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Development, Morphology and Functions of Mucoid Glands in Cercariae of *Paragonimus miyazakii*

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Abstract

Formation of mucoid glands and secretion of mucoid substance was correlated closely with development of cercaria of *Paragonimus miyazakii* during the intramolluscan phase of the life cycle. Cercariae in free-swimming phase were surrounded by a complete mucoid coat that probably protects the organism from adverse environmental conditions. Cercariae in free-swimming phase also had a mucoid strand that probably aids attachment and penetration of the parasite during infection of the second intermediate host. Morphological features of the mature mucoid glands of *P. miyazakii* were homologous to those of *P. kellicotti,* but differed in some morphological details from those of *P. westermani* and *P. ohirai.*

Key words: *Paragonimus miyazakii,* Cercaria, Mucoid gland

Introduction

Mucoid glands of the cercarial stages of trematodes stain metachromatic red with toluidine blue O and secrete mucoid substances onto the outer surface of the cercaria. These glands are believed to 1) protect cercaria from adverse environmental conditions, 2) play a role in the infection of the second intermediate host and/or 3) contribute to formation of the cyst wall (Erasmus, 1972). The morphology and development as well as the primary functions of these glands differ among cercarial species and consequently are important characters for studies of cercarial taxonomy and ecology (Kruidenier, 1951, 1953a, 1953b, 1953c, 1953d; Yokogawa and Yoshimura, 1956, 1958; Ito and Watanabe 1957, 1958, 1959; Kamo *et al.*, 1967b). More detailed knowledge about the functions of these structures should provide important clues about the ecological requirements of each species of cercaria.

The larvae of the lung fluke, *Paragonimus miyazakii* Kamo *et al.*, 1961, develop in several species of snails, including *Bythinella nipponica akiyoshiensis,* (Kamo *et al.*, 1967a; Sano *et al.*, 1979). It is confirmed by the experimental results that mature cercariae are released from the snail host, *B. n. akiyoshiensis* into water (Kawanaka M. *et al.*, 1979) and penetrate a crab second intermediate host, *Geothelphusa dehaani,* where they develop into metacercariae (Gyoten, 1986). The mechanisms by which cercaria infect crabs are unknown.

Consequently, studies of the mucoid glands may provide a better understanding of this phase of the life cycle. The present study was designed to investigate relationships between morphological changes in the mucoid glands and stages of cercarial development.

Materials and Methods

Crabs, *G. dehaani,* infected with metacercaria of *P. miyazakii* were collected in Kuma-cho, Ehime Prefecture, Japan. Cercariae of *P. miyazakii* were obtained from *B. n. nipponica* which had been infected experimentally with miracidia. The snails were collected in a small stream at Hoino in Tambara-cho, Ehime prefecture, and maintained in the laboratory prior to infection with miracidia.

Rediae and cercariae were dissected from

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infected snails and placed on a slide glass with a small quantity of 0.4% saline. The tissue was flattened with a cover glass, fixed with alcohol and corrosive sublimate, stained with 1% toluidine blue O, dehydrated with alcohol, mounted with balsam and examined under a light microscope (Yokogawa and Yoshimura, 1956). Mature cercariae in free-swimming phase that had recently emerged from infected snails were also placed on a slide glass in a small drop of water and treated as described above. Some cercariae were fixed without pressure from a cover glass and processed in the same manner.

Results

Changes in the morphology of mucoid glands were followed in three developmental phases of the cercaria of *P. miyazakii* (Table).

Intraredial Phase

A total of 790 cercariae in stages of development ranging from germ balls to fully formed cercariae were examined in 166 rediae. Out of the 790 cercariae, 296 stained metachromatic red with toluidine blue O dyes. All specimens that stained with toluidine blue O contained mucoid glands in various stages of development, however, secretion of mucoid substances by the glands was not observed.

Development of the mucoid glands began with the appearance of a few cells anterior to the acetabulum that stained metachromatic red. As development progressed, additional cells appeared in positions that were anterior, lateral and posterior to the acetabulum, eventually forming a reticulum composed of tangled dendrites (Figs. 1, 2, 3). The reticulum increased in size and complexity, developing several deeply staining portions and a few ducts (Fig. 4). Deeply stained portions of the reticulum developed into 6 pairs of mucoid glands which were distributed parallel to the mid-ventral axis of the cercarial body. Ventral mucoid reservoirs (vmr) and lateral mucoid reservoirs (lmr) formed in ducts that were extended anteriorly and anterio-laterally from the first pair of mucoid glands. Similar "lmr" developed in association with the second pair of mucoid glands. Lateral mucoid reservoirs from the third pair of mucoid glands were located along both margins of the cercaria (Fig. 5). As the glands reached maturity, ducts from the first and second pairs of mucoid glands extended further along the dorsal border of the oral sucker and formed dorsal mucoid reservoirs (dmr) at the anterior extremity of the ducts (Fig. 6).

To correlate development of the glands with maturation of the cercaria, specimens with cells that stained metachromatic red were divided into three groups. A total of 94 specimens (31.8%) had mucoid glands with no ducts or reservoirs (early "mg" stage), 87 specimens (29.4%) had mucoid glands with "lmr" and "vmr" (reservoir-forming "mg" stage), and 115 specimens (38.7%)

Figs. 1–6 Toluidine blue O-stained cercariae of *Paragonimus miyazakii* fixed during development of the mucoid glands. All cercariae were contained within rediae. Scale bar = 50μm. Figs. 1–3: Cercariae in the early mucoid gland (mg) stage. Figs. 4–5: Cercariae in the reservoir-forming (r-forming) "mg" stage. Fig. 6: Cercaria in the mature "mg" stage.

Fig. 1 Mucoid cells (→) appear first anterior to the acetabulum (a). Note oral sucker (os) and tail (t). A stylet is not present.

Fig. 2 Additional mucoid cells (→) appear anterior to those in Fig. 1 and in positions lateral and posterior to the acetabulum.

Fig. 3 Mucoid cells eventually develop into a reticulum composed of tangled dendrites.

Fig. 4 The reticulum increases in size and complexity, eventually developing several deeply stained portions and a few ducts (→).

Fig. 5 Deeply stained areas of the reticulum develop into 6 pairs of mucoid glands. The first pair of glands develops ducts with ventral and lateral mucoid reservoirs (vmr, lmr). The second and third pairs develop "lmr". The remaining mucoid glands do not develop ducts. A stylet (arrowhead) is present in 52.8% of cercariae in this stage of development.

Fig. 6 The final stage of glandular development occurs during formation of dorsal mucoid reservoirs (dmr) at the anterior extremity of the cercaria. All cercariae in this stage of development have a stylet (arrowhead).

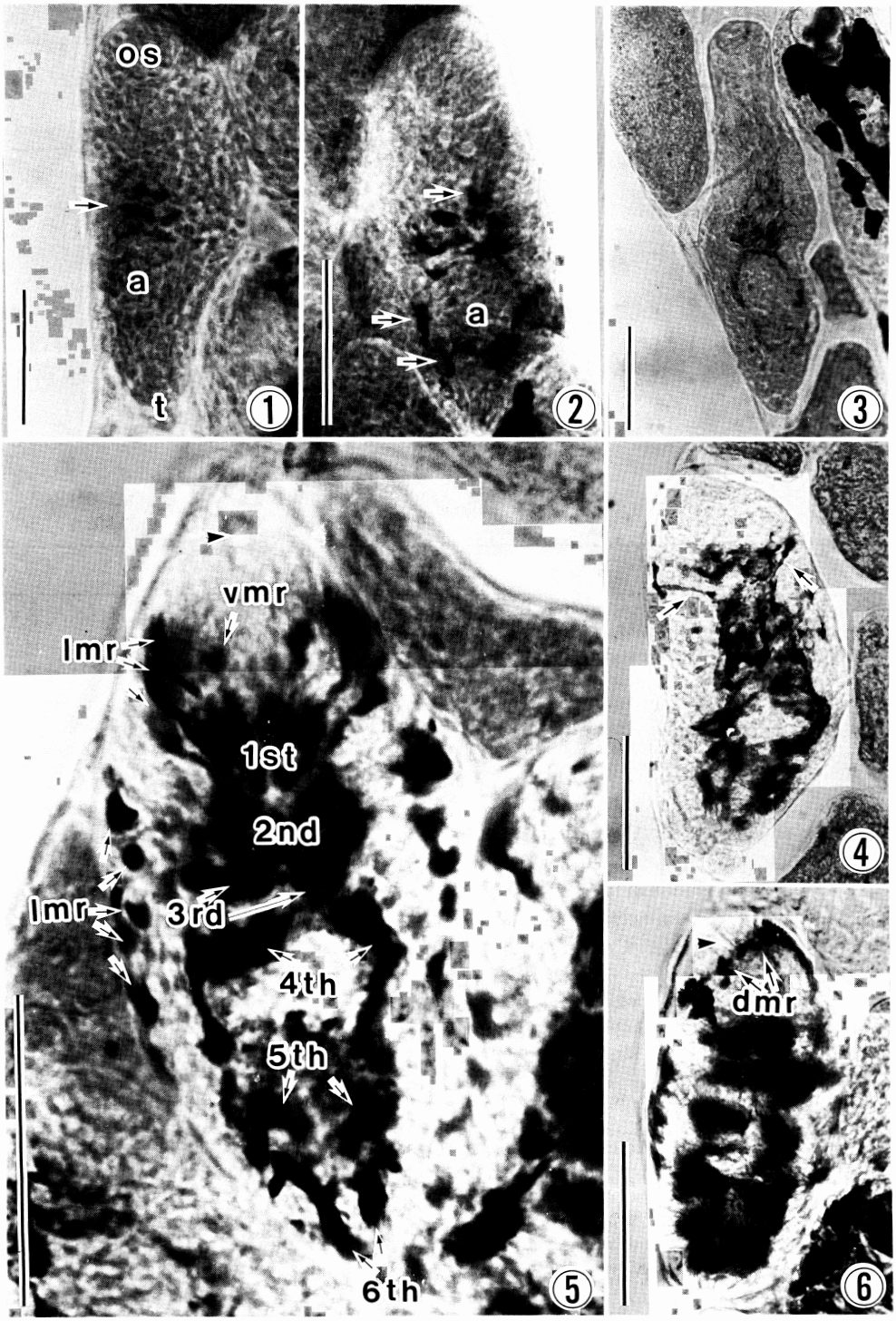
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Table Changes of mucoid glands related with the cercarial development of *Paragonimus miyazakii*

Developmental phase	Number of cercariae with					Total
	early mag,	r-forming mg,	mature mg,	ms-secreting mg,	Complete mc	
Intra-redial phase						
stylet (–)	94(100*)	41(47.2*)	0	0	0	
stylet (+)	0	46(52.8*)	115(100*)	0	0	
subtotal	94(31.8†)	87(29.4†)	115(39.6†)	0	0	296
Intra-molluscan migratory phase						
stylet (–)	0	0	0	0	0	
stylet (+)	0	0	20(100*)	98(100*)	88(100*)	
subtotal	0	0	20(9.7†)	98(47.6†)	88(42.7†)	206
Free-swimming phase						
stylet (–)	0	0	0	0	0	
stylet (+)	0	0	0	0	20(100*)	
subtotal	0	0	0	0	20(100†)	20
Grand total	94	87	135	98	108	522

mc: mucoid coat, mg: mucoid gland, ms: mucoid substance, r: reservoir
stylet(+): No. of cercariae with a stylet,
stylet(–) : No. of cercariae without a stylet,
*: Rate(%) of cercariae with a stylet or no stylet,
†: Rate(%) of cercariae in each development stage of mucoid gland.

had mucoid glands with "dmr" in addition to the "lmr" and "vmr" (mature "mg" stage). Cercariae with mucoid glands in the early "mg" stage had an oral sucker, an acetabulum and a tail, but did not have a stylet. Cercariae in the reservoir-forming "mg" stage were more developed with 52.8% having a stylet at the anterior extremity of the body. All cercariae in the mature "mg" stage had stylets and appeared mature (Table).

Figs. 7–13 Toluidine blue O-stained cercariae of *P. miyazakii* fixed after release from rediae, during migration in the snail first intermediate host. Scale bar = 50μm. Fig. 7: Cercaria with mature mucoid glands which have not secreted mucoid substance. Figs. 8–12: Cercariae fixed during the mucoid substance-secreting (ms-secreting) "mg" stage.

Fig. 8 Secretion of mucoid substances first occurs around the oral sucker (os). The "vmr" disappear at the same time.

Fig. 9 The outer surface of the cercaria is enveloped with secretion of the mucoid glands.

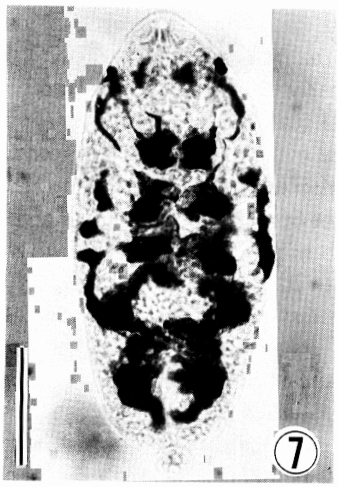
Fig. 10 Mucoid substances accumulated mainly around the oral sucker (os) and ventral groove (vg). The third pair of mucoid glands becomes rudimentary (→).

Fig. 11 Accumulation of mucoid substance around the oral sucker and ventral groove becomes more extensive. The first pair of mucoid glands also become rudimentary (→) after their contents have been secreted.

Fig. 12 The first and third pairs of mucoid glands have disappeared and the second pair has become rudimentary (→).

Fig. 13 Cercariae in the complete mucoid coat (mc) stage are completely enveloped with mucoid substance. At completion of secretion, the mucoid glands can no longer be detected.

Fig. 14 Cercaria in free-swimming phase after fixation and staining with toluidine blue O. When cercariae are smeared on a glass slide, mucoid strands (→) extend from the posterior extremity of the body. Scale bar = 50μm.



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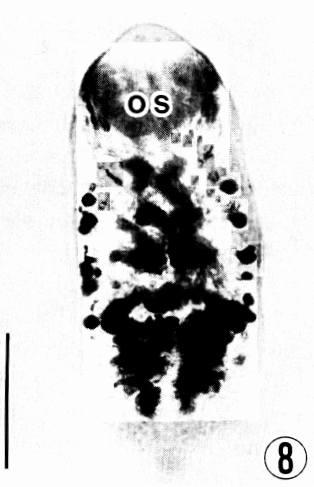

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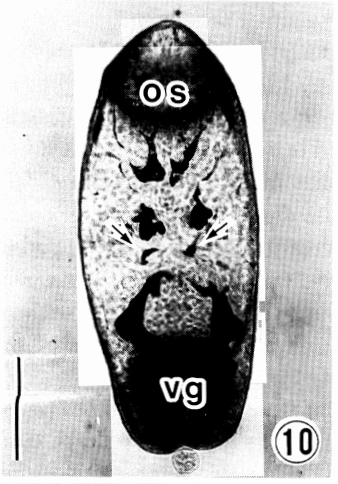

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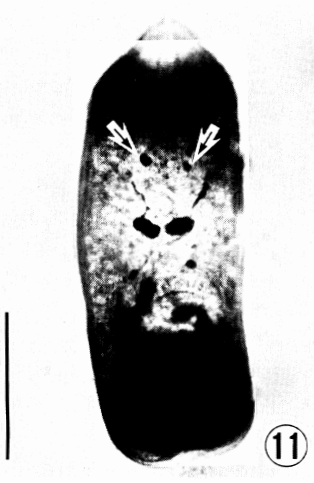

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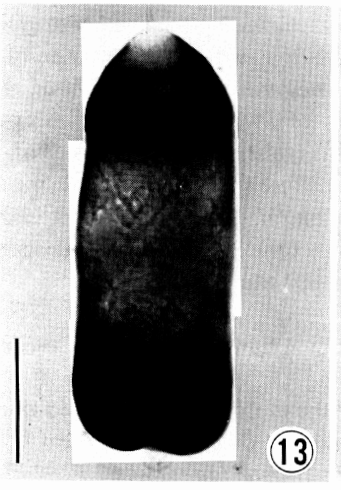

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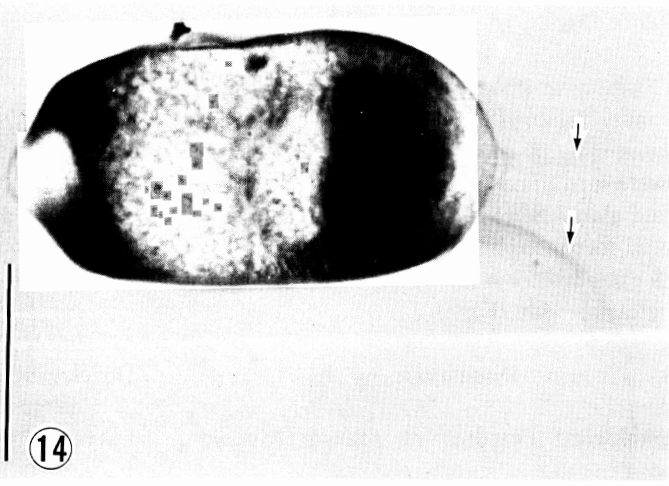

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Intramolluscan Migratory Phase

Cercariae collected from the tissues of infected snails after they had been released from rediae stained metachromatic red with toluidine blue O. All of the 206 specimens that were examined had mature mucoid glands. Twenty cercariae (9.7%) were in the mature "mg" stage of development (Fig. 7). An additional 98 specimens (47.6%) had atrophic mucoid glands and were covered with mucoid substance on their outer surfaces (mucoid substance-secreting "mg" stage). Mucoid glands could not be detected in the remaining 88 cercariae (42.7%). These specimens were completely enveloped in mucoid substance (complete mucoid coat stage).

Secretion of mucoid substance was first observed around the oral sucker (Fig. 8). After the disappearance of "vmr", secretion of the glands progressed continuously from the anterior to the posterior portion of the cercaria (Fig. 9). The third pair of mucoid glands were the last to discharge their content, eventually becoming rudimentary. After release from the glands, the mucoid substance accumulated around the oral sucker and ventral groove (Fig. 10). Each pair of glands became progressively smaller and more rudimentary in size with the second pair being the last to disappear (Figs. 11, 12). As the last vestiges of the glands disappeared, the cercarial body was completely enveloped in mucoid substance (Fig. 13).

Free-Swimming Phase

Twenty flattened and 10 smeared cercariae in free-swimming phase stained metachromatic red and were surrounded by complete mucoid coats. Mucoid glands were not detected. Eight of the smeared specimens had formed a mucoid strand which extended from the posterior extremity of the cercarial body (Fig. 14).

Discussions

Morphological Features of Mature Mucoid Glands

Yokogawa and Yoshimura (1956, 1958) carried out morphological studies of the cercarial mucoid glands of *P. westermani* and *P. ohirai* and compared them to mature cercarial glands of *P. kellicotti.* The mucoid glands of these species are nearly homologous to each other in morphological features, however, a few minor differences are evident. For example, cercariae of *P. westermani* have caudal mucoid reservoirs which have not been detected in cercariae of *P. kellicotti.* The mucoid glands of *P. ohirai* are more irregular in shape and their ducts and reservoirs are less developed than those of *P. kellicotti* and *P. westermani.* The presence of these species specific morphological differences suggests that studies of remaining species of *Paragonimus* may identify additional morphological characters that can be used in taxonomic studies of the genus *Paragonimus.*

Mucoid glands of the cercariae of *P. miyazakii* were observed by Kamo *et al.* (1967b), but their morphological features have not been described in detail. Accurate determination of the number of mucoid glands in species of *Paragonimus* is difficult, because the two most posterior pairs of glands in the cercarial body, i.e. the 5th and 6th, overlap and are difficult to separate. Kurudenier (1953c), however, was able to distinguish the 5th and 6th pairs after partial discharge of their contents.

In the present study, the 5th and 6th pairs of mucoid glands of *P. miyazakii* could be clearly distinguished (Fig. 5) in the reservoir-forming "mg" stage, indicating that the number of glands in this species is the same as in cercariae of *P. kellicotti, P. westermani* and *P. ohirai.* The mucoid glands of *P. miyazakii* were morphologically indistinguishable from those of *P. kellicotti,* but differed from those of *P. westermani* and *P. ohirai.* The former species has caudal mucoid reservoirs while the latter species has less developed mucoid reservoirs.

Morphological Changes During Cercarial Development

Changes in the morphology of mucoid glands have been described during development of *P. kellicotti, P. westermani* and *P. ohirai* (Kruidenier, 1953c; Yokogawa and Yoshimura, 1956, 1958), but the changes have not been correlated closely with stages of cercarial develop-

ment. In the present study, mucoid glands of *P. miyazakii* were first detected in cercariae with a visible oral sucker, acetabulum and tail. As cercariae developed a stylet, most glands were in the reservoir-forming "mg" stage. Development of a stylet and final maturation of the mucoid glands occurred at approximately the same time.

Mature cercariae remained within rediae for a short time, but were eventually released into tissue of the snail body. After cercariae were released, they were exposed to some stimulus within the snail tissues that activated secretion of mucoid substance. After the cercarial body was completely enveloped in this material, the cercariae remained within the host tissues for a short time and then emerged into the water. Cercariae in free-swimming phase formed mucoid strands, when they made contact with solid substances.

Functions of the Mucoid Glands

The mucoid coat which completely envelopes cercariae of *Paragonimus* spp. and extends from the posterior extremity of the cercarial body as a mucoid strand is believed to protect the organism from adverse environmental conditions during intramolluscan and free-swimming phase of the life cycle, (Kruidenier, 1953c; Yokogawa and Yoshimura, 1956, 1958). In the present study, 57.3% of cercariae isolated from snail tissues did not have complete mucoid coats and another 9.7% with mature mucoid glands failed to secrete any mucoid substance. By contrast, all cercariae in free-swimming phase completely enveloped with mucoid coats. These observations indicate that this coat has important functions only in the free-swimming phase of the life cycle, e.g. protection against the low osmotic pressure.

The mucoid strand is believed to be important during infection of the second intermediate host. Cercariae of *P. kellicotti* emerge from their snail hosts and then penetrate the thin chitinous integument of their crayfish second intermediate hosts (Ameel, 1934). The mucoid strand is believed to be important in attachment of the cercariae to the surface of the integument (Kruidenier, 1953c). By contrast, cercariae of *P. westermani* penetrate crabs, the second intermediate host, periorally when snails harboring the cercariae are ingested by the crabs (Yokogawa, 1953). These cercariae do not emerge from the snail tissue into the external environment and do not form mucoid strands (Yokogawa and Yoshimura, 1956). Consequently, Yokogawa and Yoshimura (1956) concluded that mucoid substances does not play a role in infection of the second intermediate host during the life cycle of *P. westermani.* By contrast, cercariae of *P. miyazakii* emerge from their snail intermediate hosts, completely enveloped in mucoid substance and typically develop mucoid strands. As is true with cercariae of *P. kellicotti,* these strands appear to be important for attachment and penetration into the crab second intermediate host. Here, the cercariae transform into mature metacercaria within 60–75 days after infection (Gyoten, 1986). Thus, the function of the mucoid substance appears to differ among species that share the same basic life cycle.

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