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Studies on the Life History of *Chordodes japonensis*, a Species of Gordiacea III. The Mode of Infection

With 3 Text-figures

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(*Communicated by* T. FUJII)

On the basis of observations on the ecology of gordiacean larvae in relation to their host insects, some authors came to the conclusion that the gordiaceans might reach the hosts directly, while others considered that the intermediate hosts would be necessary for the complete life cycle of the worms (Villot, 1874; Linstow, 1884, 1891, 1898; Camerano, 1892; Dorier, 1925; Müller, 1926 *et al.*). On the other hand, May (1919), Blunck (1922) and Thorne (1940) carried out some experiments to study the life history of this group of animals. May succeeded in injecting *Gordius* larvae into the mouth or the abdominal cavity of grasshoppers and observed that in 17 of 64 cases infection took place. From these results he suggested that *Gordius* might directly infect the hosts in the field. In contrast with this, Blunck who fed beetles with tadpoles of *Rana* and *Bufo* collected from the pond which was inhabited by adults of *Gordius aquaticus*, succeeded in recovering some *Gordius* from the beetles. This observation led him to the conclusion that the tadpoles were the intermediate hosts. However, Blunck did neither examine larvae in the tadpoles nor ascertain whether the larvae found in the tadpoles (Villot *et al.*) were those of *G. aquaticus*. Thorne reported that 2 out of 30 hosts (the Mormon cricket, *Anabrus simplex*) which had been fed *Gordius* larvae were later found infected with the larvae. However, since the hosts used by Thorne were collected in the field, it is not certain whether the worms found in them were derived from the ingested larvae. Furthermore, from data of both May and the present writer, it seems unlikely that minute larvae could attain 25 cm in length in 17–25 days after having been ingested. Thorne failed to obtain infected hosts by placing a drop of water containing *Gordius* larvae on the intersegmental membrane of the abdomen of the insects.

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Materials and Methods

Mantises (*Tenodera sinensis* S.) hatched out from the eggs in the laboratory were reared by feeding living flies of laboratory-reared *Drosophila virilis*. In all series of experiments except for the first one, mantises were inoculated with *Chordodes* larvae when they reached about 20–30 mm in length. In series 1, smaller mantises, 15–20 mm long, were used as materials. The inoculated mantises and their intact controls were reared by feeding flies of *Drosophila virilis, Lucilia* sp. and *Sarcophaga* sp.. At different intervals, the inoculated specimens were carefully dissected in a 0.7% salt solution under a low power microscope or a dissection-microscope (for specimens more than 21 days after inoculation), or sectioned in paraffin (for younger ones). It should be added that in none of the controls gordiacean parasites were found.

Experiments

1. *Examination of ability of Chordodes larvae to penetrate into the interior of mantises through the body wall*

Five young mantises were fixed each on a slide glass by loops of hairs or thread and a small amount of water containing 500–2000 *Chordodes* larvae was applied on its abdomens and legs, care being taken not to immerse the mouth parts of the insect in water. After about one hour, the mantis was washed in clean water, dissected and its various tissues examined for *Chordodes* larvae. It was observed that *Chordodes* larvae coming into contact with or coming near a mantis showed no behavior whatever to penetrate its body wall. Moreover, no *Chordodes* larvae could be found in the tissues of the mantises.

2. *Injection of Chordodes lavae into mantises*

In 1954, 13 young mantises were given injections of a drop of a 0.7% NaCl solution containing about 15 *Chordodes* larvae. Since the larvae were found to shrink slightly in the salt solution, in 1955 and 1957, a drop of water containing 5–12 larvae was injected into the abdominal cavity of 23 mantises. Seven mantises which died within 10 days after inoculation were discarded. Examination of the mantises at different intervals showed that in no case infection with *Chordodes* had taken place.

3. *Feeding mantises with Chordodes larvae*

Flies of *Drosophila virilis* reared in the laboratory were injected intra-abdominally with *Chordodes* larvae by means of a capillary pipette. Each fly received about 10 larvae. Immediately after this, mantises were fed such flies, one fly per mantis. Of 12 mantises, 3 were found to be infected, yielding 5 young parasites, 15 to 30 mm in body length (26–40 day stage) (Table 1).

4. *Feeding mantises with Chordodes-infected insects*

In the hatching season of *Chordodes*, ranging from June to July, mantises are about 1.5–3.0 cm in body length. Consequently, it is conjectured that they can capture only small sized animals. On the other hand, judging from the habits of *Chordodes* larvae (Inoue, 1960a), insects serving as the intermediate

Table 1

Inoculation of mantises by feeding active larvae

Mantis					Number of ingested larvae	Infection	State of parasites
No.	Sex	Hatching	Inoculation	Examination			
1	♀	IV 26	VI 11	VII 21	about 8	+	2 young, 25 and 30 mm
2	♂	〃	〃	〃	〃	—	
3	♀	〃	VI 15	VII 18	about 10	—	
4	♀	〃	〃	VII 14	〃	—	
5	♀	〃	〃	VII 20	〃	—	
6	♂	IV 27	VI 18	VII 25	〃	+	1 young, 30 mm
7	♀	V 4	〃	VII 20	〃	—	
8	♀	V 23	VI 23	VII 22	〃	—	
9	♂	〃	〃	〃	〃	—	
10	♀	VI 1	VI 26	〃	〃	+	2 young, 15 and 20 mm
11	♀	〃	〃	〃	〃	—	
12	♂	〃	〃	〃	〃	—	

40 control mantises were free from *Chordodes*.

Table 2

Inoculation of mantises by feeding imagines of *Cloëon* bearing *Chordodes* cysts

Exp. No.	Mantis					Cysts in *Cloëon*		Infection	State of parasites
	No.	Sex	Hatching	Inoculation	Examination	Days after encyst.	Estimated No.		
I (1954)	1	♀	V 13	VI 30	VIII 3	4.5	7–22	+	17 young
	2	♀	〃	〃	IX 25	〃	〃	+	5 adults emerged
	3	♀	V 21	VII 4	VIII 16	17	11	+	7 young
	4	♂	V 13	VII 6	VII 19	3	6–17	—	
II (1955)	5	♂	VI 8	VI 25	VII 2	3	11–27	+	3 minute
	6	♀	VI 11	VI 26	VII 1	4	〃	+	3 minute
	7	♀	VI 7	VI 28	VII 4	6	〃	+	7 minute
	8	♂	VI 3	VII 2	VII 9	13	2–26	+	10 minute
	9	♀	VI 7	VII 5	VII 10	6	11–33	+	5 minute
	10	♀	VI 3	VII 6	VII 26	17	2–26	—	
	11	♀	VI 7	〃	〃	7	11–33	+	1 young
III (1957)	12	♂	V 23	VI 13	VII 22	3	3–10	+	1 young
	13	♂	〃	VI 17	〃	7	〃	+	2 young
	14	♀	〃	VI 22	VII 17	12	〃	+	8 young
	15	♀	〃	VI 24	VII 21	10	7–21	+	4 young
	16	♀	〃	VI 25	VI 29	15	5–16	—	
	17	♂	VI 1	VI 26	VII 10	16	〃	+	6 young
	18		〃	〃	VI 29	〃	〃	—	
	19	♀	V 23	VI 29	VII 25	15	7–21	+	3 young
	20	♀	〃	VI 17	VII 19	7	3–10	+	9 young
	21	♂	〃	VI 19	VII 28	9	〃	+	2 young
	22	♀	〃	VI 23	IX 10	3	4–12	+	2 adults emerged
	23	♀	〃	VI 26	VIII 18	12	7–21	+	6 young
	24	♂	〃	VI 30	VII 11	10	4–12	+	3 minute
	25	♀	〃	VII 1	VII 5	11	〃	+	2 minute
	26	♀	〃	VII 4	VII 9	14	〃	—	

53 control mantises were free from *Chordodes*.

Table 3

Inoculation of mantises by feeding imagines of *Chironomus* bearing *Chordodes* cysts

Exp. No.	Mantis					Cysts in *Chironomus*		Infec-tion	State of parasites
	No.	Sex	Hatch-ing	Inocu-lation	Exami-nation	Days after encyst.	Estimated No.		
I (1954)	1	♂	V 4	VI 14	VII 3	3	7-17	−	
	2	♀	〃	VI 18	IX 26	3	1-17	−	
	3	♀	IV 26	VI 19	VII 18	4	〃	−	
	4	♂	〃	〃	VII 12	4	10-22	−	
	5	♀	V 13	VI 21	IX 26	6	1-17	−	
	6	♀	V 4	VI 22	VII 23	7	〃	−	
	7	♂	IV 26	VI 24	VII 16	9	10-22	−	
	8	♀	V 13	VI 25	IX 26	10	〃	+	1 male adult
	9	♀	〃	〃	VII 18	〃	〃	−	
	10	♂	〃	VI 26	VIII 18	〃	4-17	+	1 young
	11	♀	〃	VI 27	IX 6	12	10-22	+	6 young
	12	♀	〃	VII 1	VII 22	6	10-20	−	
	13	♀	IV 26	〃	VII 24	15	4-17	−	
	14	♀	〃	〃	VII 25	〃	〃	−	
	15	♂	V 13	VII 11	IX 26	6	15-37	−	
II (1957)	16	♀	V 23	VI 14	VII 31	4	1- 6	−	
	17	♀	〃	VI 15	VII 5	5	〃	−	
	18	♀	〃	〃	VII 31	5	〃	−	
	19	♀	〃	VI 19	VI 22	9	〃	−	
	20	♀	〃	VI 21	VII 31	11	〃	−	
	21	♀	〃	VI 24	VI 29	4	3-11	−	
	22	♂	VI 1	VI 28	VII 5	8	〃	−	
	23	♂	〃	VI 29	〃	9	〃	−	

41 control mantises were free from *Chordodes*.

Table 4

Inoculation of mantises by feeding imagines of *Culex* bearing *Chordodes* cysts

Mantis					Cysts in *Culex*		Infec-tion	State of parasites
No.	Sex	Hatching	Inocu-lation	Exami-nation	Days after Encyst.	Estimated No.		
1	♀	V 4	VII 2	VIII 9	3	6-31	+	1, young
2	♀	〃	〃	VIII 21	3	〃	+	10, young
3	♀	V 13	VII 3	VIII 1	6	6-23	+	3, young
4	♀	V 25	〃	IX 26	6	〃	−	
5	♀	V 13	VII 6	VII 13	7	6-31	−	
6	♂	V 25	〃	VII 26	7	〃	−	

17 control mantises were free from *Chordodes*.

hosts must have aquatic larval or nymphal stages. Therefore, in this series of experiments, mantises were fed imagines of *Cloëon dipterum*, *Chironomus dorsalis* and *Culex pipiens pallens*. These insects were actually infected with gordiacean larvae in the field (Linstow, 1891, 1898; Mühldorf, 1914, Müller, 1926 *et al.*) and, moreover, their nymphs or larvae were abundantly found in the water where the adults of *Chordodes japonensis* were readily collected. Nymphs or larvae of these insects were placed together with *Chordodes* larvae in a glass tube for several hours. This allowed the *Chordodes* larvae to be ingested and to penetrate into the interior of the insect (Inoue, 1960b). The insect larvae or nymphs were then washed thoroughly and transferred to another vessel containing some water free from *Chordodes* larvae. When imagines emerged from the infected larvae or nymphs, they were given to mantises, one per mantis. Just before feeding, each insect to be given to the mantis was examined as to whether it contained *Chordodes* cysts or not. To count all cysts contained in each insect, different tissues of the body must be teased and carefully examined (Inoue, 1960b). However, since mantises do not eat crushed tissues, the insects were slightly pressed between a cover glass and a slide glass so that a part of its intestine was squeezed out. If at least one *Chordodes*-cyst was observable in it, the whole insect, still alive, was given to the mantis by hanging it at the tip of a hair. In some other experiments, the number of *Chordodes*-cysts in inoculated insects was estimated by random sampling. The number of cysts contained in each specimen in the same inoculation-group was estimated to be in the following confidence interval with 95% reliability:

$$\bar{x}+\frac{t_0}{\sqrt{n-1}}u > m > \bar{x}-\frac{t_0}{\sqrt{n-1}}u$$

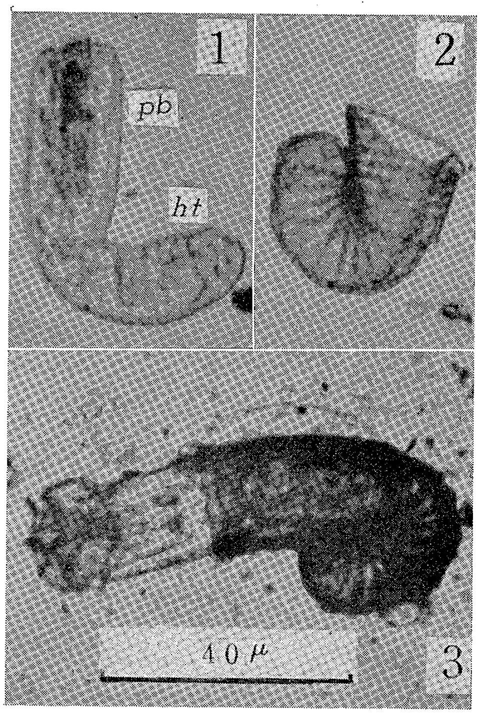
where m is population mean (the number of cysts contained in one specimen), $\bar{x}$, the sample mean, n, the size of the sample, u, square root of unbiased estimate of population variance, and t_0, the 5%-point of t-distribution of degree of freedom n-1. The number of cysts ingested by each mantis, estimated in this way, is shown in Tables 2–4.

Data from the experiments, together with those from the controls, are shown in Tables 2–4. In each of the three experiments, the mantises became infected with *Chordodes*, while none of the control specimens showed signs of infection.

DISCUSSION

In the experiments reported in the present paper, infected *Cloëon* was the most effective medium in transferring *Chordodes* larvae to the final host of the worm (Table 2) and *Chironomus* was the least (Table 3), *Culex* being intermediate between the two (Table 4). In the infection-experiment with *Cloëon*, nearly all mantises became infected if they ingested *Chordodes* cysts which had been in *Cloëon* within 2 weeks, while the infection took place in only about 50% of the mantises which had ingested the cysts older than 2 weeks (No. 10, 16, 18 and No. 26 in Table 2). In a previous paper, the present writer reported that *Chordodes* cysts can be alive in *Cloëon* for about 2 weeks (Inoue, 1960b).

Two types of *Chordodes* cysts are distinguishable in the intermediate hosts, i.e., the *Cloëon*-type and the *Chironomus*-type (Inoue, 1960b). The most marked difference between the two types is the presence of chitinous cyst-wall in the *Chironomus*-type cysts and the presence of the cellular envelope and mucus layer surrounding the encysted larva in the *Cloëon*-type ones. Examination of the faeces ejected by mantises (No. 19, 21 and 23 in Table 3; No. 24–26 in Table 2) seems to indicate that (1) the cellular envelope and the mucus layer surrounding encysted larva of the *Cloëon*-type cyst are easily digested in the alimentary canal of the mantis so that the freed larva can penetrate the intestinal wall to reach a suitable site of the body for further development, while (2) the cyst-wall of the *Chironomus*-type cyst is made of a substance indigestible by the mantis and consequently the cysts are thrown out with faeces (Fig. 2). It seems likely that this nature of the cyst-wall of the *Chironomus*-type cysts accounts for, at least in part, the low incidence of infection following inoculation with *Chironomus*. However, it should be added here that *Chordodes* larvae freed from cysts or partly covered with broken cyst-walls (Figs. 1 and 3) were found in faeces of mantises. This suggests that the walls of some ingested cysts are broken while passing through the fore-intestine of mantises. In such cases, *Chordodes* larvae may be freed in the mantises to undergo final development. In infected specimens of *Culex*, some have the *Chironomus*-type cysts alone, while others have the *Cloëon*-type cysts alone or both types of cysts (Inoue, 1960b). This fact is in good harmony with the higher incidence of infection following inoculation with *Culex* than with *Chironomus*. The results of the present experiments also indicate that the infection with *Chordodes* may occur in the mantis irrespective of the sex of the host, and that it takes about 3 months for *Chordodes* larvae to attain the adult stage in the mantis.



Figs. 1–3. *Chordodes* larvae and pieces of cyst-wall found in faeces from mantises fed with *Chironomus*-type cysts. 1. Naked, dead larva. 2. Piece of broken cyst-wall. 3. Dead larva partly covered with broken cyst-wall. *ht*, head-trunk; *pb*, proboscis.

From Experiments 3 and 4, it may be concluded that, at least under the conditions of the experiments, the mantis becomes infected with *Chordodes* following the ingestion of *Chordodes* larvae or of *Chordodes* cysts in the intermediate hosts. However, it is highly probable that the natural infection in the field takes place mainly, if not solely, via the intermediate hosts. This conjecture is supported by different observations. The present author has reported elsewhere (Inoue, 1960a) that *Chordodes* larvae cannot stand desiccation continuing for more than 20 seconds. It is unlikely, therefore, that the larvae get out of water creeping about on land or on grass leaves. On the other hand, the mantis does not go down to water (personal communication from Dr. Furukawa) and if it falls accidentally into water it swims actively on the surface never sinking to the bottom. Since *Chordodes* larvae are slow creepers and cannot swim at all, the larvae have little chance of meeting mantises. Furthermore, it was observed that

Chordodes larvae coming into contact with mantises did not try to penetrate into the interior of the insects (Experiment 1).

It seems highly probable that the mantises become infected with *Chordodes* larvae when they eat insects with *Chordodes* cysts. The mantis is carnivorous and chances are that it eats insects whose nymphs or larvae are exposed during their aquatic life to the danger of infection by *Chordodes* larvae (Linstow, 1891, 1898; Mühldorf, 1914; Müller, 1926). In the laboratory, *Chordodes* larvae are easily taken by the larvae of Ephemeroptera and Diptera and bore their way into the body of these larvae. Feeding mantises with infected insects containing *Chordodes* cysts, especially those of the *Cloëon*-type, was far more effective in causing infection than giving *Chordodes* larvae to mantises directly per os. The fact that a large number of eggs are laid in one place (Inoue, 1940), and that it takes considerable time for *Chordodes* larvae to get completely out of the chorion at the time of hatching may facilitate the meeting of *Chordodes* larvae and aquatic insect-larvae. In quiet water, newly hatched *Chordodes* larvae are crowded together just beneath the egg-strings and consequently they will be easily taken by aquatic insects which happen to come to the larvae.

From the results of the experiments and the considerations described above, it appears likely that mayflies and probably other insects of appropriate size in which *Chordodes* cysts of *Cloëon*-type are produced are the most effective intermediate hosts. Dipterous insects may also serve as intermediate hosts.

May (1919) was of the opinion that there is no change of hosts in the life history of Gordiacea. However, he was prudent enough to add that different species of the group might differ in this respect. Comparative studies in different species of the group are highly desirable.

SUMMARY

1. *Chordodes* larvae suspended in water were brought into contact with the mantis. The larvae never tried to penetrate into the interior of the body of the mantis. Moreover, later examination revealed no *Chordodes* larvae in the tissues of the mantis. Therefore, direct infection through the body wall is not likely to occur. Injection of *Chordodes* larvae into the abdominal cavity of the mantis also yielded negative results.

2. Three of 12 mantises which had been fed active *Chordodes* larvae together with food were found to be infected.

3. Only 3 out of 23 mantises which had been inoculated by feeding *Chironomus* flies bearing *Chordodes* cysts became infected, while 21 out of 26 mantises fed with *Cloëon* imagines carrying cysts were found to be infected. Three of the 6 mantises fed with *Culex* having *Chordodes* cysts were infected. Fifty-three control mantises were invariably free from the parasites.

4. From these and other results it appears likely that indirect infection through intermediate hosts is more important than that following direct ingestion of the larvae.

REFERENCES

Blunck, H. 1922 Zool. Anz., **54**, 111.
Camerano, L. 1892 Atti R. Accad. Sci. Torino, **27**, 598.
Dorier, A. 1925 Compt. Rend. Acad. Sci. Paris, **181**, 1098.
Inoue, I. 1940 Bot. a. Zool., **8**, 39 (In Japanese).
———— 1960a Sci. Rep. Tokyo Gakugei Univ., **11**, 21.
———— 1960b Annot. Zool. Japon., **33**, 132.
v. Linstow, O. 1884 Arch. f. Nat., **50**, 125.
———— 1891 Arch. mikr. Anat., **37**, 239.
———— 1898 Ibid., **51**, 745.
May, E. G. 1919 Illinois Biol. Monogr., **5**, 120.
Mühldorf, A. 1914 Zeitsch. wiss. Zool., **111**, 1.
Müller, G. W. 1926 Zeitsch. Morph. Ökol. Tiere, **7**, 134.
Thorne, G. 1940 Jour. Washington Acad. Sci., **30**, 219.
Villot, A. 1874 Arch. Zool. expér. gén., **3**, 39 and1 81.