Studies on the Larval Aggregation of *Nordmaniana trachydelta* Meyrick (Lepidoptera : Yponomeutidae)*

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Introduction

The larval aggregation is frequently observed in many species of insect. In some species, aggregation may persist throughout the larval life, however in the majority of the species, it is observed only in the early part of larval life and it gradually diminishes with larval development advances.

In a decade, aggregative habit of larva has been the subject of many investigators (see Morimoto, 1972). And it is commonly said that larval aggregation may influence upon its survival. When larvae are kept in isolation, they frequently fail to survive. In addition, aggregation may be essential for some characters such as larval development, i. e. aggregation accelerates development.

However, the adaptive meaning of aggregation to the biology and the mechanism of aggregation should differ in each systematic group. Furthermore, larval behaviour concerning with formation and maintenance of aggregation still remains uncertain.

Adult of *Nordmaniana trachydelta* deposits her eggs in lump of 100—200 on a leaf of *Euonymus alatus*. The hatchlings mine a leaf that she oviposited eggs and feed between the upper and lower epidermal layer (mesophyll). After the first moult, they come up on the leaf and make a single nest web forming a dense aggregation. The aggregative habit persists throughout the larval life in the nest web, and strong aggregation is observed even in pupal stage.

The present experiments were undertaken to examine the effect of larval aggregation on the survival and development of larva of *Nordmaniana trachydelta* and also dealt with larval behaviour concerning with the formation and maintenance of aggregation. The ecological meaning of aggregation in terms of its life being specific to the species will be discussed.

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Methods

Egg masses were collected from the campus of our faculty in early June, 1973 and 1974.

In 1973, the second instar larvae which just came up on a leaf were divided into the size of 1, 2, 10, 30 and 73 with replications of 30, 15, 3, 2 and 1, respectively. On the other hand, in 1974, egg masses of 5 days before hatching were divided into the size of 1, 5 and 30 with replications of 40, 8 and 3, respectively.

Larval rearings were carried out under the room temperature and natural daylength in plastic vessel with 9.3 cm in diameter and 4.5 cm high keeping sufficient humidity in it. The larvae were transfered to fresh leaf of *Euonymus alatus* at the interval of every day, 2 and 3 days at the early, the middle and the last instars, respectively. Censuses were carried out every day on the mortality and the development of larva.

Further, in order to elucidate how the larval aggregation forms and maintains, following observations were designed to examine larval behaviour in the laboratory.

Observation 1

Observation on hatchlings.

Observation 2

Observation on the second instar larvae which came up on leaf.

Observation 3

Observation on larval migration to their feeding sites.

Observation 4

Observation on the process of the formation and maintenance of larval aggregation in the early and the middle instars.

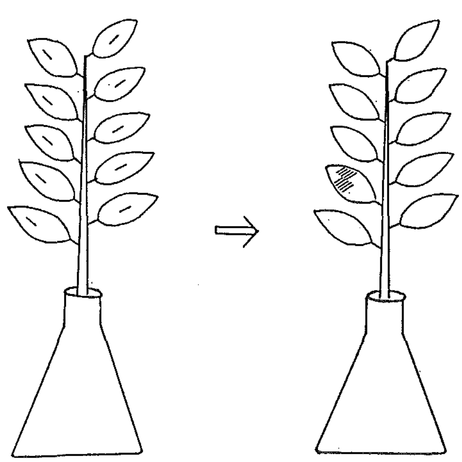

Fig. 1. Method to test the process of the formation and the maintenance of larval aggregation.

From the Observation 1 to the Observation 3, *Euonymus* twigs with many leaves were put in a water flask, while in the Observation 4 the twig with 10 leaves was put in a water flask and individual larva was put on each leaf (Fig. 1).
Censuses were carried out at regular interval and the number of larvae on each leaf were recorded with time elapses.

Results

1) Number of eggs in an egg mass and percentage of hatching

It may be important to examine the number of eggs in an egg mass in terms of the larval size of aggregation derived from it in the field.
Variation of eggs in a mass and percentage of hatching were shown in Table 1.

Table 1. Variation of the number of eggs in an egg mass and the percentage of hatching.

No. of eggs in a mass	No. of eggs unhatched	No. of eggs hatched	Percentage of hatching	No. of eggs in a mass	No. of eggs unhatched	No. of eggs hatched	Percentage of hatching
154	0	154	100 (%)	37	9	28	75.68
82	0	82	100	184	1	183	99.46
73	3	70	95.89	146	8	138	94.52
149	3	146	97.99	86	0	86	100
183	21	162	88.52	175	0	175	100
82	2	80	97.56	63	0	63	100
123	0	123	100	194	0	194	100
171	14	157	91.81	95	20	75	78.95
84	1	83	98.81	※126.89	※4.56	※122.33	※96.40
203	0	203	100				

※ Mean

The number of eggs varied from 37 to 203, but percentage of hatching was relatively high irrespective of the size of egg mass.

2) Larval mortality

As mentioned in the Methods, two series of experiments were designed in order to examine the larval mortality in relation to the size of aggregation and to examine what period is the most susceptible to the aggregation.

Experiment 1 (1973)

Larvae from which came up the leaf at the beginning of the second instar were divided into different size of aggregation. Fig. 2 shows the survival from separation to pupation. When the larvae were reared in isolation or in the group of 2 larvae, they suffered from high mortality and finally it reached to 57–60 percent. up to pupation. On the other hand, in the group of more than 10 larvae almost all larvae could survive. Mortality in each instar was shown in Fig. 4–A and it was clear that the mortality became higher at the second instar in the isolated or small grouped rearing.

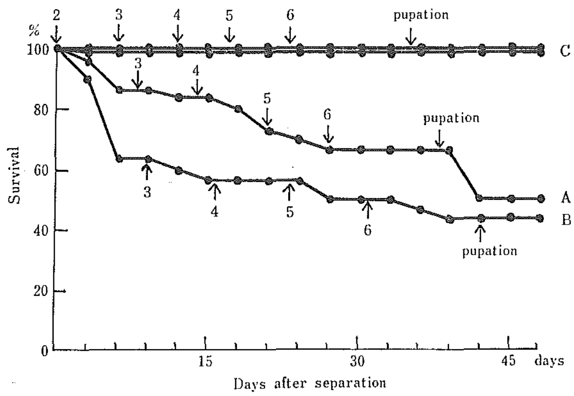

Fig. 2. Survivorship curve in different rearing groups which were separated from the beginning of second instar. Each figure on the curve represents the time of moult.
A : Isolation B : 2 group C : 10, 30 and 73 groups

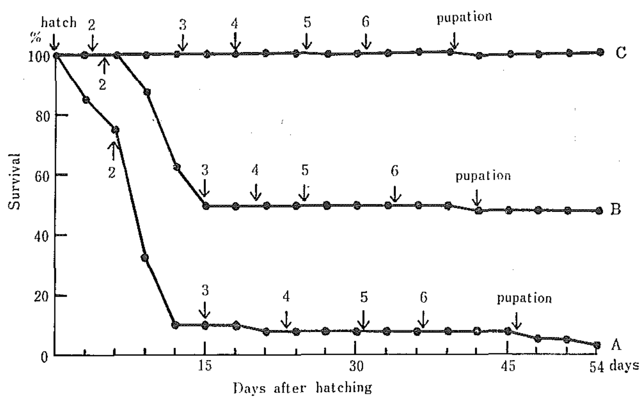

Fig. 3. Survivorship curve in different rearing groups which were separated at 5 days before hatching. Each figure on the curve represents the time of moult.
A : Isolation B : 5 group C : 30 group

Experiment 2 (1974)

An egg mass was divided into different size at 5 days before hatching. Fig. 3 shows the mortality throughout the larval life in each size. No any mortality was found in the size of 30 and it became higher with a decrease in the size of aggregation.
In the isolated rearing, almost all larvae died out till the third instar. Mortality in each

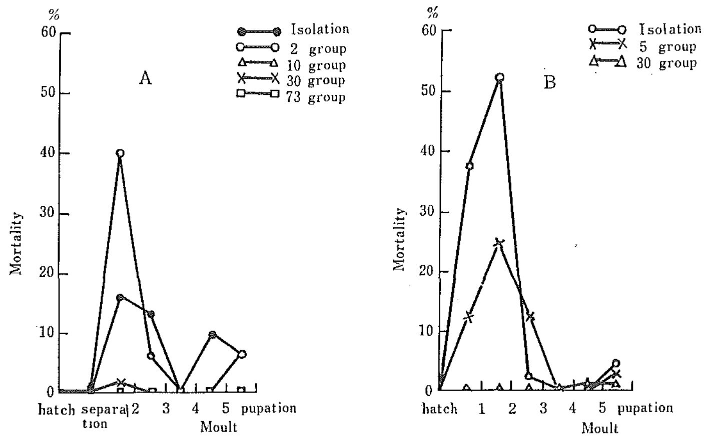

Fig. 4. Larval mortality during each instar in different rearing groups.
A : Separation from the beginning of second instar. (1973)
B : Separation at 5 days before hatching. (1974)

instar was shown in Fig. 4–B and it would be clear that mortality became high during the first and the early stage of the second instars.

From these two experiments, it is evident that the larger the size of larval group, the lower the mortality becomes and that the most critical period to the survival is the time from hatching to the early stage of the second instar, especially aggregation of the first instar should be important to the survival.
It is also interesting that the early larval period which is living without nest web is the most susceptible to the survival.

The mechanism of the high mortality in the isolated rearing will be discussed later.

3) **Duration of larval life and synchronism of moult**

The duration of larval life and the synchronism of moult relative to the size of group were examined. The results were shown in Table 2 and Fig. 5. Total length of larval life was 49.7 and 42.7 days in the isolation and the size of 30 larvae, respectively, though the length of the last instar was almost equal in each group. The duration of larval life was shorter and its coefficient of variation was smaller as the size of group became larger.

The good synchronization was observed in each moult as the larval group became larger.

4) **Width of head capsule**

The width of head capsule was also measured as an indicator of larval development in each instar except for the first and the sixth instars. The capsule in the first instar

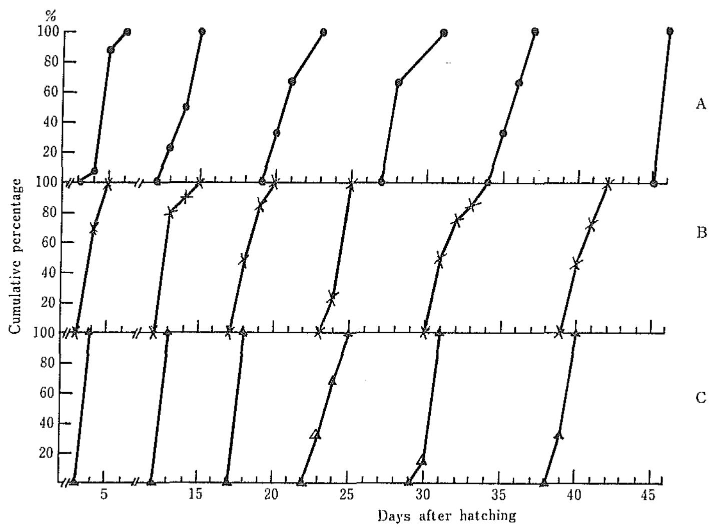

Fig. 5. Synchronism of moults in different rearing groups.
A : Isolation B : 5 group C : 30 group

Table 2. Larval duration of each instar in different rearing groups.

	Size of larval group	Replication	Instar of larva												Total length	
			1		2		3		4		5		6			
			Mean	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
1973	1	30	—	—	9.2	11.1	6.9	19.6	7.5	14.4	7.7	11.6	10.8	11.3	42.3	6.1
	2	15	—	—	8.3	7.1	5.8	6.9	6.9	20.4	5.9	10.5	10.8	10.4	36.8*	4.0
	10	3	—	—	6.0	0	6.0	0	5.0	16.6	5.7	8.4	12.0	0	34.7*	1.4
	30	2	—	—	6.0	0	6.0	0	4.0	0	5.5	13.3	12.5	5.8	34.0*	0
	73	1	—	—	5.0	0	6.0	0	5.0	0	6.0	0	12.0	0	34.0*	0
1974	1	40	5.0	10.2	9.0	12.7	7.7	7.5	7.7	7.5	8.3	13.9	12.7	4.6	49.7	3.1
	5	8	4.3	10.9	8.3	8.0	5.9	6.3	6.1	10.5	7.2	13.8	12.1	2.6	44.1*	2.9
	30	3	4.0	0	7.0	0	5.0	0	6.0	13.5	6.8	9.9	11.8	3.1	42.7*	11.0

(Mean : days ; C.V. : %)
* Each asterik indicates that the difference with the isolation is significant with 95% confidence limit.

was too small to measure and that in the sixth was crushed out at the time of moult. Table 3 shows the width in each instar in relation to the size of group. There was no significant difference between the size of groups from the second up to the fourth instar, however, it became apparent in the fifth instar, i. e. it was larger and its coefficient of variation was smaller as the size of aggregation became larger.

5) Observation of larval behaviour on the formation and the maintenance of aggregation

As mentioned in the Methods, four series of observations were conducted.

i) **Observation 1** : Observation on hatchlings.

After incubation for 7—10 days, larvae hatched synchronizingly from an egg mass, thereafter they mined into the leaf. After spending only 1 day, they came up once on the leaf and tend to move to the petiole spinning the silk threads in order to fix the leaf to a twig and mined again into it.
It seems that the larvae fixed the leaf to prevent defoliation due to the larval feeding.

ii) **Observation 2** : Observation on the second instar larvae which came up on the leaf.

After moult to the second instar, they could come up on the leaf and continued wandering around here and there as long as 5 hours, then began to move to a new fresh leaf.

The small group for the first feeder wandered around and reached to the new fresh leaf with spinning the silk trail. The larvae coming up later followed the silk trail already made and they were confluent with first feeding group. They subsequently could establish their feeding sites with making a nest web around them. It was observed that the several larvae could obtain the qualification for the first feeding group as leader. Any special leader inherently decided may not exist in the aggregation. The silk trail was gradually thickened and enlarged by other members that move later.

iii) **Observation 3** : Observation on larval migration to their feeding sites.

The small group for the first migration living in the margin of the nest web seems to provide certain signal to the whole members in the aggregation and they suddenly began to move around. The first migrating group span the silk and made a direction trail for all other larvae to the new fresh leaf,

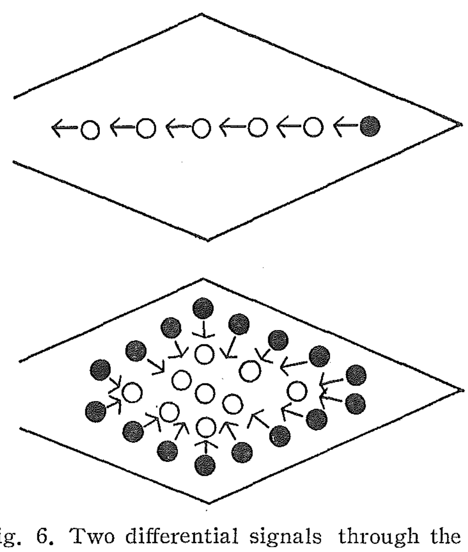

Fig. 6. Two differential signals through the nest web observed in larval aggregation.
Above : Resting or emergency signal was spread over as waving type.
Below : Migrating signal was spread over as convergent type.

Table 3. Width of head capsule of each instar in different rearing groups.

Size of larval group	Replication	Instar of larva							
		2		3		4		5	
		Mean	C. V.	Mean	C. V.	Mean	C. V.	Mean	C. V.
1	30	0.284	5.63	0.433	5.96	0.719	7.54	1.052	7.04
2	15	0.286	5.59	0.454	4.47	0.757	3.74	1.133*	4.79
10	3	0.280	2.36	0.462	5.70	—	—	1.179*	3.03
30	2	0.281	3.91	0.468	5.47	—	—	1.217*	3.96
73	1	0.266	5.86	0.432	5.07	0.769	4.42	1.226*	4.24

(Mean : mm ; C. V. : %)

* Each asterik indicates that the difference with the isolation is significant with 95% confidence limit.

then several larvae in the group turned around and followed the twig back to the old nest to guide others, where other larvae are still wandering. Any larva dropped out could not be found during the course of the marching.

The two differential signals were recognized in the larval aggregation as in Fig. 6. One was the signal informed them to rest or to approach of danger. When the several larvae living in the margin of the nest felt dangerous, they shaked themselves to make the nest web vibrate, so that the signal spreaded over to all other members as a wave. While, another was to move out of the nest web. The larvae living in the margin of

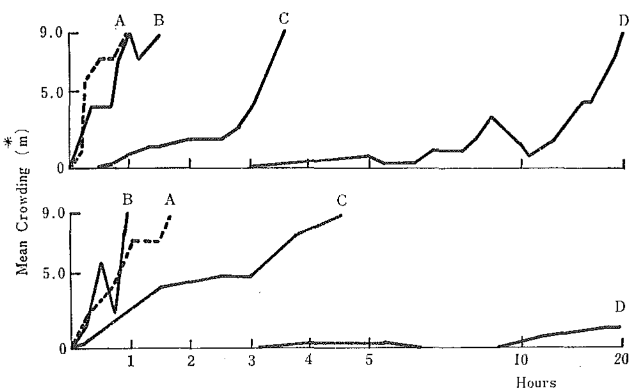

Fig. 7 The time required for the formation of larval aggregation when the aggregating larvae were scattered.
Each curve was represented by the index of Mean Crowding ($\overset{*}{m}$). Above : First or second instar larvae. Below : Third instar larvae.
A : *Nordmaniana trachydelta* B : *Spiralctia imparilis*
C : *Phalera flavescens* D : *Dictyoploca japonica*

the nest began to move and shaked themselves, so the vibration spreaded over to its center.

iv) **Observation 4** : Observation on the process of the formation and the maintenance of larval aggregation in the second and third instars.

Ten individual larvae were put on each leaf on a twig and the number of larvae on each leaf were examined with time progresses. The tendency to aggregation was represented by the index of *Mean Crowding* ($\overset{*}{m}$) (Lloyd, 1967) in comparison with those in another three lepidopterous species; the mulberry tiger moth, *Spiralctia imparilis*, the black-marked prominent, *Phalera flavescens*, and the camphor silk moth, *Dictyoploca japonica*, in which live compact aggregation till the middle of larval life (Fig. 7). *Spiralctia imparilis* is a nest maker, but another two species do not make any nest at all, though they spin the silk trail to form and maintain the larval aggregation.
The strong tendency to aggregation was observed in the larvae which make the nest web and they could aggregate much faster than other two species within 1.5 hours when the aggregation was disturbed in the early instar. On the contrary, the time required for the formation of aggregation was remarkably prolonged in *Phalera flavescens* and *Dictyoploca japonica*. It took more time to complete aggregation.

Discussion

Nest making insects were commonly observed in several species such as *Hyphantria cunea* and *Malacosoma neustria testacea*, however, their aggregating larvae gradually disperse out as the larval development advances.

In *N. trachydelta*, the larvae showed a strong tendency to aggregation in a nest web throughout the larval life. It is important, therefore to consider the adaptive meaning of aggregation to its biology and physiology.

In the present experiments of *N. trachydelta*, isolated and small grouped rearings caused high mortality during the early instar due to the failure of establishment of their feeding sites and also caused prolonged larval development. This fact indicates that the first establishment of feeding is responsible for the further survival. So, it may be considered that the first and early stage of the second instar are the most critical period in respect to survival and the development of larva, because of the lack of protective means against the environmental resistances. The effect of aggregation takes place during the early larval stage though aggregation persists throughout the larval life.

Therefore, it can be suspected as an adaptive manner for vulnerable period that the larvae live in a leaf during the first instar and aggregation makes the duration of the second instar shorten in this species.

Several investigators had emphasized on the importance of the first feeding in some species, i. e. *Euproctis pseudoconspersa* (Mizuta, 1960), *Neodiprion sertifer* (Henson, 1965), *Dictyoploca japonica* (Morimoto, 1967) and *Hyphantria cunea* (Umeya and Watanabe, 1973).

According to Henson (1965), he termed the larvae which could feed directly in the first instar as "first-biters". From the facts that the larvae of *N. trachydelta* could aggregate immediately when the larval aggregation was disturbed and that the second instar larvae wandered around here and there and made a nest, thereafter they establish their feeding sites. When the larval aggregation became sufficiently larger, it became apparent that the silk trail may be responsible for the formation of aggregation. The larvae tend to follow silk trail laid by other individuals and they move within a limited range or travel along a similar route (e. g. Long, 1955; wellington, 1957; Umeya and Watanabe, 1973).
By contrast, when the larval aggregation was small, they could not feed and died out resulted from wandering around for a long time without making nest web. It indicates that the larvae which live in small group may not be able to feed by the lack of mutual interaction. Nest web should play a role to keep the larval interaction and makes larvae to feed.

Two differental signals were found such as "resting or emergency signal" and "migrating signal". These signals were provided by the first group through the nest web to maintain the larval aggregation.

According to the present observations, it was clear that the silk trail and the nest web makes the larvae to establish their feeding sites and subsequently they restrict the larval behaviour.

Qualitative difference among individuals in the aggregation as reported by Wellington (1957 and 1960) could not be found in this species and larvae coming up earlier or larvae in which live the margin of the nest may certainly play a role of leader in an aggregation. Effect of the silk trail and nest web on the behaviour as well as the mutual interaction through the nest may be important to maintain larval aggregation.

The present studies suggest that the role of the nest web is to establish the group feeding caused by mutual interaction during the early instar and to protect against attack by the natural enemy in the later instars.

Summary

The larvae of *Nordmaniana trachydelta* Meyrick hatched from an egg mass persist the dense aggregation throughout the larval life with making a nest web.

The laboratory experiments and observations were carried out to make clear the effect of larval aggregation on the mortality and development of larva, and also to know the larval behaviour being responsible for the formation and the maintenance of larval aggregation.

Discussion will be involved the ecological meaning of the insect aggregation in terms of life being specific to the species.

From the results, it was evident that the larger the size of larval aggregation, the lower the mortality became. Aggregation may bring about a positive effect to the other characters than expected merely from the mortality.

Turning to the mechanism of larval behaviour on the formation and the maintenance of aggregation.

The silk trail spun by the first group may play a role for the formation of aggregation during the early instars.
Two differential signals were found through the nest web when they rest or danger approached (resting or emergency signal) and when they begin to move out of the nest (migrating signal). The silk trail and the nest web on the behaviour as well as the mutual interaction through the nest web may be important to the formation and maintenance of larval aggregation such as for feeding and migration.

From the results of the present studies, it may be suggested that the role of the nest web is to establish the feeding site during the early instar larvae and to protect against attack by the natural enemy in the later stage.

Acknowledgement

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ホソスガ幼虫の集合性に関する研究

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摘　　要

ホソスガ *Nordmaniana trachydelta* Meyrick はニシキギの葉に成虫が卵塊で産卵し，ふ化した幼虫は1令期間をその葉の葉肉を摂食して生活する。2令化脱皮を終えた幼虫は直ちに葉から脱出して巣網を作り，その中で強い集合生活を行なう。この集合生活は終令までそのまま続き蛹においてさえ強い集合を維持するのが普通である。巣網を作りしかも幼虫期間を通じて集合が維持される本種の幼虫の集合性の生態的意義を解明するために，卵期および2令期からいろいろの大きさの集団に分離して室内で飼育した。

1）幼虫期の死亡率は大きい集団ほど低かった。

2）幼虫の発育期間も大きい集団ほど短縮される傾向がみられた。

3）幼虫各令の頭幅にはほとんど顕著な差はみられなかった。

4）幼虫期の生存に最も重要な時期は1令と2令のごく初期であり，この時期は巣網を作らない。

5）集合内の個体に特定のリーダー個体はみられなかったが，2令になって葉から先に出て来た個体が最初の摂食時の集団を形成する時のリーダー役を果す。また幼虫各令の集団移動の際には巣網の周縁部に生息している幼虫がリーダーとして先に行動を開始することが明らかになった。

6）巣網を用いての集団内の個体の行動を律する信号が2種類観察された。1つは"休止または危険信号"であり，他は移動の時の"行動開始信号"であった。

7）若令幼虫の吐糸と巣網は摂食場所での集団摂食のための集合形成と維持に意義をもっているし，中令以降はむしろ天敵の攻撃に対する防禦のためと考えるのが妥当である。