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GROWTH AND REGENERATION OF THE SECOND ANTENNAE OF *ASELLUS AQUATICUS* (ISOPODA) IN THE CONTEXT OF ARTHROPOD ANTENNAL SEGMENTATION

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ABSTRACT

The production of new articles in the flagellum of the second antennae of *Asellus aquaticus* was studied in both undamaged and regenerating antennae. Segmentation is an iterative process in two phases: 1) the first proximal flagellomere (the meristematic article) repeatedly gives off distally a new primary article; 2) each primary article divides into four secondary articles (a quartet). To a certain extent, production and development of different quartets are independent processes. Evidence is provided that the formation of new articles and their setae are partly decoupled. During regeneration from the preferred breakage point (the so-called 'autotomy plane'), the flagellum is generated by the same mechanism of two-phase segmentation. The regenerated flagellum has a normal segmental composition, except for the tip (the apical complex), which has four flagellomeres rather than the normal five. The similar segmental pattern observed also in other malacostracan crustaceans and in insects, supports a close phylogenetic relationship among the two groups; if the latter proves not to hold, that similarity would provide an example of parallel evolution of developmental mechanism. The difference between 'true' articles and annulations, defined on the structure of the muscular system, is discussed on the basis of comparative developmental data. In general, annulations are produced more sequentially, compared with the almost simultaneous emergence of true articles.

INTRODUCTION

The study of segmentation of arthropod appendages is an interesting chapter of evolutionary developmental biology (Williams and Nagy, 1996; Williams, 1999, 2004; Abzhanov and Kaufman, 2000; Minelli et al., 2000; Bitsch, 2001; Schram and Koenemann, 2001). Nevertheless, very little attention has been paid to date to those arthropod appendages, like most antennae, where the number of articles increases during postembryonic development. Few reviews are available on this subject. Imms (1940) summarised knowledge about the postembryonic development of hexapod antennae. More recently, Boxshall and Huys (1998) proposed a general model of segmentation for the first antennae (antennules) of copepods.

Segmentation of this kind of appendages usually involves the production of new annulations and not of 'true' articles (sensu Boxshall, 2004). The structural difference between these two segmental units has been often overlooked in the discussion about arthropod limb evolution (Boxshall, 2004), and few data for comparative developmental analysis are available.

The old descriptive literature, however, is full of insightful detail that deserves reappraisal from a modern viewpoint. For example, by comparing the morphology of several species of aselloid isopods, Racovitza (1925) hypothesized a general model of antennal segmentation, but also raised problems, mainly concerning the formation of new articles during regeneration. However, his model was not supported by experiments, and lacked longitudinal studies of individual ontogenies.

The second antennae of the freshwater isopod *Asellus aquaticus* (Linnaeus, 1758) are uniramous appendages composed of a proximal peduncle and a distal flagellum. The peduncle is composed of six articles with intrinsic muscles, and has a preferred breakage point (PBP), often called the autotomy plane, between the fourth and the fifth article. The flagellum is devoid of muscles (Wege, 1911), and its articles (flagellomeres) increase in number during the whole life, as this species never stops moulting. Along the flagellum there are sensory setae of uniform shape, with characteristic distribution on a few, different positions on the distal part of each article (with few exceptions, see below). Each article bears one row of setae (Wege, 1911; Racovitza, 1925), and the number and position that the setae can occupy are distinctive of specific articles along the flagellum.

Antennal regeneration of *A. aquaticus* is well known (Przibram, 1899; Zuelzer, 1909; Wege, 1911), but there is no study of segmentation during regeneration. In this work we studied the segmentation of the second antennae of *A. aquaticus* during normal development as well as during regeneration following breakage at the PBP. Regeneration following cuts at other positions along the flagellum will be treated in a forthcoming article.

MATERIALS AND METHODS

Specimens of *A. aquaticus* were collected in a stream in locality Polegge (Vicenza, northern Italy) from May 2002 to May 2003, and reared in the laboratory with water and plant debris from the same site. Observations and experiments were performed from May 2002 to June 2003.

Females with brood pouch were isolated and checked daily to collect juveniles. Juveniles released from the brood pouch were moved to individual Petri dishes and checked regularly to collect exuviae. The collected exuviae were mounted in ethylene glycol immediately or after storage in 70% ethanol. Fixed specimens were also studied. As needed, specimens were cleared in lactophenol, duration of treatment depending on size.

Slides were observed in bright field, phase contrast or Nomarski interference. Additional in vivo observations were performed by manipulating the specimens as described below for regeneration experiments.

The schedule of production of new articles was tracked by the help of morphological markers (setae). Faint article boundaries, often characteristic of articles in the course of division, also contributed to the assessment of the segmentation schedule. Observations on the postembryonic development of 88 specimens were performed. For every specimen several exuviae were collected, thus allowing to study individual ontogenies. Additional adult and subadult specimens were also observed.

Regeneration experiments were performed on young (0-60 days) specimens, as these moult and regenerate faster than later stages. Specimens were placed on a slide in a drop of water, the slide was then placed above a Petri filled with ice to reduce the specimen's activity. Cuts were performed under a dissection microscope using fine scissors or a microtome knife. Only one antenna was cut, usually the right one. After amputation the specimens were moved back to their Petri dishes and subsequent exuviae were collected and studied as described.

Regeneration experiments were performed on 33 specimens. For 19 specimens the antenna was cut at the PBP; for the remaining 14 the cut was also in the peduncle, but distal to the PBP. In the latter cases the stumps were subsequently reduced (by an undetermined mechanism: autotomy, autospasy or autotilly; see Bliss, 1960) at the PBP without any further intervention.

RESULTS

Structure and Segmentation of the Flagellum

The general morphology of the second antennae is shown in Fig. 1A.

Most of the flagellar length can be described as a series of sets of four articles having an individually specific setal pattern (number and position of setae), we call these units complete quartets (Fig. 1B, C). The whole flagellar portion composed of complete quartets is called central region. A different sequence of flagellomeres is found in the proximal meristematic region and in the distal region of the flagellum (apical complex) (for a detailed morphological description of the whole flagellum, see Racovitza, 1925).

The meristematic region is where new quartets are formed. The production of new quartets follows a stereotyped scheme of segmentation as first hypothesized by Racovitza (1925) on the basis of the morphology of the flagellum. Segmentation proceeds in two phases. First, the most proximal flagellar article (the meristematic article) divides into two articles: a proximal copy of itself and a distal article. This distal article is the founder of a future quartet (one-article incomplete quartet; Fig. 2). Second, the one-article incomplete quartet produces a complete quartet by growing and dividing in successive steps, thus passing through the conditions of two- and three-article incomplete quartet. This pattern of division follows a precise sequence (Fig. 3): the one-article incomplete quartet divides in two articles, of which the distal one divides before the proximal one. The meristematic region is composed of the meristematic article, followed by a variable number of incomplete quartets arranged in a proximo-distal gradient of 'maturity', those closer to the state of complete quartet being always distal to the less complete ones (Fig. 4).

The meristematic region is followed by a variable number of complete quartets forming the central region. The number of complete quartets increases during ontogeny.

The apical complex is composed of the distal article (apex) which is cone-shaped and differs in the setal pattern from all other articles in the flagellum. The apex is preceded by four articles whose setal pattern does not match with that of a quartet. Undamaged antennae thus have an apical complex of 5 articles, with a specific setal pattern (Fig. 5). The three proximal articles of this apical complex have the same setal pattern as the first, second and fourth article of a complete quartet (see Figs. 1B-C). The penultimate article has a setal pattern intermediate between those of the first and the third article of a quartet.

Morphogenesis during Normal Development

At hatching, the second antennae of *A. aquaticus* are composed more frequently of 12 flagellomeres, but newly hatched specimens with 11 or 10 flagellomeres are common (27% and 18% of the observed cases, respectively). From proximal to distal, a 12-flagellomere flagellum has the following composition: the meristematic article, one two-article incomplete quartet, one complete quartet, and the apical complex.

The postembryonic development of the flagellum then involves both the production of new one-article incomplete quartets by the meristematic article, and their division to produce new complete quartets. Once a new one-article incomplete quartet has been produced, its segmentation proceeds autonomously, invariably following the stereotyped pattern just described. However, there must be some form of control over the schedule of production and division of the several incomplete quartets at the level of the entire meristematic region, because there are limits to the range of the number of incomplete quartets and because there is a proximo-distal gradient of maturity among incomplete quartets. This limited set of segmental patterns suggests the existence of developmental constraints on the relative timing of segmentation among different incomplete quartets.

In early postembryonic stages, only one or two incomplete quartets are present. In later stages, as the number of flagellomeres increases, the mean number of incomplete quartets also increases (linear regression, two-tailed *t*-test, $P < 0.001$, $n = 40$, Fig. 6). In flagella with a short meristematic region, the proximo-distal gradient of maturity is tight, so there is no repetition of equally developed incomplete quartets, e.g., two one-article incomplete quartets are not found. As the flagellum gets longer, the number of incomplete quartets increases and some repetition of equally developed incomplete quartets occurs. Two consecutive one-article, two-article, and three-article incomplete quartets were found in flagella with at least 41, 27, and 53 flagellomeres respectively.

The detailed segmentation schedule of the flagellum varies among specimens and, to a lesser extent, even between the two flagella of the same specimen. This variation in time produces a variation in segmental arrangement, so that flagella with the same number of articles can actually exhibit different quartet composition. During normal development from 11 to 16 flagellomeres, flagella with 11, 13 or 15 articles are found with either of two alternative quartet compositions, whereas flagella with 12, 14 or 16 articles present only one. This pattern can be easily explained as a by-product of the constraints on segmentation timing outlined above (Fig. 7).

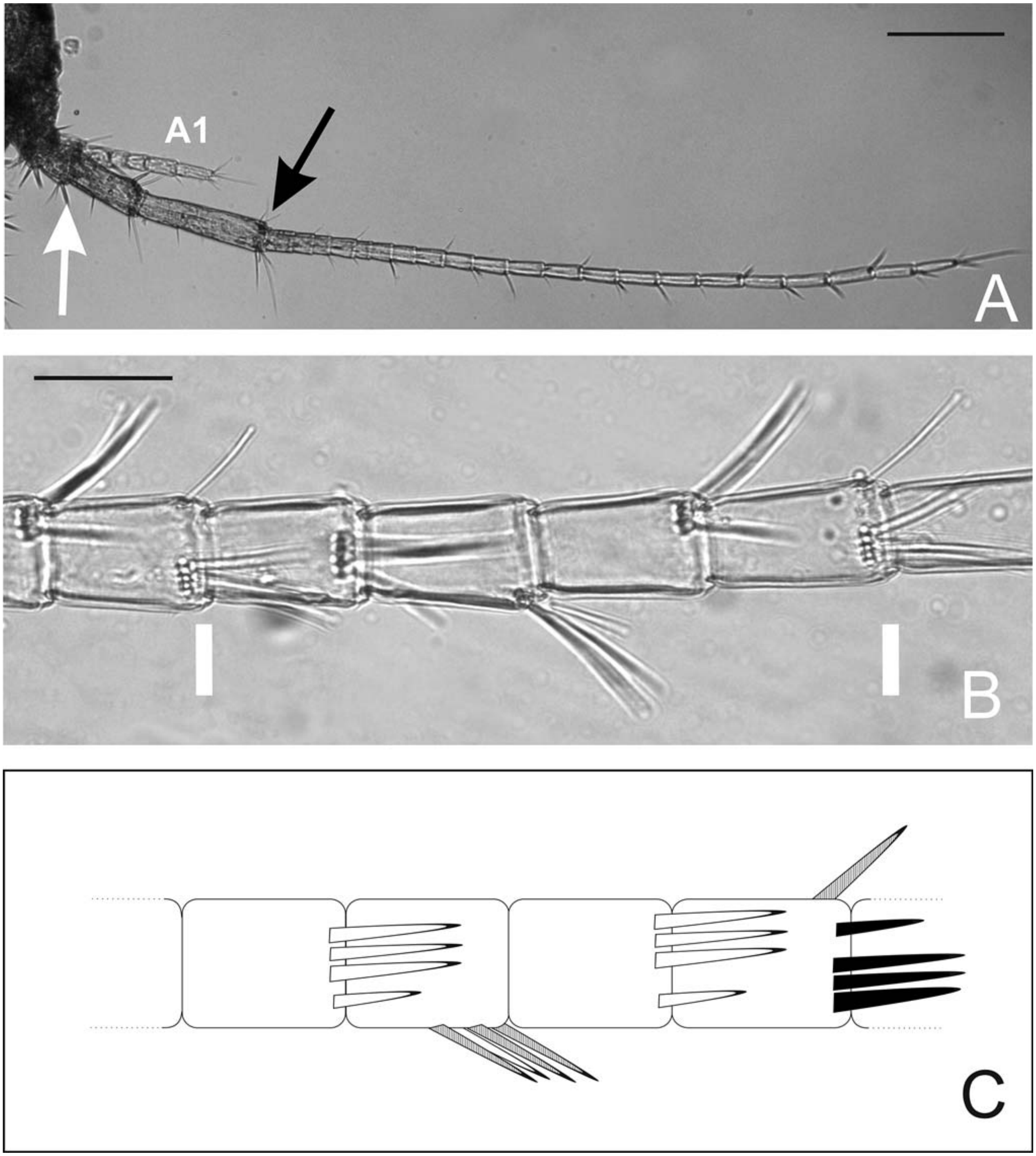

Fig. 1. Second antenna of *Asellus aquaticus*. A, dorsal view. White arrow points at the preferred breakage point (PBP), black arrow at the edge between peduncle and flagellum. A1, first antenna. Scale bar 200 µm. B, a complete quartet, bracketed within the white marks, of a left antenna. Scale bar 50 µm. C, schematic representation of a complete quartet. Dorsal setae are represented in black, lateral setae in grey, ventral setae in white.

The frequency at which different quartet compositions are found is different. This is because some quartet compositions may persist for more than one moult cycle, while others are both produced more rarely and do not last for long (Table 1). During the early postembryonic development the number of articles added at each moult follows some regularities. About 50% of the antennae with an even number of flagellomeres do not increase article number at the next moult, while about 30% acquire two flagellomeres. No regularity is found in longer antennae.

Morphogenesis during Regeneration

To cut the antenna at the PBP means to remove the two distalmost articles of the peduncle and the entire flagellum

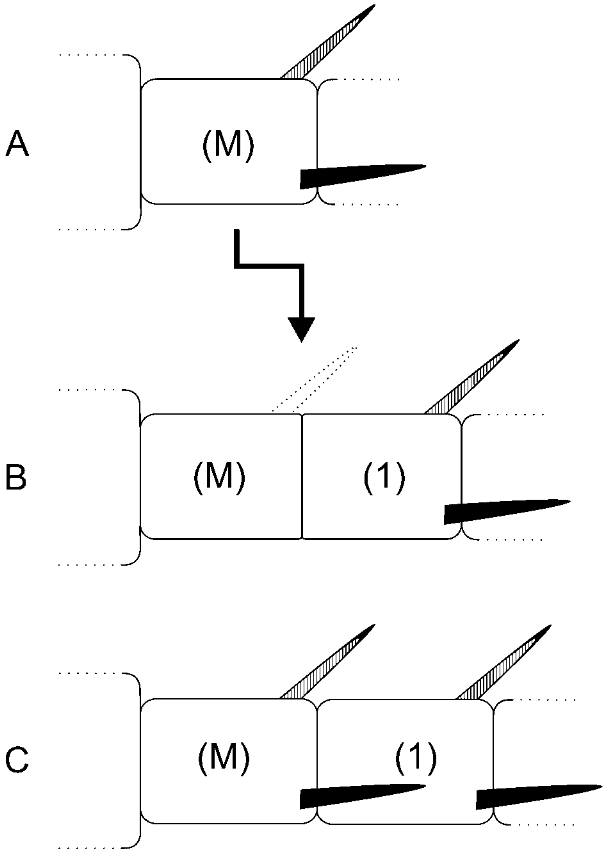

Fig. 2. Schematic representation of the division of the meristematic article in the flagellum of the second antennae of *Asellus aquaticus* (left antenna seen from above, distal end to the right). Orientation and symbols as in Fig. 1C, setae whose first appearance does not necessarily coincide with the first appearance of the next distal article boundary are dashed. Arrow points to the new article boundary. (M), meristematic article; (1), one-article incomplete quartet. The meristematic article can be unarmed or bear one or two seta(e), depending on its developmental status; these setae will be 'inherited' by the one-article incomplete quartet that will form after the division. The A to B to C sequence may be accomplished in one to three moults.

(Fig. 1A). When the regenerate first appears (at the first or at the second post-operative moult) it is already composed of the two distalmost articles of the peduncle, plus between 4 and 15 new flagellomeres.

In comparing segmentation schedules of undamaged and regenerating antennae, regenerating antennae with N flagellomeres must be compared with undamaged antennae with N-1 flagellomeres, because a flagellum regenerated from the PBP always has an apical complex of four articles instead of the normal five. The production of this regenerated apical complex follows very closely the stereotyped division sequence of a complete quartet during normal development, except for the last article which assumes the morphology of the apex (Fig. 8).

In the meristematic region, new quartets are produced by the same mechanism observed in normal development. However, the segmentation scheduling is more variable (Fig. 9). Comparing regenerating antennae with N articles to the corresponding undamaged ones with N-1 articles, during regeneration the meristematic region may include a larger number of incomplete quartets (compare for instance the antennae with 11 flagellomeres in Fig. 7 with the ones with 10 in Fig. 9, and the antenna with 16 flagellomeres in Fig. 7 with the one with 15 in Fig. 9). Also, incomplete quartets with the same article number are found in the same flagellum earlier in the developmental schedule, i.e., in shorter flagella (see antenna with 10 flagellomeres in Fig. 9). This is reflected in the fact that, in most cases, a given number of flagellar articles may correspond to two different quartet compositions.

Formation of Articles and Formation of Setae

The time of formation of new articles does not correlate strictly with the time of formation of their setae. The meristematic article (or an article of an incomplete quartet) can divide when it has already one or more setae at mid-length, or not. For the dividing articles of incomplete quartets these mid-length setae will be 'inherited' by the proximal one of the two 'offspring' articles. It is not rare, anyway, to find unarmed articles, i.e., articles that will develop their setae only following one more moult.

Racovitza (1925) recorded 'incomplete quartets' with four rows of setae in a sequence comparable to that of a normal complete quartet. This implies some articles having more than one row of setae. Racovitza interpreted these cases as the product of a regeneration process. Three comparable cases were observed in our material (regenerated antennae only). These articles are the product of a normal setal development associated to a failure of the article boundary formation (Fig. 10).

DISCUSSION

The Development of the Flagellum

The process of segmentation of the flagellum of the second antennae in *A. aquaticus* is both iterative, as the two-phase process is repeated several times, and intercalary, since new elements are inserted between older ones. Primary articles are produced by the meristematic article, and these are subsequently divided into a fixed number of secondary articles.

Contrasting with the production of primary articles, which is a sequential, virtually open process, the subsequent subdivisions of each primary article into four final segmental units is closed and stereotyped, the distalmost article of a two-article incomplete quartet being always the first to divide.

A proximo-distal gradient of maturity is present along the series of incomplete quartets. When the number of incomplete quartets is small enough (short meristematic region), the gradient is tight, not allowing the repetition of equally developed incomplete quartets. The number of incomplete quartets is correlated with size and/or segmentation of the whole antenna, both in normal development and regeneration.

The main exception to this segmentation process is the apical complex. Paradoxically enough, there are more problems in trying to explain the formation of the normal apical complex than the regenerated one, as it is the former that does not follow the usual segmentation path of the flagellum.

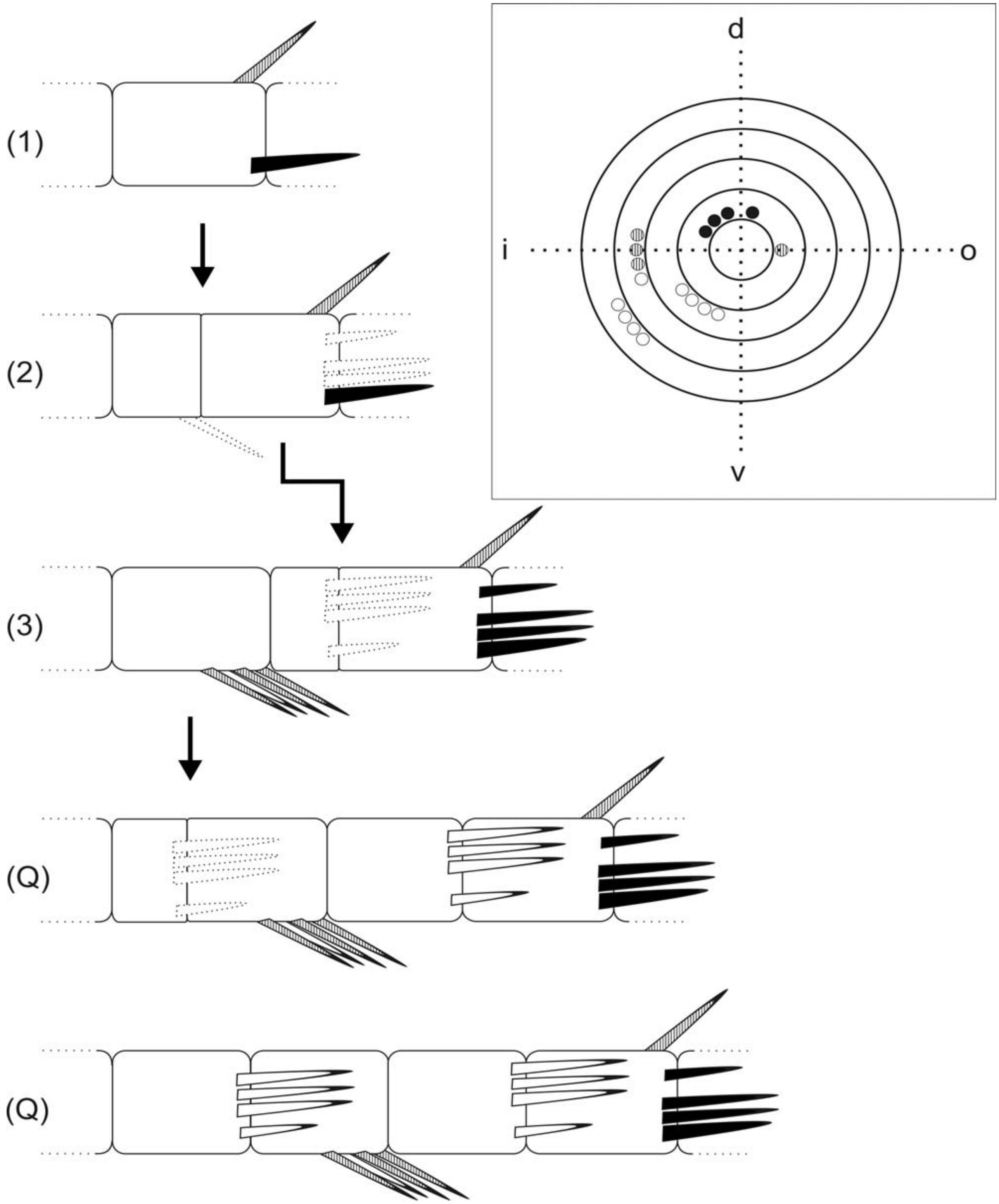

Fig. 3. Schematic representation of the stereotyped division pattern of one-article incomplete quartet. (1), one-article incomplete quartet; (2), two-article incomplete quartet; (3), three-article incomplete quartet; (Q), complete quartets, here shown with two different degrees of development of article size and setae. Orientation and symbols as in Figs. 1-2. In the frame, an apical view of a complete quartet; locations of the setae around the articles is indicated by the circles, black, grey, or white according to the coding in Figs. 1-2. d, dorsal side; v, ventral side; i, inner side; o, outer side.

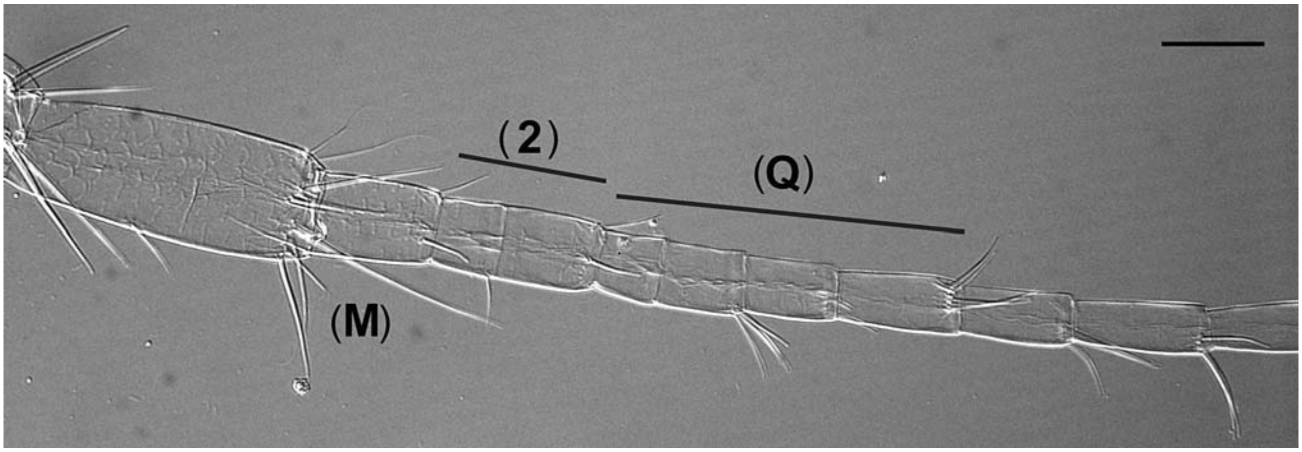

Fig. 4. An example of meristematic region and proximal part of the central region of a second antenna flagellum of *Asellus aquaticus*. (M), meristematic article; (2), two-article incomplete quartet; (Q), complete quartet. Scale bar 50 μm.

Comparative Development of Isopod Antennae

The segmentation model illustrated here for the second antennae of *A. aquaticus* can be possibly applied to most of other aselloid isopods, because their second antennae are patterned in quartets. Racovitza (1925) already provided examples for the Asellidae (several species of *Asellus* and *Caecidotea stygia* Packard, 1871 (sub *Caecidothea stygia*)) and the Stenasellidae (*Protelsonia gjorgjevici* (Racovitza, 1924) and *Stenasellus virei* Dollfus, 1897). However, among the aselloids studied by Racovitza (1925), there were also species expressing a systemic deviation from this model. The central part of the flagellum of the second antennae of *Mancasellus* (now *Lirceus*) is composed of units of two articles, rather than four. However, these two-article units bear the regular aselloid quartet configuration of four rows of setae, as each article of the central region of the antenna bears two rows of setae, one at mid-length of the article and one on its distal part.

Among valviferan isopods, new articles of the second antenna flagellum are produced by the division of the first flagellomere only. This was reported for several species of *Idotea* (Naylor, 1955) and for *Synisoma nadejda* Rezig, 1989 (El Hedfi-Bel Haj Khelil, 2002; and references therein).

The flagellum of the first antennae of the asellids develops in a way similar to that of the second antennae. New primary articles are produced by the fifth antennomere. In *A. aquaticus* these primary articles divide only once, producing two secondary articles (M. Ronco, unpublished data), while in *Lirceus* they remain undivided (Zeleny, 1907). Thus, the 2:1 ratio between quartets in *Asellus* second antennae and two-article units in *Asellus* first antennae mirrors the 2:1 ratio between two-article units in *Lirceus* second and one-article units in *Lirceus* first antennae. On the basis of morphological observations of the first antennae in many isopods, among which was *A. aquaticus*, Wägele (1983) considered the fifth antennal article (the article where we see meristematic activity) as the second flagellomere. This would imply that the meristematic article is not the first flagellomere. We do not have original data on that, but, on the basis of anatomical observations (muscles and tegument), von Kaulbersz (1913) long proposed to consider the fifth antennal article of the first antennae of *A. aquaticus* as the first flagellomere.

Since the condition here described for *A. aquaticus* is found in the second antennae of other species of Asellidae as well as Stenasellidae (see above), we can infer that this is the plesiomorphic condition for the Asellidae. The different condition found in *Lirceus* (Asellidae) is thus a derived one. Due to the limited taxonomic sampling on isopod antennal development, it is not possible to infer a plesiomorphic developmental condition for isopods as a whole. There is, however, variation (see above). It is interesting to note that all kinds of evolutionary variation in the antennal flagellum segmentation process reported above deal only with the second phase of the process, i.e., with the subsequent divisions of articles produced by the meristematic one.

Comparative Structure and Development of Arthropod Antennae

Although there is currently no consensus about the phylogenetic relationships of the major arthropod groups, according to some phylogenetic hypothesis, malacostracan crustaceans and insects are phylogenetically closely related groups (see Wilson et al., 2000; Harzsch, 2002; Fanenbruck et al., 2004). The antennal flagellum of both these groups is characterized by being completely devoid of muscles (Imms, 1939). This kind of structure is what Boxshall (2004) named ‘terminal annulation’. Terminal annulations are not usually found in the antennae of other arthropods (Imms, 1939). Exception are some springtails, e.g., *Sminthurus viridis* (Linnaeus, 1758) (Imms, 1939; see also Boxshall, 2004), the first antennae of the anostracan *Caenestheriella australis* (Loven, 1847) (see Boxshall, 2004), and the first antennae of the ostracod *Uncinocythere occidentalis* (Kozloff and Whitman, 1954) (see Smith and Tsukagoshi, 2005).

It seems reasonable to assume that terminal annulations in the antennae (both first and second) of malacostracans is an apomorphy of this group, or of a clade of malacostracans plus insects, if the latter will turn out to be their sister group. If the first and second antennae of malacostracans are homologous to the first and second antennae of ‘entomostracans’,

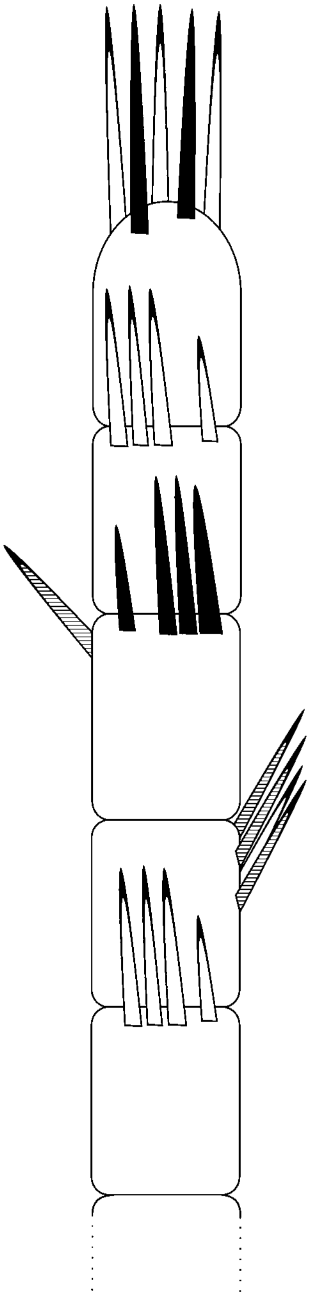

Fig. 5. Schematic representation of the normal apical complex (symbols as in Figs. 1-2).

we can conclude that terminal annulations in the antennae emerged at the same time in both first and second antennae in malacostracans. This means that first and second antennae did not evolve independently, a supposition also supported by the similarities in the segmentation process of the flagellum in these two pairs of appendages (see above for isopods, below for other arthropods).

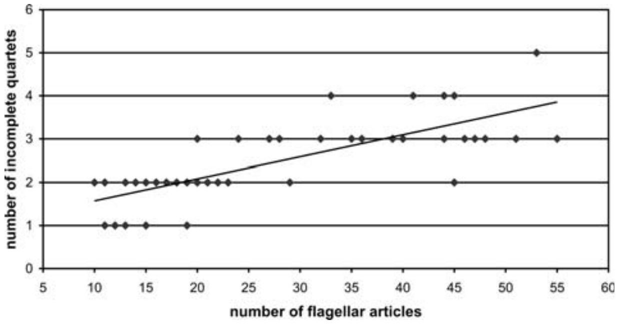

Fig. 6. Relationship between the number of incomplete quartets and the number of flagellar articles in undamaged flagella. Regression line is shown.

Few data are available for the segmentation of antennal flagella in other malacostracan crustaceans. The number of flagellar articles in the first antennae of the amphipod *Gammarus chevreuxi* Sexton, 1913 increases by division of the first and, sometimes, also of the second flagellomere (Sexton, 1924; quoted in Sutcliffe and Carrick, 1981). In the lateral flagellum of the first antennae of the decapods *Cherax destructor* Clark, 1936 and *Panulirus argus* (Latreille, 1804) new articles are always produced at the base of the flagellum (Sandeman and Sandeman, 1996; Steullet et al., 2000), and in both species divisions in articles other than the first one are evident.

In hemimetabolous insects the first flagellomere was long recognised as the main producer of new articles (Imms, 1940), and Henson (1947) actually named it 'meriston' for this reason. In the antennae of cockroaches (Quadri, 1938; Haas, 1955; Campbell and Priestley, 1970; Schafer, 1973) and termites (Fuller, 1920) the primary articles divide once, producing two secondary articles each. In Campbell and Priestley's (1970) nomenclature, the articles produced by the 'meriston' are called 'meristal annuli' and the two articles produced by the division of one meristal annulus are called a 'doublet'.

Similar developmental processes are thus found in the antennal flagellum of malacostracan crustaceans and insects. There is always a particular article (usually the first flagellomere) that is a specific 'center of production' of new articles. However, as far as we are aware, regularity in secondary divisions has been found only in Asellota and in Dictyoptera. Diversity among the different crustacean and insect lineages may thus depend on the degree and regularity of secondary divisions. In the antennae of both 'entomostracan' crustaceans and myriapods, no such developmental similarities can be found (Boxshall and Huys, 1998 for copepods; Smith and Tsukagoshi, 2005 for ostracodes; Minelli et al., 2000 for centipedes). In addition, as noted above, if we take into account the structure of the muscular system in the antennae, 'entomostracan' crustaceans and myriapods clearly do not align with malacostracans and insects. If these similarities in flagellar structure and development in the antennae of malacostracans and insects are not due to the close phylogenetic relationship among the two groups, this would be an example of parallel evolution of a largely similar developmental mechanism for the segmentation of the flagellar structure with termial annulations.

Development of Annulations versus 'True' Articles

Boxshall (2004) clarified the difference between true articles and annulations in arthropod appendages. Annulations can be of two different kinds: terminal, if they are completely devoid of muscles (as are the antennal flagella studied in the present

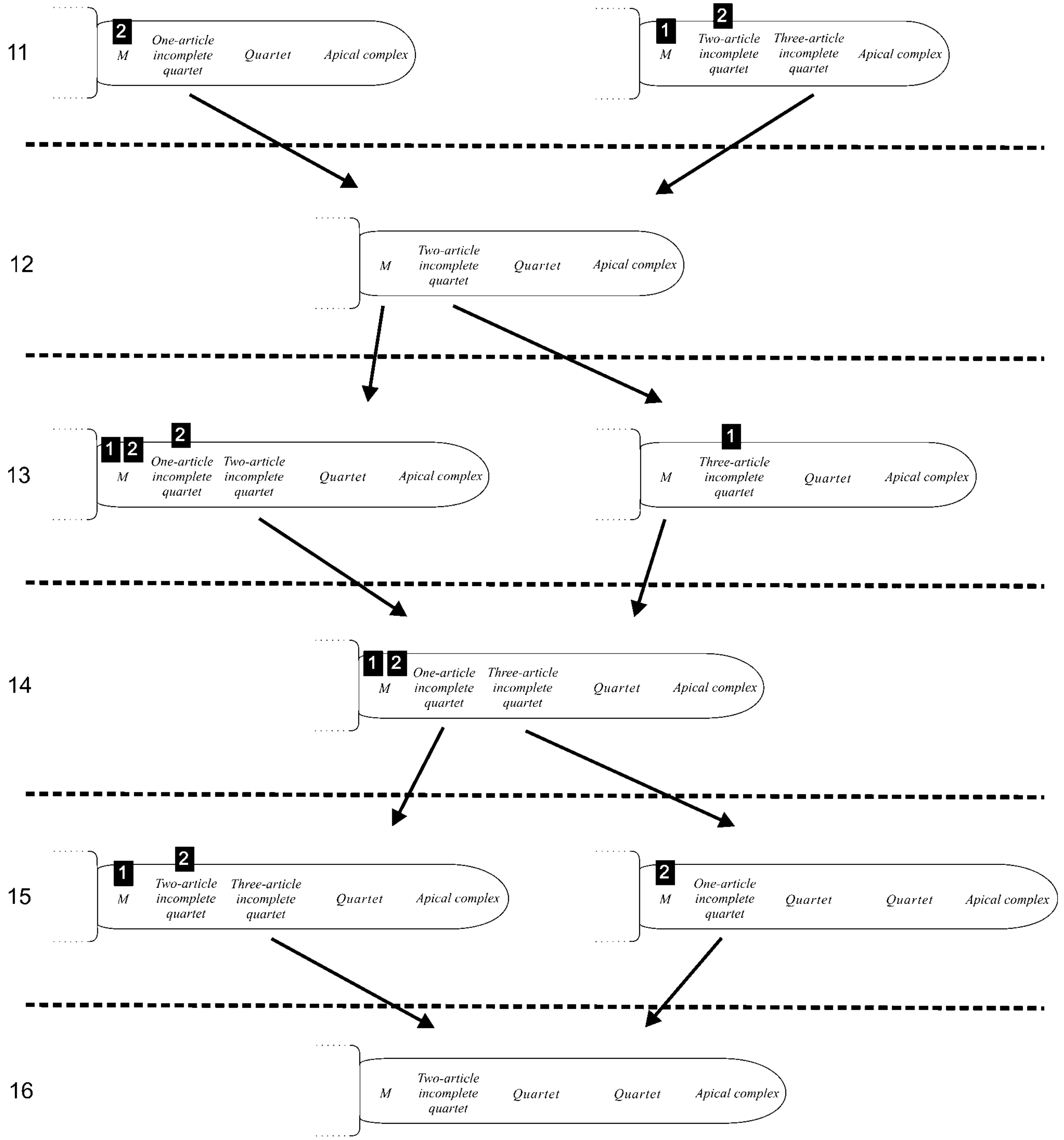

Fig. 7. Schematic representation of the changes in segmental composition in the flagellum during normal development from 11 to 16 flagellomeres (numbers on the left). Black tags indicate the meristematic article and the incomplete quartets that do not divide because: [1] divisions would produce a too short or a too long meristematic region (outside the observed range); [2] divisions would produce two consecutive incomplete quartets with the same number of articles, defeating a tight gradient of maturity.

work), or intercalary, if they have a muscle or a tendon that pass through the annulations but does not insert on them. Boxshall also noted that annulations tend to appear later in the development of a limb if compared to true articles.

Furthermore, during postembryonic development, new appendages (or parts of appendages) composed of true articles are usually described as becoming completely segmented within a short period of time, e.g., during metamorphosis, in the case of the legs of a fly, after which they do not segment further. In these appendages, the formation of article boundaries is almost simultaneous. On the contrary, parts of appendages with annulations may, in general, increase article number over a longer period of time, across several moults: that is, the production of new

Table 1. Individual flagella in subsequent developmental stages of early postembryonic development. Pre-moult segmental composition and the corresponding post-moult status are shown; the total number of flagellomeres is given in square brackets. Segmental composition is summarised as follow: (M), meristematic article; (1), one-article incomplete quartet; (2), two-article incomplete quartet; (3), three-article incomplete quartet; (Q), complete quartet; (AC), apical complex. Percentages have been calculated only where at least 10 cases were available.

Pre-moult segmental composition		Post-moult segmental composition		N. of observed cases	%
[11]	(M) (1) (Q) (AC)	[12]	(M) (2) (Q) (AC)	2	—
N = 2					—
[11]	(M) (2) (3) (AC)	[12]	(M) (2) (Q) (AC)	3	—
N = 7		[13]	(M) (3) (Q) (AC)	3	—
		[14]	(M) (1) (3) (Q) (AC)	1	—
[12]	(M) (2) (Q) (AC)	[12]	(M) (2) (Q) (AC)	13	52.0%
N = 25		[13]	(M) (3) (Q) (AC)	4	16.0%
		[13]	(M) (1) (2) (Q) (AC)	1	4.0%
		[14]	(M) (1) (3) (Q) (AC)	7	28.0%
[13]	(M) (3) (Q) (AC)	[13]	(M) (3) (Q) (AC)	2	—
N = 3		[14]	(M) (1) (3) (Q) (AC)	1	—
[13]	(M) (1) (2) (Q) (AC)	[14]	(M) (1) (3) (Q) (AC)	10	100.0%
N = 10					
[14]	(M) (1) (3) (Q) (AC)	[14]	(M) (1) (3) (Q) (AC)	17	50.0%
N = 34		[15]	(M) (1) (Q) (Q) (AC)	2	5.9%
		[15]	(M) (2) (3) (Q) (AC)	2	5.9%
		[16]	(M) (2) (Q) (Q) (AC)	12	35.3%
		[18]	(M) (1) (3) (Q) (Q) (AC)	1	2.9%
[15]	(M) (1) (Q) (Q) (AC)	[16]	(M) (2) (Q) (Q) (AC)	1	—
N = 1					
[15]	(M) (2) (3) (Q) (AC)	[15]	(M) (2) (3) (Q) (AC)	2	—
N = 6		[16]	(M) (2) (Q) (Q) (AC)	3	—
		[17]	(M) (1) (2) (Q) (Q) (AC)	1	—
[16]	(M) (2) (Q) (Q) (AC)	[16]	(M) (2) (Q) (Q) (AC)	12	63.2%
N = 19		[17]	(M) (1) (2) (Q) (Q) (AC)	1	5.3%
		[18]	(M) (1) (3) (Q) (Q) (AC)	6	31.6%
[17]	(M) (1) (2) (Q) (Q) (AC)	[17]	(M) (1) (2) (Q) (Q) (AC)	2	16.7%
N = 12		[18]	(M) (1) (3) (Q) (Q) (AC)	6	50.0%
		[19]	(M) (2) (3) (Q) (Q) (AC)	4	33.3%
[18]	(M) (1) (3) (Q) (Q) (AC)	[18]	(M) (1) (3) (Q) (Q) (AC)	9	47.4%
N = 19		[19]	(M) (2) (3) (Q) (Q) (AC)	5	26.3%
		[20]	(M) (2) (Q) (Q) (Q) (AC)	3	15.8%
		[21]	(M) (1) (2) (Q) (Q) (Q) (AC)	2	10.5%
[19]	(M) (2) (3) (Q) (Q) (AC)	[19]	(M) (2) (3) (Q) (Q) (AC)	2	14.3%
N = 14		[20]	(M) (2) (Q) (Q) (Q) (AC)	2	14.3%
		[20]	(M) (1) (2) (3) (Q) (Q) (AC)	3	21.4%
		[21]	(M) (1) (2) (Q) (Q) (Q) (AC)	6	42.9%
		[22]	(M) (1) (3) (Q) (Q) (Q) (AC)	1	7.1%
[20]	(M) (2) (Q) (Q) (Q) (AC)	[21]	(M) (1) (2) (Q) (Q) (Q) (AC)	1	—
N = 2		[22]	(M) (1) (3) (Q) (Q) (Q) (AC)	1	—
[21]	(M) (1) (2) (Q) (Q) (Q) (AC)	[22]	(M) (1) (3) (Q) (Q) (Q) (AC)	4	—
N = 6		[23]	(M) (2) (3) (Q) (Q) (Q) (AC)	2	—

annulations is more distinctly sequential than the production of true articles.

Insect legs are produced during embryogenesis or during metamorphosis, the last articles to be produced being those of the tarsus (Bitsch, 2001; Kojima, 2004): these articles are intercalary annulations. Furthermore, in some species, e.g., in the hemipteran *Oncopeltus fasciatus* (Dallas, 1852), the final tarsal segmentation is acquired during postembryonic development (Shaw and Bryant, 1974). In crabs and lobsters, appendages that are formed during postembryonic development appear with their definitive segmental pattern, but for the annulated part of a limb, e.g., the exopod of the pereiopods in spiny lobster's phyllosoma (Duggan and McKinnon, 2003). A similar condition was reported also to occur during the regeneration of arthropod appendages. Here, true articles are all usually formed already when the regenerate first appears, while annulations increase in number over several moults (Maruzzo et al., 2005).

In the present study, we analysed a terminal annulated structure with indeterminate article number. This structure actually never stops adding new articles as long as there are moults available. A comparable behaviour has never been reported for parts of appendages composed of true articles.

However, there are appendages composed of true articles that increase in article number over sever moults. The first antennae of copepods do not have annulations, and new articles are produced during all the larval moults (Boxshall and Huys, 1998). The musculature of this limb is rather simple, being composed of few muscles running from the base of the limb to the tip, and inserting in each article in between (Boxshall, 1985). New, intermediate, muscle insertions are assumed to be produced when new article boundaries are formed (Boxshall, 2004). In lithobiomorph centipedes, new antennal articles are produced during postembryonic development (Lewis, 1981) and, again, their musculature is rather simple, with few muscles running from

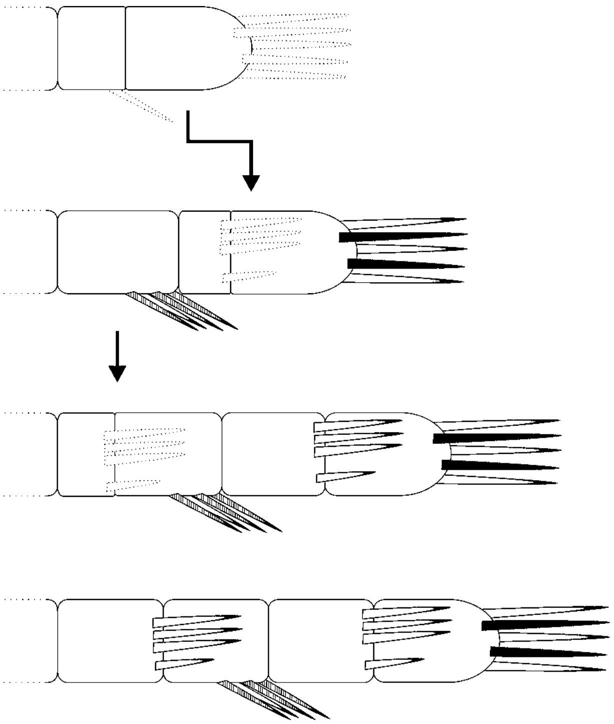

Fig. 8. Schematic representation of the genesis of the apical complex following regeneration from the PBP. Number and location of the setae in the apex show intraspecific variability (orientation and symbols as in Figs. 1-2).

the base to the tip and inserting in each article in between (Imms, 1939). In copepods, thoracopods are produced during the copepodid phase. They usually appear first as limb buds and later as incompletely segmented limbs. All the few subsequent additions of articles, anyway, involve only the rami (Ferrari, 1988). Thoracopod rami have also few muscles running from the base to the tip and inserting in each article in between (Boxshall, 1982, 1985).

So, despite the limited data available at present, we can conclude that the anatomical differences between true articles and annulations are matched by major differences in their development. The structure of the muscular system in a limb, in fact, correlates very well with its development. True articles are produced earlier in development and, usually, 'all at once', while annulations are produced later and in an 'anamorphic-like' fashion. An intermediate condition is found in those (parts of) appendages where few muscles run along the whole length and insert in each article.

Formation of Articles versus Setae

In the present study, we used the setae as markers to track the formation of new articles, a method that has been widely used in developmental studies based on morphological techniques. However, even if the correlation between the formation of articles and the formation of setae is close enough for this purpose, the timing of production of setae and of articles of the second antennae of *A. aquaticus* are not as closely correlated. A newly formed article can be unarmed or bear a few setae. Also, following regeneration, an undivided article with a double rows of setae is sometimes formed. This is equivalent to two normal consecutive articles without a segmental boundary in between.

It should be noted that during the embryogenesis of *A. aquaticus* the antennal setae are formed simultaneously, just before hatching, when the flagellum has 10-12 flagellomeres (C. Biffis, personal communication). A similar event has been also recorded for the first antennae of *Lirceus* (Zeleny, 1907).

In different species of copepods, different correlations between homologous article boundaries and homologous setae have been described (Ferrari and Benforado, 1998; Ferrari and Ivanenko, 2001). Also within the aselloids the correspondence between articles and rows of setae can be different: in most aselloids the structure is as described here, but there are exceptions, as *Lirceus*.

It seems safe to conclude that the positional information for the epidermal cells that will produce article boundaries and for those that will produce the setae is at most only partially derived by reciprocal interactions among these cells.

Regeneration of the Antennae: Similarities and Differences with respect to Normal Development

In those few arthropods where antennal segmentation has been observed during both normal development and regeneration, it was noted that the two processes proceed in a similar way, e.g., in isopods (Zeleny, 1907; present study), in diplurans (Imms, 1940), in cockroaches (Schafer, 1973), in centipedes (Lewis, 2000), and in lobsters (Steullet et al., 2000; Harrison et al., 2003).

In the regeneration of *A. aquaticus* second antennae, after the formation of the blastema and regeneration of the distalmost articles, segmentation of the flagellum proceeds in a way very similar to normal development. New articles are acquired by the same mechanism of two-phase segmentation. However, due to an obvious acceleration of the morphogenetic process during regeneration (see Schafer, 1973 and Harrison et al., 2003 for a comparable increased activity of the 'meristematic' region in cockroach and lobster respectively), the relationship between the number of flagellomeres and the number of incomplete quartets differs from that observed during normal development. Meristematic regions are comparably longer during regeneration with respect to undamaged antennae with the same numbers of articles, and it is rare to find a unique quartet composition for a given number of flagellomeres. Thus, even in short antennae, the gradient of maturity of the quartets is not tight, but allows for the repetition of equally developed incomplete quartets.

In the regeneration of the second antennae of *A. aquaticus* from the PBP, a specific 'new' phenotype of the apical complex, with one flagellomere less than the normal one, was always obtained. In arthropods, regeneration abnormalities in the segmentation of the tegument, possibly produced by similar developmental mechanisms, are common. The hypomorphic regeneration very often observed in the insect tarsi (reviewed in Maruzzo et al., 2005) is one of these. In cockroaches, regeneration from the PBP produces a tarsus with four articles instead of the normal five, the normal

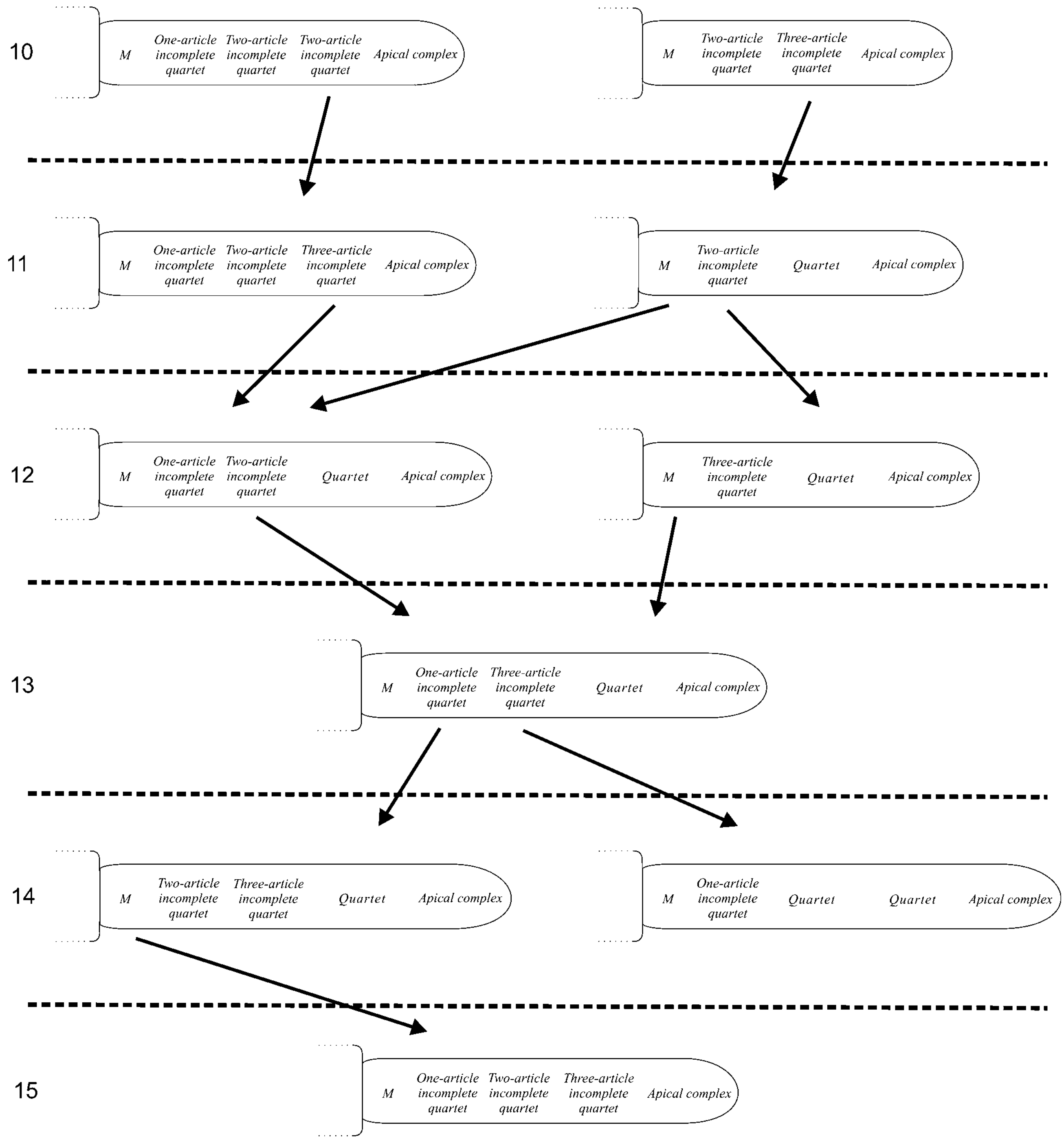

Fig. 9. Schematic representation of the changes in segmental composition of the flagellum during regeneration from the PBP from 11 to 16 flagellomeres. The apical complex is hypomorphic, with only four articles (see text). Numbers on the left are the total number of flagellomeres.

number is restored only if the amputation involves just one or two distalmost tarsal article(s) (Tanaka et al., 1992).

CONCLUSION

Excluding a few distalmost articles, the flagellum of the aselloid second antennae develops by iterating a two-phase segmentation process: a meristematic article produces new 'units' that grow and divide largely independently. This mechanism can also be hypothesized, with differences in the degree and regularity of secondary segmentation, for the segmentation of the flagella in other malacostracan crustaceans and in insects. In *Asellus*, following regeneration from the preferred breakage point, all articles are produced by the same two-phase mechanism. Since the distalmost articles are also produced by this mechanism, the result is a regenerate with a hypomorphic apical complex, with one article less than the normal one. A gradient of maturity persists during regeneration, but it is not tight; and it is more

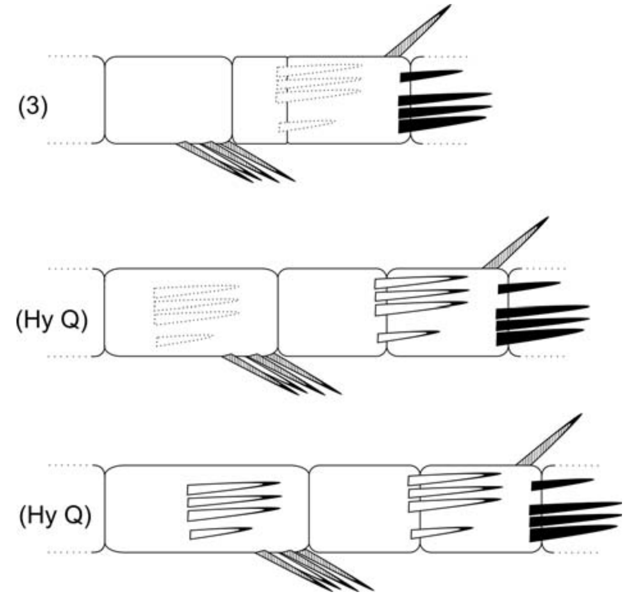

Fig. 10. Schematic representation of one of the rarely occurring articles with a double row of setae. In this example a three-article incomplete quartet (3) produces a hypomorphic quartet (of three articles only (Hy Q) here shown with two different degrees of development of article size and setae) with the setal pattern typical of a complete quartet (orientation and symbols as in Figs. 1-2).

similar to that observable during comparably later developmental stages in normal development. We conclude that the positional information used by the epidermal cells that will produce a new article boundary and by those that will produce the setae is largely independent, and is not based on reciprocal signalling among these cells. Instead, the positional information that these cells use is determined along the proximo-distal axis of the flagellum/antenna and/or within a quartet.

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