The flaccid cuticle and spiny eggs of *Myolaimus* sp. (Myolaimidae, Nematoda) serve as protection against predation by *Koerneria histophora* (Diplogastridae, Nematoda)

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Summary. Feeding experiments with *Koerneria histophora* as a predator and *Myolaimus* sp. as prey, both from the same habitat, sap exudate of a chestnut tree, showed that in contrast to control experiments with other nematodes from the sap exudate community, *K. histophora* is not able to cut open the cuticle of *Myolaimus* sp. although many attempts to pierce *Myolaimus* were observed. I conclude that this is due to the flaccid cuticle, which is an apomorphic feature of the taxon *Myolaimus*. Furthermore, preliminary results suggest that also the spiny eggs of *Myolaimus* sp. are protected against predation of *K. histophora*. The formation of the spines on the egg shell by the zygote is described.

Key words: adaptation, biodiversity, egg-shell, functional morphology, sap exudate.

Recording biodiversity is one of the major tasks of current research in biological systematics. Another task is to find approaches to explain biodiversity. At first glance biodiversity appears as a huge richness of possible morphologies that can be described taxonomically. Of course, this richness of morphologies reflects the richness of different functions in the interactions between organisms of the species and their enviroment (mainly the interactions with organisms of other species within a given biocenosis). So another major question which, at least when dealing with small invertebrates like nematodes, is seldom tackled is: Why do they all look so different? This question concerns the functional morphology of characters. As the shape of characters evolved in an ecological context, the functional morphology of a given structure should ideally be studied by observing the organisms within their natural habitat. With nematodes this is an almost impossible requirement. However, in the present work, a quasi natural habitat could have been established for culturing the nematode community of sap exudates of decidious trees.

This study concerns the odd outer layer of the body cuticle in *Myolaimus* that is not firmly attached with the inner layer (arrow; Fig. 2H), but is flaccid and has a wrinkled appearance (De Ley & Blaxter, 2002). The possible function of this peculiar feature could hitherto only be guessed: "It might help, to escape attacks of carnivorous nematodes..." (Sudhaus & Koch, 2004). However, preliminary experiments with *Mononchus* specimens proved the contrary, the predators readily fed on *Myolaimus* specimens (personal observations). However, the important question is: Are Mononchidae as predators present in enviroments inhabited by *Myolaimus* species? According to the present study, the answer turned out to be no. The only carnivorous nematode species that frequently was present in sap exudate samples was the diplogastrid *Koerneria histophora*. This paper presents the results of feeding experiments with *K. histophora* as a predator and *Myolaimus* sp. as possible prey. The *Myolaimus* species found in the sap exudate samples is new to science and hitherto the only one that reproduces as a protandrous self-fertilizing hermaphrodite. As preliminary results suggested that the eggs of *Myolaimus* sp. are protected against predation of diplogastrid nematodes by possessing spines on the egg shell, it seems appropriate to also present observations on the formation of these spines within the uterus.

MATERIAL AND METHODS

Examined species: *Acrostichus dendrophila* (Weingärtner, 1955), isolated from sap exudate of a chestnut tree, Groß Ziethen, Brandenburg, Germany.

Caenorhabditis elegans (Maupas, 1899), strain CB4088, kindly provided by Prof. Dr. D. Fitch.

Diplogasteriana schneideri (Paesler, 1939), isolated from sap exudate of a chestnut tree, Groß Ziethen, Brandenburg, Germany.

Halicephalobus sp., isolated from sap exudate of a chestnut tree, Groß Ziethen, Brandenburg, Germany.

Koerneria histophora (Weingärtner, 1955), isolated from sap exudate of a chestnut tree, Groß Ziethen, Brandenburg, Germany.

Myolaimus sp., strain SB 421, isolated from sap exudate of a chestnut tree, Groß Ziethen, Brandenburg, Germany.

Rhabditella octopleura (Steiner, 1929), isolated from sap exudate sampled by Dr. S. Wirth near the Teufelssee-lake in Berlin, Germany.

Culturing. The nematodes were isolated by putting substrate from the samples in a Petri dish (5 cm diameter) provided with a 0.5 cm thick layer of agar (0.2 %) made from tap water without any additional nutrients. The sap exudate nematode community emerged from the substrate and colonised the agar, following the growth of microflora that spread over the agar. The spread of microflora was assisted by histiostomatid mites from the samples that moved over the agar surface. Pieces of agar from old cultures were used to inoculate fresh agar plates. By this procedure cultures could be maintained over many generations.

Notation of feeding behaviour. Observations of reactions after contacts between *Koerneria histophora* specimens and prey nematodes in the culture dishes were noted in the following way. Every observed encounter of an adult with another nematode or an egg was counted as a contact if the predator touched the other nematode with its entire frontal plane which ideally means all labial sensillae at once. Three possible responses to contacts were classified: i) no reaction, ii) unsuccessful attempts to attack, and iii) successful attacks. In addition to 'natural' contacts, contacts were provoked by placing nematodes taken from a different culture in front of *K. histophora* specimens.

Control for feeding experiments. To show that *K. histophora* is an effective predator, experiments were also carried out with other sap exudate nematodes as potential prey (*Acrostichus dendrophila*, *Diplogasteriana schneideri*, *Halicephalobus* sp., *Rhabditella octopleura*) as well as *Caenorhabditis elegans* fourth-stage juveniles (J4), which correspond to adult *Myolaimus* specimens in size, as a control.

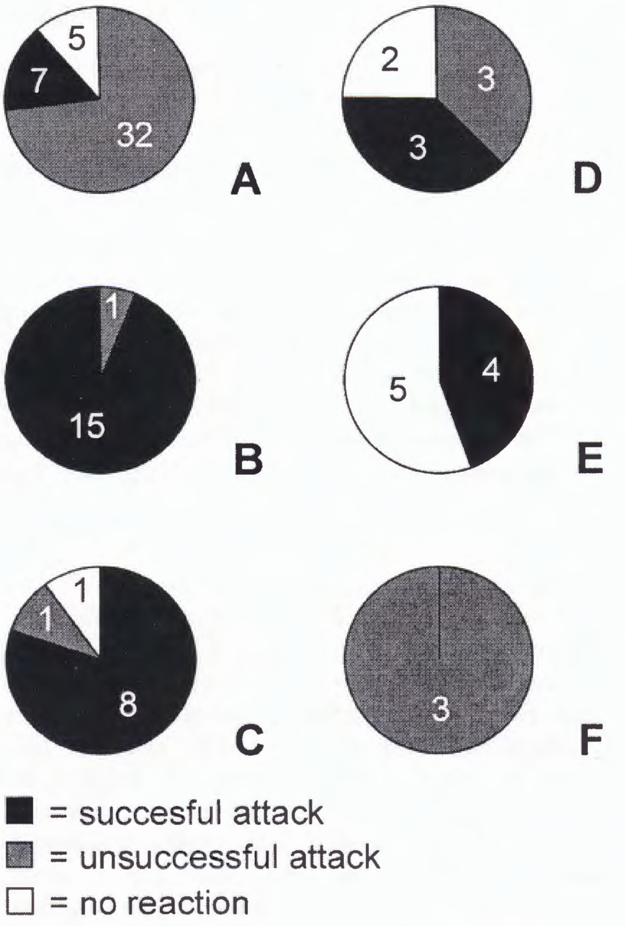

Fig. 1. Feeding experiments. A. Responses of 27 *K. histophora* specimens to 'natural' contacts (n = 44) with specimens of *Myolaimus* sp. Six out of the 7 successful attacks were on J1 specimens, one was on a J2 specimen. B. Responses of 16 *K. histophora* specimens to provoked contacts (n = 16) with *C. elegans* specimens (J4 and younger). C. Responses of 10 *K. histophora* specimens to 'natural' contacts (n = 10) with *Rhabditella octopleura* specimens. D. Responses of one *K. histophora* male to 'natural' contacts (n = 8) with *Halicephalobus* sp. specimens. E. Responses of one *K. histophora* female to 'natural' contacts (n = 9) with *Halicephalobus* sp. eggs. F. Responses of 3 *K. histophora* specimens to 'natural' contacts (n = 3) with *Myolaimus* sp. eggs.

Videomicroscopy. Video sequences were shot using a CANON XL H1 HDV video camcorder mounted on a ZEISS Axioplan2 light microscope or a dissection microscope (LEITZ MZ16). HDV Video sequences were captured on a PC using the HD-down convert mode and edited with ADOBE Premiere 6.0 Software to obtain still frames for the plates.

RESULTS

Feeding experiments. The quantitative analysis of the responses to contacts, wether 'natural' or provoked between specimens of *Korneria histophora* and specimens of other species are shown in Figs 1A-F.

'Unsuccessful attacks' of *K. histophora* on *Myolaimus* sp. specimens sometimes lasted for up to 5 min where *Koerneria* specimens were observed trying to cut open the cuticle of *Myolaimus* specimens. In contrast to other sap exudate nematodes, such as *A. dendrophila*, *D. schneideri* and *R. octopleura*, *Myolaimus* sp. specimens do not flee rapidly after contacts with the predatory *K. histophora.* Instead, if they flee at all, *Myolaimus* sp. specimens move comparatively slowly away from the site of the attack.

Spine formation in *Myolaimus* sp. eggs (Fig. 2). *Myolaimus* sp. is a protandrous hermaphrodite. Its ovotestis produces sperm in late J4 stage that is stored in a spermatheca (arrow; Fig. 2A) at the junction between ovary and oviduct. After the J4/adult moult the ovotestis has switched to oogenesis. During ovulation (Fig. 2B), mature oocytes pass the spermatheca and get fertilised. As is characteristic for the genus, the gonad in *Myolaimus* sp. is prodelphic and antidromously reflexed (Lorenzen, 1994). That means that the flexure of the gonad is between ovary and oviduct. During ovulation in *Myolaimus* (see Fig. 2B), the oocyte enters the oviduct with its proximal pole (different from what was proposed by Lorenzen, 1994) as in diplogastrids (Fürst von Lieven & Sudhaus, 2000). The egg shell is formed by the zygote soon after fertilisation (Figs 2C-E). At the onset of egg shell formation, spines start to grow from the egg shell surface (arrows; Figs 2D, E), also in regions where there is no contact between egg shell and uterus wall (arrow; Fig. 2C). This indicates that egg shell and spines originate from the zygote rather than from the uterus. The spines (arrow; Fig. 2F) are already formed when the egg shell is still flexible enough to allow the zygote to pass the constriction that is formed between the spermatheca and the uterus proper (Fig. 2F).

DISCUSSION

The quantitative results as well as the observations on *Koerneria* specimens that unsuccessfully try to cut open the cuticle of *Myolaimus* specimens show convincingly that *Myolaimus* specimens are protected against predation of the diplogastrid species by possessing a flaccid cuticle that does not allow for the diplogastrid teeth to get a grip. Other sap exudate nematodes, such as *Halicephalobus* sp. and *R. octopleura*, were eaten by *Koerneria.* The diplogastrid mouthparts that are designed for cutting and sucking (see Fürst von Lieven & Sudhaus, 2000; Fürst von Lieven, 2002, 2003) obviously work only on taut surfaces and are incapable of cutting a hole into a flaccid surface. *Koerneria* specimens were only occasionally able to feed on juvenile *Myolaimus* specimens that they swallowed whole from the tip (tail or anterior end). By the same technique, species of Mononchidae that are larger in size as compared to *K. histophora* specimens, are able to feed on *Myolaimus* adults (personal observations). However, mononchs did not occur in the observed sap exudates, so sap exudate inhabiting *Myolaimus* species do not have to fear predation by nematodes.

An interesting question is, if that also holds true for *Myolaimus* eggs. As can be seen in Fig. 1E, *K. histophora* feeds on nematode eggs. Unfortunatedly, only three contacts between *Koerneria* and *Myolaimus* eggs were observed (Fig. 1F). In all three cases the *Koerneria* specimens tried to suck on the eggs but were not able to open them, whereas all observed attacks were succesful in the case of *Halicephalobus* eggs (Fig. 1E). These results point to a possible protection of *Myolaimus* eggs against predation by diplogastrids which could be due to the spines that cover the egg shell in *Myolaimus* sp.

To conclude, a possible function for two peculiarities of *Myolaimus* morphology (the flaccid cuticle and the spiny eggs) are suggested. The flaccid cuticle definitely protects *Myolaimus* against attacks of diplogastrid nematodes, and *Myolaimus* eggs are possibly also protected against such predation by being covered with spines. I am not arguing that both structures evolved in response to predation by diplogastrids, but that they function to escape it. The characters discussed here were

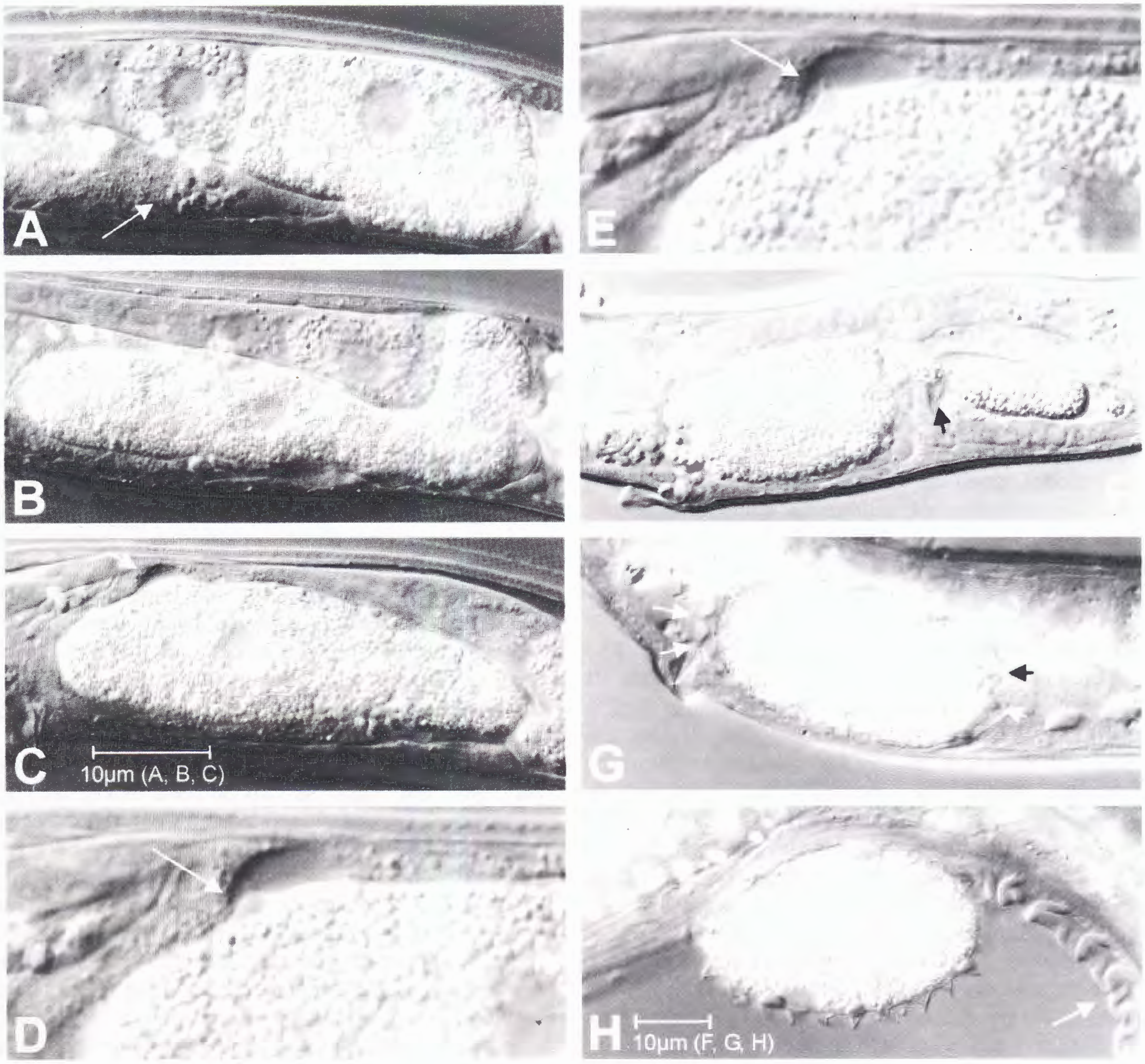

Fig. 2. *Myolaimus* sp., hermaphrodite, right lateral aspects. A-C: Ovulation. A: Arrow points to spermatheca; B: Oocyte entering spermatheca; C: Zygote within uterus. Arrow points to region where uterine wall is detached from egg shell; D-E: Enlargements (2.2x) of region where arrow points at in C, showing growth of a spine (arrows); F: Egg passing constriction between spermatheca and uterus proper. Spines are already formed (arrow). Sperm at left hand side from the zygote is stored within the postvulvar uterine sack (the homologon of the reduced posterior gonad branch); G: Egg within uterus just before oviposition. Arrows point at spines; H: Egg after oviposition Arrow points at flaccid cuticle of hermaphrodite.

present in the stem species of *Myolaimus*, and we do not know if diplogastrids are typical for the habitats of *Myolaimus* in general. As a rule morphological structures can possess many functions and it is wise at first to detach the question of recent functions from that of adaptation. A further possible function of the flaccid cuticle could be protection against desiccation, and there are preliminary observations that *Myolaimus* can withstand desiccation to a considerable degree (V. Bärmann, pers. com). We need to test the linkage between morphological structures and all possible functions we can think of before creating a scenario of selection pressures that led to the origin of a structure. But without knowing something about *some* possible functions we can never address that kind of question. The question of adaptation depends on the question of function; the question of function depends on live observations, possibly in the natural context. Therefore, it is clear that without live observations we will never know why all these nematodes look so different.

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Fürst von Liven A. Дряблая кутикула и шипастые яйца *Myolaimus* sp. (Myolaimidae, Nematoda) служат для защиты от нападения хищных нематод *Koerneria histophora* (Diplogastridae, Nematoda).
Резюме. Хищничество *Koerneria histophora* на *Myolaimus* sp. было исследовано экспериментально в среде их общего обитания – жидких выделениях каштановых деревьев. Было показано, что, в отличие от хищничества на других нематодах из того сообщества выделений каштановых деревьев, нематоды *K. histophora* не были способны разрушить целостность кутикулы *Myolaimus* sp., хотя наблюдали их многочисленные попытки проткнуть поверхность жертв. Предполагается, что этому способствует дряблая кутикула *Myolaimus*, представляющая собой апоморфию данного таксона. Предварительные наблюдения показывают, что яйца *Myolaimus* sp. с поверхностью, покрытой шипами, также являются защитой от *K. histophora*. Описан процесс формирования шипов на поверхности оболочки яйца.