The Change in Reproductive System of the Southern Green Stink Bug, *Nezara viridula*, and its Application to Forecasting of the Seasonal History

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INTRODUCTION

An application of changes in morpho-functional condition of adults to predict winter mortality and also population density of the following year was demonstrated on a noxious pentatomid, *Eurygaster integriceps* (cf. Fedotov, 1955, 1960; Brown, 1962). It is conceivable that in an insect species of which adults live long as compared with the duration of immature stages, biology of the adults is important to understand population dynamics of the species. Moreover, when this species is multivoltine and oviposition takes place recurrently, overlapping of successive generations usually occurs. In the present paper, it is suggested that the change in the reproductive system of the southern green stink bug, *Nezara viridula*, provides one of the promising tools in the study of population dynamics as well as in the prediction of seasonal history of the bug.

MATERIALS AND TECHNIQUE

The number of females dissected in 1962 and their host plants were shown in table 1. Dissection of the insects was made at four to five day intervals and the following observations were made (see Fig. 1).

1. Degree of maturation of ovaries: The five stages of ovarial maturation were distinguished as follows, the ovaries without any differentiated chambers and visible eggs (−), the eggs were immature (+), some or most of the eggs matured, but not in the oviducts (+), eggs matured, and some of them were found in the oviducts (++) and both ovaries and eggs degenerated (±).

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Table 1. Design and schedule of the dissection

| Month     | Hibernacula | Wheat | Rape | Potato Crucifer | Peas | Beans & Smart- | Rice | Total no. dissected | Frequency of dissection |
|-----------|-------------|-------|------|-----------------|------|common weed    | haricot beans |                  |                           |
| March     | 55          | 57    | 41   | 7               | 29   | 3              | 149  | 36                | 55                         | 2                           |
| April     | 6           | 35    | 7    | 109             | 85   | 227            | 211  | 7                 | 98                         | 6                           |
| May       | 35          | 7     | 17   | 156             | 144  | 139            | 144  | 6                 | 156                        | 7                           |
| June      | 17          | 109   | 85   | 144             | 139  | 32             | 32   | 5                 | 139                        | 4                           |
| July      | 17          |       |      |                 |      |                |      |                   | 19                         | 4                           |

(2) Evidence of mating: A muscular room containing the duct of spermatheca becomes corpulent and opaque after mating, whereas that of virgin one is slender and rather transparent, and the light green duct leading to spermatheca can be seen through a wall of muscular room. It was impossible, however, to distinguish those mated once from those mated repeatedly.

(3) Evidence of oviposition: After the ovulation, the ovariole stalks and oviducts which are first transparent and white turn to light yellow. But it was unable to see the colour change of the membranous wall of oviducts among the females of the stage (+), because yellow matured eggs were fully packed in their ovarioles and oviducts.

(4) Symptom of cease of oogenesis: The females that oviposited and near to the end of oviposition period showed invariably a "black spot" in each ovariole usually at the lower part of germarium irrespective of the stage of ovaries but for the stage (−). The females of the stage (±) always have these black spots in the ovarioles. The origin of the spots was uncertain, but it was consisted of black yellow substance which was attached to the wall of ovariole forming a ring like shape.

(5) Fat body: The amount of fat body is designated as little −1, medium −2, and much −3. The size and type of fat body cells of the adults which were just before hibernation, during hibernation and after hibernation were examined respectively under a microscope.

(6) Total number of the eggs in the ovaries: As the females designated as (+) have eggs that can be counted individually, the number of the eggs in a ovarioles was assessed.

CHANGES IN PERCENTAGES OF FEMALES IN RELATION TO MATURITY, MATING, OVIPosition AND BLACK SPot.

To check the results obtained from the dissection of the females, censuses of the egg masses were conducted in the potato field and in the several paddy fields that were different in the date of ear formation to determine the oviposition process of each generation (ref. HOKYO & KIRITANI, 1963).

Seasonal change in the degree of ovarial maturation in the bug population was expressed by the percentage of the females designated as stages (+) and (+++) in each collection (Fig. 2B). The same method was used in calculating the percentages of females with regard to the mated, oviposited and black spotted (Fig. 2C). The percentage curve of mature females was composed of three parts, and each part represented the hibernated generation, the first and the second, respectively. Saw-toothed like fluctuation of the curve of the hibernated generation was attributable...
Fig. 2. Capture record of the light trap (A). Percentage of matured females (●) and frequency histogram of egg masses deposited in the fields (B). Percentages of females that were mated (……), oviposited (——) and black spotted (---) (C). The amount of fat body in active phase (○), during hibernation (○) and the mean number of eggs in the ovaries of (+) stage females (●) (D).
to the repeated egg layings by the females. The reduction in percentage of mature females in the late period of each generation was largely responsible for the entry of newly emerged adults of the following generation. On the other hand, the curve of the first generation as well as the frequency histogram of egg masses (Fig. 2B) was unimodal, suggesting that the females laid the eggs in a short period, namely in about two weeks. The curve of the second generation was bimodal, but the second peak of the curve was rather accounted for the existence of a population that produced the fourth generation than for the repeated ovipositions by females of the second generation. Adults of the third and the fourth generations though the latter being minority began to emerge in late September, and in late October, respectively, and hibernate without ovarial development until next spring. Consequently, the percentage of the matured females fell into nil for the pre-hibernating populations.

It is logically assumed that if all of the females in a population are of an age, percentages of the females with regard to the mated, oviposited and black spotted would increase in turn as the time elapses in the foregoing order. Such a sort of sequential incident of these curves was demonstrated in the population of hibernated adults. However, due to a rise in temperature with time, the intervals of these curves became short as the generation went on and finally in the second generation, all of the curves changed contemporaneously. As the life span of the adults is fairly long, overlapping of the adults of different generations occurs. Accordingly, these percentage curves after passing the maximum descended as immature adults of the following generation emerged. Overlapping of generations was responsible to shallow valleys in the curve for the periods between consecutive generations (Figs. 2B & 2C). Therefore, a figure showing more exact relation of these curves with respect to the individual

![Fig. 3. Showing the interrelation among the percentage curves of the females mated(-x-), oviposited(-o-), and black spotted(-o-), and the accumulated percentage curve(-●-) of the oviposited egg masses with regard to the different generations.](image)
Table 2. Intervals between dates of 50 per cent occurrence of the mated, oviposited and black spotted females and the date on which 50 per cent of the total egg masses have deposited.

<table>
<thead>
<tr>
<th>Interval between</th>
<th>Generation of adult</th>
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<tbody>
<tr>
<td></td>
<td>hibernated</td>
</tr>
<tr>
<td>Mated and oviposited</td>
<td>21 days</td>
</tr>
<tr>
<td>Oviposited and black spotted</td>
<td>29 days</td>
</tr>
<tr>
<td>Mated and deposited 50% of egg masses</td>
<td>37 days</td>
</tr>
<tr>
<td>Oviposited and deposited 50% of egg masses</td>
<td>16 days</td>
</tr>
</tbody>
</table>

generations was prepared (Fig. 3). The oviposition curves expressed in terms of accumulated percentage of the eggs that were laid in the fields were inserted also in Fig. 3 to show the relationship with those three curves.

The three percentage curves of the hibernated generation rose steeply and ran almost parallel to each other. Intervals measured as those between 50 per cent occurrence showed respectively three weeks and four weeks from the mated to oviposited and oviposited to black spotted. All of the females had deposited eggs at least once until May 22, but a fraction of eggs deposited until this time amounted only 23 per cent of the total eggs in that generation. On the other hand, when the percentage of the black spotted reached to 100 per cent level about 97 per cent of the eggs had been laid down by the hibernated females. The date on which 50 per cent of the eggs deposited was May 29 corresponding to the second period of the egg laying activity (cf. Figs. 2B and 2C). This point of 50 per cent egg laying was preceded by those points of mated and oviposited in five weeks and in more than two weeks, respectively (table 2). Termination of egg laying was indicated fairly well by the appearance of black spotted individuals. In the present case, when 13 per cent (on June 9) of the hibernated females showed the symptom of black spots in their ovarioles, 90 per cent of the eggs had been deposited by the hibernated females.

But unlike the hibernated generation, intervals among the curves were much shortened in the first generation (table 2 and Fig. 3). Furthermore, the percentage curve of oviposited females overlapped with the accumulated percentage curve of the deposited eggs. This fact suggested that each female that survived to the time of egg laying deposited approximately one egg mass under natural conditions. The effective oviposition period during which about 90 per cent of the egg of the first generation were laid was about two weeks or in the late half of July. The situation was somewhat different in the second generation, because the intervals of the curves were much shortened and almost coincided with each other. The starting point of accumulated percentage curve of the deposited eggs delayed somewhat in time as compared with those of the curves of the oviposited and black spotted. This deviation seemed to be partly due to the incompleteness of sampling of eggs and partly due to the fact that those eggs attributed to the oviposition by females of the second generation mixed partly with those laid by the reproductive females belonging to the third generation. Otherwise, the accumulated percentage curve of the deposited eggs in the second generation would have a similar relation with other three curves as observed in the first generation. The surviving females of the second generation therefore would deposit approximately one egg mass per female.

As the laboratory experiments showed that the egg laying of females was preceded by mating behaviour at least by a week (Kiritani, 1963a), the relationship between the degree of ovarian development and mating was examined. About 20 per cent
of the hibernated females which were designated as the stage (−) mated at least once, whereas both in the first and the second generations, the mated females were invariably at least at the stage (+) of ovarian development. On the other hand, those females designated as (‖‖) were all mated irrespective of the generation. The different nature of the hibernated adults in respect to the relation mentioned here seems to be attributable to their long life span as compared with the successive generations.

NUMBER OF GENERATIONS IN A YEAR

An electric lamp of 40W was used as a

Fig. 4. Duration of different generation was represented in proportion of old and new adults(A). Current representation of the emergence period of the adults producing four generations(B). Change in percentage of the newly emerged adults which were involved in the individual collections(C).
light trap and daily examinations were made on the number of catches from the beginning of April to the last date of November when adults cease to be trapped. The capture records in 1962 were represented in Fig. 2A. The number of insects trapped by an electric lamp is affected by such factors, intensity of the light source, weather conditions, position of the trap and physiological condition of the insects. Hence, the relative abundance of each generation obtained by the light trap is not comparable to each other without remark.

The light trap used in the present survey was situated on a marginal footpath of the paddy field where censuses of oviposited eggs of the second generation were conducted. It was considered that the relative abundance of hibernated adults was likely to be underestimated. On the other hand, that of the first generation was overestimated due to the mass migration of adults to the paddy field concerned. Most of the adults of the third generation emerged in the middle part of October as indicated by the trap records. These adults would have to be produced from the eggs deposited during the early part of September taking the duration of immature stages into account. Decline of the first peak in the percentage curve of the matured females of the second generation in Fig. 2B was entirely due to the appearance of individuals preparing for hibernation. These individuals began to appear on September 11, thereafter most of the females that emerged newly remained in a state of immaturity, and mating as well as ovarian development did not occur until next spring. In spite of this, there observed a second peak of the curve concerned on September 25 and the females designated as the stage (++) and (+++) persisted up to October 25. It is considered that these females emerged as the third generation in the early part of September when others were in the second. This can be perceived also from the capture records that there was yet a continuous small number of adult flight or emergence mainly in November after passing the period of mass flight. A schematic illustration of the seasonal histories of adult populations with different numbers of generations was given in Fig. 4. It must be mentioned, however, that the emergence periods of the population having four generations is current ones, because it was assumed that these individuals had originated from the eggs deposited in early part of the oviposition period by the hibernated females.

**FAT BODY**

An index of fat volume in each sampling was calculated by giving numerals 1, 2 and 3 for the amount of fat body little, medium and much, respectively. Generally speaking, changes in volume and shape of fat body cells were remarkable in the third generation (Figs. 1 and 2D). Before hibernation, females of the third generation showed no sign of mating as well as ovarian development, but increased in amount of fat body as time went on. The index of amount of the fat body reached to the maximum on October 13 which corresponded to the time when the light trap recorded the maximum catch (cf. Fig. 2A). Thereafter, the index decreased again successively for a period in consequence of the emergence of the fourth generation. But it is certain that these individuals of the fourth generation would have resumed the same trend as in the third generation before going to their wintering quarters. The volume of fat body decreased as they resumed resumption of activity in spring. In May when eggs began to be laid, the index almost fell to unity or little, and it continued to fluctuate within a narrow range over the successive generations until the emergence of the third generation. There were three peaks in the middle of May, middle of July and early part of September, each corresponding to an early period of reproductive activity of each generation.

It was observed that the fat body inde
Table 4. Number of eggs in the ovaries in relation to the generation and actual size of egg masses observed in the fields in 1962.

<table>
<thead>
<tr>
<th>Parent generation</th>
<th>Hibernated</th>
<th>1st</th>
<th>2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissection</td>
<td>No. of females dissected</td>
<td>119</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Average no. of eggs</td>
<td>73.2</td>
<td>79.7</td>
</tr>
<tr>
<td>Field observation</td>
<td>No. of egg masses observed</td>
<td>47</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>Mean size of egg masses</td>
<td>73.5</td>
<td>86.3</td>
</tr>
</tbody>
</table>

was closely related to the ovarial development and egg laying (Table 3). The amount of fat body was relatively small among the newly emerged adults and increased until the beginning of ovarial development, but again decreased as the ovaries developed. Such a relation, however, was not maintained among the females that had oviposited at least once. An increase in the amount of fat body among the pre-hibernating females was accompanied by a change of the size of fat body cells. The hypertrophy of fat body cells was illustrated in Fig. 1. Deformation of the fat body cells was observed from the middle part of October and this condition lasted until next spring when oviposition began. 

NUMBER OF EGGS IN OVARIES IN RELATION TO GENERATION

The number of eggs contained in the ovaries was assessed for the females designated as stage (++]=. Only a number of eggs in an ovariole was examined and the number was multiplied by 14 which is the number of ovarioles as to give the total number of eggs. The mean number of eggs in the ovaries fluctuated showing three apparent peaks which increased in value from generation to generation (Fig. 2D). Such a sort of fluctuation could not be explained sufficiently by the change in the mean size of egg masses relative to the date of oviposition in the fields. Comparison was made between the numbers of eggs retained in the ovaries and the numbers per egg mass actually observed in the census fields (Table 4). The mean size of egg masses deposited by the hibernated females was almost the same with the mean number of eggs obtained by the dissection. But in the first and the second generations, the mean numbers of eggs found in the ovaries were somewhat less than those obtained from the egg masses in the fields. A rather discontinuous increase in the mean size of egg masses with regard to each generation might be explicable assuming that the mean number of eggs developed in each ovariole increases by unity in successive generations, viz. five per ovariole in the hibernated, six in the first and seven in the second, thus the total number of eggs would be 70, 84 and 98, respectively.
DISCUSSION

There is a considerable evidence to show that a shift in population density of an insect species is associated with or preceded by a change in physiological condition with or without a change in external appearance. The phase variation observed in the locusts and noctuids is one of the most outstanding response of the insect to the level of their population density. Aside from the phase variation, there is an evidence suggesting that a change in the physiological condition is responsible to the population fluctuation among species of the forest insects (ref. Tischler, 1955; Utida, 1957; Graham, 1963). It has been claimed by Russian workers that numerical fluctuations in the population of Eurygaster integriceps are associated with changes in the physiology or morpho-functional condition of the individuals. Recently, Brown (1962) has made an extensive review in regard to the Russian system of forecasting on the basis of morpho-functional changes. Fedotov and his coworkers postulated that fall in numbers of E. integriceps resulted from a decline or depressed condition in certain of the internal organs, especially the fat and food reserves, which determine the resistance of the insect to adverse conditions during the overwintering period. It is possible frequently to forecast the strength of invasions at least once a year in advance by studying the morpho-functional conditions, because, if the physiological condition is good, the population may continue to maintain itself at a high level for a longer time even in the face of unfavourable environmental conditions (ref. Brown, 1.c.). Prediction of the time of occurrence of the first generation of the rice stem borer, Chilo suppressalis, suggested by Fukaya (1951) on the basis of annual variation in intensity of diapause of the hibernated larvae is another example on the same line.

It was elucidated that the dissection method was useful in predicting some phases of oviposition process of the bug, especially in the hibernated generation (table 2).

Movement of the females between oviposition plants and feeding plants (Kiritani, 1963 b), and their polyphagous habit (Oho & Kiritani, 1960) made it difficult to determine the oviposition process of the bug by censuses in a specified field. Under these circumstances, prediction of the oviposition process by dissection is more effective than conducting laborious surveys in the field. Furthermore, in decision of the date or period when overall control measures should be applied, or in decision of the sampling date to assess the percentage parasitism or and density of the egg mass, the dissection of adults obtained from different places would provide more accurate and unbiased decision than the method based upon the censuses in some specified fields. By regular dissections of adults, it can be determined the number of the generation a year even when different generations overlapped. Moreover, the emergence of an additional generation or the fourth was possible to forecast nearly before six weeks by dissecting its parents.

A new suggestion was made estimating the fecundity of natural populations by incorporating the result of dissection into that of field census on egg masses. There are yet some points of inconsistency among the estimates with regard to the fecundity obtained from laboratory experiment and experimentation in the field cages (Kiritani, 1963 a; Kiritani et al., 1963; and unpublished data). The method proposed here, though it leaves much room for improvement, would be useful in studying the fecundity of natural population of insects. The fact that the mean number of eggs in the ovaries of mature females well corresponded with the mean size of egg masses collected from the field, suggested that the former could be used in study of the variation in egg mass size with regard to the generation and year.

Arrest of the ovarial development and accumulation of the fat body being accom-
panied with the hypertrophy of fat body cells was observed among the pre-hibernating females. This suggested that the females pass the winter as imaginal diapause. But about half of the male adults collected from hibernacula had active sperms in their testis. This fact offers a problem on the difference between quiescence and diapause as elucidated by Hodek and Cerkasov (1960) in Coccinellidae. A similar phenomenon in regard to differential maturity between the sexes was also observed in E. integriceps by Brown (1962). The results obtained in the present study suggested that the dissection method would provide one of the useful means not only for prediction, but also for the study of population dynamics of the insects.

**SUMMARY**

A total of 1,161 females of the southern green stink bug, *Nezara viridula* were dissected throughout its active season from April to December in 1962 at an interval of four or five days. Each individual was examined in regard to the following points; (1) degree of maturation of the ovaries discriminating five stages, (2) virgin or mated, (3) non-oviposited or oviposited, (4) appearance of black spots in the ovarioles as an indication of termination of oogenesis, (5) number of eggs contained in the ovaries of mature female and (6) amount of fat body. To check the results obtained from the dissection, censuses were conducted during the oviposition period of each generation to determine the egg laying process relative to time. Capture records of adults by a light trap were employed in interpretation of the seasonal history of the bug.

It was suggested that by regular dissections of females, it was possible to predict the process of oviposition knowing the intervals among percentage curves in regard to the mating, oviposition and black spotted, respectively. The number of generations in a year can be assessed by the dissection even when generations overlap, and the occurrence of an additional generation can be forecasted by dissecting its parents. The relation between the percentage curve of oviposited females and the accumulated percentage curve of the number of egg masses which were laid in the field was available for estimating the fecundity of the natural population. The number of eggs in the ovaries of the mature females corresponded well with the size of the egg masses observed actually in the field. The amount of the fat body considerably increased before and during hibernation. This seemed to be responsible for the hypertrophy of the cells. The differential maturity between the sexes in the hibernating population was suggested.

It was concluded that the changes in the reproductive system provide a useful means predicting the changes of seasonal history in the population of the southern green stink bug and application of this method is promising to other insect pests.

**ACKNOWLEDGEMENT**

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摘　要
ミナミアオカカメシの生殖組織の変化とその季節的消長の予察への応用

桐谷　圭治

和歌山県農業試験場朝来試験地

総計 1,161頭のミナミアオカカメシ Nezara viridula の雌成虫を 1962 年の 4 月から 12 月にかけて 4 ～ 5 日間隔で 1 回隔て 20 頭ずつ解剖し、(1) 卵巢の成熟度 (5 段階), (2) 交尾状況と未交尾か, (3) 育児経験の有無, (4) 卵卵発育の経過について現われる卵巣小管内の “黒点” の有無, (5) 成熟卵の卵数, (6) 脂肪体の多少とその細胞の形状などを観察した。また各世代の産卵期間における野外での産卵状況を調査するとともに誘が灯にによる捕殺虫数の記録もあって参考にした。

交尾・産卵・黒点率曲線の関係から産卵開始日や産卵盛期あるいは終期を越冬世代成立では数週間前に予察し得る。また各世代の成虫は重なりあって出現するが解剖によれば多くの場合世代間の区別がつくうえ、年間世代数もわかる。また部分的に第 4 世代を生じる場合も現世代の産卵発育曲線の傾向から予測できる。

産卵卵の割合と野外における累積産卵曲線の関係から自然における成虫個体群の産卵回数も推定できる。すなわち産卵期まで生存した越冬世代成虫では 3 ～ 4 卵塊、それ以後の世代では平均 1 卵塊しか使わない。成熟卵の卵数は各世代の野外における卵塊サイズとよく一致した。脂肪体の量は越冬前には増加し越冬後活動の開始とともに著しく減少する。これはおもに脂肪体細胞の肥大によると考えられる。

以上の結果から雌の生殖組織の変化はミナミアオカカメシの季節的消長を予察する上でたいへん有効な手段となりうることがわかった。この方法はひろく他の害虫にも応用できると思われるが、ときに他のカメシ類については直ちに適用しうると思われる。

抄　録
アワノメイガの個体群動態における降雨の役割


従来、多くの研究者によって降雨がアワノメイガの発生に影響していることが言われてきたが、降雨量や降雨の頻度が個体群の動態にどのような役割をはたしているかを実験的に確かめた例はない。

このために成虫を放し育育箱を野外と実験室内に設置して、人工的に雨を降らせて、種々の降雨の条件下での成虫の寿命 (50% 死亡日数)，産卵数，増殖率 R0 を求めた結果，降雨量の多い場合に寿命，産卵数，R0 ともに低下していた。このことは実験室内での結果でも確かめられ，降雨量が過少の場合，次世代の個体数は減少することが推察された。

次に，降雨の頻度は多いほど，また降雨時の温度は高いほど産卵期は多くなり，R0 は高まることも確かめられれた。この原因は，降雨によって成虫の摂取する水が与えられ，また温度が高まるので体内外から食料が失われ，このことが産卵期や寿命に影響するものと思われる。もちろん，降雨量の多いとき，成虫の死亡による減少もありはあるが，全体的に見ると，降雨は次世代の個体数を増加させる方向に働くものと考えられる。

（農技研　中村　和雄）