Recent Advances in Insect Embryology in Japan and Poland
Edited by H. Ando and Cz. Jura
Arthropod. Embryol. Soc. Jpn.
(ISEBU Co. Ltd., Tsukuba) 1987

Position of Germ Rudiment and Rotation of Embryo in Eggs of Some Dragonflies (Odonata)

Kozo MIYAKAWA

Synopsis

Changes of the dorso-ventral axis of embryos of seven dragonfly species during development are described, on the base of the dorso-ventral axis of the egg when deposited, corresponding to the axis of the mother. In Zygoptera, Anisozygoptera, and aeshnid Anisoptera, so far studied, the germ rudiment is formed on the dorsal side of the egg. The embryos rotate through 180° in Zygoptera, 90° in Anisozygoptera, and 0° in an aeshnid Anisoptera. In exophytic eggs of libellulid Anisoptera, the embryos exhibit a gravity dependent rotation to take a venter-up position at about revolution stage. Time and cause of the rotation are divergent according to taxa, even in individuals of the same species. Possible four different causes of the rotation are suggested. (1) Bilaterally asymmetric fusion of amnion and serosa probably brings about a rotation during revolution. (2) Zygopteran embryos, after revolution, rotate to fit themselves to the dorso-ventrally asymmetric egg space through change of their form with growth or occasional muscular movements. (3) Libellulid gravity dependent rotation is caused by only difference of weight between the embryo and the yolk. (4) Irregular rotations seen in grown embryos are attributed to occasional activities of muscles in trunk region.

Introduction

Since Wheeler's terminology (1893), the term "blastokinesis" is currently used for movements of the embryo in the egg of insects. There are two main movements in hemimetabolous embryos: the anatrepsis, the movement which occurs during formation of the germ band and amnion, and the katatrepsis (revolution) occurring after rupture of the embryonic envelopes (amnion and serosa) and before the dorsal closure of the embryo. These movements occur in general as a dorso-ventral turning, to change the flexure of the embryo. The blastokinesis is remarkable especially in primitive orders of the insects (Johannsen and Butt, 1941; Ando, 1962; Cobben, 1968; Anderson, 1972).

In addition to these dorso-ventral turns, the embryo sometimes performs rotation around the egg long axis. The rotations were observed in several hemimetabolous orders, *e. g.*, Odonata (Seidel, 1929), Plecoptera (Miller, 1939), Orthoptera (Slifer, 1932), Isoptera (Striebel, 1960), Cheleutoptera (Anderson, 1972), Thysanoptera (Haga, 1985), Homoptera (Sander, 1956), and Heteroptera (Cobben, 1968). Among these, the best investigated, from comparative and systematic points of view, is Heteroptera by Cobben (1968).

In Odonata, Seidel (1929) showed a 180° rotation of zygopteran *Platycnemis pennipes* embryo at katatrepsis or revolution. This was later widely quoted as a representative of the order (Krause, 1939; Weber, 1954; Ando, 1962; Anderson, 1972). Ando (1962) carried out an elaborate work on odonate embryology with special reference to *Epiophlebia superstes*, but he did not show any detailed data on the rotation. Recently Prof. H. Ando asked me to write a short joint-paper on the dragonfly embryology for a general publication (not yet published), and we decided to use *Calopteryx atrata* for this purpose. In examination of *C. atrata* embryo, we confirmed a 180° rotation similar to that of *P. pennipes* (Seidel, 1929).

Since then I was interested in the rotation of odonate embryo and obtained data for seven species, of six different genera, referable to five families, covering three suborders of Odonata. These brought me a view somewhat different from the previous understanding of the odonate embryo rotation.

This paper describes brief outlines of embryogenesis, in living condition, with special reference to the rotation for seven odonate species, and gives a discussion on the rotation of the embryo from embryological and ecological aspects.

Materials and Methods

Species studied were as follows:

- Zygoptera
  - *Calopteryx atrata* Selys (Calopterygidae)
  - *Cercion sieboldii* Selys (Coenagrionidae)
- Anisozygoptera
  - *Epiophlebia superstes* (Selys) (Epiophlebiidae)
- Anisoptera
  - *Aeshna nigroflava* Martin (Aeshnidae)
  - *Orthetrum albistylum speciosum* Uhler (Libellulidae)
  - *Sympetrum frequens* Selys (Libellulidae)
  - *S. parvulum* Bartenef (Libellulidae)

Eggs of *A. nigroflava* collected by Mr. K. Naraoka from Aomori Prefecture, and of *S. parvulum* collected by Mr. Y. Arai from Yorii, Saitama Prefecture were given by Mr. Y. Arai. Eggs of other species were collected by myself from Saitama Prefecture.

For investigation of rotations of the embryo, it was inevitable to keep the eggs in a constant direction on the bottom of a watch-glass during development. In endophytically ovipositing species of Zygoptera, Anisozygoptera and aeshnid Anisoptera, the eggs were extracted from the host plant tissue and transferred to the watch-glass on which bottom vaseline was spread as a bond, thereby the initial dorso-ventral axis of the egg relative to that of mother insect was recorded. These eggs developed in normal state.

In exophytically ovipositing libellulids, the dorso-ventral axis of the eggs could not be determined, because the eggs were collected into water of a vial, when the mother insects were captured while ovipositing, were completely of radial symmetry. These eggs were transferred to watch-glass within 1 or 2 days after oviposition. They attached to the bottom of the glass with their gelatinous covering and their position was constantly kept until hatching. Thus, the direction of dorso-ventral axis of libellulid eggs was unknown, and direction of the eggs relative to the glass bottom may have been determined at random or by gravity when arranged on the bottom.

Results

Zygoptera

Calopteryx atrata Selys (Calopterygidae)

The eggs were obtained from populations inhabiting Koma river, Hidaka, Saitama Prefecture (Miyakawa, 1982) in late summers of 1981 and 1984.

The egg just deposited was nearly cylindrical, 1.25 × 0.25 mm in size, with a membranous funnel around the anterior pole. As development proceeded, its volume increased to 0.28 × 0.29 mm and its shape changed into a banana form with convex

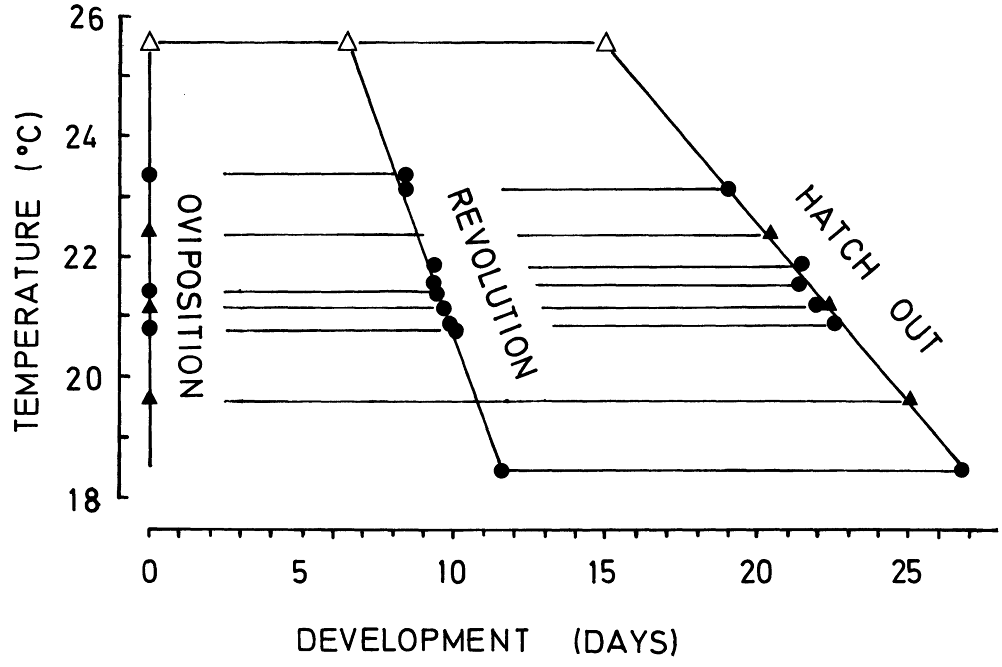

Fig. 1. Durations of embryonic development of *Calopteryx atrata* in various temperature. Filled circles showing pre- or post-revolution period; filled triangles, total embryonic period; open triangles, from data of Ando (1962).

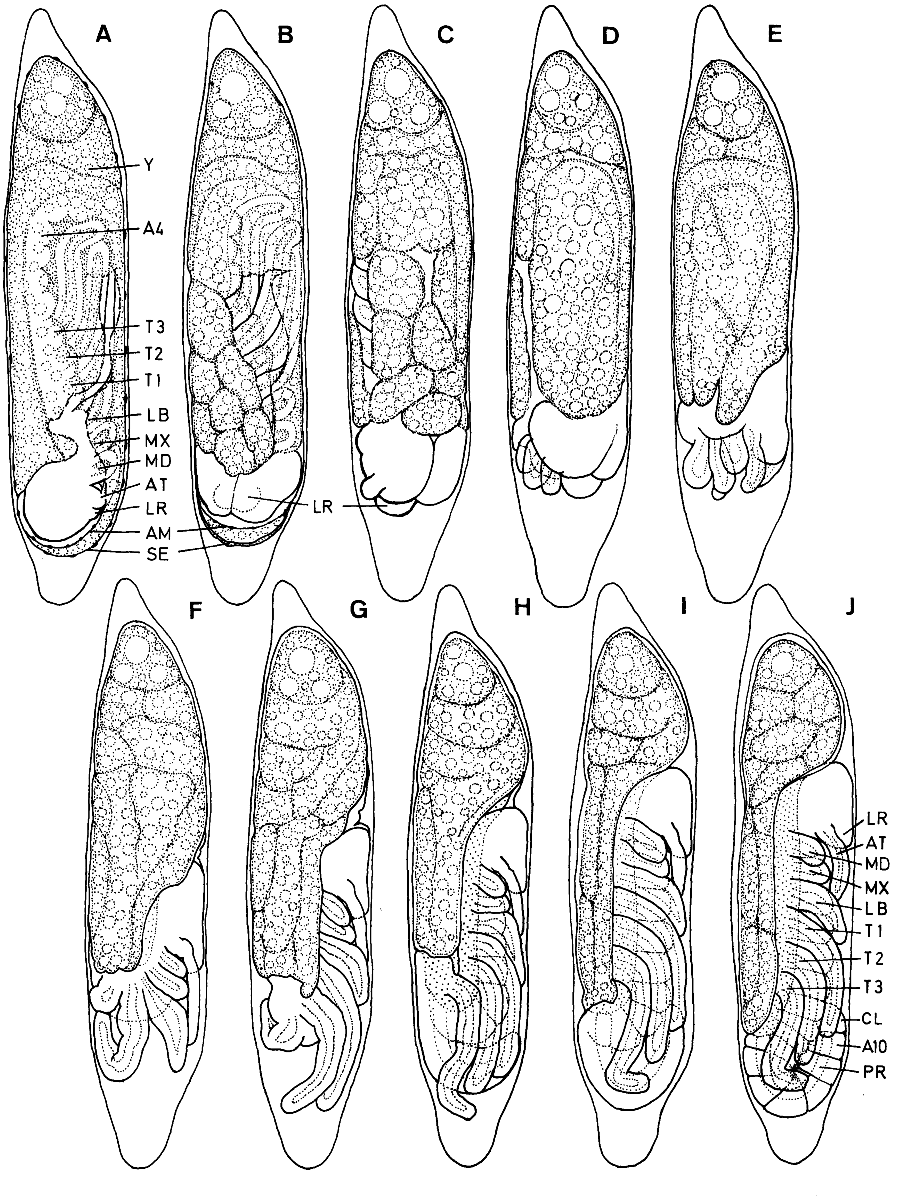

Fig. 2. Revolution with a 180° rotation of an embryo of *Calopteryx atrata* at 24℃. A, 23: 00 (24. IX. 1981); B, 00: 00 (25. IX. 1981); C, 01: 15; D, 02: 00; E, 02: 40; F, 03: 25; G, 04: 45; H, 07: 25; I, 09: 50; J, 11: 00. For further explanation see the text. A4, 10, fourth and tenth abdominal segments; AM, amnion; CE, cephalic lobe; CL, caudal lamella rudiment; DO, secondary dorsal organ; EY, compound eye rudiment; LB, labium rudiment; LR, labrum rudiment; MD, mandible rudiment; MG, mid-gut; MX, maxilla rudiment; PR, proctodaeum; RG, rectal gill rudiment; SE, serosa; ST, stomodaeum; T1—3, pro-, meso- and metathoracic segments or legs; Y, yolk.

ventral side. Dorso-ventral axis of this egg corresponds to that of the mother insect.

The germ rudiment was formed at the dorsal side of 1/5 egg length from the posterior pole. The protocorm developed from the posterior end of the germ rudiment and sank into the yolk extending toward anterior pole of the egg. Thus the embryo became J-shaped in side view. When the embryo became segmented and beared appendages, it became S-shaped with dorsally curled gnathal region and ventrally bent abdomen. At this stage the embryo-yolk complex surrounded by the serosa detached from the chorion at both anterior and posterior poles of the egg. Embryo rotation never occurred till just before revolution or katatrepsis.

Time length of embryonic development differed according to temperature as shown in Fig. 1, and Q_{10} was calculated as 2.13.

At the end of pre-revolution period, a space between the embryo and the amnion, in front of the head (amniotic cavity), enlarged and the united membranes of amnion and serosa became clearly observed as two lines between the embryo and the chorion. Revolution began when the united part of the two membranes ruptured.

Figure 2 shows processes of revolution of an embryo at 24℃. Two hours before rupture of the membranes (RM), the embryo was in a position unchanged in dorso-ventral axis as before (A). Then clockwise rotation of the embryo began, as viewed from the posterior pole of the egg, and it proceeded about 90° in 1 hr (B). RM occurred when the embryo rotated about 100° (C), and the rotation proceeded further (D) and reached 180° when the embryo's head turned toward the anterior pole 1. 5 hr after RM (E). About 2.5 hr after RM, the head reached a mid-way of the egg length and all the gnathal and prothoracic segment escaped from the yolk (F). Further processes, G, H, I, and J of Fig. 2 show stages at 3.5, 6.5, 9, and 10 hr after RM, respectively. No further rotation occurred during these period.

Then, other 36 eggs were re-examined. The results are shown in Fig. 3. Embryonic stages are here roughly divided into pre-revolution stage (A), revolution stage (B), and later stage after dorsal closure (C). No embryos rotated in pre-revolution stage, but in an early stage of revolution 75% of embryos rotated 90° to 180°. At the end of revolution stage 180° rotated embryos increased in number, and almost all the embryos rotated 180° by the end of embryonic period. Examination of the other, more than 60 eggs of full-grown stage in the plant tissue, revealed that almost all the embryos were in a position 180° rotated from that of pre-revolution stage.

From these observations, it was concluded that, in *Calopteryx atrata*, the embryo does not rotate during pre-revolution stage, but it rotates, in most cases, during revolution stage and in some cases, during post-revolution stage, and completes 180° turn along the antero-posterior axis of the egg by hatching.

Cercion sieboldii Selys (Coenagrionidae)

Eggs examined were collected from some floating roots of the reed, *Phragmites japonica* in which oviposition was made by a female in tandem at Ichino river, Saitama, on 9. August 1985 (n = 20). Embryonic period was 17-18 days at a mean temperature of 29℃ (26℃ − 32℃). Revolution occurred at 12 − 13 days after oviposition.

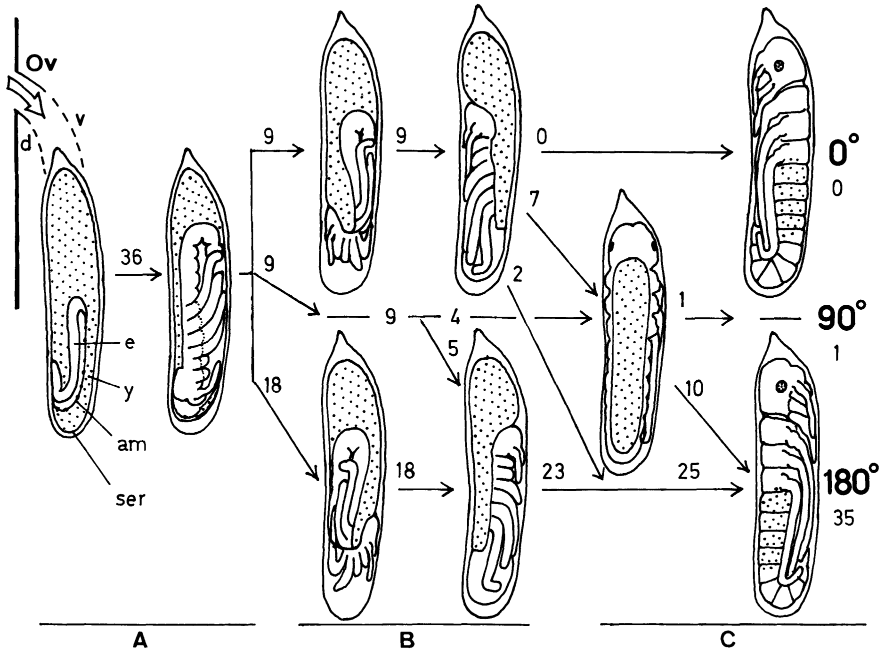

Fig. 3. Orientation of the egg at oviposition (Ov) in the host plant (anterior pole upward; dorso-ventral axis, dv) and orientation of the embryos at successive stages in the egg of *Calopteryx atrata* (n = 36). Numerals indicate number of embryos at each stage rotated at 90° and 180° from the initial position (0°) in the egg shell. A, anatrepsis stage; B, katatrepsis stage; C, post-revolution stage.

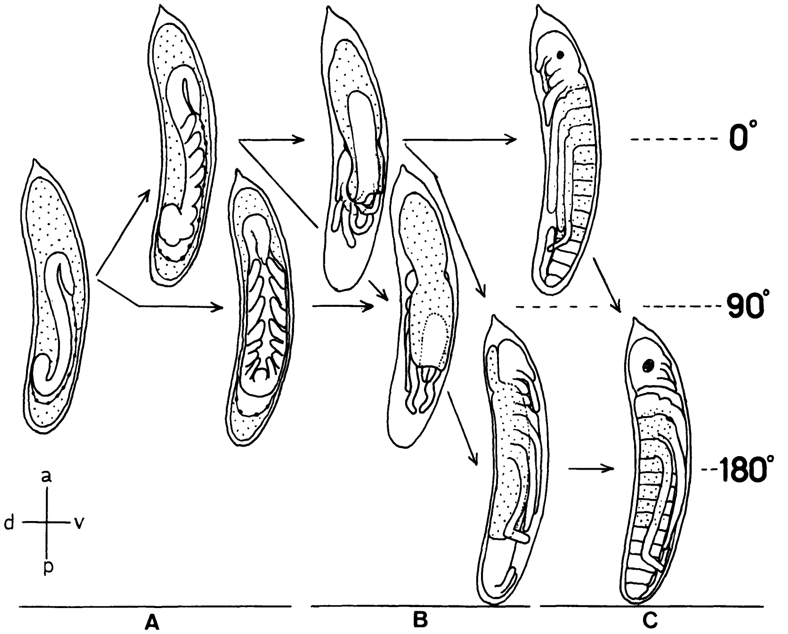

Fig. 4. Rotation of *Cercion sieboldii* embryos. Egg axes are indicated as a-p (antero-posterior) and d-v (dorso-ventral). Developmental stages the same as Fig. 3.

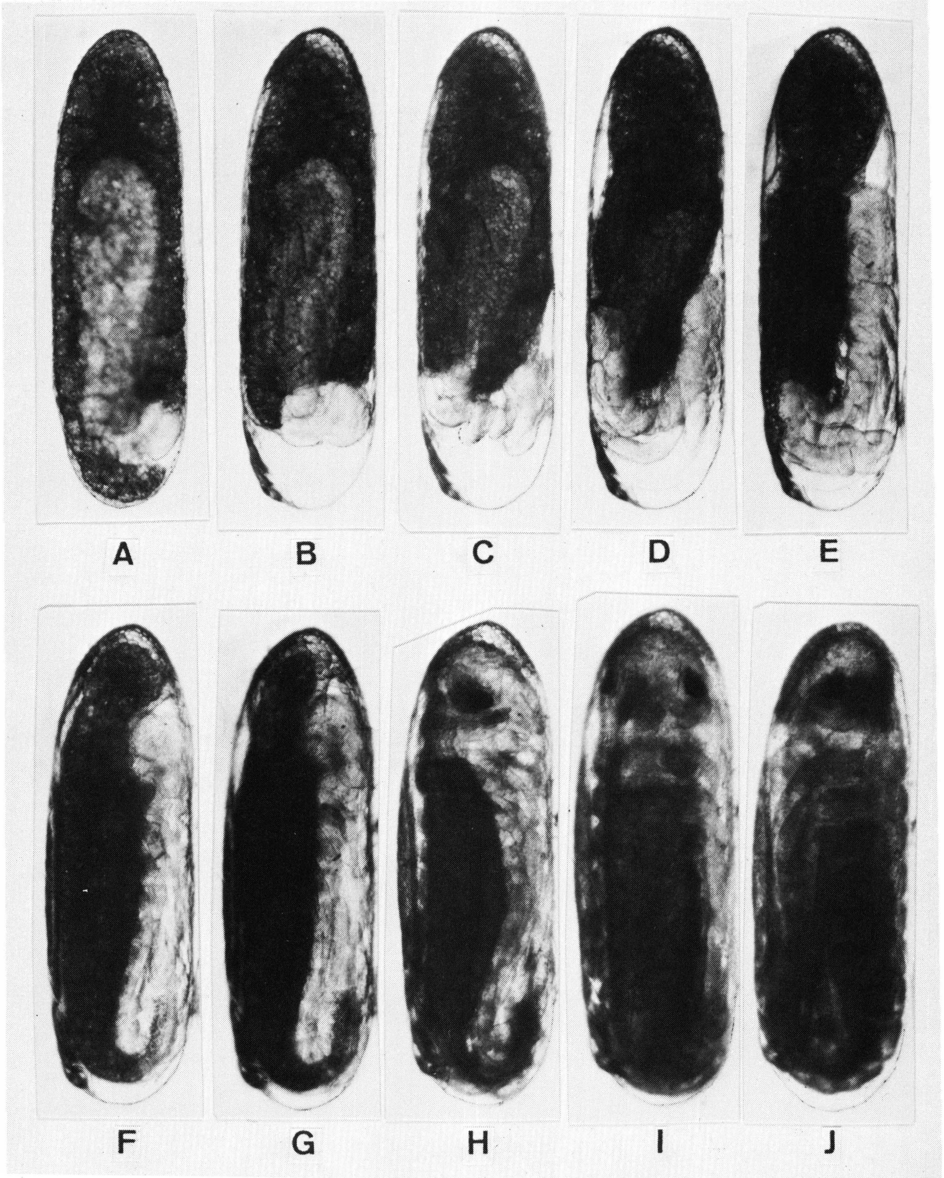

Fig. 5. Revolution and rotation of an embryo of *Epiophlebia superstes* in the egg, left side view with the anterior pole upward. A, 1408 hr (21. V. 1985); B, 1100 hr (22. V. 1985); C, 1302 hr; D, 1453 hr; E, 1649 hr; F, 0820 hr (24. V. 1985); G, 0820 hr (25. V. 1985); H, 0930 hr (29. V. 1985); I, 0830 hr (31. V. 1985); J, 0820 hr (3. VI. 1985). For further explanation see the text.

The egg of a middle age was 1.1 × 0.2 mm in size, and banana-shaped with convex ventral side. Its curvature was larger than in *Calopteryx atrata*.

The embryogenesis was quite similar to that of *Calopteryx atrata*, except in the posture of full-grown embryo. The embryo of *Cercion sieboldii* had a posture of whole the abdomen straight with a flexure at the departure of the caudal lamellae in the egg (Fig. 4), while in *Calopteryx atrata* the embryo of this stage had a posture with a flexure at the eighth abdominal segment (Figs. 2, 3).

Figure 4 shows positions of the embryo in the egg through development (A) before, (B) during, and (C) after revolution. No rotations were observed in early embryonic stage, but all the embryos rotated 180° by hatching. Of the embryos examined, 14% began rotation before the rupture of envelopes, 62% in an early revolution stage, 16% in a later revolution stage, and 8% after completion of dorsal closure. Thus the time of rotation was variable as observed in *Calopteryx atrata*.

Anisozygoptera

Epiophlebia superstes (Selys) (Epiophlebiidae)

Eggs observed were those laid into thalli of the bryophyte, *Conocephalum conicum* at a mountain stream of Saitama Prefecture in 1985 — 1986 (*cf.* Tamiya and Miyakawa, 1984). The thalli were easily kept in live condition in moisture, and were convenient to extract the eggs from them and also to observe the eggs *in situ* through transmittent light. Ages of embryos, when collected, were estimated according to descriptions of Ando (1962), and four series of eggs, in which the embryos were in early stages before the revolution, were used in this study.

The egg was newly cylindrical with dorso-ventral symmetry and 1.05 — 1.15 × 0.33 mm in size.

Of 18 eggs extracted, 16 eggs developed normally in water of watch-glasses under room temperature of 23℃ (16.5℃ —33.5℃).

Figure 5 shows the positional change of an embryo through development from just before revolution to full-grown stage. The embryo, before revolution, was in a position with its dorsum directed to upper surface of the thalli, and was S-shaped in lateral view. The orientation of the embryo in the egg agreed dorso-ventrally, but reversed antero-posteriorly with that of mother insect which laid (Fig. 5 A). Figure 5 B shows the embryo at the beginning of revolution, *i. e.* just after the rupture of embryonic envelopes. No rotation occurred until this stage. Then revolution of the embryo proceeded as shown in Fig. 5 C — G. After revolution of the embryo, yolk was taken into the mid-gut, and dorsal closure was completed. Compound eyes were heavily pigmented and other organs were formed (Fig. 5 H). The embryo showed occasional muscular movements and turned its direction at 90° for the first time (Fig. 5 I), and reached full-grown stage (Fig. 5 J).

Figure 6 shows rotations of 10 of these embryos during and after revolution stages. One embryo (10%) rotated 90° as early as in the revolution stage, but the other (90%) commenced rotations after the dorsal closure. All the embryos rotated by 7 days after commencement of revolution. It was interesting to note that some embryos rotated nearly 180° in a day after the occasional muscular movements began, and 80% of the embryos took a final position 90° rotated from the original position.

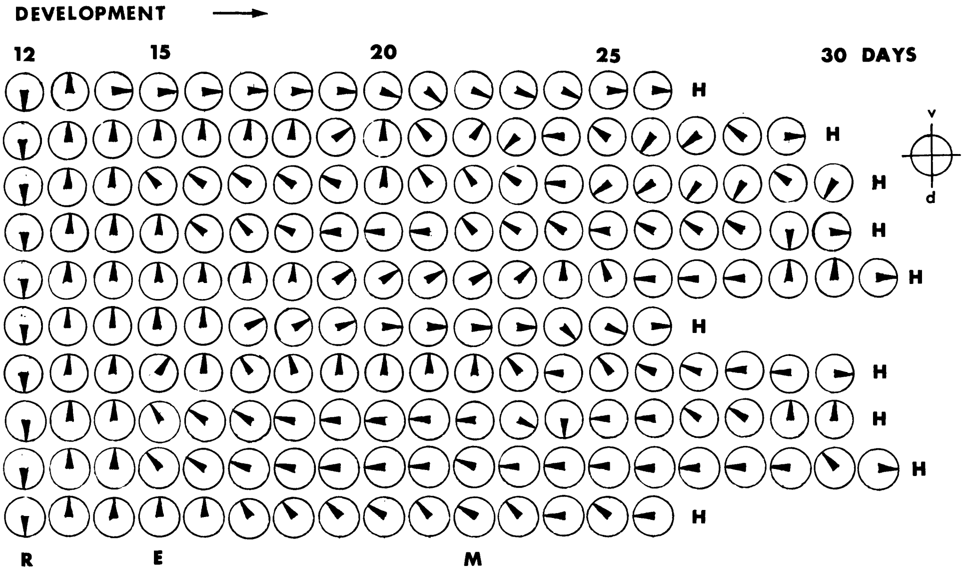

Fig. 6. Rotatograph for *Epiophlebia superstes* embryos in the eggs extracted from the host plant ($n = 10$). Arrow tops indicating ventral side of the embryos. Circles enclosing the arrow top showing egg shells in posterior view with ventral side downward. Developmental time is indicated by days after oviposition. E, stage when eyes pigmented; H, hatching; M, stage when muscles became functional; R, revolution stage.

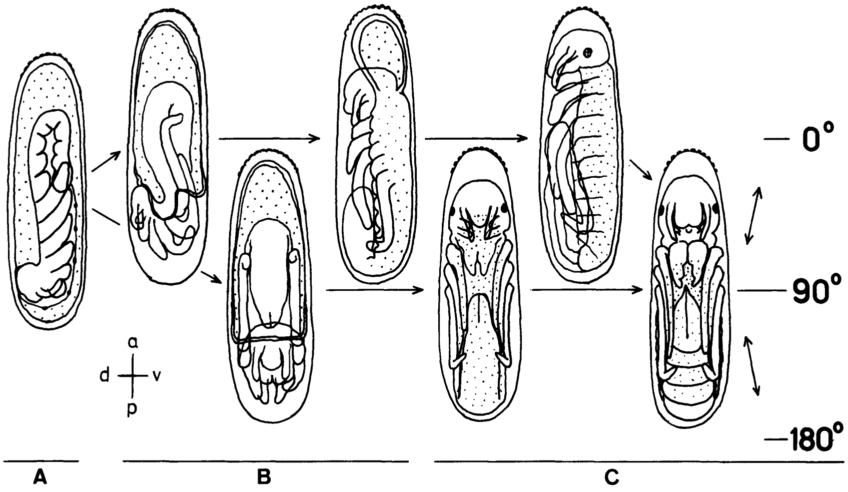

Fig. 7. Schematic expression of rotations for *Epiophlebia superstes* embryos in the eggs free from the host plant. *cf.* Figs. 3, 4.

Analysis of these 16 embryos revealed that 10 (62%) rotated 90°, three (19%) rotated 45° or 150°, and three (19%) rotated 0° or 180° at the last day of embryonic life. The final directions of these embryos revealed that they rotated clockwise (62%), anticlockwise (19%), and 0° and 180° (19%) as a result of rotations. From these data, rotations of the embryos are schematically shown in Fig. 7.

A question arose as whether the same is true for the embryo in the egg inserted in the thallus of *Conocephalum conicum*. To prove this, observations were made on the eggs from the underside of the thallus through a transmittent light. The pre-revolution embryos were in a position as their venter directing toward the underside of the thallus, the same as those occurred in extracted eggs. From this stage onward, 68 eggs were individually followed every day. At a revolution stage, corresponding to Fig. 5 F, 34 embryos (50%) were found to have rotated 90°, of which 24 (67%) were anticlockwise, whereas 10 (36%) were clockwise when viewed from posterior pole of the egg. Two embryos (3%) were non-rotated and 32 (47%) were undetermined because of opacity of the thallus. As development proceeded, all of observable embryos kept their direction unchanged. At a post-revolution stage, corresponding to Fig. 5 H or I, 52 embryos were found to have rotated at 90°, of which 37 (71%) were anticlockwise and 15 (29%) were clockwise (Fig. 8). In later stage, all the observable embryos were rotated at 90°. As compared to the case of extracted eggs, embryos in plant showed somewhat different feature in that 90° rotation occurred as early as revolution stage and further repeated rotations did not occur. These differences may have been caused by presence or absence of surrounding plant tissue which suppressed expansion of the egg space in cross section. This suppressed condition may have brought about rotation during revolution and inhibited rotations during post-revolution period. This point will be discussed later in detail.

Behaviour of the pronymphs, how to escape from the host plant was observed (n=10). After hatching (*cf.* Ando, 1962), all the pronymphs escaped from the thallus through the oviposition pore by peristalsis, without rotation, with their dorso-ventral axis parallel to the plant surface. After escaping from the oviposition pore, the pronymphs performed a twisting movement with the caudal appendages attaching to the oviposition pore. When their head touched to the plant surface, the pronymphs moved there abandoning the caudal support, forming a bridge, and they changed their posture to a state with ventrally flexed abdomen beneath them. After this, the pronymphs rose their head and jumped ahead by sudden extension of the abdomen (*cf.* Tokunaga and Odagaki, 1939) within a second or more. If failed to jump, the pronymphs repeated the same movement until success of jump. From this observation, it was suggested that the 90° rotation of the embryo in the egg would have a perspective advantage to cost the pronymph the lowest energy in forming the bridge just after escaped from the oviposition pore.

Anisoptera

Aeshna nigroflava Martin (Aeshnidae)

Eggs used were laid into wet filter paper by a captive female collected by Mr. S.

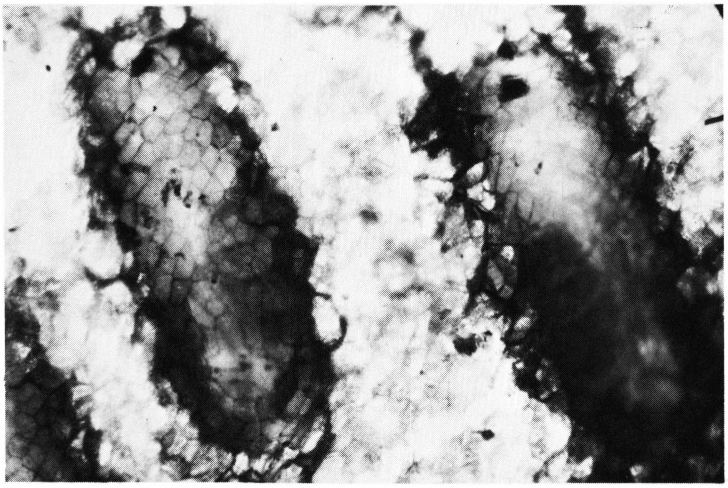

Fig. 8. *Epiophlebia superstes* embryos at 90° rotated from the initial position in the eggs in the host plant, *Conocephalum conicum*, viewed from the underside of the plant. The left embryo rotated clockwise and the right one anticlockwise, in posterior view.

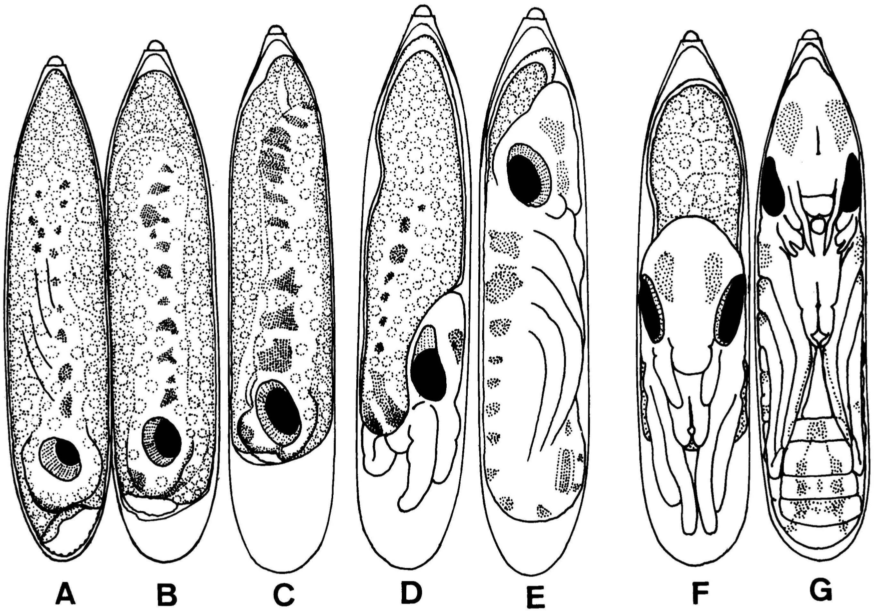

Fig. 9. Development of *Aeshna nigroflava* embryos. A — B and D — E are of the same embryos, respectively. A — E are in the eggs located as their left side upward and F, G are in those located as their dorsal side upward in the watch-glass. All the eggs in upper view. A, 9. XI. 1985; B, 14. I. 1986; C, 17. III. 1986; D, 12. III. 1986; E, 13. III. 1986; F, 28. III. 1986; G, 28. III. 1986. Note that embryos of this species never performed rotation throughout development.

Naraoka on 11. October 1985.

The egg was nearly cylindrical with somewhat pointed anterior pole and a round posterior pole and 1.9—2.15 × 0.4—0.43 mm in size.

Eggs were fixed to a watch-glass, employing the method previously mentioned (n =16). These were divided into two groups, the one fixed in a side-up position, another in a dorsum-up.

As feature of early stage was quite similar to that of *Epiophlebia superstes*, it is omitted here.

The embryos developed and reached a stage before revolution, during first month. In this stage they had developed compound eyes with deep pigmentation and red-brownish spots on each side of the thoracic and abdominal segments. The embryos overwintered in this state (Fig. 9 A, B). Detailed descriptions of hibernating stage will be given later under a separate heading.

After hibernation, the embryos increased in size, and changed bending of their head and abdomen. Pigmented parts of compound eyes and body segments enlarged. In the mean time, yolk decreased in volume, and embryos, as a whole, decreased in size forming a large space at the posterior pole of egg. No rotation occurred during this period. As development proceeded, the embryo-yolk-serosa complex decreased in size further, and the space at the posterior pole enlarged extending to 1/5 egg length. The head apex bent more ventrally and the abdominal bending became acuter (Fig. 9 C). This was the final state of pre-revolution stage of the embryo. No rotation occurred till this stage in all the embryos observed.

Revolution occurred in March of the next year. All the survived embryos (n=14) of the two groups performed revolution along each sagittal plane which was determined at the pre-revolution stage (Fig. 9 DE, FG). Thus, in this species, rotation never occurred with revolution. After rotation the embryos developed also without rotation.

Hatching occurred in a period from mid-March to early April. Although hatching of the pronymphs was not directly observed, directions of the pronymphs at hatching were known from the directions of the split of the chorion, which was made by the egg tooth or egg burster present on the frons of the embryo (*cf.* Ando, 1962). Consequently the split line was corresponding to the mid-ventral line of the embryo. The split lines showed that all the embryos never rotated until hatching.

Orthetrum albistylum speciosum Uhler (Libellulidae)

The egg used were collected from a female captured when ovipositing on a vinyl sheet above the vegetable farm, just after copulation, at Kawagoe, on 17. September 1985 (*cf.* Miyakawa, 1979). In order to observe daily change of the embryos, 22 eggs were transferred to a watch-glass. After half a day they attached to the bottom with their own gelatinous covering in a direction, which, however, was unknown in dorso-ventral polarity. Of these, 21 eggs developed normally, and pronymphs hatched 9 days after oviposition under a mean room temperature of 22℃ (17℃—29℃).

The newly laid egg was ellipsoid and 0.43 × 0.27 mm in size. The egg increased in size to 0.49 × 0.36 mm after development of 7 days (Fig. 10 E). The chorion was elastic, and in some eggs the anterior pole of the chorion was found in a position devi-

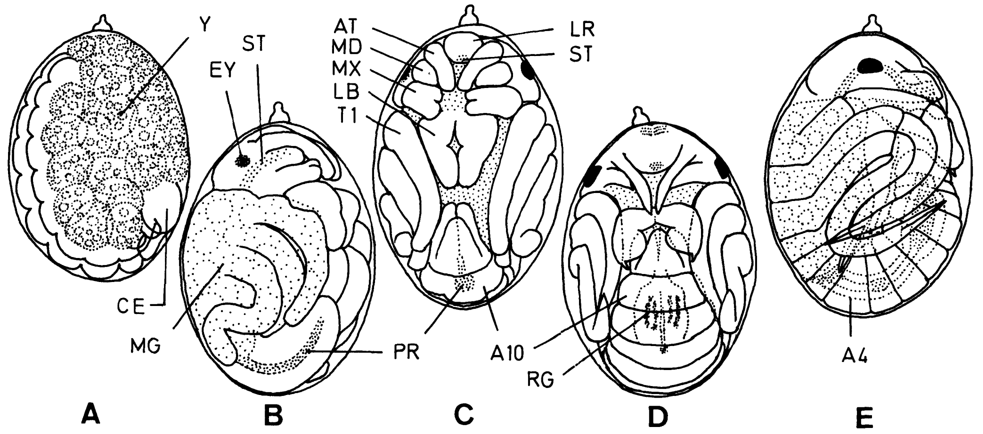

Fig. 10. Development of *Orthetrum albistylum speciosum* embryos in the eggs attached to a watch-glass, all upper view. A, 3 days old; B, 4 days old; C, 4 days old; D, 7 days old; E, 7 days old. Note directions of the embryos. C is in a venter-up position resulted from a gravity dependent rotation. Abbreviations the same as Fig. 2.

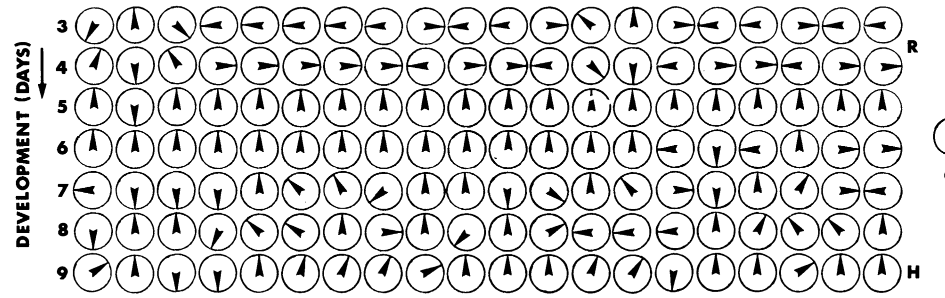

Fig. 11. Rotatograph for *Orthetrum albistylum speciosum* embryos (n = 20). Encircled arrow tops show directions of the ventral side of the embryos. Developmental time is indicated by days after oviposition. G, gravity; H, hatch; R, revolution.

Table 1. Results of upside-down reversal experiment *Orthetrum albistylum speciosum* (n = 160).

Time after reversed	Dorsum-up	Inter-mediate	Venter-up
0 *hr*	148	12	0
12 *hr*	38	37	85
1 *day*	25	44	91
2 *days (7 days old)*	52	74	34

ated from the embryo's long axis (Fig. 10 D).

The germ rudiment was recognized through a transmittent light as a transparent layer on the surface of the yolk at the posterior or postero-lateral part of egg as early as 1 day after oviposition. From these observations, directions of the dorso-ventral axis of the eggs were presumed as to be perpendicular ($n=8$) and nearly horizontal ($n=14$) to gravity, if the blastoderm did not rotate. The germ band, in large part, developed on the periphery of the yolk. The protocorm extended peripherally, passing the posterior pole, to the opposite side of the egg and reached near the anterior pole, from where, however, it extended sinking into the yolk (partially invaginated type, Ando, 1962). The position and direction of the protocephalon relative to the egg was the same as in *Epiophlebia* and *Aeshna*.

On the third day of development, the germ band was segmented (Fig. 10 A). At this stage, 14 of 22 embryos took a position lateral to, seven nearly perpendicular to gravity, and one stopped development.

On the fourth day of development, revolution occurred, but rotation was not observed.

On the fifth day of development, most of the embryos took a position as their ventral side upward ($p < 0.001$) (Fig. 10 C). This venter-up position may have been brought about through rotation probably caused by difference of weight between the embryonic area and the yolk on the one hand, and by size of the embryo-yolk complex smaller enough than the egg space to allow slide of the complex and the chorion on the other.

In order to prove this presumption, other 160 simultaneously laid eggs were reversed upside down at the end of the fifth day of development. The results were as shown in Table 1. From these data, changes of the ratio through the first day after reversed supported the above presumption. The ratio of the second day, however, opposed it. To solve this question, changes of the embryos through development must be considered.

Figure 11 shows the daily change of directions of the 20 embryos of the watch-glass through the development. In 3—4 days after oviposition, most of the embryos lied in a position lateral to gravity ($p<0.01$), but without a significant difference in dextro-sinistral polarity ($p>0.05$). Revolution occurred in this period (Fig. 10 B). In 5—6 days after oviposition, most of the embryos took the venter-up position ($p<0.05$). The embryos of 7 days old became large enough to fill the egg space and their muscles became functionable to change direction of the embryo. Direct observation on some embryos at this stage revealed rotations of this type. Thus, the question arisen in the above experiment was solved.

Hatching occurred on the ninth day after oviposition. When hatching, the pronymphs burst the chorion mid-ventrally and escaped from the egg with peristalsis. Most of the pronymphs, at this time, were in venter-up or latero-venter-up position whereas few were in dorsum-up position ($p<0.01$).

Sympetrum frequens Selys (Libellulidae)

The eggs used were collected from a female captured when ovipositing in tandem on a temporary water at Kawagoe on 30. September 1985. As the shape and other characters of the egg resembled those of *O. albistylum speciosum*, they were treated as same as the former. Important difference from the former was that *Sympetrum frequens* embryo paused its development in winter and hatched in the spring of next year (*cf.* Ando, 1962). This will be decribed later in a separate heading and shown in Fig. 17.

The egg was ellipsoid and 0.56 × 0.32 mm in size just after laid. It increased to 0.60 × 0.41 mm at full-grown stage.

The germ rudiment appeared on the second day of development at the posterior pole of the egg as a transparent area on the periphery of the yolk. This area was narrow in some eggs and wide in others, suggesting that their initial directions of dorsoventral axis were variable as observed in *O. albistylum speciosum*.

On the fourth day, the germ bands became segmented and their directions appeared much certainly. On the fifth day, some laterally lied embryos showed their abdomen extended to a point about 1/5 egg length from the anterior pole of the egg. The remnant of the abdomen was sunk into the yolk. On the seventh day, the proctodaeum was pigmented in red-brownish colour in some precocious embryos. It was this stage that the directions of the 39 embryos were known in certainty. Sixteen embryos lied laterally and 23 embryos lied medially to gravity. On the 15th day, the stomodaeum became red-brownish in precocious embryos, so that these embryos had both stomo- and proctodaeum red-brownishly coloured. On the 22nd day, the embryos reached a stage with compound eyes pigmented, but no sign of revolution appeared yet. Directions of 39 embryos were venter-up (31), intermediate (6) and dorsum-up (2). On the next day they changed 38, 0 and 1, respectively. This type of rotation was quite similar to that of *O. albistylum speciosum*, which, however, occurred in post-revolution stage. Then the upside-down reverse test was carried out, using 80 other eggs of the same age during a period of 28—29. October (28th—29th day).

Figure 12 shows results of the upside-down reverse test. This proved that the rota-

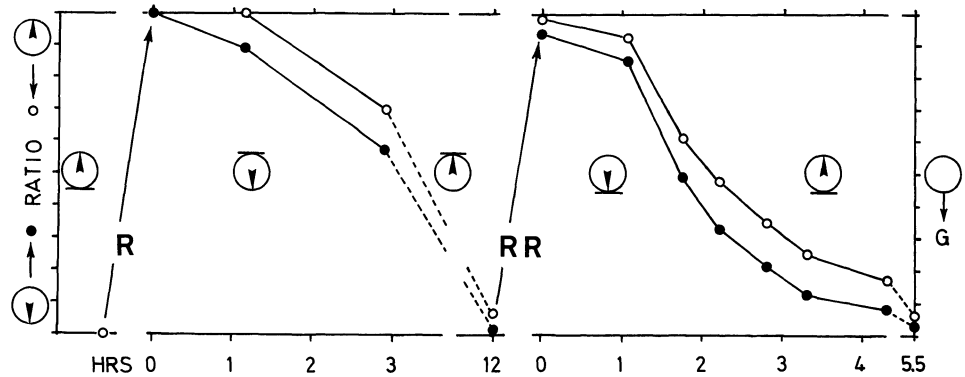

Fig. 12. Results of upside-down reversal experiments in *Sympetrum frequens* egg (n = 80). All the embryos were in a venter-up position before reversal (R). Just after reversal the embryos naturally took a dorsum-up position, but they rotated through *ca.* 180° during half a day to take a venter-up position. Reversal again of the eggs (RR) resulted in further 180° rotation of the embryos during 5. 5 hr to take again a venter-up position.

tion of *S. frequens* embryos was also gravity dependent. But, in this species, the rotation proceeded with slide of the chorion and the serosa with the aid of an egg fluid as a lubricant between them. The 180° rotation in the second turning test (RR) required a period of about 5.5 hr.

Revolution (Fig. 13) occurred in November and December in 82.5% embryos. The remnant embryos revoluted in January to March of the next year. Duration of revolution differed according to temperature. It lasted 2 days at 16℃ (8℃−24℃), but it lasted nearly half a month at 8℃ (1℃−17℃).

Rotations were observed in 62% embryos (n=40) just before, during, and just after revolution. The rotation at this period was apparently different from the gravity dependent nature as mentioned above, because it was divergent in the angle and final direction according to individuals. In post-revolution stage, all the embryos rotated irregularly with their occasional muscular movement.

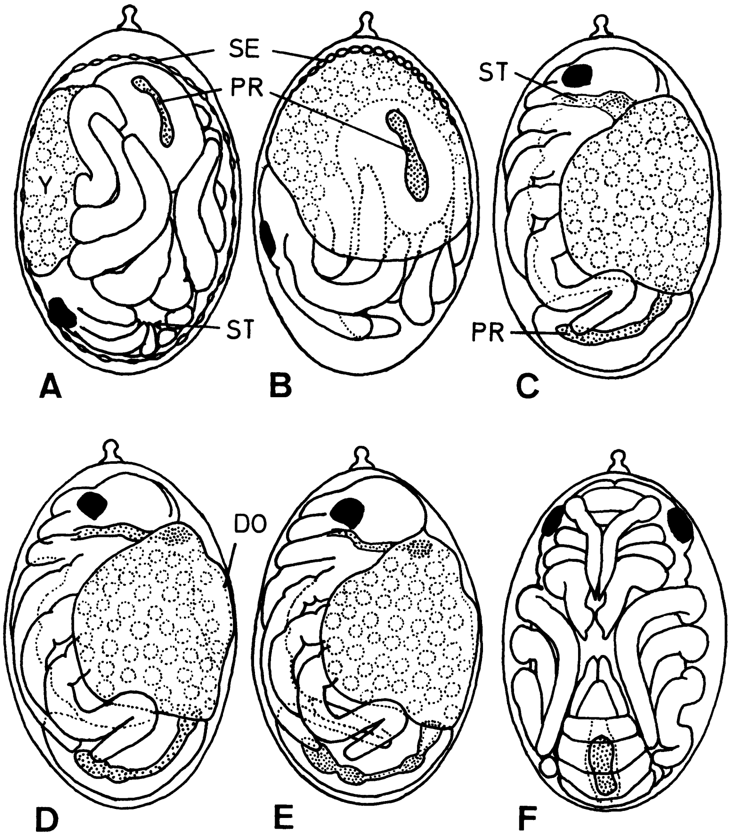

Fig. 13. Positional change of a *Sympetrum frequens* embryo in the egg during a period before and after revolution, upper view. A, 4. XI. 1985; B, 9−10. XI. 1985; C, D, E, 11. XI. 1985; F, 12. XI. 1985. Note that the embryo rotated *ca.* 90° during revolution from a venter-up position (A) and then returned again to the venter-up position (C). Abbreviations the same as Fig. 2.

When hatching, the full-grown embryos or pronymphs burst the chorion antero-ventrally along their mid-line. Examination of chorion's burst line revealed that the positions of the pronymph at hatching (n=28) were venter-up (60.7%), intermediate (35.7%), and dorsum-up (3.6%). Thus, 96.4% of the pronymph escaped from the chorion at directions free from the bottom of the watch-glass on which the eggs attached.

Figure 14 shows, as a summary, rotations of 20 embryos of *S. frequens* during their development. All the embryos simultaneously took the venter-up position through the gravity-dependent rotation at a period after compound eyes had pigmented (E) and before revolution (R). During the course of revolution they rotated variously or did not rotate at all. Later, after muscles had become functionable some of them rotated variously. Finally, almost all the embryos rotated just before hatching.

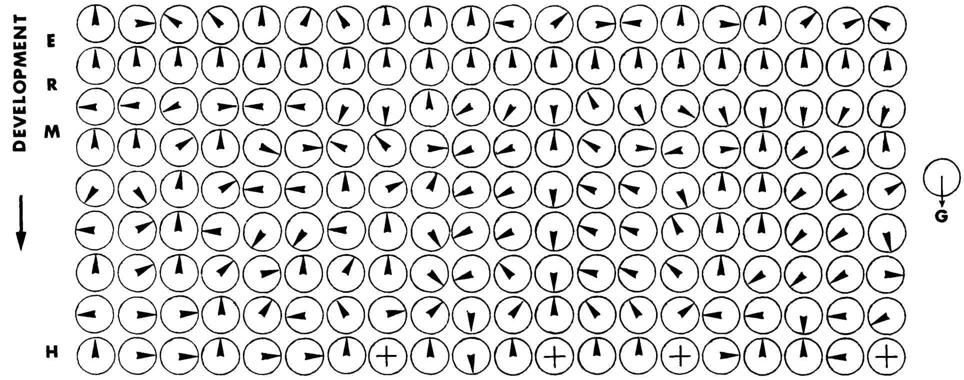

Fig. 14. Rotatograph for *Sympetrum frequens* embryos (n = 20). As this species had a long embryonic period and the proceeding of development widely differed according to individuals, the period from muscles functioned (M) to hatch (H) was divided into six stages at intervals for each embryo. Embryos with a cross symbol did not hatch till 10. IV. 1986. E, stage when eyes pigmented; R, revolution.

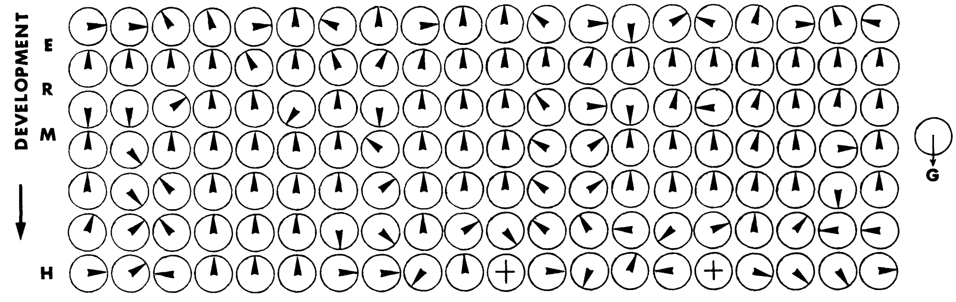

Fig. 15. Rotatograph for *Sympetrum parvulum* embryos (n=20). In this species, a period from M to H was divided into four stages. Other explanations and abbreviations the same as Fig. 14.

As compared to the case of *O. albistylum speciosum*, the time of the gravity-dependent rotation differed. In *S. frequens*, it occurred before revolution whereas in *O. albistylum speciosum*, after revolution. However appearance of both embryos at this period resembled each other in having well-pigmented compound eyes and well-developed body parts. In other words, it may be said that *S. frequens* embryos proceeded much more precocious development relative to occurrence of revolution than *O. albistylum speciosum* embryos.

Sympetrum parvulum Bartenef (Libellulidae)

Egg used were taken by Mr. Y. Arai at Chichibu, Saitama Prefecture on 26. September 1985 and were kept at 25℃ till 14. November 1985. After receipt from him, the eggs were reared under the room temperature as shown in Fig. 17.

The egg was ellipsoid and 0.59 × 0.45 mm in size at a grown pre-revolution stage (Fig. 16). It was thicker than that of *S. frequens*, as seen in the ratio of width/length, 0.76 contrary to 0.68 for the latter.

Figure 15 shows rotations of 20 embryos through development. The first line of Fig. 15 shows directions of the embryos on 17. November 1985. They were at a stage with rudimentary segmental appendages and faintly coloured rudimentary proctodaeum (Fig. 16 A). As is seen in Fig. 15 the embryos directed variously to gravity. After a week the embryos reached a stage with pigmented compound eyes and developed appendages (Fig. 16 B). At this stage all the embryos lied in the venter-up position

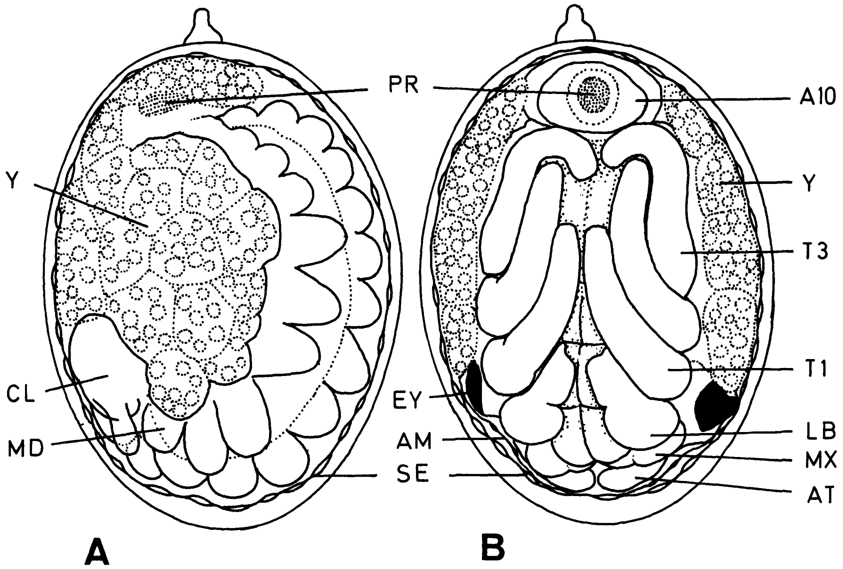

Fig. 16. Positional change of a *Sympetrum parvulum* embryo in pre-revolution stage. A, laterum-up position, 17. XI. 1985; B, venter-up position, 24. XI. 1985. Abbreviations the same as Fig. 2.

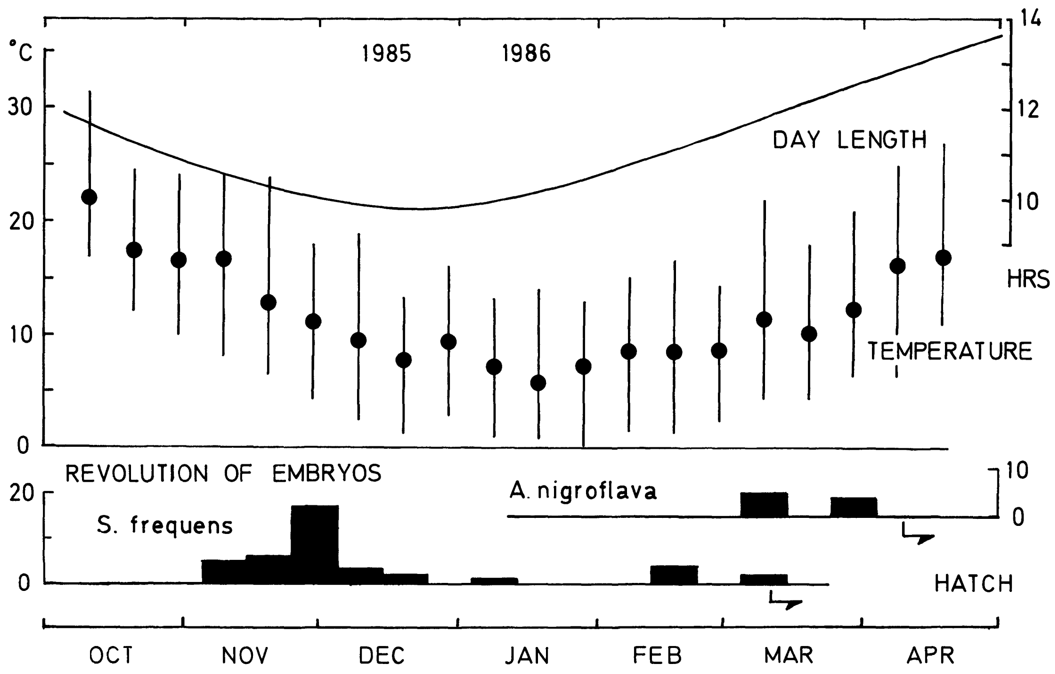

Fig. 17. Seasonal range of revolution and hatch in the hibernating embryos of *Aeshna nigroflava* (oviposition: 11. X. 1985) and *Sympetrum frequens* (ovipos. : 30. IX. 1985) with the environmental factors, temperature and photoperiod. Note that *S. frequens* embryos, developed in the eggs simultaneously laid from the same mother, showed a wide range in time of revolution, whereas *A. nigroflava* embryos had a short range of revolution time in spring.

similarly to *S. frequens* embryos of the same age.

The embryos overwintered in a pre-revolution stage keeping the venter-up position.

Revolution occurred in a period from 1. February 1986 to 28. March 1986. Just after revolution, a considerable number of embryos (75%) turned at 90° — 180° (Fig. 15, line 3). This number was significantly higher than in *S. frequens* at the same stage ($p < 0.001$). The same was true for a subsequent stage (Fig. 15, lines 4, 5) till just before hatching. In this period, 70 — 80% of embryos usually took the venter-up position, whereas in corresponding period of *S. frequens* only 20 — 30% of the embryos did so (Fig. 14, lines 4 — 7). This feature of *S. parvulum* embryo resembles rather *O. albistylum speciosum* one than *S. frequens* one.

Hatching occurred in a period from 12. March to 5. April 1986. When hatching, all the embryos ($n = 18$) took their position at least 30° apart from gravity where their egg shells attached on the bottom of the glass.

Development of hibernating embryos of Aeshna and Sympetrum

Aeshna nigroflava embryos overwintered in a pre-revolution stage (Fig. 17) as previously stated by Ando (1962). The embryos did not exhibit any major morphological changes from 9. November 1985 to 10. January 1986 (Fig. 9 A). A remarkable change appeared on 14. January 1986 in the cephalic region of the embryo (Fig. 9 B). In this region, the serosa commenced shrinkage and the head bent somewhat ventrally. This change occurred at the minimum temperature of the season (*ca.* 5.5℃ in average) while the day length was increasing. Then, the embryos developed to prepare revolution for 1.5 months under a mean temperature of 8℃ (0.5℃ − 17℃). Revolution occurred in March at 11℃ (4.5℃ − 18℃). Hatching began 10. April and ended 21. April 1986. The temperature of this period was 16℃ − 17℃. The post-revolution period of the embryos, therefore, was a month or less.

Sympetrum frequens embryos performed revolution in November and December 1985 (82.5%) during which the day length was decreasing to the minimum. This agrees with Ando's result (1962). However remnant of the embryos (17.5%) overwintered in a pre-revolution stage and they did revolution in January to March of the next year (Fig. 17). Of 40 embryos, 28 (70%) hatched by 10. April 1986. No difference of haching rate was found between the two groups which were revoluted in 1985 (70%) and in 1986 (71%).

Sympetrum parvulum embryos overwintered exclusively in a pre-revolution stage, and they performed revolution in February and March of 1986. Of 20 embryos examined, 18 (90%) hatched in a period from 12. March to 5. April 1986.

Seasonal type of the embryonic development of *S. parvulum*, therefore, is similar to that of the latter group of *S. frequens* and somewhat resembles that of *A. nigroflava.* Hibernating stage of *S. parvulum* embryos, however, was much more advanced (Fig. 16 B) than that of *A. nigroflava* embryos (Fig. 9 A). It must be added here that *A. nigroflava* embryos did not completely pause development during hibernation period. Minor changes are detectable in development of compound eyes and segmental patches (*cf.* Fig. 9 A, B). Among these three species, *S. frequens* seems to have the most plastic nature in response to environmental factors, the temperature and the photoperiod.

Common character of the hibernating embryos of *Aeshna* and *Sympetrum* is seen in a precocious development of some ectodermal organs, such as the compound eyes, the segmental patches and the stomo- and proctodaeum, which reach an advanced stage before revolution. This was never observed in other non-hibernating species so far known.

Discussion

Position and orientation of the germ rudiment in the insect eggs

In hemimetabolous insect eggs, the germ rudiment differentiates from the blastoderm in a definite region, usually near the posterior pole of the egg as an area composed of thicker cells. The cells then aggregate to the prospective ventral midline of the embryo and form the vermiform germ band with elongation of the protocorm invaginating into, or on the surface of the yolk. Its antero-posterior axis, at least in the protocephalic region, agrees with the antero-posterior axis of the egg (*cf.* Ando, 1962, 1970; Cobben, 1968; Anderson, 1972). The dorso-ventral polarity of the germ disc, however, relative to that of the egg has previously been paid little attnetion, when the egg was dorso-ventrally symmetric.

Brandt (1869) showed the fine figures of *Calopteryx virgo* germ rudiment (his Figs. 4—6, on Pl. 1). Judged from the dorso-ventrally asymmetric egg of the species, the germ rudiment was apparently formed on the dorsal side of the egg. Seidel (1929) demonstrated clearly that the germ rudiment of *Platycnemis pennipes* was formed on the dorsal side of the egg (his Fig. 10). The same was true for zygopteran *Cal. atrata* and *Cer. sieboldii*, anisozygopteran *E. superstes*, and anisopteran *A. nigroflava* of Odonata. These facts show that in the primitive taxa of Odonata the germ rudiment is formed on the dorsal side of the yolk surface relative to the egg axis.

Cobben (1968), in Heteroptera, found that there were many positional types of the germ band, but the early germ band was formed usually on the dorsal side of the egg (his Fig. 276). If the above observations not exceptional, it can be said that in primitive taxa of both hemimetabolous orders the germ rudiment, or early germ band, is formed on the dorsal side of the egg with orientation as its anterior end (protocephalon) pointing to the anterior pole of the egg.

On the other hand, Cobben found that in heteropteran Miridae, Acantosominidae, Tingidae and probably also Cydrinidae, the early germ band was formed on the lateral side of the egg. In these cases the dorso-ventral axis and position of the germ band were about 90° rotated from the usual ones. Similar cases were found also in holometabolan insects. According to Okada (1960), the germ band was formed in various positions in cross section of the flattened ellipsoid egg of *Chilo suppressalis* (Lepidoptera), *i. e.* the mid-line of the germ band was located on a flattened side of the egg in some examples, but it was located on a edged side of the egg in the others (his Text-Fig. 2). In thrichopteran *Stenopsyche griseipennis*, the germ rudiment was formed on a dorso-lateral or ventro-lateral side of the egg which attached to the stone in water as its ventral side directing to the stone surface when oviposition (Miyakawa, 1973).

Before going further, Hallez's law must be touched here. Cobben (1968) quoted Hallez's original French description (1886) and discussed on the law in detail. From his quotation, Hallez's law can be summarized as follows: There are exact agreements in their principal (cephalo-caudal and dextro-sinistral) axes between the mother and the egg on the one hand, between the egg space and the embryo on the other. According to Cobben, Hallez found his law based on *Tettigonia veridissima* (Orthoptera), *Hydrophilus piceus* (Coleoptera) and others, and his observations were probably made on their ripe eggs. Cobben's modification of Hallez's law, applied to Heteroptera, is as follows: The orientation of the longitudinal and transverse axes of the released egg shell and the enclosed embryonic rudiment correspond with those of the depositing mother, unless eggs are rotated during delivery or the longitudinal axis is reversed in relation to that of the female by tumbling.

In my present view, the position and orientation of the germ rudiment, in primitive taxa of Hemimetabola, seem quite reversed dorso-ventrally to the egg axis. But, axes of the germ rudiment and the egg correspond antero-posteriorly. This assumption, and above quoted facts, led me to have an idea that the current understanding of difference between lower taxa of hemimetabolous and higher taxa of holometabolous germ rudiments in the position and orientation relative to the egg may have been arisen by shift from dorsal to ventral, through rotation, on the one hand, and from invaginated to superficial, relative to yolk, on the other (*cf.* Johannsen and Butt, 1941; Ando, 1962; Cobben, 1968). At present time we have no information to support this assumption concerning the dorso-ventral polarity of the chorion and yolk system in oogenesis stage (Mahowald, 1972), nor reliable information on rotation of the egg during oviposition. Recent review by Counce (1973) on the causal analysis of insect embryogenesis gave an account, quoting Seidel's results on *Platycnemis* (1929), that differentiation center induces the germ rudiment formation in a restricted area of yolk periphery. Her account, however, did not give any information on relationship between the blastoderm-yolk system and the chorion concerning their dorso-ventral axis.

Finally, the term "ventral plate" must be discussed here. This is used as an equivalent to the germ rudiment, germ primordium or pregerm, *i. e.* a thickened area of blastoderm to form future embryonic body. This term involves the polarity in itself, showing dorso-ventral axis of the plate and it is quite reasonable for itself. However if my present assumption based on the egg shell is adopted, the ventral plate is formed on the dorsal side of the egg in primitive taxa of Odonata and also in primitive Heteroptera.

Rotations of the odonate embryos in further development

From the germ band stage onward, the embryonic period is here divided into three stages, since mechanics of rotation seem to differ according to stages:

(A) Anatrepsis stage — during which basic pattern of organs, except those of dorsal region, is formed in a condition enclosed with the amnion and serosa.

(B) Katatrepsis (revolution) stage — during which the amnion and serosa (embryonic envelopes or membranes) rupture, and the embryo turns dorsoventrally to acquire its position as its head points the anterior pole of the egg, enclosing the yolk on its back.

(C) Dorsal closure of the embryo to hatching.

In Heteroptera Cobben (1968) found that there were rotations, if not all, in the above stages A, B, C and he named them band rotation, embryonic rotation or spiral, and prolarval rotation, respectively. Most of the rotations were through 180°, but the band rotation (A) was limited to 90°, and the prolarval rotation (C) was irregular and through several angles. He considered that the embryonic rotation (B) was brought about principally by intrinsic action of the serosal-amniotic complex, but the other rotations (A and C) were of a quite different nature.

In Odonata, rotations were found also in all the stages A, B, C, but the rotation in so early stage as when the germ band started metamerism or limb buds just appeared, was not observed.

In Zygoptera, all the embryos performed a 180° rotation in the dorso-ventrally asymmetric egg, but feature of the rotation was different according to individuals. The rotation took place in most cases during stages A—B and in some cases during stages B—C. Seidel's statement on the rotation in *P. penippes* (1929) is the former case. These are separately considered as follows: (1) Rotation, which occurred in a period bridging stages A and B, seems probably to be caused by a bilaterally asymmetric fusion of amnion and serosa above the embryonic head, which brings about a twist of the embryo when the serosa, amnion and yolk system constrict to prepare and complete the revolution (Fig. 2). This type of rotation was previously suggested by Cobben (1968) in saldid Heteroptera. (2) Rotation, which began in stage B, seems probably to be caused by a bilaterally asymmetric constriction of the serosa and yolk system in major part and change of form of the embryo in minor. The latter resembles the next item. (3) Rotation, which began in stage C, seems to be caused by irregular and partial changes of form of the embryo by occasional muscular movements of trunk region to fit the embryo in the dorso-ventrally asymmetric egg space.

Thus in Zygoptera so far known, occurrence of a 180° rotation is certain, though time and cause of rotation are various. The full-grown embryo lies in orientation corresponding to that of mother insect (*cf. Calopteryx virgo*: Degrange, 1974).

Brandt's (1869) Figs. 1 – 19 on the embryonic development of *Calopteryx virgo* are errorneously referred to in text books (Tillyard, 1917, Fig. 109 A – K; Imms *et al.*, 1971, p. 330). Brandt illustrated them quite exactly in right side view of the embryo (not the egg !), but he did not mention on occurrence of the rotation. Judged from the dorso-ventrally asymmetric shape of the egg and orientation of the embryo in his figures, the 180° rotation apparently occurred in a stage between Fig. 14 and Fig. 15 similarly to the case of *C. atrata* as shown in Fig. 2.

In Anisozygoptera, *Epiophlebia* embryos did not rotate in stage A, but they turned more or less 90°, clockwise or anticlockwise, by the end of embryonic period as a result of a 90° rotation in stage B and/or later irregular rotations in stage C. This feature was somewhat different between the eggs in the plant tissue and those extracted from it. In the former case, almost all the embryos performed a 90° rotation at stage B, but did not show further rotation in stage C (Fig. 8), whereas in the latter, rotations took place in both stages B and C (Figs. 6, 7). This difference may be attributed to: (1) Radial symmetry of the egg, which unlike zygopteran eggs, never restricts the final orientation of the embryo during rotations in stage C. Rotation in stage C takes place more easily in the unnaturally expanded chorion of the egg free from plant tissue. (2) In the expanded egg, on the contrary, the connection of the chorion and the serosa loosens and allows more easily a contradictory rotation when a 90° rotation takes place during stage B.

The 90° rotated position of the *Epiophlebia* embryo may have an adaptive value for subsequent movements of the hatched pronymphs on the plant surface to reach the water, as described previously.

In Anisoptera, there are two types of oviposition, the endophytic and the exophytic. The true endophytic oviposition is seen in only Aeshnidae in the suborder. Aeshnid eggs morphologically and ecologically resemble those of Zygoptera and Anisozygoptera and therefore are suitable to compare with them.

Aeshna nigroflava embryos never performed rotation throughout the embryonic period. Thus, after revolution, the embryos took a position dorso-ventrally 180° reversed to the egg axis. This is only the case among the odonate embryos so far studied. Orientation of the full-grown *A. nigroflava* embryos, therefore, differ 90° from that of anisozygopteran *Epiophlebia* embryos and 180° from that of zygopteran embryos.

Libellulid eggs are deposited exophytically. The dorso-ventral axis was unable to be determined as stated previously. Consequently these are not comparable to those of above mentioned odonate taxa. However libellulid embryos exhibited a different type of rotation. Embryos of *Orthetrum* and *Sympetrum* performed a gravity dependent rotation before or after the revolution stage. This type of revolution exclusively resulted in the venter-up position of the embryo, probably caused by difference of the weight of the embryo and the yolk (Figs. 10—16). This is apparently different from rotations as seen in zygopteran and anisozygopteran embryos. This may be attributed to the superficial position of the embryo, and the shape and space of the egg shell which touches loose the embryo during this stage. Other than this rotation, the libellulid embryos performed also the rotation during stage B through various angles according to individuals. This is regarded as similar to the case of other odonate taxa.

In conclusion, to establish the exact relationship among the three suborders in this respect, much more aeshnid species must be examined in future study. Furthermore, it is needed to find a method to determined the exact dorso-ventral axis of newly laid egg in exophytic odonate taxa, including primitive Gomphidae, Petaluridae and Cordulegasteridae on the one hand, and also to examine Lestidae which have a habit of endophytic oviposition into the terrestrial plants like *Epiophlebia* on the other, for completion of a comparative study on the rotation of odonate embryos from phylogenetical and ecological points of view.

Acknowledgements

I am greatly indebted to Prof. Dr. H. Ando, Sugadaira Montane Research Center, University of Tsukuba, for his kind help in various ways to proceed this work and critical reading of the manuscript. My cordial thanks are also due to Dr. R. H. Cobben, Agricultural University of Wageningen, Netherlands, for his kind gift of his monograph, and to Mr. Y. Arai, Yorii, Saitama, for his helpful gift of good number of living aeshnid and sympetrine eggs.

References

Anderson, D. T., 1972. The development of hemimetabolous insects. *In* S. J. Counce and C. H. Waddington (eds.), Developmental Systems: Insects, Vol. 1, 95-163. Academic Press, London, New York.

Ando, H., 1962. The Comparative Embryology of Odonata with Special Reference to a Relic Dragonfly *Epiophlebia superstes* Selys. Jpn. Soc. Promot. Sci., Tokyo.

————, 1970. Embryonic development (in Japanese). *In* T. Uchida (ed.), Systematic Zoology, Vol. 7-III-A, Arthropoda IIIa, Insecta I, 37-130. Nakayama-shoten, Tokyo.

Brandt, A., 1869. Beiträge zur Entwicklungsgeschichte der Libelluliden und Hemipteren mit besonderer Berücksichtigung der Embryonalhülle derselben. *Mém l'Acad. Imp. Sci. St. Pétersburg, Sér.* 13: 1-33.

Cobben, R. H., 1968. Evolutionary trends in Heteroptera. 1. Eggs, Architecture of the Shell, Gross Embryology and Eclosion. Ctr. Agr. Publ. Docum., Wageningen.

Counce, S. J., 1973. The causal analysis of insect embryogenesis. *In* S. J. Counce and C. H. Waddington (eds.), Developmental Systems: Insects, Vol. 2, 1-156. Academic Press, London, New York.

Degrange, C., 1974. L'oeuf et l'eclosion de *Calopteryx virgo* L. (Odonata, Zygoptera, Calopterygidae). Considerations generals sur l'eclosion des larves des odonates. *Trav. Lab. Hydrobiol. Grenoble* 64/65: 269-286.

Haga, K., 1985. Oogenesis and embryogenesis of the idolothripine thrips, *Bactrothrips brevitubus* (Thysanoptera, Phlaeothripidae). *In* H. Ando and K. Miya (eds.), Recent Advances in Insect Embryology in Japan, 45-106. Arthropod. Embryol. Soc. Jpn. (ISEBU Co. Ltd., Tsukuba).

Hallez, P., 1886. Loi de l'orientation de l'embryon chez les insectes. *C. R. Hebt. Séan. Acad. Sci. Paris* 103: 606-608. (Cited by R. H. Cobben, 1968.)

Imms, A. D., O. W. Richards and R. G. Davies, 1977. Imms' General Textbook of Entomology (10th ed.), Vol. 1. Chapman & Hall, London.

Johannsen, O. A. and F. H. Butt, 1941. Embryology of Insects and Myriapods. McGraw-Hill, New York, London.

Krause, G., 1939. Die Eitypen der Insekten. *Biol. Zbl.* 59: 495-536.

Mahowald, A. P., 1972. Oogenesis. *In* S. J. Counce and C. H. Waddington (eds.), Developmental Systems: Insects, Vol. 1, 1-47. Academic Press, London, New York.

Miller, A., 1939. The egg and early development of the stonefly, *Pteronarcys proteus* Newman (Plecoptera). *J. Morphol.* 64: 555-609.

Miyakawa, K., 1973. The embryology of the caddisfly *Stenopsyche griseipennis* MacLachlan (Trichoptera: Stenopsychidae). I. Early stages and changes in external form of embryo. *Kontyû* 41: 413-425.

————, 1979. Vinyl shield of vegetable farm as a releaser of dragonfly reproductive behaviour (in Japanese with English summary). *Tombo* 22: 24-26.

————, 1982. Reproductive behaviour and life span of adult *Calopteryx atrata* Selys and *C. virgo japonica* Selys (Odonata: Zygoptera). *Adv. Odonatol.* 1: 193-203.

Okada, M., 1960. Embryonic development of the rice stem-borer, *Chilo suppressalis*. *Sci. Rep. Tokyo Kyoiku Daigaku, Sec. B* 9: 243-296.

Sander, K., 1956. The early embryology of *Pyrilla perpusilla* Walker (Homoptera), including some observations on the later development. *Aligarh Musl. Univ. Publ. (Zool. Ser.), Indian Insect Types* 4: 1-61.

Seidel, F., 1929. Untersuchungen uber das Bildungsprinzip der Keimanlage im Ei der Libelle *Platycnemis pennipes*. *Roux Arch. EntwMech. Org.* 119: 322-440.

Slifer, E. H., 1932. Insect development III. Blastokinesis in the living grasshopper egg. *Biol. Zbl.* 52: 223-229.

Striebel, H., 1960. Zur Embryonalentwicklung der Termiten. *Acta Trop.* 17: 193-260.

Tamiya, Y. and K. Miyakawa, 1984. On the oviposition habitat of *Epiophlebia superstes* (Selys) (Anisozygoptera: Epiophlebiidae). *Odonatologica* 13: 301-307.

Tillyard, R. J., 1917. The Biology of Dragonflies (Odonata or Paraneuroptera). Cambridge Univ. Press, Cambridge.

Tokunaga, M. and S. Odagaki, 1939. Ecological and morphological studies on a dragonfly, *Epiophlebia superstes* Selys (in Japanese). *In* Vol. Jubil. Prof. S. Yoshida, 472-496. Osaka.

Weber, H., 1954. Grundrisse der Insektenkunde. Gustav Fischer, Stuttgart.

Wheeler, W. N., 1893. A contribution to insect embryology. *J. Morphol.* 8: 1-160.

Author's address: Dr. K. Miyakawa
Imafuku 1024, Kawagoe, Saitama 356,
Japan