The Emergence of Winged Viviparous Female in Aphid

III. Critical Period of Determination of Wing Development in Rhopalosiphum prunifoliæ

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INTRODUCTION

The problem of wing development by parthenogenetic aphid females has received a great deal of attention. Alate forms can be caused to appear among the apterous viviparous aphids by various external factors. However in the study of any factor responsible for the wing development in aphids, it is of primary importance to know precisely the critical period during which wing development is most readily affected, because the factor which is to act upon it may not have any influence before and after that period.

The present article deals with the results of a series of experiments to show that the destiny of future wing development is determined in the apple grain aphid, Rhopalosiphum prunifoliæ, during the period extending from birth to the early stage of the second instar.

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MATERIAL AND METHOD

The aphid, Rhopalosiphum prunifoliæ, was fed with the leaves of Holcus Sorghum L. var. japonicus under condition of darkness throughout the experiment. In the control series the food was given at the rate of 4 insects or individuals per 10 cm² of leaf piece to avoid the effect of high population density. The breeding temperature was always kept at 25°C.

Until the larvae develop into the third stage at which the wing buds become visible, it is exceedingly difficult to discriminate only by external appearance the larvae to be winged from those to remain unwinged. As a matter of fact, it seems that there is no exact method of discovering the critical period of wing development other than to see the actual growth of the wing. Therefore the actual development of wings was examined in the third instar after having added stimuli to the larvae in their early stages of development. High population density and starvation, both of which had been proved highly effective for the development of alate form in this species (Noda, 1954 and 1956), were adopted throughout the investigation as the stimuli.

RESULT

Experiment I. A large number of newly born larvae, by parthenogenesis from the wingless adults, were divided into 4 lots. (1). The first lot was fed with leaves cut into pieces of 10 cm² at the rate of 40 individuals per piece for 40 hours immediately after birth. After the 40 hours, the density was decreased to 4 per 10 cm² and they were fed until the development of wing buds could be distinguished by the usual method. This is called “40-4” lot. (2). The second lot was treated by just the reverse method of (1), i.e., the young were fed with 10 cm² leaves at the rate of 4 individuals per leaf from the beginning, then the density was increased to 40. This is represented by “4-40”. (3). In the third lot, named “40-40”, the young
were reared throughout the investigation by the amount of food leaves equal to (1) or (2) at the rate of 40 individuals. (4). In the control lot, "4-4", the food was given constantly at the rate of 4 individuals per leaf of 10 cm².

Experiments were repeated five times in each lot. The total numbers of the winged and the unwinged forms produced in each case are shown in the following table.

Table 1. Effect of population density on the development of winged form.

<table>
<thead>
<tr>
<th>Lot</th>
<th>Total</th>
<th>Unwinged</th>
<th>winged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>40-4</td>
<td>194(6)</td>
<td>88</td>
<td>106</td>
</tr>
<tr>
<td>4-40</td>
<td>198(2)</td>
<td>185</td>
<td>13</td>
</tr>
<tr>
<td>40-40</td>
<td>196(4)</td>
<td>94</td>
<td>102</td>
</tr>
<tr>
<td>4-4</td>
<td>200(0)</td>
<td>185</td>
<td>15</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate those which died during the investigation.

According to the ƒÔ2-test, it seems that there are no significant differences in the development of the winged form between "40-4" and "40-40", and between "4-40" and "4-4". However the differences between "40-4" and "4-40", between "40-4" and "4-4", between "40-40" and "4-40" and between "40-40" and "4-4" are of significance at the level of 1% respectively. It is seen from the table that in the lots where the larvae are reared in higher population density for 40 hours from the newly born stage, the percentage values of the emergence of the winged form are larger than in those with lower density, whether they are treated thereafter at higher or lower densities. This also proved to be true in the following experiment.

**Experiment II.** The newborn larvae were fed at the rate of 4 individuals per 10 cm² of food leaf during the first instar, but after completion of the first ecdysis the density was raised to 40 individuals and they were reared until the wing buds became visible. Experiments were repeated four times and the sum-up values are indicated in Table 2.

It is evident that the high population density during the period of feeding following the first molting also yields a higher percentage of emergence of the winged form of statistical significance, although the effect is somewhat smaller, when compared with Experiment I.

**Experiment III.** The larvae were starved at 25°C for 10, 15 and 20 hours respectively without intermission after the first molting, and the effect on the development of the winged form was examined with the following result.

**Experiment IV.** The larvae of second instar, after having been reared by the standard method throughout the preceding instar, were starved for 5 hours, immediately after, and 5, 10 and 15 hours after the first ecdysis respectively, and the future destiny in the
wing formation was examined (Table 4).

Table 4. Effect of starvation started from different hours after the first ecdysis on the wing development.

<table>
<thead>
<tr>
<th>Form</th>
<th>Hours after first ecdysis</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>119</td>
</tr>
<tr>
<td>Unwinged</td>
<td>72</td>
<td>88</td>
</tr>
<tr>
<td>Winged, number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, %</td>
<td>36.8</td>
<td>26.1</td>
</tr>
</tbody>
</table>

Differences in the percentages are significant at 1% level between 36.8—26.1—5.1 and 3.3—1.9, and between each value of 36.8, 26.1 and 5.1, but not so between 5.1 and 3.3, and between 3.3 and 1.9. Thus it seems reasonable to assume that the earlier the starvation takes place in the second instar, the larger the percentage value for future wing development.

Experiment V. More detailed combination was adopted in this case. Larvae were isolated from the food plant and starved for 5, 10 and 15 hours respectively, after they had spent their normal feeding life for 5, 10, 15, 30 and 35 hours immediately following birth (see Table 5).

From the accompanying table it may be seen that, although the death rate increases with the duration of starvation, the rate of development of the alate form also becomes higher with the increase in the duration of starvation. But as for the starting time of starvation, the rate of wing development increases gradually with the elapse of time from which the starvation starts, attaining a maximum at a certain point, then is followed by a gradual decrease.

To make these relations clearer some statistical considerations were made. The distribution analysis was tried with the numbers of the two upper lines and the left half of the third line in the table. It was shown from these tests that there are significant differences of 1% level not only in “within groups variance” but also in “between groups variance”.

The lowest percentage of the wing development in this series of investigation is 24.5% as found at the left uppermost position of the Table. It may, therefore, be said that the percentage may always be raised at least to this level by a minimum 5 hour starvation, starting within 35 hours after the birth. So taking this lowest value, 24.5%, as the base line, I drew a frequency polygon (Fig. 1) which represents the relation in the 5 hour starvation between the percentage of wing development and the hours after birth. As the polygon seems to be almost an ideal normal curve, $\chi^2$-test was tried using the concept of the null-hypothesis of normal distribution.

The result showed that the level of significance lies between 10 and 20% (df=7, $\chi^2$=10.922), proving that the hypothesis can not be rejected. Therefore, the curve in Fig. 1 may be recognized as that of true normal distribution. From this curve, theoretical

Table 5. Effect of starvation of various durations started from different hours after birth, on the winged form development.

<table>
<thead>
<tr>
<th>Duration of starvation (hours)</th>
<th>Hours after the birth whence the starvation started</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immediately</td>
</tr>
<tr>
<td>5 Number</td>
<td></td>
</tr>
<tr>
<td>% of winged</td>
<td>74 24</td>
</tr>
<tr>
<td>% of winged</td>
<td>24.5</td>
</tr>
<tr>
<td>10 Number</td>
<td></td>
</tr>
<tr>
<td>% of winged</td>
<td>69 33</td>
</tr>
<tr>
<td>% of winged</td>
<td>32.4</td>
</tr>
<tr>
<td>15 Number</td>
<td></td>
</tr>
<tr>
<td>% of winged</td>
<td>72 44</td>
</tr>
<tr>
<td>% of winged</td>
<td>37.9</td>
</tr>
</tbody>
</table>

U=Unwinged, W=Winged, T=Total.
percentage of alate form to be brought about by the starvation of 5 hours may be computed for any hour in the development after birth, whence the starvation begins.

**DISCUSSION**

The results of the investigations just described throughout the preceding chapter appear to support that the "time of determination" theory of wing development is also true in this aphid, *Rhopalosiphum prunifoliæ*. According to this theory there is a critical period during which wing development is most readily affected by external factors.

It was shown in Experiment I that the viviparous young aphids maintained at high population density on the food plant for 40 hours after birth transform into the winged form in high percentage, regardless of later density. So it may be imagined that the critical period during which wing development is most readily affected by external factors.

Since larvae end their first instar in an average of about 28.5 hours following birth, when kept at the temperature of 25°C, the 10th hour of the second instar corresponds to the 38.5th hour following birth. As is seen from Table 5, however, the larvae, when food is not ingested for 5 hours from the 35th hour after birth, transform into the pterous form which takes place in a percentage showing significant difference from that of the control. Therefore it may be said that the limit of the critical period must exist within a relatively narrow space between 35th and 38.5th hour following birth. As the minimum duration of the first instar of this aphis was found to be about 26 hours at 25°C, the future form of the young will be determined at the latest by the 36th hour following birth.

Experiment V was undertaken for the purpose of finding the time of determination at which the maximum rate of wing development may be expected. The following facts may be observed from Table 5 and Figure 1.

1. If the larvae are removed from the food plant for a short number of hours such as 5, 10 and 15 immediately after birth, and at the 5th, 10th, ······30th and 35th hour after birth, there is always a greater percentage of the winged form produced, i.e., greater than 24.5%, as compared with the
larvae that were not starved. From this, it may safely be concluded that the critical period of determination of winged form development extends during the development of the larvae ranging from birth to the 35th hour of age.

2. The alate form produced increases in number in proportion to the duration of starvation from 5 to 15 hours, if the starvation occurs at the same stage of development.

3. The development of the larvae from emergence to the age of 35 hours is accompanied by a significant change in the percentage production of future winged form as affected by starvation. The changes are quite similar in the three cases of 5, 10 and 15 hour starvations. The percentage values which are relatively small in the newly emerged larvae shows a gradual increase with the development, reaching the maximum in each case at the 15th hour. They then decrease again to the former values at the 35th hour.

An examination of Fig. 1 shows that, if the larvae are starved for 5 hours, the maximum percentage of wing development is to be expected theoretically in the larvae at the age of 21 hours after birth. As the duration of the first instar of this aphis is an average of 28.5 hours at a temperature of 25°C, that is to say, the starvation has its highest effect on the future wing development in the latter stage of the first instar. During the period both prior to and following critical period, the earlier or later the starvation takes place, the less becomes the rate of wing formation. Percentage values of more than 50% will be obtained during the period extending from 14.5th to 27.5th hour following birth, namely, from middle to the end of the first instar.

Thus it may safely be summarized that the period for the determination of wing development in the future aphid, *Rhopalosiphum prunifoliae*, extends from the newly emerged to the larvae of 38.5 hours of age, i.e., 10 hours from the beginning of the second instar, and the time at which wing development is most readily affected is at the 21th hour in the later stage of the first instar.

There are a number of studies that support the "time of determination" theory of the alate form development in aphids, but the time doubtless varies from one species to another. For instance, Neils (1912) found that the aphid, *Nectarphora rosae*, determines its future development of wings during the first day of its existence, while according to the investigation of Shull (1928), whether the parthenogenetic aphid, *Macrosiphum solanifolii*, is to be winged or not is determined about 34 hours before birth. Shull (1938) later noted that the wing buds start their definitive thickening perhaps 22 to 24 hours before birth.

Finally it cannot be ignored that, as seen from Table 5, the percentage values of wing development are always far smaller in the individuals from which food was removed at the early stage of development, compared with those starved at a later stage, even if the duration of starvation is longer in the former. For instance, in the larvae starved for 15 hours at the beginning of larvae or after 5 hours from birth, the rate of wing development is far lower than in those starved for only 5 hours after 15 or 20 hours from emergence. I suppose that this is attributed to the disturbances in the rate of the developmental processes involved in the early stage, such as those which fix the time of determination and those which control the secretion of some hormones affecting the wing development, as was suggested by Shull (1942) in the development of wing buds in *Macrosiphum solanifolii*.

**SUMMARY**

1. The critical period of determination of future wing development of the apple grain aphid, *Rhopalosiphum prunifoliae* was determined experimentally at a constant temperature of 25°C, using high population density and starvation as the stimuli.

2. The action of starvation is in general far stronger stimulus for wing development than that of high population density.

3. In the larvae maintained at high population density during 40 hours following the birth or during the earliest stage of the
second instar, the percentage values of the winged individuals produced in the third instar are very much larger than those in low density at the corresponding stage.

4. In the larvae forcibly removed from the food plant for 5 to 15 hours immediately following birth and after various hours from 5 to 35 hours following the emergence, high percentages of wing development are always obtained.

5. Maximum rate of wing development caused by the effect of 5 hour starvation is obtained in larvae of 21 hours old which correspond to a later stage of the first instar. And the percentage values greater than 50% are produced during the period extending from 14.5th to 27.5th hour after birth, i.e., from the middle to the end of the first instar.

6. The increase in the rate of wing formation can not be seen in the larvae starved at ages beyond the 10th hour of the second instar.

7. The increase in the rate of wing development is not so remarkable, when the starvation occurs in the earliest stage of larval development.

LITERATURE CITED

SHULL, A. F. (1939) Ibid. 73: 256.

摘 要

アブラムシの有翅型胎生雌の出現について

III. キビクビアブラムシ Rhopalosiphum prunifolias における型決定の臨界期

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アブラムシにおける胎生雌の型決定には温度、光その他の要素が関係しているようであるが、ムキ類の害虫であるキビクビアブラムシ Rhopalosiphum prunifolias の場合には高検密度と絶食が大きな影響力を持っている（野田, 1954, 1956）。これら諸要素の作用と有翅型出現の関係を明らかにするためには、まず型決定の臨界期を明確にしておくことが先決問題である。本実験においては上述の2要素（高検密度と絶食）の作用を利用して、このアブラムシにおける前記臨界期と生翼の最盛期を明らかにすることができた。実験はすべて暗黒下定温 25℃ で行った。その結果を要約すると次のとおりである。

1) 有翅型は胎生された直後から生後38.5時間目（これは第1回脱皮直後から起算すると10時間目にあたる）以内の間に決定される。この臨界期を経過した後においては外部からの刺激の影響を受けることがない。

2) 理論上の生翼の最盛期は生後21時間目である。すなわち幼虫第1令後半期の半ばころである。

3) 50%以上の幼虫が5時間の絶食によって有翅型に変わり得る時期は、理論的にには生後14.5時間目から生後27.5時間目までの間である。この時期は幼虫第1令の中程から後期に相当する。

4) 絶食の有翅型出現に対する影響力は、高検密度のそれよりも一般に大きいようである。

5) 同一の育成途上にある幼虫を絶食させた場合には、絶食期間の長いほど有翅型出現率が高くなる。

6) しかるに生後15時間または20時間経過した幼虫を5時間絶食させた場合と、生直後の幼虫または生後5時間経過したものを15時間絶食させた場合とを比較すると、後者のほうがはるかに絶食時間が長いにもかかわらず、有翅型出現率はかえって低位である。これは生育初期に絶食の刺激を加えると、Shull (1942) が暗示したように生育に関係あるホルモンの分泌などに、変調をきたすためではないかと考えられる。