THE PHYLOGENY OF THE GENUS *LEUCORRHINIA* AND THE EVOLUTION OF LARVAL SPINES (ANISOPTERA: LIBELLULIDAE)

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A cladistic analysis of the genus *Leucorrhinia,* based on adult morphological characters, found one most parsimonous tree with a consistency index of 0.35. The evolution of large dorsal larval spines was mapped on the resulting tree. This mapping suggests that the presence of spines is the primitive state within *Leucorrhinia* and that they have disappeared on five different occasions, or have disappeared twice on lower branches and reappeared three times higher up in the tree.

INTRODUCTION

Odonate larvae are common prey of many predator groups: birds (KENNEDY, 1950) fish (CROWDER & COOPER, 1982; RASK, 1986), and other invertebrates (PRITCHARD, 1964). As prey they show a variety of behavioural (PIERCE, 1988; McPEEK, 1990), and morphological adaptations (JOHANSSON & SAMUELS-SON, 1994; McPEEK et al., 1996) against predation.

The presence of spines on the body has been shown to provide an effective protection against predators in many animal groups (EDMUNDS, 1974; HAVEL & DODSON, 1984; ABRAHAMS, 1995). Many odonate genera, especially among the anisopterans, have larvae with prominent lateral and dorsal spines on the abdomen (WALKER, 1958; WALKER & CORBET, 1975; ASKEW, 1988). In odonates, the larval spines may function as an aid in climbing and sprawling in vegetation and on the substrate (DA SILVA AGUIAR, 1989) and as protection against predators (JOHANSSON & SAMUELSSON, 1994). Currently there is some support for the predator defence hypothesis in *Leucorrhinia dubia* larvae. Larvae of *L. dubia* grow longer spines in habitats with fish, fish have longer handling times when eating long-spined larvae, and fish induce differences in spine shape under laboratory conditions (JOHANSSON & SAMUELSSON, 1994; ARNQVIST

& JOHANSSON, 1998). Considering the potential use of these spines it would be interesting to know how they have evolved in a genus. Being potentially highly adaptive and connected to habitat shifts, convergent evolution of spines is expected.

In this study we focus on the evolution of the abdominal spines in larvae of the genus *Leucorrhinia* (Anisoptera, Libellulidae, Sympetrinae), in which some species have prominent spines while others have very small spines on the abdomen (WALKER & CORBET, 1975; ASKEW, 1988). The genus consists of 14 species and several subspecies (DAVIES & TOBIN, 1985). The purpose of our study was to provide a phylogeny of the genus and map the evolution of larval spines on the resulting tree.

MATERIAL AND METHODS

We used 14 taxa in the genus for our analysis (Tab. I). *L. ussuriensis* Bartenef was excluded from the study since no specimens were available. In addition, recent results suggest this species to be a synonym of *L. orientalis* Selys (MALIKOVA, 1995). Since *L. dubia orientalis* Belyshev has been

Table I

The species used in this study, their geographic distribution and information about the specimens' sex and where they were collected. – [Vb=Västerbotten; – Vrm=Värmland; – Upl=Uppland; – Dlr=Dalarna; – Got=Gotland; – Vstm=Västmanland; – Vg=Västergötland]. – *Celithemis* and *Sympetrum* were used as outgroups

Species	Distribution	Number and origin of specimens
L. albifrons (Burm.)	Europe	1♂, Vb; 1♂, Vrm; 1♂, Uppl; 2♀, Got, Sweden
L. borealis Hag.	Canada-N. USA	2♂, Yukon Ter; 1♀, Saskatchewan; 1♀, Manitoba, Canada
L. caudalis (Charp.)	Europe	2♂, 2♀, Norway
L. dubia dubia (Van der L.)	Eurasia	2♂, 1♀, Vb, Sweden
L. dubia orientalis Sel.	Siberia-Japan	2♂, 2♀, Hokkaido, Japan
L. frigida Hag.	N. America	1♂, 1♀, Ontario, Canada
L. glacialis Hag.	N. America	1♂, 2♀, Ontario, Canada; 1♂, 1♀, California, USA
L. hudsonica (Sel.)	N. America	3♂, 3♀, Quebec, Canada; 2♂, California, USA
L. intacta Hag.	N. America	1♀, Ontario; 2♂, 2♀, Quebec, Canada
L. intermedia Bart.	Asia	2♂, 1♀, Hokkaido, Japan; 1♀, Russian far East (Helsinki Museum)
L. patricia Walker	Canada	2♂, 2♀, Ontario, Canada
L. pectoralis Charp.	Europe	1♂, Dlr; 1♀, Uppl; 1♂, 1♀, Vstm; 1♂, 1♀, Vg, Sweden
L. proxima Calv.	Canada-N. USA	2♂, 1♀, Ontario, Canada
L. rubicunda (L.)	Eurasia	2♂, 2♀, Vb, Sweden
Celithemis eponina (Dru.)	N. America	1♂, 1♀, Florida, USA
C. ornata (Ramb.)	USA	1♂, 1♀, Florida, USA
Sympetrum danae (Sulz.)	Holarctic	3♂, 1♀, Vb, Sweden
S. obtrusum (Hag.)	N. America	4♂, 2♀, Ontario, Canada

treated as a valid species by some authors (BELYSHEV, 1973; ISHIDA, 1996), we included this taxon in the analysis. To generate a hypothesis about the phylogenetic relationship among the species we performed a cladistic analysis using 24 adult characters (Tab. II). Of these characters, 7 were examined in both sexes, 14 in males only and 3 in females only. States for most characters were obtained from multiple specimens (Tab. I). We included four species in the outgroup (Tab I): the nearctic *Celithemis eponina* and *C. ornata* (Leucorrhiniini), and from Sympetrini the two species *Sympetrum danae* and *S. obtrusum*.

Character state distribution was entered in MacClade 3.03 (MADDISON & MADDISON, 1992), and thereafter the matrix was further analysed in both MacClade and PAUP 3.1 (SWOFFORD, 1993). All multistate characters were treated as unordered. A general heuristic search was done in PAUP in order to find the most parsimonious tree(s). Branch support was tested with bootstrapping in PAUP (FOREY et al., 1992).

The occurrence of spines was taken from the literature (WALKER & CORBET, 1975; CANNINGS & STUART, 1977; ASKEW, 1988; NORLING & SAHLÉN, 1997), and some unpublished observations were provided by S. Cannings.

<center>Table II</center>
<center>Character and character states used in the cladistic analysis</center>

<center>Characters present in both sexes</center>

(1)   Labium, colour: (0) at least outer margin light; (1) all black
(2)   Fore wing, number of cell rows between IR3 and Rspl: (0) 1; (1) 2
(3)   Fore wing, MA and CuP: (0) parallel; (1) divergent
(4)   Base of hind wing: (0) without distinct black spot; (1) with distinct black spot
(5)   Metatibia, dense row of spines below the knee: (0) absent; (1) present
(6)   Abdominal segments 2-4: (0) normal; (1) white powdered
(7)   Abdominal superior appendages colour: (0) dark; (1) white

<center>Characters present in males</center>

(8)   Thorax, colour of setation: (0) light; (1) mixture of light and dark
(9)   Thorax, colour: (0) not metallic; (1) metallic
(10)   Mesepisternum adjacent to mesepimeron, colour: (0) brown; (1) black
(11)   Mesepimeron, shape of brown field: (0) not wider than tall; (1) wider than tall
(12)   Metepimeron, colour: (0) predominantly black; (1) predominantly brown
(13)   Anterior lamina, posterior edge: (0) straight; (1) split or excavated
(14)   Hamulus, lateral outgrowth: (0) absent; (1) present; (2) present with an indentation
(15)   Hamulus: (0) without spines; (1) with spines
(16)   Hamulus, anterior tips: (0) bent outwards; (1) not bent outwards
(17)   Hamulus, posterior edge: (0) bent upwards; (1) not bent upwards
(18)   Genital lobe: (0) without spines; (1) with spines
(19)   Abdominal segment 7, colour of dorsal spot: (0) red; (1) yellow; (2) absent
(20)   Abdominal segment 9, ventral placement of gonopore: (0) displaced forward; (1) in the middle of the segment
(21)   Inferior appendage, form: (0) sides converging posteriorly; (1) sides parallel; (2) sides diverging posteriorly

<center>Characters present in females</center>

(22)   Vulvar lamina, form: (0) longer than wide; (1) as long as wide; (2) wider than long
(23)   Vulvar lamina, direction of the median gonapophyses: (0) backwards; (1) sidewards
(24)   Vulvar lamina, surface behind the median gonaphyses: (0) no hairs; (1) with hairs

RESULTS

The character matrix is shown in Table III. The cladogram presented in Figure 1, is the single shortest tree found by PAUP (TL 79, CI 0.354, RI 0.545). The bootstrap test provided statistical support only for the ingroup (79%), and the *L. albifrons* + *L. caudalis* clade (51%).

The presence of large larval spines is not restricted to a single branch on the tree (Fig. 1). Applying Farris optimization (FARRIS, 1970) to the tree gave several equally parsimonious solutions, in all of which the spines are part of the *Leucorrhinia* groundplan. Either the spines have disappeared on five different occasions within the genus, or they have disappeared twice on lower branches and reappeared three times higher up in the tree. A single disappearance of the spines would increase the tree length by at least seven steps.

DISCUSSION

It is clear from the phylogeny, that the presence of large larval spines is not restricted to a single clade. McPEEK (1995) suggested that changes in morphology of damselfly larvae were associated with habitat shifts. After such a habitat shift, selection pressure exerted by the environment should favour the morphologies best adapted to the new environmental conditions. Under the assumption that large spines are beneficial in some environments but not in others, the multiple disappearance of spines in the tree suggests several such habitat shifts. These shifts might have triggered a reduction of the spine length. Currently we do not know what environmental conditions that cause the reduction of large spines. We know that fish predators have difficulties in handling *L. dubia* larvae with large spines (SAMUELSSON & JOHANSSON, 1994), but we do not know the disadvantage of having long spines, besides a general cost in producing them.

The fact that *L. dubia* larvae show very low abundance in the presence of fish compared to in the absence of fish (HENRIKSON, 1988; SAMUELSSON & JOHANSSON, 1994), suggests that spines are not very effective against fish predators. However, a relevant comparison in this context would be between spine and spineless species with regard to abundance with fish. It is interesting to note that the longitudinal dark bands present on the ventral side of the abdomen is negatively correlated with spine length in the genus. While the correlation is less clear among the palaearctic species, a strong negative correlation exists for the North American species (WALKER & CORBET, 1975; NORLING & SAHLÉN, 1997). Cryptic coloration has been shown to be an effective defence against predators in many species (EDMUNDS, 1974), but as far as we know, no studies have looked at the eventual function of cryptic coloration in odonate larvae. More work is clearly needed, especially with regard to abundance patterns of spined and spineless species, larval coloration and how these are related to environmental conditions.

Table III

The distribution of the character states among the examined species. Characters that could not be determined with certainty are denoted by a question mark

| Species/Charact. no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
S. obtrusum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?
S. danae	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	2	0	0	2	0	0
C. eponina	0	0	0	1	0	0	?	0	0	0	0	0	0	1	1	0	1	0	?	0	?	1	?	1
C. ornata	0	1	0	1	0	0	0	0	0	1	0	1	0	1	1	1	1	0	1	1	0	0	0	0
L. albifrons	0	0	1	1	1	1	1	0	1	1	1	0	1	1	0	0	0	0	2	1	0	2	1	1
L. borealis	1	0	1	1	1	0	0	0	0	1	1	0	1	1	0	1	0	0	0	0	1	1	1	1
L. caudalis	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	2	0	0	0	0	0
L. d. dubia	1	0	1	1	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	1	1	1	0
L. d. orientalis	1	1	1	1	1	0	0	1	0	1	1	0	0	1	1	1	0	0	0	1	0	0	1	0
L. frigida	1	0	1	1	1	1	0	0	0	?	?	?	1	0	0	0	0	0	2	1	0	0	1	1
L. glacialis	1	1	1	1	1	0	0	1	0	0	1	1	0	0	1	1	0	0	2	1	1	2	1	1
L. hudsonica	1	0	1	1	1	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	2	1	0	1
L. intacta	0	0	1	1	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	1	2	0	1	1
L. intermedia	1	0	1	1	1	0	0	1	0	1	1	0	0	2	0	0	1	0	0	0	1	1	1	1
L. patricia	1	0	1	1	1	0	0	1	0	0	1	1	1	2	1	1	1	1	0	0	1	1	1	0
L. pectoralis	1	0	1	1	1	0	0	1	0	1	1	0	0	2	0	1	1	0	1	1	1	1	1	1
L. proxima	1	0	1	1	1	0	0	1	0	0	1	1	1	1	0	0	1	1	0	0	1	2	1	1
L. rubicunda	1	0	1	1	1	0	0	1	0	1	1	0	1	0	0	0	1	0	0	0	1	2	1	1
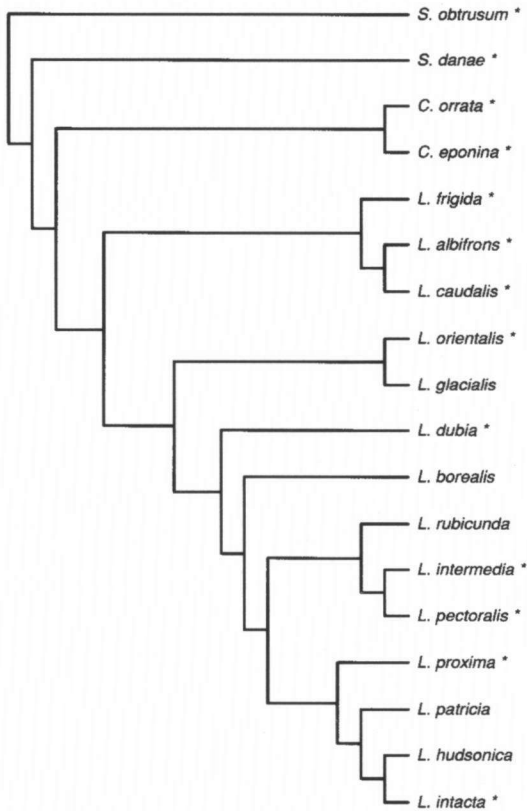
Fig. 1. The shortest tree found. The presence (*) of large abdominal spines in larvae is shown for each terminal taxon.

As seen in the low CI and lack of strong statistical support, our phylogenetic hypothesis is weak. A more firmly based phylogenetic hypothesis would probably be possible to find by adding also characters from larvae to the analysis. However, this was not possible at this stage of our investigation due to the lack of larval material of several species. Adding only the abdominal spine character to the analysis resulted in four shortest trees, one of which was the same as the tree in Figure 1. The other three trees differed only in the position of *L. borealis* and *L. rubicunda*, showing the same dynamics in the evolution of the larval spines as our first tree.

The best supported mono-phyletic group within the genus is the *L. albifrons* + *L. caudalis* clade, supported by characters no. 7 (abdominal superior appendages), 9 (thorax colour) and 16 (hamulus anterior tips). This clade is grouped together with *L. frigida* by character no. 13 (posterior edge of the anterior lamina) and no. 6 (abdominal segments 2-4 white-powdered). As *L. albifrons* and *L. caudalis* look very much alike in their overall appearance with white-powdered abdomens, it is interesting to note that they also have genital similarities. An interesting thing to note in the tree is that *L. dubia dubia* and *L. dubia orientalis* did not group together as suggested in some odonata lists e.g. DAVIES & TOBIN (1985), HARITONOV & MALIKOVA (1998). Instead *L. dubia orientalis* appeared as the sister-taxon of *L. glacialis*. The monophyly of this group depends on character no. 2, saying that these two species are the only ones that have double rows of cells between IR3 and Rspl., and character no. 15 (spines on hamulus).

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