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Leg deformation during imaginal ecdysis in the downy emerald, *Cordulia aenea* (Odonata, Corduliidae)

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ABSTRACT

A dragonfly larva migrates from the water to the shore, perches on a plant stem and grasps it with strongly flexed legs. Adult legs inside the larval exoskeleton fit to the larval legs joint-to-joint. The adult emerges with stretched legs. During the molt, an imaginal leg must follow all the angles in exuvial joints. In turn, larval apodemes are withdrawn from imaginal legs. We visualized transient shapes of the imaginal legs by the instant fixation of insects at different moments of the molt, photographed isolated exuvial legs with the imaginal legs inside and then removed the exuvial sheath. Instant shapes of the imaginal tibia show sharp intrapodomere bends copying the angle in the larval femoro-tibial joint. The site of bending shifts distad during the molt. This is possible if the imaginal leg is pliable. The same problem of leg squeezing is also common in hemimetabolous insects as well as in other arthropods, whereas holometabolous insects overcome problems of a tight confinement either by using leg pliability in other ways but not squeezing (cyclorrhaphan flies, mosquitoes) or by pulling hardened legs out without change of their pupal zigzag configuration (moths, ants, honey bees). The pupal legs are not intended to grasp any external substrate.

1. Introduction

The exodus of dragonflies (Odonata-Anisoptera) from the water to the ground, and from the ground to the air, has been attracting the attention of professional and amateur naturalists for three centuries: a robust, dark and awkward larva turns into a bright, elegant and swift flier.

The first attentive observer was Réaumur (1742). He described in detail the order of events during the transformation of a larva to a flying adult: the way out of the water to the shore, the search for a convenient perch on plant stems, the grasping of the stem, the change in eye coloration, the standstill at the chosen place, the formation of slits on the larval thorax and head, the swelling of the adult head and thorax and their emergence above the larval integument, withdrawal of legs and wing vestiges, strange bending of the anterior body part and passive drooping in this position, the sudden upright turn, grasping with the legs at the perch above the exuvia, withdrawal of the abdomen, spreading of the wings and their activation before the first flight. The duration of some steps in this schedule was also indicated by Réaumur (1742). Postures during these stages are depicted (Réaumur, 1742: Plate 39) for two anisopteran species (presumably *Aeschna* sp. and *Sympetrum* sp.; the study was done before Linnaeus).

Later on, the imaginal molt in dragonflies was examined many times, beginning with de Bellesme (1877) who observed *Libellula depressa*. We may also cite observations of Tillyard (1917) on *Petalura gigantea*, Corbet (1957) on *Anax imperator*, Wildermuth (2003) on *Epitheca bimaculata*, Andrew and Patankar (2010) on *Pantala flavescens*. These observations were better illustrated than the first ones, but they reveal the same sequence of behavioural acts as established by Réaumur.

The emergence of the free adult out of the larval integument proceeds in three main stages: (1) a preparatory stage, including grasping of the substrate with the flexed legs and sharp claws, separation of the imaginal integument from the larval integument, and increase of internal pressure; (2) release out of the larval integument, or *ecdysis*; (3) maturation of the free imago (postecdysis) including expansion of the body, spreading of the wings, tanning and hardening of the new cuticle, vibration of the wings. Postecdysis is over when the adult starts its first flight. Formal classification of substages in the ecdysis, which was established for the cricket *Teleogryllus oceanicus* (Carlson, 1977a), the crab *Carcinus maenas* and the crayfish *Orconectes limosus* (Phlippen et al., 2000), was not undertaken for dragonflies. The sequence and the timetable of events is mostly the same for all studied dragonflies.

The quiescent preparatory stage lasts about 10 – 20 min; it includes peristalsis of the abdomen which inflates the whole postcephalic body and causes divergence of the wing vestiges. Ecdysis begins with the split

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of the larval integument above the thoracical terga and the head (3 – 5 min); withdrawal of the head and the thorax and, later on, of the legs also lasts about 5 min. The passive crooked interval lasts about 20 – 30 min. Erection of the body and withdrawal of the abdomen take only a few minutes. Maturation lasts 1 – 2 h, depending on the temperature, whereas it occurs overnight in *A. imperator* which molts before midnight and waits until the morning. A very quick ecdysis was recorded in *Hemigomphus heteroclitus* before a thunderstorm: the active ecdysis, expansion of wings and pigmentation of the cuticle took only 10 min (Tillyard, 1917).

When the adult dragonfly flies away, the exuvia remains on the spot. It is hard enough to withstand wind and rain and may be found after weeks in the same configuration as when the larva grasped the stem. Leg positions in the exuvia during the ecdysis are stable. The grasping legs are elevated in the coxo-trochanteral joints, usually strongly flexed in the femoro-tibial joints (FTJ), the front coxae are protracted, whereas the middle and hind ones are retracted. Wildermuth (2003, see Fig. 1b and c) demonstrated a strong flexion of the FTJ (by 90 – 160° in the kinematic sense) in the quiescent larva of *Epitheca bimaculata*.

The imaginal legs are formed inside the larval ones almost with the same length of podomeres (Leipelt et al., 2010). Leg muscles in the larval and adult dragonflies are also identical (Maloeuf, 1935). Hence, the pharate legs inside the larval legs copy the shape of the latter one-to-one. Nevertheless, the imaginal legs become stretched ventrad and posterad at the end of their withdrawal. Presumably, the imaginal legs are passively pulled out of the larval ones and any point of the imaginal leg must pass through all the more proximal parts of the exuvial leg, including joints. Initially straight imaginal podomeres must follow all the angles formed by the exuvial joints, at any moment copying the shapes of the angulate tunnel inside the exuvial leg.

This process of transient deformation is concealed *in vivo* inside the opaque exuvia. In the present article, we demonstrate the shapes which the pharate legs attain inside the exuvial ones, and how these deformations are corrected during withdrawal of the imaginal legs.

2. Materials and methods

2.1. Definitions

Standard nomenclature for the leg-fixed coordinate system, irrespective of real leg posture, was proposed for an idealized leg, stretched laterad in a plane, perpendicular to the fore-aft body axis: here one can discern dorsal, ventral, anterior and posterior sides and the proximal–distal directions (Gutsevich et al., 1970).

The angle of flexion–extension in the femoro-tibial joint (FTJ) is measured either in the common (colloquial) sense as α between the femur and the tibia or in the kinematic sense as β between vectors along two podomeres, both pointing distad; $\alpha + \beta = 180°$. Kinematic values are used in mechanics and robotics.

Ecdysis is the release of a specimen out of the previous instar integument. The shed empty integument is called exuvia (pl. exuviae). The adult, formed inside the larval body, is called the pharate adult; the free adult is the exarate one. Until an adult leg is completely free, we still regard it as a pharate leg.

2.2. Specimens

Larvae of *Cordulia aenea* (Linnaeus, 1758) were captured on riverside plants, at a lake in the landscape preserve “Koncha Zaspa”, in the outskirts of Kiev (Ukraine), on 8 – 12 May 2017. Larvae were placed on sticks in a box for continuous surveillance. As soon as one of them stopped to move, ecdysis could be expected to start within 10 – 15 minutes. Emerging insects were immobilized at different grades of progress by injecting 0.1 – 0.2 ml of 96 % ethyl alcohol into the thorax. Movements stopped within 2 – 3 s. Specimens were stored in 70 % ethyl alcohol.

The general view of the fixed specimens was photographed with a Canon EOS 550D camera (Canon Inc., Tokyo, Japan) either with a macro lens or with a lens attached to the ocular of the dissection microscope MBS-9 (manufactured in the former Soviet Union). Specimens were submerged in 96% ethyl alcohol in order to neutralise nitidulous light reflection from the body surface. They were photographed against a blue background for better contrast.

Several adults of *Epitheca bimaculata* (Charpentier, 1825) (Corduliidae), *Libellula quadrimaculata* Linnaeus, 1758 and *Orthetrum cancellatum* (Linnaeus, 1758) (Libellulidae), captured in the field, were dissected for checking the leg muscles and joints.

2.3. Dissection

Specimens were dissected under the same microscope on an elastic white gum scaffold with the aid of fine forceps and a sliver of a brittle razor. During dissection, the specimens were periodically moistened with drops of 70 % ethanol. Dissection included two stages: rough cutting of the body and fine preparation of the legs.

The goal of rough cutting was to isolate a pharate leg inserted inside an exuvial one. For this purpose, it was necessary to isolate the thorax, cut off wing vestiges, cut off the exuvial integument dorsad of the larval coxae and isolate each leg, chopping body walls and muscles outside of the pharate coxa. Legs, prepared in this fashion, were stored in tagged drops of glycerol. Afterwards, they were rinsed in 96 % ethanol, photographed and fixed in Bouin’s solution for several days in order to preserve the shape of the pliant pharate legs.

For fine preparation, the fixed legs were washed in distilled water, rinsed in ethyl alcohol and dissected very carefully on the same scaffold as described above. The goal was to remove the exuvial shell piece by piece in small steps. One must be especially careful during the preparation of pharate tarsi inside the exuvial tarsomeres at the early ecdysial substages. Pharate legs, extracted from the exuvia, were stored in drops of glycerol and photographed at the same orientation as in the whole mount inside the exuvial leg.

Long apodemes in the empty exuvial femur were exposed by cuts down the side walls of this podomere, by a section around the trochantero-femoral joint (TFJ) and by the separation of apodemes from the dorsal and ventral walls of the femur. Later on, the walls of the exuvial femur were removed up to the distal edge of the femur. Most accurate is the preparation of exuvial apodemes between the pharate FTJ and the exuvial FTJ at early substages of exuviation. Apodemes are transparent. Usually, we stained the whole mount in 0.05 % water solution of $KMnO_4$ for 5 – 10 min. Apodemes inside the exuvial tibia were prepared in the same fashion.

2.4. Processing of photographs

Separated legs were photographed submerged in 96 % ethyl alcohol against a blue background. Standard orientation of a leg for photography was as in a right leg observed from behind. For the sake of consistency, photographs of left legs were flipped horizontally.

Photographs were edited in the software Adobe Photoshop 5.5 (Adobe Systems, Inc., San Jose, CA, USA). The background was retoushed with the tool “magic wand” in the standard blue hue.

2.5. Angular measurements

We evaluated the angles in exuvial FTJs roughly by eye, by comparing them with a template with radially protruding angles from 10° to 120° (in steps of 10°; see Fig. 1, inset). A dry exuvia was turned manually in front of the monitor with the template image. When one leg was positioned in the monitor plane, the template angle best fitting the angle α between the femur and the tibia could be determined. These angular values were transformed in the kinematic sense to β ($\beta = 180°$