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Percent Developmental Time (%DT); an Index to Indicate Numerically Sequence of Events Occurred in the Course of Embryogenesis in Insects

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Synopsis

Percent developmental time (% DT) is an index which indicates numerically chronological sequence of the events occurring during the embryogenesis in insects. The time of occurrence of an embryonic event in reference to developmental time may be expressed in percentage of the entire developmental time, with 0 % DT at oviposition.

By selecting events for % DT calculation, the author laid emphasis on; 1, morphogenetic movement which seems to have special meaning on organogenesis, and 2. histological differentiation occurring within the embryos. Information necessary to calculate % DT of these events was collected from the works on the embryogenesis of several insects belonging to hemipteroid and orthopteroid orders.

The author suggests that introduction of % DT is rewarding and helpful to reveal basic developmental program which may likely govern insect embryogenesis, and may further shed more lights on the understanding of evolution of Insecta.

Introduction

Previously published descriptive works on insect embryology used to give chronological information on the events with the style that such and such an event may be observed to occur some hours or days after oviposition (Anderson, 1972a, 1972b). On the time-table of embryogenesis appeared in these articles several embryonic events were seen to occur "collectively" at a certain date after oviposition; detailed chronological sequence of the events in due course of the embryogenesis however may not usually be known. Furthermore, cultural conditions under which these eggs were observed were different from material to material and from author to author. As the situation being so, comparison of these information may not so easily be done especially when one wishes to reveal basic developmental program which may likely govern the embryogenesis of taxonomically different materials.

The author therefore intends to introduce an index, percent developmental time (% DT) to indicate numerically chronological sequence of the events occurring during the course of embryogenesis. The time of occurrence of certain embryonic event in reference to developmental time may be expressed in percentage of the entire developmental time, with 0 % at oviposition and 100 % at hatching.

An attempt to indicate numerically the occurrence of the embryonic events in relation to developmental time was however first appeared as early as in 1960 (Striebel,); his idea is exactly the same as that of the present author, who proposed the term, % DT. Striebel (1960) compared the embryogenesis of two species of Isoptera (*Kalotermes flavicollis* and *Zootermopsis nevadensis*), two species of Orthoptera (*Tachycines asynamorous* and *Gryllus domesticus*), one species of Odonata (*Platycnemic pennipes*), and indicated that chronological sequence of the events was identical among the materials compared, but % DT of each event was sometimes considerably different from material to material.

The author believes that Striebel's idea is very important and promising as an approach to reveal basic developmental program. However, insect embryologists seem to have paid very little attention to his work, and further attempt to consider the insect embryogenesis from this viewpoint was not known so far (see also Bentley *et al.* 1979). In this article the author will attempt to indicate quantitatively diversities observed in the embryogenesis of insects belonging to hemipteroid as well as orthopteroid orders. Information necessary to calculate % DT of several embryonic events was collected from the works by previous authors. Striebel (1960) seemed to have put emphasis on gross morphological features of the embryos on selecting the embryonic events to calculate % DT, whereas the present author laid more emphasis on; 1. morphogenetic movements which seem to have special meaning on organogenesis, and 2. histological differentiation occurring within the embryos.

Embryonic events chosen in this article are;

1. inner layer formation; this indicates interblastemic differentiation in the germ band (GB) resulting in mesoderm formation.
2. stomodaeum formation; this morphogenetic movement occurred in the ectoderm indicates the beginning of alimentary canal formation from the anterior end of the embryo in most insects.
3. proctodaeum formation; this indicates the beginning of the alimentary canal formation from the posterior end of the embryo.
4. neuroblast differentiation; this indicates intrablastemic differentiation within the ectoderm, which may lead to the ventral nerve cord formation.
5. katatrepsis; this indicates rupture of the extraembryonic membranes and subsequent movement of the embryos over the posterior egg pole.
6. yolk engulfment; this indicates zipper-like fusion of the external epithelium of the embryos has completed.

Results and Discussion

1. Percent developmental time of embryonic events observed in several hemipteroid insects

Information of the embryogenesis of four species belonging to four families of Heteroptera, one species of Homoptera and one species of Psocoptera was analyzed. Besides the em-

bryonic events noted in introduction, invagination of the blastoderm and cardioblast differentiation were employed; the former event indicates the beginning of GB formation, and the latter event shows intrablastemic differentiation occurred within the mesoderm which leads to the dorsal vessel formation. It should be here noted that % DT may fluctuate considerably for various reasons, the most significant one of which is interval of observation. Long observation interval such as 24 hr may not give us accurate information necessary to calculate correct % DT when duration time of the embryogenesis was from five to seven days; for this reason observation on *Pyrrhocoris* (Seidel, 1924) was not included in this article.

As seen in Table 1, chronological sequence of the embryonic events occurring in these hemipteroid insects is identical. Inner layer formation was seen to occur following invagination, and neuroblast differentiation prior to stomodaeum formation; this was also true in case of *Gerris.* In *Gerris* the neuroblasts were seen to appear in the ectoderm within six hr (25°C) after embryonic rotation around the longitudinal egg axis which occurred at about 48 hr (20°C) after oviposition, and stomodaeum formation occurred subsequently, between six and nine hr (25°C) after embryonic rotation. This indicates that % DT of neuroblast differentiation in *Gerris* is not smaller than 17.4. The same sequence of neuroblast defferentiation and stomodaeum formation may also be seen from observation on *Oncopeltus* (Bütt, 1949). However, in *Pyrilla* (Sander, 1956) no conclusive evidence was obtained, which indicates that the neuroblast differentiation occurred prior to stomodaeum formation. Ectodermal cells in *Pyrilla* that are destined to become the neuroblasts were already observed in 35-hr embryo, but the cell division which actually produced the neuroblasts seemed to have occurred by 41 hr, whereas the stomodaeum was already formed in 41-hr embryo.

Although % DT diversity of neuroblast differntiation and that of stomodaeum formation were insignificantly small throughout these hemipteroid insect embryos, invagination of GB in *Gerris* was seen to occur earlier than that in other hemipteroid insects; this means that comparatively long time is necessary in *Gerris* embryos from invagination to neuroblast differentiation. This may be as due to either the rate in the developmental speed in *Gerris* embryos has suddenly reduced after inner layer formation, or a short quiescent period of development has included in the course of the embryogenesis.

The author is now inclined to support this second interpretation for follwing reason.

Embryonic rotation around the longitudinal egg axis in *Gerris* which occurred almost immediately before neuroblast differentiation is the type of morphogenetic movement which is not known to occur in other hemipteroid insect embryos chosen in this article. This embryonic movement in *Gerris* began at about 40 hr (20°C) after oviposition (% DT = 14.5) and finished within next six to eight hr (% DT = 17.4); no significant histological differentiation was seen to occur during this embryonic movement (Mori, 1969). Thus it is very likely that this embryonic rotation is the quiescent period specifically included in the developmental program of *Gerris* embryogenesis. A posture taken by the embryo at the time of hatching might have necessitated this morphogenetic movement, but true reason of inclusion of this step in the course of *Gerris* embryogenesis is not yet known.

The cardioblast differentiation which may lead to dorsal vessel formation was an embryonic event which occurred considerably later than the neuroblast differentiation. In *Gerris* the cardioblasts were seen to differentiate from the somatic mesoderm immediately before katatrepsis; this was also true in the case of *Hygia* (Unpublished observation). Although % DT of the cardioblast differentiation obtained in these embryos was not so close with each

Table 1. Percent developmental time of embryonic events observed in several hemipteroid insects.
* % DT may not be calculated as the time of hatching was not noted.
** % DT is underestimated (see also text).

Order	Hemiptera-Heteroptera				Homoptera	Psocoptera
Family	Lygaeidae	Reduviidae	Coreidae	Gerridae	Fulgoridae	Liposcelidae
Genus/species	*Oncopeltus fasciatus*	*Rhodnius prolixus*	*Hygia opaca*	*Gerris paludum*	*Pyrilla perpusilla*	*Liposcelis divergens*
Author(year)	Butt(1949)	Mellanby(1936)	Mori(Unpub.)	Mori(1969)	Sander(1956)	Goss(1953)
Temp. (°C)	20	21	25	20	31	31
Invagination	(35)*	11.5	—	8.9	12.7	14.3
Inner layer formation	(54)	12.4	13.2	11.1	16.2	16.1
Neuroblast differentiation	(59)	17.2	17.5	+17.4**	+18.9**	18.5
Stomodaeum formation	(65)	20.7	21.3	+17.4	+21.6	+21.4**
Proctodaeum formation	(65)	24.1	21.3	+17.4	+21.6	+21.4
Cardioblast differentiation	—	44.8	42.3	34.8	—	—
Katatrepsis	(100)	39.7	46.1	39.9	42.2	53.0
Yolk engulfment	(110)	51.7	53.8	47.7	53.0	59.5

Table 2. Percent developmental time of embryonic events observed in several orthopteroid insects.

Order	Orthoptera		Isoptera	
Family	Acrididae	Rhaphidophoridae	Kalotermitidae	Termopsidae
Genus/species	*Locusta migratoria*	*Tachycines asynamorus*	*Kalotermes flavicollis*	*Zootermopsis nevadensis*
Author(year)	Roonwal(1937)	Ibrahim(1957)	Striebel(1960)	Striebel(1960)
Temp.(°C)	33	26	26	26
Inner layer formation	13.5	13.0	12.9	12.5
Stomodaeum formation	16.7	24.0	31.5	35.7
Proctodaeum formation	18.9	—	44.4	—
Neuroblast differentiation	20.5	29.7	40.7	—
Katatrepsis	46.2	59.5	57.5	56.0
Yolk engulfment	69.3	70.3	74.0	73.5

other, the time from the occurrence of cardioblast differentiation to katatrepsis was almost the same for both species. In *Rhodnius* the cardioblasts were said to differentiate after katatrepsis had began (Mellanby, 1936), but this information should be reconfirmed. In passing, the cardioblasts in *Locusta* (Orthoptera : Acrididae) were also seen to differentiate before katatrepsis (Roonwal, 1937)*.

All of these hemipteroid insect embryos undergo katatrepsis; only those embryos which underwent katatrepsis successfully are allowed to have larval structure with normal three-dimensional sequence. Experimental suppression of katatrepsis has resulted in formation of the everted embryos, in which three-dimensional sequence among the embryonic tissues was reversed (Ando, 1955; Mori, 1975). Percent developmental time of this important morphogenetic movement in these hemipteroid insect embryos distributed from 39.7 to 53.6 and that of yolk engulfment from 47.7 to 59.5. This seems to indicate that the rate in the developmental speed of the embryos has become different from material to material after the stomodaeum formation had occurred, but entire duration time from katatrepsis to yolk engulfment was approximately the same throughout these hemipteroid insect embryos.

This study of % DT of several embryonic events occurring in these hemipteroid insects may suggest that they are embryologically rather uniform group. That is, they share basic developmental program and only minor modifications of the program could be observed, which are species-specific; the different rate in the developmental speed from one embyonic event to another may also reflect embryological species-specificity.

* % DT = 35.8

2. Percent developmental time of embryonic events observed in several orthopteroid insects

Information on the embryogenesis of two species belonging to two families of Orthoptera and two species belonging to two families of Isoptera was analyzed (Table 2).

Comparison of % DT information of the two termite species has indicated that % DT diversity was insignificantly small (see also Striebel, 1960). However, % DT diversity of the embryonic events observed in two species of Orthoptera was significantly large. Available information was very fragmentary, but the cause of this diversification between these two species might be as largely due to different rate in the developmental speed of these insect embryos.

In these orthopteroid insect embryos stomodaeum formation was seen to occur before neuroblast differentiation. Occurrence of the proctodaeum was seen to follow the stomodaeum formation, but it was difficult to determine whether the proctodaeum formation had started before the neuroblast differentiation or not; this should be considered as due to the difficulty in determining the exact time of occurrence of proctodaeum formation.

Katatrepsis in these orthopteroid insect embryos was seen to occur later than that in the hemipteroid insect embryos (Table 1). Although the identical style of embryonic movement was observed in both groups of these insects at the time of katatrepsis, the time necessary from katatrepsis to yolk engulfment in orthopteroid insect embryos was much longer than that in hemipteroid insect embryos. The author has obtained an impression that considerably long time necessary to finish the zipper-like fusion of the external epithelium observed in these orthopteroid insect embryos brought about this prolongation of yolk

engulfment procedure.

Difference of % DT of the embryonic events obtained from these orthopteroid insects may suggest that the insects included in the orthopteroid group are embryologically more diverse than those belonging to the hemipteroid group.

3. Discussion

The striking difference found between the results obtained by the present author and those by previous author (Strievel, 1960) is that chronological sequence of stomodaeum formation and neuroblast differentiation in the course of embryogenesis is reversed between the insects belonging to different orders. In the most hemipteroid insects observed, neuroblast differentiation occurred prior to stomodaeum formation, whereas stomodaeum formation in orthopteroid insects occurred earlier than neuroblast differentiation. This might suggest that the style of GB formation is influential in the chronological sequence of the events which occur subsequently, as hemipteroid insect embryos employ immersion type GB formation, whereas orthopteroid insect embryos employ superificeal type GB formation.

However, what the author intends to emphasize here is that % DT could be employed as one of the most useful information in representing diversifications of the embryogenesis among different insect orders, and at the same time, may help us to understand basic developmental program most likely govern insect embryogenesis.

It is reasonable to assume that insect embryogenesis may proceed under the direction of basic developmental program, in which all developmental events (steps) are arranged sequentially. However, when we observe individual material we are only allowed to know various modifications of this program, each of which is specific for each insect species. These modifications on basic developmental program must have occurred in the course of evolution of Insecta either as a change in the chronological sequence of the events, as seen in case of neuroblast differentiation and stomodaeum formation described in this text, or change in the pattern of cytodifferentiation, i. e., morphological diversification. Appearance as well as disappearance of events occurred in the developmental program, as seen in the case of embryonic rotation specifically included in the developmental program of one group of Hemiptera, may also be considered as another cause of modifications. It is very likely that one type of modification occurs in close association with another.

Up to present insect embryologists tended to evaluate results of morphological observation as the only available and worthy information to interprete embryogenesis in insects. This is especially true when previous authors considered phylogeny of Insecta. However, it is very difficult to decide which one of the morphological features is basically important and which one has occurred as a result of diversification. From this viewpoint the author considers that accumulation of quantitative information such as % DT is very rewarding and helpful to reveal basic developmental program of insect embryogenesis, and consequently contribute to the understanding of evolution of Insecta.

Finally, it should be pointed out that % DT may only indicate sequence of occurrence of the events in due course of the embryogenesis; this index however is useless to indicate the heritage of individual cells consisting each embryonic organs. Cell lineage which has been used to indicate developmental history of cell differentiation, on the other hand, failed to include chronological information; this is the critical shortcoming in considering embryonic

developmental program. The author therefore suggests that cell lineage and % DT should be integrated in the near future to describe embryogenesis in insects.

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