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PHYLOGENETIC SIGNIFICANCE OF ANTENNAL GROWTH PATTERNS AT TWO LEVELS OF DRAGONFLY TAXONOMY: THE EXAMPLE OF *CORDULEGASTER* (ANISOPTERA: CORDULEGASTRIDAE)

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In the course of postembryonic development of an odonate larva, the number of antennal segments increases from 3 in newly hatched larvae to 7 in most full-grown larvae. New segments are formed through division of existing segments with the construction of new joints. 2 growth or division centers in the odon. antenna are responsible for this joint formation. A third growth center does not form new joints, but stimulates extension of existing segments. — Ontogenetic studies on 2 spp. of *Cordulegaster* demonstrate the phylogenetic significance of antennal growth pattern at 2 levels of dragonfly taxonomy. At the family level, differences in the operating sequence of the 2 division centers determine a variety of basic antennal growth patterns. The sequence of divisions observed in *C. insignis* and *C. pictus* is in agreement with the position of the Cordulegastridae in the currently accepted genealogy of the order. — Minor but significant interspecific differences in antennal development among W. Palaearctic *Cordulegaster* constitute a key character to differentiate between taxa belonging to the *C. boltonii* and *C. bidentatus* species groups. Biometric analysis of immature larvae of *C. insignis* (*C. bidentatus* group) and *C. pictus* (*C. boltonii* group) explain these differences as being due to a subtle difference in the original position of the division centers, rather than to allemetric growth of antennal segments during larval development.

INTRODUCTION

The antenna of a newly hatched odonate larva is composed of three segments: the scapus, the pedicellus, and a one-segmented flagellum. The antenna of full-grown larvae and the dragonfly imago are principally composed of seven segments: scapus, pedicellus, and a five-segmented flagellum. Reduced numbers of antennal segments occur in the Calopterygidae and some Coenagrionidae (Zygoptera), Epiophlebiidae (Anisozygoptera), Petaluridae and Gomphidae (Anisoptera).

A new antennal segment can be built only during ecdysis from one larval stage (instar) to the next. During this event, one of the existing flagellar segments divides into two new segments through formation of a joint. New joints can originate in one of two growth centers present in the odonate antenna. The first growth center is originally located in the proximal half of the one-segmented flagellum, and later in the proximal (first) flagellar segment. The second growth center is originally located in the apical half of the one-segmented flagellum, later in the most apical flagellar segment. In the course of postembryonic development, the proximal or basal growth center will form three new joints, the apical growth center only one.

MIYAKAWA (1977) described three prominent types of antennal growth pattern, which he named the B.B.B.A, B.A.B.B. and B.B.A.B. types, that differ from each other in timing of the single apical division (A) relative to the three basal divisions (B). He also demonstrated that these three types are linked to higher taxa in the Odonata, reflecting the genealogy of the order as constructed by FRASER (1957) on a selection of imaginal characters. The B.B.B.A. pattern, in which the apical division comes last, is considered to be the original, most primitive type of antennal development. It is found in the Coenagrionidae, Petaluridae and Aeshnidae. The B.A.B.B. type occurs in Corduliidae and the more primitive Libellulidae, and the B.B.A.B. type in more evolved members of Libellulidae; a transitional type between B.A.B.B. and B.B.A.B. is found in a few species of *Sympetrum* and related genera. Reduced numbers of segments as occurring in the Calopterygidae, Epiophlebiidae and Gomphidae can be attributed to the suppression of one or more division events. Similar suppressions are also found in a number of species belonging to families that usually display a seven-segmented antenna. In *Tanypteryx pryeri* (Petaluridae) and several Coenagrionidae species, for example, six-segmented antennae of full-grown larvae result from suppression of the unique division in the apical segment (MIYAKAWA, 1977). Full-grown larvae of *Caliaeschna microstigma* (Aeshnidae) possess no more than five antennal segments (BUCHHOLZ, 1954). This paper presents the first data on antennal growth pattern in the Cordulegastridae.

VERSCHUREN (1989) studied interspecific relations in West-Palaearctic *Cordulegaster* species and subspecies. It was concluded that, on the basis of larval morphology, West-Palaearctic representatives of the genus can be divided into two major species groups: the *C. boltonii* group and the *C. bidentatus* group. Constant differences in length ratio of the flagellar segments were found to constitute a prominent character to differentiate between members of these two major species groups. In full-grown larvae belonging to the *C. boltonii* group, the combined length of the two apical flagellar segments (annulus 4 + annulus 5) exceeds the length of the proximal flagellar segment (annulus 1). In members of the *C. bidentatus* group, the opposite is usually found. VERSCHUREN et al. (1987) used this character, among others, to recognize the larva of *C. mzymtae* as belonging to the *C. bidentatus* species group.

The need for diagnostic characters that are adequate for identification of full-grown larvae (and exuviae) as well as immature larvae, stimulated the present ontogenetic study of antennal development. The two species of *Cordulegaster* chosen for this purpose, *C. insignis* (*C. bidentatus* group) and *C. pictus* (*C. boltonii* group), are assumed to exemplify the general trends occurring in their corresponding species group.

MATERIAL AND METHODS

This study is based on biometric analysis of larval specimens of *Cordulegaster insignis* Schneider, 1845 and *C. pictus* (Selys, 1854). The antennae of 56 full-grown and 111 immature larvae were measured, resulting in totals of 143 data points for *C. insignis* and 113 for *C. pictus*. Measurement of head width is used as a substitute for measurement of total body length in ranking larvae by size.

The material was collected at various localities in Greece and Bulgaria (H. Malicky leg. Lunz; coll. H. Dumont, Gent), and Turkey (L. Brendonck, H. Dumont & D. Verschuren leg.; coll. H. Dumont, Gent). The locality details are listed in VERSCHUREN (1989).

Segments of the flagellum are sometimes denoted as annuli, viz. annulus 1 to 5 (cf. VERSCHUREN, 1989; VERSCHUREN et al., 1987). IMMS (1940) considers the pedicellus as the first annulus, followed by annulus 2 to 6 of the flagellum. To avoid confusion, the term "annulus" will not be used in this paper, in favour of "flagellar segment" (Fig. 2).

IDENTIFICATION OF INSTARS

Controlled breeding from egg to imago has not yet been achieved for any *Cordulegaster* species. Consequently, it could never be assessed exactly how many larval stages, or instars, have passed when an individual larva completes larval development and engages in metamorphosis. Based on measurements of full-grown larvae and the 6 preceding instars, ROBERT (1958) estimated that the total number of instars in *C. boltonii* should be 12. For the purpose of analysis and discussion of instar-dependent events in antennal development, it is necessary to achieve instar number identification of any individual larva. First, we will try to produce a reliable estimate of the total number of instars comprised in larval development of *Cordulegaster* species.

As with all arthropods, larval growth in the Odonata proceeds stepwise, and significant size increases only happen during moulting events. Starting from the uniform size of the egg, and arriving at the fairly uniform size of the dragonfly imago, we expect larval sizes in a sufficiently large sample to cluster in a number of distinct components, equal to the number of larval stages in between moults. In our material of *C. insignis*, however, such clustering of values is only obvious at the beginning and at the end of development (Fig. 1). Experimental breeding efforts (e.g. PILON & MASSEAU, 1983; PILON & RIVARD, 1979; RIVARD & PILON, 1977) have shown that often intrastadial size variation results in an overlap of size range of successive instars. In our study, additional increase of intrastadial variation may be expected from the heterogeneous origin of the *C. insignis* material (104 specimens from 25 different localities). However, intrastadial variation alone is unlikely to be responsible for the irregular and semi-continuous distribution of values along the size gradient during most of development. Rather, we may attribute the observed distribution of values in our *C. insignis* data set to the occurrence of several types of development, each characterized by a specific number of instars.

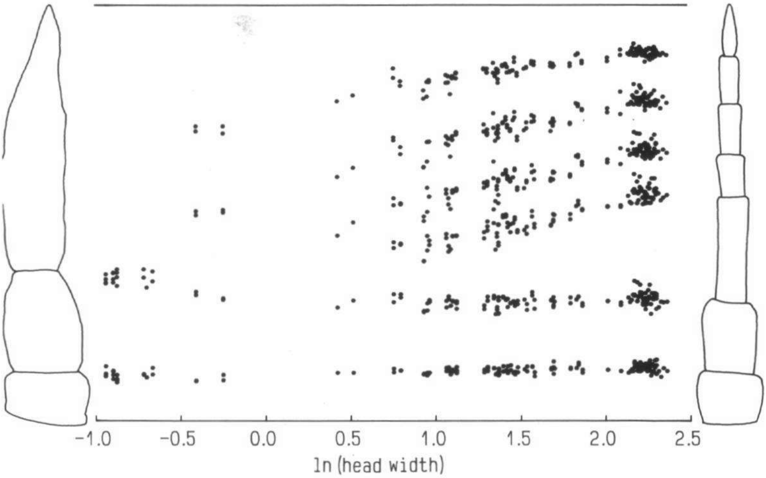

Fig. 1. Antennal development in *C. insignis*, raw data. The location of joints between antennal segments are presented as replicate Y-values. Head-width values are used as a measure of larval body size; logarithmic transformation enhances spacing of values.

The occurrence of more than one type of postembryonic development in a single dragonfly species was first documented by BALFOUR-BROWNE (1909), and later found to be a common feature in experimental rearing programs. SCHALLER (1960, 1972) and SCHALLER & MOUZE (1970) suggested that the number of moults during larval life may be determined epigenetically, and stressed the importance of temperature conditions during embryogenesis for the typology of postembryonic development. LEGGOTT & PRITCHARD (1985) describe an increase of instar number with temperature in series of *Argia vivida* larvae raised at different temperatures, while MATHAVAN (1990) reports increase of instar number with temperature in *Orthetrum sabina*, and decrease in

Brachythemis contaminata. The co-occurrence of several types of development has also been observed within a single experimental population, reared in uniform conditions (PILON & FRANCHINI, 1984; PILON & MASSEAU, 1983; PILON & RIVARD, 1979; RIVARD & PILON, 1977). The phenomenon is interpreted as follows: intrapopulation variation in larval growth rate causes a fraction of the larvae of a particular instar to become retarded in size. In order to reach the required size for successful metamorphosis, these larvae are forced to include one or more additional moults near the end of their development. The intermediate sizes of such larvae do not fit in the general pattern shown by larvae following the normal type of development, which results in the observed continuity of values.

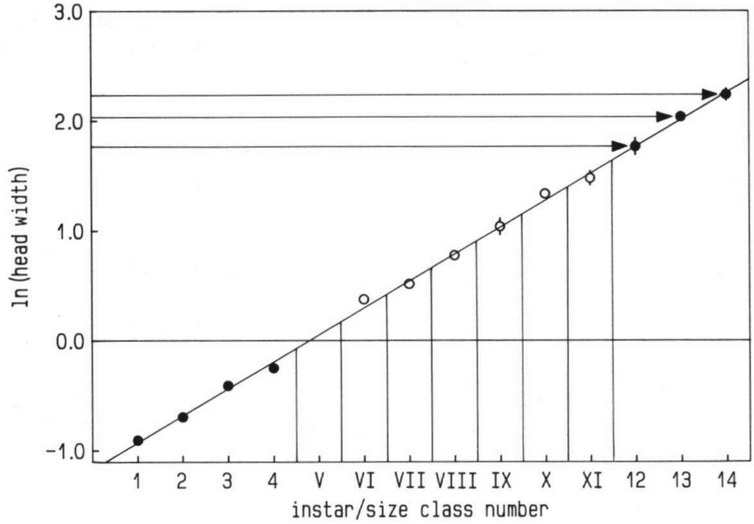

Fig. 2. Transformation of individual larval size values to size classes: (a) Regression of mean ln (head width) to instar number, instars 1 to 4; — (b) Allocation of F_{-2}, F_{-1} and F_0 larvae to instars 12, 13 and 14; — (c) Definition of size classes V-XI to accommodate for larvae with intermediate sizes. —[Closed circles: sample means, morphologically differentiated instars only; — open circles: size class means, other instars].

The occurrence of several developmental types in the studied material has now shifted our original concern of instar number identification towards an attempt to estimate the number of instars that corresponds with the normal, unimpeded larval development of *C. insignis.*

Although a growth factor from one instar to the next may show considerable variation in individual cases, larval growth in Odonata is basically exponential (DUNKLE, 1985; HASSAN, 1977; LEGGOTT & PRITCHARD, 1985;

PICKUP et al., 1984). For the purpose of instar identification, we here assume larval growth in the usual type of development to proceed in a perfectly exponential manner. Following this assumption, the linear relation between instar number and logarithmically transformed larval size values can be employed to construct a regression line through the logarithmically transformed means of measured headwidths for larvae belonging to instars, 1, 2, 3 and 4 (Fig. 2). In the *Cordulegaster* species studied, these instars can be differentiated morphologically: instar 1 larvae still contain a large mass of yolk in their gut, while instars 2, 3 and 4 can be differentiated on the basis of tarsomere number and eye development (Verschuren, unpublished). Full-grown larvae (F_0) and larvae of the penultimate instars F_{-1} and F_{-2} can be morphologically differentiated on the basis of wing-bud length and, for females, of the development of the external genitalia. The fact that mean head-width values for the latter three instars appear to fit the constructed regression line at x=14, x=13 and x=12, respectively (Fig. 2), strongly suggests that the instar number associated with perfect exponential growth in *C. insignis* is 14, prolarva not included. Larvae that become retarded early in their development and have to perform one or more additional moult(s), would reach a total of 15 or even 16 instars. In the following discussion of interspecific differences in antennal growth, we will concentrate on the type-14 development, associated with perfect exponential growth. Referring to this type of development, the instar numbers which are assigned to specific phases in antennal growth are assumed to be the typical, i.e. most frequently occurring numbers. Restriction to one type of development as conceived here creates the possibility to group the continuously distributed larval sizes in 14 size classes, each associated with a particular instar number. We hereby assume that the majority of larvae belonging to any particular instar will indeed be classified in the corresponding size class. This manipulation of data is necessary in view of our comparison between antennal development in *C. insignis* and *C. pictus*. It is justified by the notion that critical events in dragonfly development, such as metamorphosis, appear to be size-dependent rather than instar-dependent, because they will proceed only when the larva has reached adequate size, rather than a particular instar number. Consequently, size classes may be considered an appropriate tool to situate these events in the whole of larval development.

Calculated mean headwidth values for instar 1 (n=12) and 14 (n=49) are chosen as class medians. The logarithmic width of each class is set constant, and the mean of values in a particular size class is taken to approximate the mean of the corresponding instar in the type-14 development. These mean values, and the standard deviations derived from them, are then addressed to the respective size class medians (Fig. 2). In the following discussion, we will maintain a distinction between the morphologically differentiated instars 1-4 and 12-14, and the intermediate larvae belonging to size classes V-XI. Data on *C. pictus* are transformed in a similar way.

THE BASIC PATTERN OF ANTENNAL GROWTH IN *CORDULEGASTER*

The first two divisions in the antenna of *C. insignis* are executed simultaneously: at ecdysis to the third instar, the basal and apical growth centers both form a new joint, which results in the direct increase from three to five antennal segments (Fig. 3). The second division of the basal center occurs at ecdysis to instar V or VI (no larvae of instar V available), and the final division is achieved at ecdysis to instar VIII. Specimens belonging to instar I to V of *C. pictus* were not available for this study, but similar to *C. insignis,* the final division takes place in the basal growth center, at ecdysis to instar VIII. Information on antennal growth patterns in other species of *Cordulegaster,* in the related genera *Anotogaster* and *Neallogaster,* or in members of the other subfamily, the Chlorogomphinae, is presently lacking.

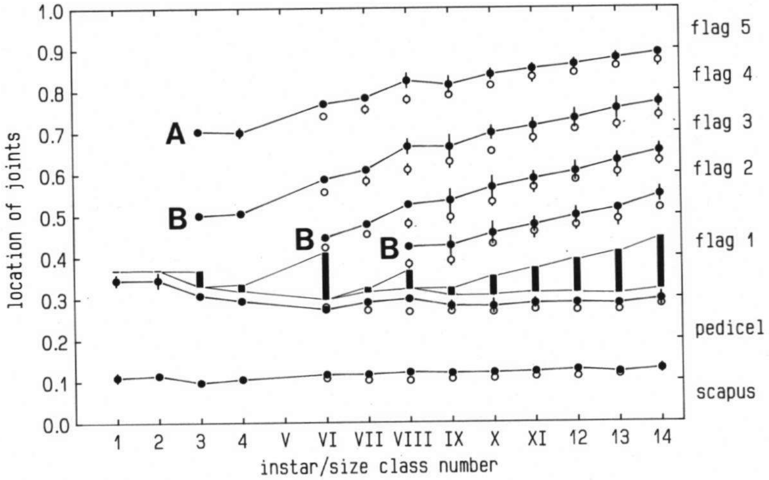

Fig. 3. Antennal growth pattern in *C. insignis* (closed circles) and *C. pictus* (open circles). — [A, B: operation events of the apical and basal division centers; — vertical bars: estimation of excess growth due to activity of the extension center, at its assumed position].

The type of antennal growth pattern found in *C. insignis* also occurs in *Epitheca bimaculata sibirica* (Corduliidae; MIYAKAWA, 1977). It is identified as the B:A.B.B. type pattern and considered a variation of the B.A.B.B. type, which is the pattern most commonly found in Corduliidae and the more primitive Libellulidae. In both cases, the apical growth center is operative at an early stage of larval development, simultaneously with or shortly after the first division in the basal growth center, but prior to its second division. In the more evolved Libellulidae, the apical growth center starts operating only after the second basal division, but before the third (B.B.A.B.). According to MIYAKAWA (1977), the difference between the B.A.B.B. and B.B.A.B. types is phylogenetically probably unessential, since both types occur in the genus *Sympetrum*, along with the transitional type B.B:A.B. In Zygoptera and those families of Anisoptera considered as primitive (Aeshnidae, Petaluridae), on the other hand, the apical growth center does not operate until after the third basal division (B.B.B.A.). Quite often, its activity is even suppressed completely, resulting in full-grown larvae with six-segmented antennae (B.B.B.O.).

Based on his study of antennal growth patterns, MIYAKAWA (1977) divided the order Odonata in two major groups: a rather primitive group comprising the Zygoptera, Aeshnidae and Petaluridae (B.B.B.A., B.B.B.O. and related types) and a more evolved group comprising the Corduliidae and Libellulidae (B.A.B.B., B.B.A.B. and related types). This division is in accordance with the position of the various families on the genealogical tree of the Odonata as constructed by FRASER (1957) on a series of exclusively imaginal characters. The intermediate position of the family Cordulegastridae between these two major groups in currently accepted dragonfly phylogeny lends phylogenetic relevance to the study of its antennal growth pattern. Following Miyakawa's classification, and assuming only minor variation in antennal growth pattern within the family, we can here establish that the Cordulegastridae are to be classified in the group of more evolved dragonflies, and within this group with closer affinity to the Corduliidae than to the Libellulidae. Consequently, the evolutionary shift from a more primitive to a more evolved pattern of antennal growth must have come into being prior to the separation of the Cordulegastridae from the genealogical branch leading to the Corduliidae and Libellulidae.

DIFFERENCES IN ANTENNAL GROWTH BETWEEN *CORDULEGASTER* SPECIES

Joints between flagellar segments in *C. pictus* are located proximally from corresponding joints in *C. insignis* (Fig. 3), due to interspecific differences in proportionate length of the apical and proximal flagellar segments: the two apical segments of *C. pictus* are constantly longer than those of *C. insignis*, whereas the first flagellar segment is contantly shorter. In full-grown larvae of *C. pictus*, the combined length of the apical segments exceeds the length of the first (flag 4+5 / flag 1 > 1), while in full-grown *C. insignis* the opposite is usually found (flag 4+5 / flag 1 < 1). This character has been found to hold for all West--Palaearctic *Cordulegaster* taxa belonging to the two corresponding species groups, and constitutes a prominent character to differentiate between them (VERSCHUREN, 1989). In fact, when dealing with equally aged immature larvae, the combined length of flag 4 and 5 is always longer in *C. boltonii*-related species than in *C. bidentatus*-related species, and the opposite is true for flag 1. Due to changes in the proportionate length of antennal segments during larval development, however, the equations for full-grown larvae written above are not applicable to immature larvae.

In the course of larval growth, the antennae undergo regressive development: they become progressively shorter relative to most other body parts, because their growth factor is below average. In *C. insignis,* for instance, the ratio of antennal length to headwidth decreases from 0.63 ± 0.02 (n=12) at instar I to 0.39 ± 0.02 (n=49) in full-grown larvae. Most of this regressive development is completed during early and middle stages of development. From about instar 10 onwards, the antennal growth factor approximates the average for other body parts. Changes in the proportionate length of the antennal segments after instar 10 can be attributed to allometric growth of individual segments. After that critical moment, flag 5 continues to become progressively shorter, flag 1 becomes progressively longer, and the other antennal segments keep their growth in pace with the antenna as a whole. Taking into account the regressive development of the antenna in early stages, we must conclude that during postembryonic growth as a whole, only flag 1 becomes progressively longer. Such excess growth at all times being restricted to a single segment strongly suggests the existence of an embryonic region or center of extension, located in the first flagellar segment and which does not form new joints but merely stimulates extension of that segment. From the result of its activity (Fig. 3), it must be located proximally to the basal division center, but operating more or less independently from the latter. The basal division center may become operative only when flag 1 has reached an appropriate excess of length through the extension center's activity. A similar extension center associated with the apical division center does not seem to exist.

Directly influencing the absolute length of the first flagellar segment, differences in duration or intensity of the extension center's activity between *Cordulegaster* species could have been responsible for the differences in proportionate length of antennal segments between the two species groups under consideration. However, as the location of antennal joints in *C. pictus* proximal to those in *C. insignis* remains fairly constant throughout development (Fig. 3), this is probably not the case. Joints in *C. pictus* already have their proximal position at the moment of joint formation itself, as can be seen at the 2nd (instar VI) and 3rd (instar VIII) operation event of the basal division center. On its own, this more proximal location of the division centers themselves in the originally one-segmented flagellum of *C. boltonii*-related species, relative to *C. bidentatus*-related species, can be held responsible for the observed differences in proportionate length of antennal segments between the two studied species and associated species groups. The net result of the extension center's activity, viz. the growth excess of flag 1, is about equal in both species studied (Fig. 3), so that the relation of allometry appearing between this segment and the other antennal segments may be considered as essentially similar in all *Cordulegaster* species under consideration.

CONCLUSIONS

MIYAKAWA (1977) reviewed published information and supplied new evidence for the phylogenetic relevance of antennal growth patterns in Odonata. His analysis was based on the definition of two growth centers that are responsible for the division of existing segments though formation of new joints. The present study establishes the pattern of antennal growth in *Cordulegaster* to be closely related to the pattern found in Corduliidae and primitive Libellulidae, and extends the relevance of antennal growth studies to phylogenetic relations on the species level. The provided evidence is partly based on the definition of a third antennal growth center, probably associated with the basal division center, which does not form new joints, but is responsible for the excess growth of flagellar segment 1 during postembryonic development.

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