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Transient leg deformations during eclosion out of a tight confinement: A comparative study on seven species of flies, moths, ants and bees

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A B S T R A C T

Legs in dipteran pupae are tightly packed in a zigzag configuration. Changes in the shape or configuration of long podomeres during eclosion have been overlooked because they occur rapidly (in a few minutes) and the legs are hidden inside a tight opaque confinement: the puparium in the Cyclorrhapha, the obtect pupa in mosquitoes. We fixed insects at different times during eclosion and obtained a temporal description of changes in leg shape. At the start of eclosion in *Calliphora vicina* and *Drosophila melanogaster*, femora are buckled in between the joints. Later, the chain of podomeres straightened, pointing posterad. Initial deformation and further stretching were passive, exerted by forces external to the legs. The prerequisites for this are pliability of the tubular podomeres and anchoring of the tarsi to the confinement. Each femur was strongly crooked instead of buckled in the mosquito *Aedes cantans*. The site of bending shifted distad in the course of eclosion: a sort of peeling. In contrast, other insects (the moth *Bombyx mori*, the ants *Formica polyctena* and *Formica rufa*, the honey bee *Apis mellifera*) left their tight confinements without any change in the initial zigzag leg configuration and without transient deformations of initially straight femora and tibiae.

1. Introduction

Reversible leg deformations have been found by Frantsevich (2016) in the blow fly *Calliphora vicina* extricating from the puparium: (i) before eclosion, the legs of the pharate imago inside the puparium are packed in a zigzag (Z-configuration): coxae retracted, trochanters elevated, femora pointing dorsad and anterad, front and middle tibiae and tarsi pointing posterad, hind femora and tibiae crooked about 90°; (ii) the fly gets out of the puparium with legs stretched straight and directed posterad; (iii) hence, the femora must turn by 90° or even more, but there is no space for such a turn inside the puparium. Leg straightening is concealed *in vivo* inside the opaque puparium which obstructs direct observation. Therefore flies were fixed at different moments of extrication in order to obtain a temporal series of leg configurations. Transformation of femoral position was achieved by steep bucklings in one or two sites within the pliant femur, as it is illustrated in the Graphical abstract (the left specimen). The fold among the femur behaved like a sort of a hinge: parts of the femur could rotate about the fold. Instead of turning the straight femur, the fly extended the chain of short subpodomeres. Buckling was repaired *in vivo* by stretching of all legs during the further extrication. Crooked hind tibiae were also stretched, at least partly.

Evidently, the blow fly could not get out of the puparium without leg buckling. Most probably, this species which is able to deform own podomeres and to repair the straight shape is not unique among insects. If extrication without this ability is impossible, then the peculiar deformation mechanism must be preformed before appearance of *Calliphora*, or the Cyclorrhapha, or other flies, or even earlier. Zigzag leg packing is inherent in dipteran pupae: all pupae, depicted in the monograph by Brauns (1954), demonstrate this leg configuration (60 species of 35 families). The

Abbreviations: A. c., Aedes cantans; *A. m., Apis mellifera*; *B. m., Bombyx mori*; *C. v., Calliphora vicina*; *D. m., Drosophila melanogaster*; *F. p., Formica polyctena*; *F. r., Formica rufa*; R1, R2, R3, right front, middle and hind leg.

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Definitions of terms in the text

Buckling deformation of a bent pipe due to the loss of the elastic stability, when the convex face of the pipe expands, the concave face constricts, the induced strains at both faces include force components directed to the middle line of the pipe; the pipe flattens and abruptly forms a fold

Crooking smooth bending of the pipe or the segment of the pipe, without alteration in the sign of curvature

Eclosion the emergence of an insect from a pupa

Extrication the release from entanglements, such as a hard confinement or the soil, after or during eclosion

Exuvium the empty pupal integument after release of the imago

Pharate imago the almost ripe adult insect, formed and hidden inside the pupal shell

Puparium the unshed and hardened larval integument protecting the pupa inside it

Ranking arrangement of specimens of a given sample in the order of monotonous change of some trait (traits); ordinal numbers of specimens are their ranks. If, due to approximation, two or more specimens are compared equal, they receive the same rank

same was depicted for pupae of *Drosophila melanogaster* (Bainbridge and Bownes, 1981, Figs. 19 and 20). Champions in tight packing are mosquito pupae. They accommodate the long legs of the future imago under the thorax, because the abdomen is used for swimming and must be free (Nachtigall, 1962; Brackenbury, 1999). Pupal legs in the Culicidae were depicted by Darsie (1951, Fig. 1) and Brauns (1954, Fig. 19): in addition to the zigzag arrangement of coxae, femora, and tibiae, the hind tarsus enscribes additional loops under the wing pad.

On the other hand, mosquito eclosion was demonstrated in the film "Microcosmos" (Nuridsany et al., 1996, 1:09:00–1:09:30). With permission of the cited authors and the studio, we illustrate eclosion postures as a collage of still frames (Fig. 1). All legs seen above the water are straight and point posterad. The pupal shell is a tight confinement with scarce space for leg maneuvers. How the mosquito manages straightening of its legs, is concealed underwater.

The same style of leg packing is characteristic not only for Diptera. Pupae with legs, tucked in Z-configuration, were illustrated for the noctuid moth *Barathra brassicae* by Obenberger (1964, Fig. 177), for the honey bee *Apis mellifera* by Lavrekhin and Pankova (1969, Fig. 34), for the red wood ant *Formica rufa* by Dlusskiy (1967, Figs. 1,4).

We inspected several species, close or far relatives to *C. vicina*, which encountered similar obstacles during eclosion. Taking into account the short duration of eclosion – few minutes only – we selected insects which could be cultivated or collected in the field and provide abundant samples with synchronized eclosion.

Selected candidates are listed in Table 1. Legs of pupae in these species are packed in zigzag. All candidates have been treated uniformly; their fixed and dissected legs were arranged in temporal series and compared. We show below that flies use various modes of leg deformation and further straightening during eclosion, in contrast to representatives of the Lepidoptera and the Hymenoptera, which do not straighten their legs at all, their leg podomeres are not malformed in pharate imagines.

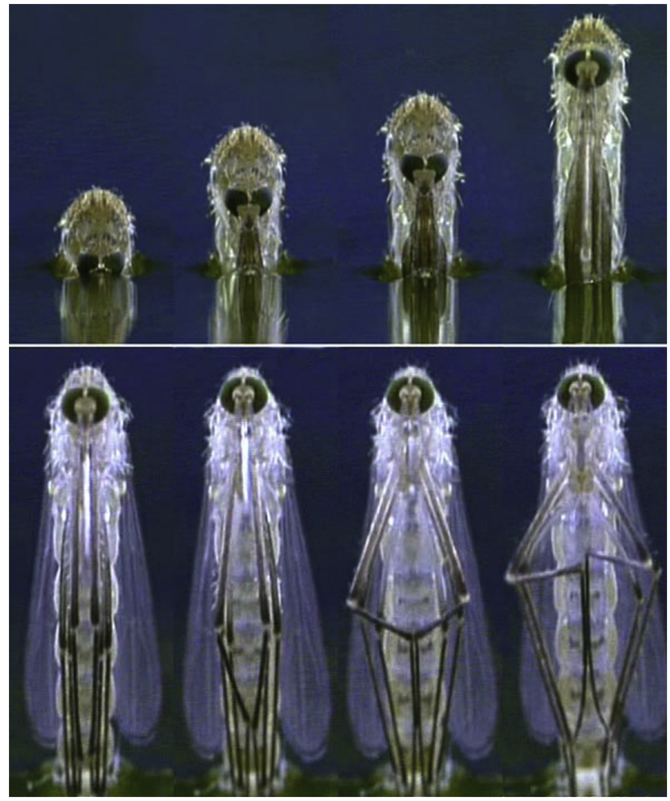

Fig. 1. Eclosion of a mosquito (collage of still frames from the film "Microcosmos"). Top panel – beginning of eclosion; bottom panel – leg extraction from the exuvium. Front leg long podomeres in the bottom right frame are clearly recognized as the femur, tibia, tarsus. In the bottom left frame, all podomeres are stretched posterad in a line.

2. Materials and methods

2.1. Rearing and pre-fixation

C.v.: postfeeding larvae were placed into a glass jar with sand for pupation. Puparia were transferred to plastic Petri dishes and kept under a wet cloth at 24–26 °C. After emergence of the first adults, puparia were under watch in order to intercept flies during extrication. Extricants were captured and placed at once in 70% ethyl alcohol: their movements stopped in 2–3 s.

D. m.: larvae were reared on the standard nutritional medium (Roberts, 1998) at 20 °C in glass tubes with transparent plastic plates, inserted vertically for pupation. Plates were transferred to Petri dishes (with few water droplets inside) for observation of extrication under a dissection microscope. Flies ready to extricate have been recognized by a swollen ptilinium. When the fly advanced out of the puparium, the plate with all attached puparia was flooded with hot water (5 ml, over 50 °C), thus the extricating specimen was momentarily immobilized together with all its neighbors on this plate. Then the plate was stored in 70% ethyl alcohol. The selected specimen was detached from the plate with the aid of a sliver of a hard steel razor blade. Alternative immobilization with ethyl alcohol caused ejection of a specimen out of its puparium.

A. c.: larvae and pupae, caught in a deep pit in the forest, were reared in the laboratory at 15–20 °C in 3 l jars filled with the water from their native water body. Pupae were transferred to 300 ml cups for observations of eclosion. Exuvia with eclosing mosquitoes were captured with a small forceps and transferred into vials with 96% ethyl alcohol.

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