carapace length.

Predictably, we found an average match of 33 % (Fig. 7A). We obtained the same average match in a comparison of the exuvial and flagellar columns with the exception of one unique

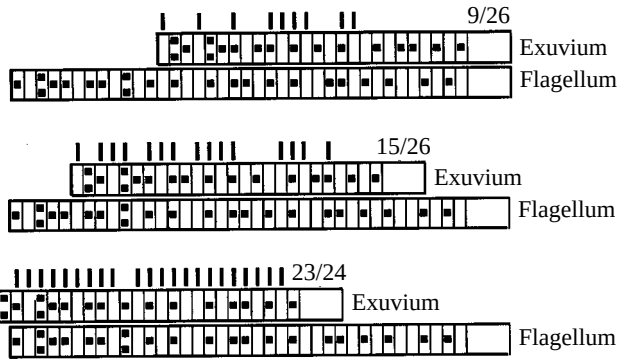

Fig. 6. Matching the exuvium and the newly moulted flagellum. The horizontal columns show the numbers (black squares) of large guard hairs in the sequential segments of the exuvium and of the flagellum. The upper pair of columns represents a comparison of the segments when the most proximal segments bearing aesthetascs are aligned. Nine of the 26 segments that could be compared have the same number of guard hairs (marked by the vertical bars). In the histograms (see text and Fig. 7), this would be entered as a 34.6 % match. The centre and lower pair of columns show what happens when the exuvial column is moved past the flagellum until the matchpoint is found and 23 out of 24 segments have the same number of guard hairs.

alignment for each pair, where matching reached between 72–85 % (Fig. 7B). Such matchpoints were not found if the exuvium was compared with the contralateral flagellum of the same animal (Fig. 7C) nor were they found if the exuvial column was moved proximally past the flagellar column.

Similar comparisons were made in which counts of the small guard hairs and aesthetascs were used. In larger animals (50–55 mm in carapace length), matchpoints were found for all three receptor types and occurred at the same alignment point in all cases (Fig. 8A,B). Comparisons of the exuvium and new flagellum in smaller animals (30 mm) produced a different result: The large guard hairs reached a 95 % match at one point, but there was no corresponding matching between the aesthetascs or small guard hairs at this or any other point (Fig. 8C). However, at the matchpoint for the large guard hairs, a comparison of the numbers of aesthetasc hairs per segment between the exuvium and the new antennule showed that, in most cases where the two did not match, this was the result of an increase in the numbers of aesthetasc hairs.

These comparisons suggest that new ORNs are being differentiated at the proximal end of the aesthetasc array. This is supported by the presence of developing clusters of ORNs and aesthetascs that are visible beneath the proximal cuticle of fixed and cleared antennules from intermoult animals (Fig. 9). These presumptive receptor organs lie beneath the cuticle of those segments without feather hairs on the ventral surface, and the developing aesthetasc hairs correspond with the small protuberances visible with the SEM on the outer surface of the segments. The possibility that older distal segments are being shed at moulting was confirmed by finding such segments remaining within the exuvia. When matched with the new flagellum, this part of the exuvium clearly protruded beyond the new distal tip of the flagellum.

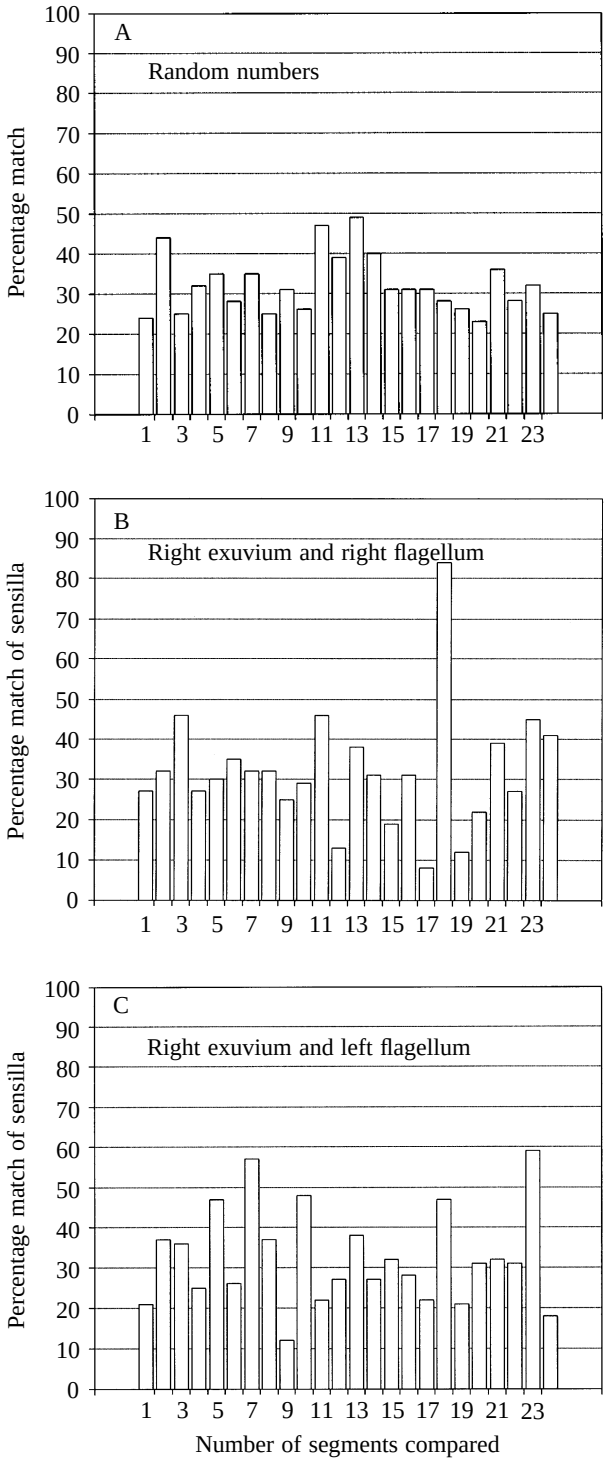

Fig. 7. Histograms recording the percentage of compared segments that have the same number of receptor hairs at one particular alignment. 1 on the abscissa indicates that the most proximal of the aesthetasc-bearing hairs on the exuvium and flagellum are aligned. The exuvial column was moved out (i.e. distally) past the flagellar column (see Fig. 6). (A) The result from the comparison of two columns of numbers between 0 and 2, chosen at random from a telephone directory. Values range between 22 % and 50 % and overall produce an average match of 33 %. (B) The right exuvium compared with the right flagellum. Values range between less than 10 % and 45 %, except at position 18 (exuvium shifted 18 places distally), where the number of segments that bear the same numbers of guard hairs rises to 85 %. A feature that was found in all comparisons was that very low numbers of segments match at the positions just before and just after the matchpoint. (C) A comparison between the exuvium of the left flagellum with the right newly moulted flagellum in the same animal. Values vary from just above 10 % to 60 % with no obvious matchpoint.

Discussion

The results of this study show that the distal end of the lateral flagellum of the antennule is the first to differentiate and produce the olfactory receptor neurones and their sensilla. Segments are then added at each moult to the lateral flagellum at its proximal end. The flagellum continues to lengthen without losing the receptors at the distal tip (unless damaged) by adding aesthetasc-bearing segments at the proximal end of the receptor array. It would appear that the chances of losing the distal tip of the flagellum increase with body size because the characteristic numerical pattern of the distal end of the postembryonic flagellum is seldom seen in animals larger than approximately 8 mm carapace length. Older animals shed the distal segments with the exuvium. New segments, in excess of those that are shed, are recruited at the proximal end of the receptor array, and the flagellum gradually increases in length.

Matching the exuvium with the newly moulted flagellum provides convincing evidence for the above. The near, but not perfect, match provides us with further insights into the way in which aesthetasc sensilla are added to the flagellum. In the smaller animals, no match could be found when aesthetasc numbers were compared, but in some larger animals the aesthetasc hairs provided the highest percentage match of all receptors compared. The explanation for this may lie in the gradual increase in number of aesthetasc sensilla per segment that is correlated with the increase in the body size (Table 1). Small animals rarely have rows of four or five aesthetascs, whereas this is common in the larger animals. The mismatch between aesthetasc numbers on the exuvium and flagellum in smaller animals, when these are aligned for the guard hairs, could therefore be produced by a local increase in ORN and aesthetasc numbers. An examination of the mismatching segments proved this to be the case. Thus, in the early stages of the animal's life, ORNs and aesthetascs are added along the entire length of the array as well as at the proximal end. This effect is enhanced by the production of segments at the proximal end of the array that have the full complement of four (or five) sensilla and the gradual loss of the distal segments that carry smaller numbers. In the older animals, a local increase in numbers of aesthetasc sensilla appears to be rare.

The imperfect match between the guard hairs is also a good indication that changes can still occur along the length of the flagellum. Where mismatches occurred at the matchpoint, they were found to be caused by an increase in the number of hairs present.

The comparison of the left exuvium with the right newly moulted flagellum sometimes produced matching that was

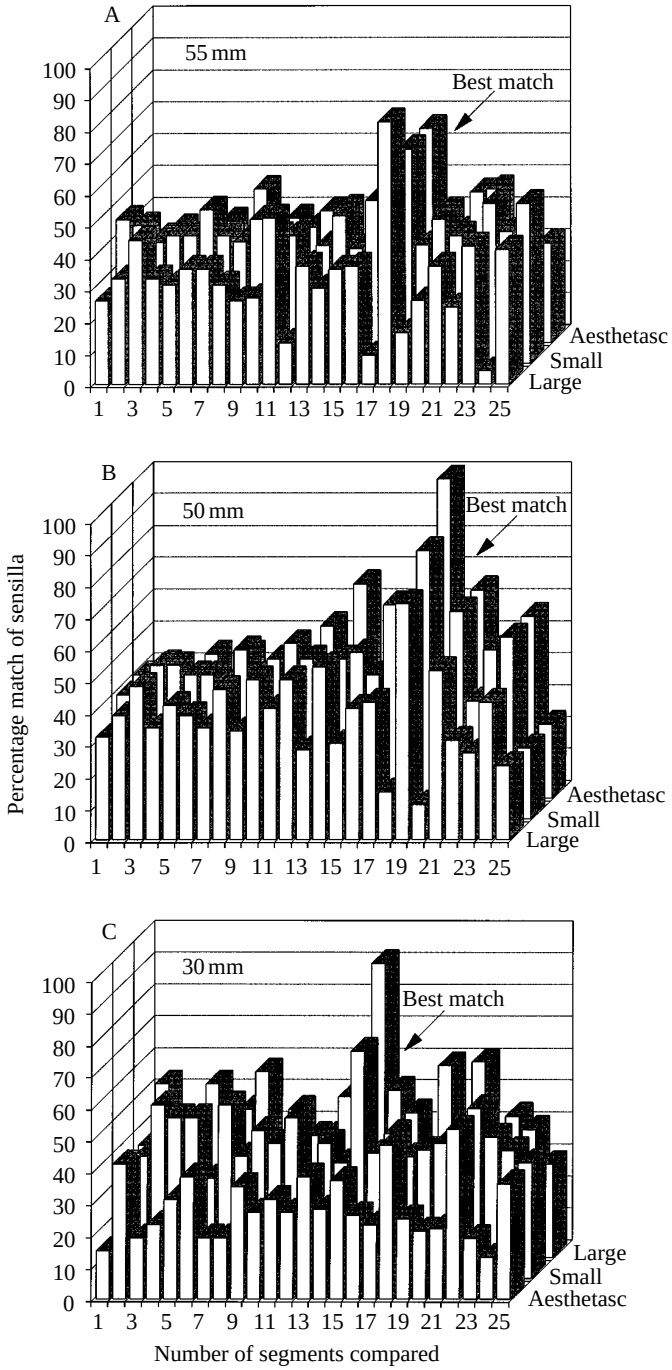

Fig. 8. Simultaneous comparisons of numbers of aesthetasc sensilla, small guard hairs and large guard hairs on each segment (see Figs 6, 7). The alignments are as described in Fig. 7, with 1 indicating that the most proximal aesthetasc-bearing segments are aligned. (A) The 55 mm carapace length animal has a matchpoint coincident for all three receptor types at position 18. The large guard hairs attain the highest match, the aesthetascs are next and the small guard hairs are the most variable. (B) In a 50 mm carapace length animal, the exuvium needed to be displaced distally by 19 segments before a match was found, again coincident for all three receptor types. The best match here was found between the aesthetasc sensilla, the match for the small and large guard hairs being about the same. The very low match immediately before and after the matchpoint in all three types of receptor hairs is pronounced in this animal. (C) Large guard hairs match well in the 30 mm animal, but the small guard hairs and the aesthetasc hairs do not have a point where their match is above values that could occur by chance.

quite high (almost 60 % in Fig. 7C). One interpretation of this result is that the flagella on different sides of the same animal are alike and that the distribution of the receptor hairs along the flagellum is not entirely random. Some evidence for a common control on the size and number of segments contained within the flagella on either side of the animal comes from observations on the regeneration of antennular flagellar in juvenile animals. After unilateral ablation, regeneration in such animals proceeds only until the regenerated antennule achieves the same number of segments as the unablated contralateral control, after which growth continues as before (R. E. Sandeman, unpublished observation).

One aspect of the flagellar growth remains unclear. The base of the flagellum in mature animals rests on the ischiopodite, and the annuli of the proximal half of the flagellum carry no aesthetasc hairs. Towards the proximal end of the aesthetasc receptor array, feather hairs are absent from the ventral surface of the segments. The feather hairs are gradually restricted to the dorsal surface of the flagellum and are absent over the proximal aesthetasc-bearing segments. From this observation and the presence of developing ORNs and aesthetascs beneath the cuticle of the undifferentiated segments, it would appear that the segments of the flagella are not being produced *de novo* at the proximal end of the chemoreceptor array but are being gradually transformed into chemoreceptive segments. How this is controlled provides us with an interesting question which may be approached through the ability of the antennules to regenerate after damage.

Given that the chemoreceptors are being shed from the distal end of the flagellum and that new receptors are being formed at the proximal end of the array, we are able to calculate an approximate rate of receptor turnover (in this case equivalent to the loss of the distal receptors) as, in all but the largest animals, more receptors will be added than are lost. Receptor turnover in crayfish can only occur at moulting, and the frequency of moulting is dependent on the amount of food they obtain, the temperature of the water in which they live and their size. Nevertheless, matching the exuvia of a 55 mm carapace length animal with its newly moulted flagellum showed it to have lost seven distal segments carrying 23 aesthetasc sensilla. The total aesthetasc count on the exuvia amounted to 150, whereas the new flagellum carried 180, i.e. a gain of 30 sensilla. Twenty-three of these can be considered as replacements, leaving a net gain in this animal of seven sensilla. The approximate total turnover of ORNs lies in the region of 2300.

The serial replacement of the ORNs along the antennule of the crayfish differs markedly from the replacement of the ORNs in vertebrates in a way that may have important consequences for the central organization of the olfactory systems of the two groups of animals. Recently, Breer and his colleagues have shown that the membranes of ORNs in

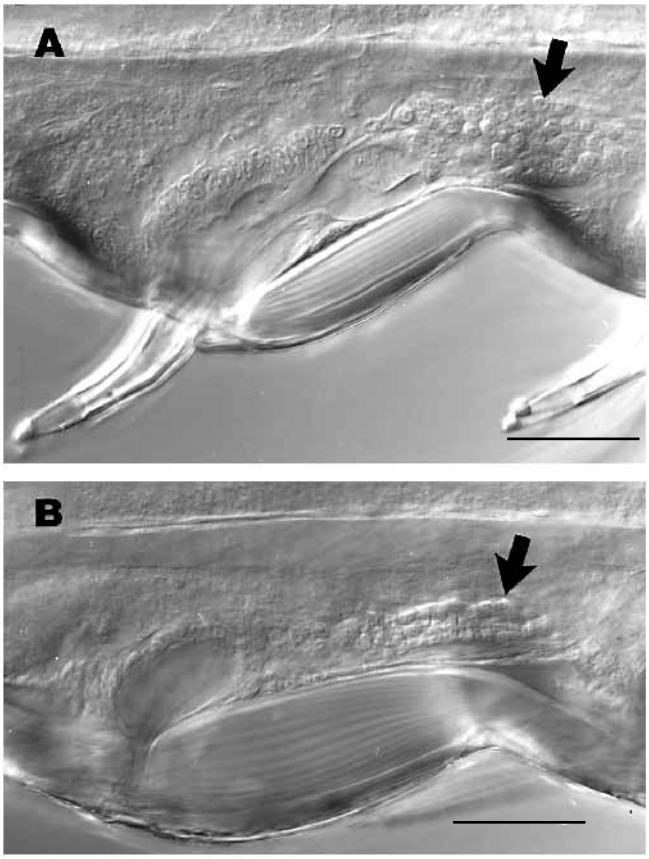

Fig. 9. Differential contrast micrographs of flagellar segments near the distal (A) and at the proximal (B) ends of the aesthetasc array. The ORNs (arrow) and the cells associated with the mature and externalised aesthetasc sensillum are clearly visible in A. In B, the aesthetasc sensillum is still enclosed in a bulb-like structure leading to a canal forming in the cuticle. Cells of approximately the same size as the ORNs of the mature sensillum lie in rows just proximal to the developing aesthetasc sensillum (arrow). Scale bars, A, 50 μm; B, 25 μm.

discrete areas within the olfactory epithelia in rats contain the same kind of receptor molecules and so are sensitive to the same kinds of odours (Breer, 1993, 1994; Strotmann *et al.* 1994). ORNs in these areas must be continually replaced with others of the same kind, otherwise the demonstrated specificity of these areas would soon vanish. The rat olfactory epithelium therefore exhibits a certain spatial organization that reflects odour selectivity which could be centrally preserved because axons from the same areas of the epithelium are likely to end in close proximity to one another in the olfactory bulb.

In contrast, the spatial concentration of sensilla with a specific odour sensitivity on the crayfish antennule would result in the sensitivity to that particular odour being severely attenuated when the animal moulted, unless the lost portion was simultaneously replaced with receptors of the same sensitivity. It is more likely that each row of aesthetasc sensilla has the entire spectrum of odour sensitivities that the animal needs and that the central projections from each row will extend over the entire olfactory lobe. Radioactive leucine tracing of the central projections of the aesthetasc sensilla from a single segment has shown this to be the case (Mellon, 1990). This notion is also supported by physiological studies of lobsters where single rows of aesthetasc sensilla and even single sensilla have the entire spectrum of odour sensitivities (Spencer, 1986).

References

ACHE, B. W. (1991). Phylogeny of smell and taste. In *Smell and Taste in Health and Disease* (ed. T. V. Getchell, L. M. Bartoshuk, R. L. Doty and J. B. Snow, Jr), pp. 3–18. New York: Raven Press.

BLEST, A. D. AND DAVIE, P. S. (1980). Reduced silver impregnation derived from the Holmes technique. In *Neuroanatomical Techniques* (ed. N. J. Strausfeld and T. A. Miller), pp. 98–118. Berlin, Heidelberg: Springer Verlag.

BREER, H. (1993). Second messenger signalling in olfaction. *Ciba Foundation Symposia* **179**, 97–109.

BREER, H. (1994). Odor recognition and second messenger signaling in olfactory receptor neurons. *Seminars Cell Biol.* **5**, 25–32.

CHASE, R. AND RIELING, R. (1986). Autoradiographic evidence for receptor cell renewal in the olfactory epithelium of a snail. *Brain Res.* **384**, 232–239.

CHASE, R. AND TOLLOCZKO, B. (1993). Tracing neural pathways in snail olfaction: From the tip of the tentacles to the brain and beyond. *Microsc. Res. Techn.* **24**, 214–230.

HILDEBRAND, J. G. (1995). Analysis of chemical signals by nervous systems. *Proc. natn. Acad. Sci. U.S.A.* **92**, 67–74.

MELLON, DEF. (1990). Evidence for non-topographic afferent projection and growth-related central reorganization in the crayfish olfactory system. In *Frontiers in Crustacean Neurobiology* (ed. K. Wiese, W.-D. Krenz, J. Tautz, H. Reichert and B. Mulloney), pp. 49–57. Basel: Birkhäuser Verlag.

MELLON, DEF. AND ALONES, V. (1993). Cellular organization and growth-related plasticity of the crayfish olfactory midbrain. *Microsc. Res. Tech.* **24**, 231–259.

MELLON, DEF., TUTEN, H. R. AND REDICK, J. (1989). Distribution of radioactive leucine following uptake by olfactory sensory neurons in normal and heteromorphic crayfish antennules. *J. comp. Neurol.* **280**, 645–662.

MOULTON, D. G. (1974). Dynamics of cell populations in the olfactory epithelium. *Ann. N.Y. Acad. Sci.* **237**, 52–67.

SANDEMAN, D. C. AND DENBURG, J. L. (1976). The central projections of chemoreceptor axons in the crayfish revealed by axoplasmic transport. *Brain Res.* **115**, 492–496.

SANDEMAN, D. C. AND SANDEMAN, R. E. (1994). Electrical responses and synaptic connections of giant serotonin-immunoreactive neurons in crayfish olfactory and accessory lobes. *J. comp. Neurol.* **341**, 130–144.

SANDEMAN, R. E. AND SANDEMAN, D. C. (1991). Stages in the development of the embryo of the freshwater crayfish *Cherax destructor*. *Roux's Arch. dev. Biol.* **200**, 27–37.

SANDEMAN, R. E., WATSON, A. H. D. AND SANDEMAN, D. C. (1995). Ultrastructure of the synaptic terminals of the dorsal giant serotonin-IR neuron and deutocerebral commissure interneurons in the accessory and olfactory lobes of the crayfish. *J. comp. Neurol.* **361**, 617–632.

SCHMIDT, M. AND ACHE, B. W. (1992). Antennular projections to the midbrain of the spiny lobster. II. Sensory innervation of the olfactory lobe. *J. comp. Neurol.* **318**, 291–303.

SPENCER, M. (1986). The innervation and chemical sensitivity of single aesthetasc hairs. *J. comp. Physiol.* **158**, 59–68.

STROTMANN, J., WANNER, I., HELFRICH, T., BECK, A. AND BREER, H. (1994). Rostro-caudal patterning of receptor-expressing olfactory neurones in the rat nasal cavity. *Cell Tissue Res*. **278**, 11–20.

TIERNEY, A. J., THOMPSON, C. S. AND DUNHAM, D. W. (1984). Site of pheromone reception in the crayfish *Orconectes propinquus*. *J. crust. Biol.* **4**, 554–559.

TIERNEY, A. J., THOMPSON, C. S. AND DUNHAM, D. W. (1986). Fine structure of aesthetasc chemoreceptors in the crayfish *Orconectes propinquus*. *Can. J. Zool*. **64**, 392–399.

WACHOWIAK, M. AND ACHE, B. W. (1994). Morphology and physiology of multiglomerular olfactory projection neurons in the spiny lobster. *J. comp. Physiol*. **175**, 35–48.