Available online at www.sciencedirect.com
SCIENCE DIRECT®
Zoologischer Anzeiger 244 (2005) 79–91
ZOOLOGISCHER ANZEIGER
www.elsevier.de/jcz

The embryonic moult in diplogastrids (Nematoda) – homology of developmental stages and heterochrony as a prerequisite for morphological diversity

Alexander Fürst von Lieven*

Institut für Biologie/Zoologie, Freie Universität Berlin, Königin-Luise-Str. 1-3, 14195 Berlin, Germany

Received 3 October 2004; received in revised form 24 April 2005; accepted 26 April 2005

Corresponding editor: M.V. Sørensen

Abstract

Video analysis of developing eggs showed that diplogastrids, in contrast to the majority of nematodes, moult from J1 to J2 before they hatch from the egg. This embryonic moult leads to a conflict between the terminology for the J1–J4 postembryonic stages and the definition for the embryonic period that ranges from fertilization to hatching. The nature of developmental stages is discussed on the occasion of this terminological problem. Stages as "embryo", J1, J2,… are defined as periods of time between certain developmental events and are called instars, whereas other stages as "tadpole stage" refer to characters. Character-defined stages refer to the temporal aspect of developmental characters. Only character-defined stages can be homologized between species. It is proposed to ignore the conflict of the J1–J4 terminology with the embryonic–postembryonic dichotomy as an embryonic stage cannot be satisfyingly defined. The biological relevance of the heterochronic shift of the first moult into the egg period is the possibility to omit secretion of the J1 stoma and pharynx cuticle as the diplogastrid J1 does not feed. Thereby the time slot for stoma morphogenesis that must be finished before cuticle deposition is prolonged. Within Diplogastridae this time slot facilitated the evolution of a tremendous diversity of complex stoma morphologies that is unique within "Rhabditida".

Keywords: Diversity; Embryo; Instar; Moults; Ovoviviparity; Pharate; Recapitulation

1. Introduction

When comparing the timing of developmental events in *Caenorhabditis elegans* ("Rhabditidae") and *Pristionchus pacificus* (Diplogastridae) Félix et al. (1999) found that diplogastrids have only three postembryonic preadult stages compared to the four usually present in nematodes. Being aware of the difficulties in homologizing developmental stages, they introduced their own terminology for diplogastrid preadult stages (J1, J2, J3, compared to L1, L2, L3, L4 in other nematodes). Félix et al. (1999) described heterochronic shifts of developmental events in *P. pacificus* compared to *C. elegans* as migration and division of lateral Pn ectoblasts, division of the larval mesoderm precursor cell (M), division of sex myoblast precursor cells, division of somatic gonad precursor cells (Z1/Z4), 1st division of vulva precursor cells, and the division of sheath and spermatheca and dorsal uterus precursor

*Tel.: +49 30 838 53838; fax: +49 30 838 53916.
E-mail address: lieven@zedat.fu-berlin.de.

0044-5231/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jcz.2005.05.001

cells. These heterochronic shifts were discussed on the assumption that no trace of a former fourth stage (as for example, an embryonic moult) can be found in *P. pacificus*. Nevertheless embryonic moults were described in the diplogastrids *P. lheritieri* (Grootaert 1976), *Butlerius degrissei* (Grootaert and Jaques 1979) and *Odontopharynx longicaudata* (Chitambar and Noffsinger 1989). The results of this study show that *P. pacificus* as all hitherto examined diplogastrids moults once within the egg (Fig. 1). According to these results, the discussion of heterochrony between rhabditids and diplogastrids must be based on a new and consistent terminology for embryonic and juvenile/larval stages in nematodes. To accomplish this, what a nematode developmental stage actually is needs to be clarified. Furthermore, the results of this study suggest that the heterochronical change of the *hatching* $=>$ *first moult*-sequence towards *first moult* $=>$ *hatching* provided the possibility to evolve complicated mouthparts in Diplogastridae. This argument is based on a detailed comparison of stoma morphogenesis in *P. pacificus* and *C. elegans*.

2. Material and methods

Examined species: All species examined in this study except for *Koerneria pararmata* are cultured in the laboratory of Walter Sudhaus at the Institut für Biologie/Zoologie in Berlin (Germany).

Caenorhabditis elegans (Maupas, 1900) (strain CB 4088)
Diplogasteroides magnus Völk, 1950 (strain SB 308)
Koerneria pararmata (W. Schneider, 1938), sampled by Dr. F. Riemann from tidelands of the Weser estuary, Bremerhaven (Germany).
Neodiplogaster tropica Cobb, 1924 (strain SB 354)
Oigolaimella n. sp. (strain SB 353)
Pristionchus pacificus Sommer et al., 1996 (strain PS 312)
Pseudodiplogasteroides sp. (strain SB 257)

Technique: To observe moults within the egg and stoma morphogenesis, batches of eggs were taken from the cultures and transferred to slides provided with a 0.5 mm thick layer of water agar (5%). A cover slip was applied and the specimens were observed with a Zeiss Axioplan. Observations on several eggs were documented by video imaging using a Hitachi KP-D20B CCD camera attached to the Axioplan. Sequences were digitized using a Sony DCR-PC110E Camcorder and edited with Adobe Premiere 6.0.

To designate the chronological sequence of certain developmental events in *Pristionchus pacificus*, hermaphrodites were transferred to fresh agar plates and removed after 2 h. The plates were kept at 20 °C. After 16–17 h, when the events of interest were expected to occur, eggs were transferred to slides provided with a layer of agar (as described above) for microscopy. Observations on chronological sequences were made on four eggs from different plates.

Terminology for stomatal compartments: For different parts of the stoma the terminology developed by DeLey et al. (1995) is used: Anteriorly the stoma is formed by inner walls of the lips and is called cheilostom. The cuticle that surrounds the lumen posterior to the cheilostom is secreted by arcade syncytia. The corresponding stomatal region is called gymnostom. The posterior-most part of the stoma is formed by pharyngeal cells and is called stegostom.

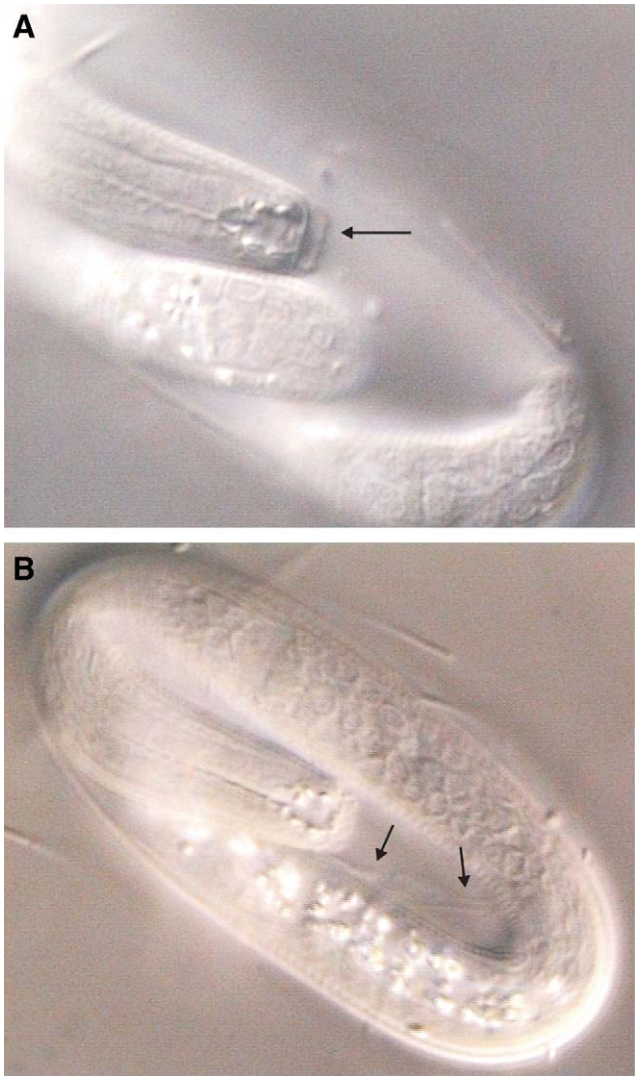

Fig. 1. J2 in the egg of *Oigolaimella* n. sp. (A) Arrow points at "cap" of J1 cuticle. (B) Arrows point at J1 exuvia.