AN EXPERIMENTAL APPROACH TO SOME QUANTITATIVE ASPECTS OF GRAZING BY SILKWORMS (BOMBYX MORI)

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Synopsis


Measurements of how much proportion of ingested or assimilated food in terms of dry matter, caloric content and nitrogen content is converted into larval tissue ("conversion efficiencies") and reserved till the pupal stage ("reserve ratios") were made with a silkworm population, for the purpose of considering what criterion is most suited to characterize
the structure and function of an ecosystem. The silkworm population was supplied sufficiently with fresh mulberry leaves (Morus alba) every six hours, being reared in an experimental room under controlled conditions.

The obtained results suggest that the form of the ecological pyramid represented by nitrogen content is clearly different from that represented by biomass or caloric content. A herbivore loses by grazing a less proportion of nitrogen from its tissues as compared with the other two criteria.

Reserve ratios are rather preferable to conversion efficiencies as a standard to discuss the values of the coefficients, like efficiency of conversion, of lepidopterous insects together with various kinds of animals, because the lepidopterous larvae, particularly silkworms, make cocoons which are not of protoplasma.

**Introduction**

In these four decades, a number of ecologists have been treating energy flow as the main criterion that characterizes the structure and function of an ecosystem. Quite reasonable as this tendency is, we do not think that the ecosystem can be described only with energy flow, since energy flow does not necessarily imply the flow of protoplasmic substances around the ecosystem. Besides the ecological pyramid of the caloric content, we consider that constructing another pyramid, namely that of nitrogen content, is one of the most indispensable tactics for depicting the distinct feature of a complex ecosystem.

Many reports from fields and laboratories have cast light on the static and dynamic states of the grazing system to some extent, and yet what has come to light are too misty to satisfy most of ecologists who expect to grasp the clear figures of food chains in the biosphere. We intend to demonstrate our experimental data regarding the trophic relation of a mulberry-silk worm system in detail.

We can find a great number of fundamental and practical works in sericultural science published since the last century, and many of them are interesting. Especially, those by KAWASE (1914) and HIRATSUKA (1917) should be appreciated. They computed the balance sheet of energy flow through a silkworm, though not from the view point of ecosystem ecology. We consider that the mulberry-silk worm system is one of the best experimental materials for the investigation of plant-herbivore systems except for the too splendid cocoon of silkworm, because (i) we can accurately estimate the quantities needed for the studies, such as the amount of intake and faeces, etc., (ii) a silkworm takes only during its larval stage and usually feeds on nothing but mulberry leaves by nature, although an artificial food has been lately made.

Furthermore, we find the studies of certain species of lepidopterous larvae from insect physiology by EVANS (1939a, b), and can compare our data with his.

**Material and Methods**

This report is based upon two experiments, one of which (Expt. A) was made in the coconery of the Department of Sericulture, Faculty of Agriculture, Tokyo University of Agriculture and Technology, from May to June, 1964, and the other (Expt. B), in a laboratory of the Department of Botany, Faculty of Science, University of Tokyo, from July to August, 1966.

The silkworm (Bombyx mori) used in both experiments was a hybrid between the female of “Japanese 106” and the male of “Daizo”; the latter had become hereditarily stable at an old time. The hybrid is one of the most resistant to every silkworm disease, though it is not used by farmers because of its small cocoon. A larva of this hybrid usually grows a pupa after the fifth moult.

The eggs at both experiments were prepared at the Laboratory of Genetics and Breeding of Silkworms of Tokyo University of Agriculture and Technology, and the larvae were hatched in a room under conditions of a constant temperature 22 ~ 24°C, nearly saturated high relative humidity and illumination of roughly 50 lux. These conditions have much influence on their modes of life during the course of not only the present generation, but also the next generation. At the spring hatching (Expt. A in this report), if only external conditions are satisfactory, no artificial operation is needed. In Expt. B (summer hatching), however, some proper treatments had to be
adopted so as to activate diapause eggs.

1. Experiment A

Soon after the larvae hatched, they were moved to a room with constant light intensity 100–200 lux, air temperature 23~25°C, and relative humidity 60~75 per cent. In the room about 2 per cent solution of formalin had been sprayed ten days before hatching to prevent the silkworms from catching pathogenic diseases.

The variety of the mulberry tree (*Morus alba*) chosen was "Ichinose", one of the most nutritive varieties. The trees used were grown under ordinary management with manure and chemical fertilizer in the farm of the university mentioned above.

A silkworm population consisting, at first, of nearly 10,000 individuals was cultured according to the current manner of rearing in Japan.

The mulberry leaves with the twigs were picked at 6 p.m. every evening when most of the stomata of the young and mature leaves were closed, and then reserved in a box with cool and humid air. Every larva in the population was supplied sufficiently with fresh leaves every six hours except during the quiescent period.

The time when each quiescent period started was determined as follows: when about 80 per cent of the population stopped to take the leaves. The time of the end of a quiescent period was similarly defined; when about 80 per cent of the population exuviated. The sampling for determining their body weight was, however, made at the time when nearly all of them had exuviated.

From the population an adequate number of individuals, 500 in the first and the second instars, 300 in the third, 200 in the fourth, and 50 males and 50 females separately in the last, were sampled at random every 24 hours except during the quiescent period. The samples were reared in the same manner as the population, so that any kind of data could be collected every day. The body of this hybrid is so small that we can discern its sex without a microscope only in the last instar.

2. Experiment B

In this experiment, the silkworms were parted into two groups soon after the fourth moult. Every member of the first group (the test group) of 10 males and 10 females could take intact leaves from the outset of the last instar. The second group (the control), consisting of the same number of silkworms as the first, was treated in the same way as those in Expt. A, except that the sampling was made only at the start of the last instar.

After the fourth moult, the mulberry variety was changed from "Ichinose", which had been used in Expt. A, to one of "Rosó" strains. Four years old trees were transplanted in pots 22cm in diameter in May, 1966. Prior to the experiment, the pots were randomly parted into two batches, namely, the one for the test group of the silkworms and the other for the control group. This experiment was carried out in a room with the same conditions as in Expt. A. In the first group, every silkworm larva was placed by us upon a twig so that they could sufficiently take the intact leaves without competition between one and another. However, once they were settled, we did not touch them except when they dropped from the twigs or leaves which they had been ingesting. When the silkworm had consumed about 70 per cent of the leaves in area, the tree was replaced by another. The other group was supplied with the leaves in the same manner as in Expt. A.

Methods for Estimation of Each Biological Value

1. Estimation of the amount of intake

(a) In the case of picked leaves: All the procedures used here had been established in sericultural science according to the following formula;

\[ w_i = aW_s - w_r, \]

where \( w \) and \( W \) mean dry weight and fresh weight respectively, and the suffixes \( i, s \) and \( r \) indicate ingested, supplied (ration) and remnant leaves. Coefficient \( a \) is the ratio of dry weight to fresh weight of the supplied leaves, and was determined with the leaves sampled at random from what should have been the members of the ration. \( W_i \) is not equal to \( w_i/a \), but \( w_i/a' \), where \( a' \) indicates the ratio of fresh weight to dry weight of edible parts (eliminating conspicuous leaf veins) of supplied leaves, since a silkworm usually would not take in leaf veins. That is;

\[ a = \frac{w_s + w_r}{W_s + W_r} \]

while \( a' = \frac{w_s}{W_s} \).
where the suffixes e and v represent the edible parts and veins of the leaves, respectively.

This method was applied to the estimation of the amount of intake in Expt. A and in control group in Expt. B.

(b) In the case of intact leaves: The mulberry trees transplanted into pots were kept in a dark room more than one day so as to make the excessive products translocated from the leaf blades and to make the deviation of the leaf weight per area as small as possible. All the leaves on the trees were copied directly onto the sheets of photographic paper as shown in Fig. 1, and the copies were cut along their outlines. According to the number of leaves, 7~15 leaves were sampled at random and dried to estimate the adequate value of the coefficient (b in the following equation) between dry leaves and the corresponding copies in weight. The rest of leaves were given silkworms as mentioned already. The coefficient, of which degree of correlation was never less than 0.96, was determined through the least square method with respect to each tree. The following equation gives the dry weight of ingested leaves;

\[ w_i = b W_r - w_r, \]

where \( W_r \) represents the weight of the copies of supplied leaves.

Fig. 1. Method for estimation of standing crop of mulberry leaves.
(See text for details).

We neglected the error, which EVANS (1939a) pointed out, caused by the respiratory loss of supplied leaves in the duration, because it must have been small enough to be neglected, though we cannot say to what extent it had influence on the estimation.

2. Body weight of silkworms

During Expt. A, the larvae were sampled from the population for measuring their live weight and dry weight. In the course of the fourth and fifth instars but the fourth quiescent period, inasmuch as a larva is occupied about a half of its live weight by the ingested food in its gut (SAKAI, 1950), we carried out an operation to remove the whole gut from its body to obtain the value of the so-called "true" dry weight of the larva, besides the "apparent" dry weight of a simply dried one. The latter includes the dry weight of the ingested leaves in the gut. The "true" dry weight does not include the dry weights of the gut itself and the body fluid lost by the operation. Until the end of the third instar, the live weight and the dry weight were measured with the larvae which had been starved for several hours after sampling to make the value as "true" as possible. The procedure depends upon SAURAT'S report (1930) which states that most of ingested leaves passes through the gut within four hours even during the third instar.

The body weight of a pupa is easily measured. As to a male imago, there is a problem; because the scales are whirled up from the wings at every flap. And yet, the error caused by this seems to be trivial.

3. Amount of faeces excreted during a period

After the beginning of the third instar, the faeces scattered among the remnant leaves are solid and can be collected without difficulty by quivering the leaves. Until the end of the second instar, because each of the faeces is too minute and too light to be separated from the leaves only by quivering leaves, it is necessary to rub the faeces with fingers or pick up each with small forceps to collect all of them. These troublesome works cannot be avoided to estimate correctly the amount of ingested leaves.

The faeces include various sorts of excretory products, of which the main component was uric acid (HATANO, 1914; HIRATSUKA, 1914 and 1917; KAWASE, 1917; FUKUDA, 1951). This was made clear by analyzing chemically not the faeces but the urine excreted at the end of the larval stage (so-called "matured state") and at emergence.
4. Amount of assimilated food during a period

Theoretically, the following equation will give the accurate value of the amount of assimilated food;

\[ w_n = w_i - w_f + w_p + w_e \]

where \( w_n \), \( w_i \), \( w_f \), \( w_p \) and \( w_e \) mean the dry weights of assimilated food, ingested food, faeces, excretory products and waste tissue from the gut. At present, however, we have no method to determine the accurate weights of the excretory products and waste tissues both of which are included in the faeces. We, therefore, cannot help making use of the following approximation;

\[ w_n \approx w_i - w_f + w_p \approx w_i - w_f \]

where \( w_n \) represents the weight of the uric acid in the faeces. Usually we are allowed to neglect \( w_e \), so that we also use the definition \( w_n \approx w_i - w_f \). To the caloric value of assimilated matter a similar definition is applicable. However, concerning the assimilated nitrogen, we must consider \( w_n/3 \) which corresponds to the nitrogen content of uric acid.

5. Amount of urine, exuviae and cocoon

Urine excreted twice during the life of a silkworm is measured in amount with high difficulty, since it is liquid. Its main component is considered to be uric acid (Hiratsuka, 1917; Fukuda, 1951), and no organic substance but nitrogenous compounds have been found. Therefore, measuring the amount of uric acid held in the body of a larva or a pupa which is going to excrete urine, we may roughly infer to the whole amount of urine substance, though Isaka (1952) has pointed out that a little amount of uric acid remains in a prepupa after excretion.

It is easy to collect their exuviae and cocoons, and there is no problem in determining their amounts.

6. Chemical analysis

(a) Total nitrogen content: Total nitrogen content was evaluated by the micro-Kjeldahl procedure. Each value is expressed by the arithmetical mean of three samples from the corresponding material.

(b) Uric acid content: Uric acid content was measured by a newly improved method through the ion exchange resin, Dowex 1×10 formate type (Tojo and Hiran, 1966). This method can distinguish uric acid from other nitrogenous compounds, e.g. purines or amino acids, better than previous methods through chemical reactions.

7. Caloric content measurement

The caloric content of the materials was measured by two kinds of bomb calorimeters according to the collected amount of materials. The one (A) was applied to the material which permitted preparation of five samples each of which was more than 1 g in dry weight, by this calorimeter the order of more than 10 cal./g being significant. The other (B) was applied to the material with less abundance and has the ability to make the order of more than 100 cal./g significant.

Scott (1965) insists that the estimate of caloric content shown in many papers does not mean the enthalpy, but the internal energy in thermodynamics, and that one should calculate the value of the former rather than that of the latter. Though we agree with his opinion, we did not calculate it since the calculation is much complicated, in addition, we would like to compare our data with those already published by other investigators.

As a rule, a material was generally burst five times. When the deviation range of the values per gram dry weight on three times' trials was less than 1 per cent of the mean of the three, further trials were omitted to save time and labour. The coefficient of variation on five times' trials was always less than 2 per cent at calorimeter A, less than 5 per cent at calorimeter B. Inasmuch as it requires heavy works to determine the caloric contents, we did not burst everyday's materials, but every other day's and the value per gram dry weight was applied to the next day's.

Results of Experiments

1. Experiment A

Tables 1, 2 and 3 give some sketches of how the silkworms grew throughout the larval stage in Expt. A.

In the mid course of the fourth and fifth instars, the difference between the mean "true" dry weight and the mean "apparent" one is rather considerable. However, at the end of the fifth instar (matured state) the difference becomes insignificant, since the larvae which have reached the matured state excrete all the matter in their guts. Inasmuch as it takes some hours for the ingested matter to go through the gut and as the matter ingested
### Table 1. Intake, assimilation and growth of silkworm in terms of biomass

<table>
<thead>
<tr>
<th>Stage</th>
<th>Hours from Hatching</th>
<th>Cumulative Amount of Ingested Matter for 100 Individuals g d.w.</th>
<th>Cumulative Amount of Assimilated Matter for 100 Individuals g d.w.</th>
<th>Assimilation Rate in Instar %</th>
<th>Growth of Silkworm Dry Weight mean×100±s.d.×100 (No. of Samples)</th>
<th>Growth of Silkworm Live Weight mean×100±s.d.×100 (No. of Samples)</th>
</tr>
</thead>
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<tr>
<td>lst Instar</td>
<td>60</td>
<td>male female mean 0 0.34</td>
<td>male female mean 0 0.24</td>
<td>70*</td>
<td>male female mean 0.031(300) 0.431(200)</td>
<td>male female mean 0.008(300) 0.068(200)</td>
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<td></td>
<td>20</td>
<td>male female mean 0.34</td>
<td>male female mean 0.24</td>
<td></td>
<td>male female mean 0.428(200) 0.059(200)</td>
<td>male female mean 0.068(200)</td>
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<tr>
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<td>80</td>
<td>male female mean 0.34</td>
<td>male female mean 0.24</td>
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<td>male female mean 1.74(200) 0.26(200)</td>
<td>male female mean 0.21(200)</td>
</tr>
<tr>
<td>2nd Instar</td>
<td>130</td>
<td>male female mean 1.08</td>
<td>male female mean 0.56</td>
<td>42</td>
<td>male female mean 1.06(300) 0.21(300)</td>
<td>male female mean 0.21(200)</td>
</tr>
<tr>
<td>Moult</td>
<td>148</td>
<td>male female mean 1.08</td>
<td>male female mean 0.56</td>
<td></td>
<td>male female mean 8.93(200) 1.2(200)</td>
<td>male female mean 0.21(200)</td>
</tr>
<tr>
<td>3rd Instar</td>
<td>208</td>
<td>male female mean 5.37</td>
<td>male female mean 2.63</td>
<td>48</td>
<td>male female mean 8.17(200) 0.93(200)</td>
<td>male female mean 0.21(200)</td>
</tr>
<tr>
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<td>male female mean 5.37</td>
<td>male female mean 2.63</td>
<td></td>
<td>male female mean 15.6(100) 1.9(100)</td>
<td>male female mean 0.21(200)</td>
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<tr>
<td>4th Instar</td>
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<td>male female mean 8.9</td>
<td>male female mean 3.9</td>
<td>41</td>
<td>male female mean 29.5(100) 3.8(100)</td>
<td>male female mean 0.21(200)</td>
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<tr>
<td></td>
<td>283</td>
<td>male female mean 17.3</td>
<td>male female mean 7.5</td>
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<td>male female mean 44.7(50) 6.5(100)</td>
<td>male female mean 0.21(200)</td>
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<td></td>
<td>303</td>
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<tr>
<td>Moult</td>
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<td>male female mean 32.0</td>
<td>male female mean 13.4</td>
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<td>male female mean 37.9(300) 43.8(300)</td>
<td>male female mean 0.21(200)</td>
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<tr>
<td>Pupa</td>
<td>520</td>
<td>male female mean 267</td>
<td>male female mean 269</td>
<td></td>
<td>male female mean 88(123) 19.8(28.8)</td>
<td>male female mean 0.21(200)</td>
</tr>
</tbody>
</table>

* This value may be overestimated.

### Table 2. Intake, assimilation and growth of silkworm in terms of caloric value

<table>
<thead>
<tr>
<th>Stage</th>
<th>Hours from Hatching</th>
<th>Cumulative Amount of Ingested Calory for 100 Individuals kcal.</th>
<th>Cumulative Amount of Assimilated Calory for 100 Individuals kcal.</th>
<th>Assimilation Rate in Instar %</th>
<th>Calorie Content of Silkworm Dry Weight mean×100±s.d.×100 (No. of Samples) kcal.</th>
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</thead>
<tbody>
<tr>
<td>lst Instar</td>
<td>60</td>
<td>male female mean 0 1.54</td>
<td>male female mean 0 1.07</td>
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<td>male female mean 0.05(300) 0.33(200)</td>
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<td></td>
<td>20</td>
<td>male female mean 1.54</td>
<td>male female mean 1.07</td>
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<td>male female mean 0.05(300) 0.33(200)</td>
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<tr>
<td>Moult</td>
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<td>male female mean 1.54</td>
<td>male female mean 1.07</td>
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<td>male female mean 0.28(200)</td>
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<tr>
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<td>male female mean 5.0</td>
<td>male female mean 2.6</td>
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<td>Moult</td>
<td>148</td>
<td>male female mean 5.0</td>
<td>male female mean 2.6</td>
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<td>male female mean 1.1(200)</td>
</tr>
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<td>male female mean 12.0</td>
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<td>Moult</td>
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<td>male female mean 25.3</td>
<td>male female mean 12.0</td>
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<td>male female mean 5.6(200)</td>
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<td>4th Instar</td>
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<td>male female mean 18</td>
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<td>male female mean 8(120)</td>
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<td>283</td>
<td>male female mean 41</td>
<td>male female mean 18</td>
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<td>male female mean 148</td>
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<td>Moult</td>
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<td>male female mean 148</td>
<td>male female mean 64</td>
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<td>male female mean 8(120)</td>
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<td>387</td>
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<td>male female mean 483</td>
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<td>male female mean 206(206)</td>
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<td>male female mean 1166</td>
<td>male female mean 483</td>
<td></td>
<td>male female mean 119(164)</td>
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</table>

* This value may be overestimated.
Table 3. Intake, assimilation and growth of silkworm in terms of nitrogen content

<table>
<thead>
<tr>
<th>Stage</th>
<th>Hours from Hatching</th>
<th>Cumulative Amount of Ingested Nitrogen for 100 Individuals</th>
<th>Cumulative Amount of Assimilated Nitrogen for 100 Individuals</th>
<th>Assimilation Rate in Instar</th>
<th>Nitrogen Content of Silkworm for 100 Individuals</th>
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</thead>
<tbody>
<tr>
<td>1st Instar Moul</td>
<td>0 60</td>
<td>male female mean 0.016</td>
<td>male female mean 0.012</td>
<td>75*</td>
<td>0.011 0.008</td>
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<tr>
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<td>80</td>
<td>male female mean 0.016</td>
<td>male female mean 0.012</td>
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<td>0.007</td>
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<td>male female mean 0.055</td>
<td>61</td>
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<td>0.13</td>
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<td>0.18</td>
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<td>0.32</td>
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<td>male female mean 0.81</td>
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<td>0.54</td>
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<tr>
<td>Moult</td>
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<td>male female mean 1.38</td>
<td>male female mean 0.81</td>
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<td></td>
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<td>6.2</td>
<td>6.1</td>
</tr>
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<td></td>
<td>1.13</td>
<td>0.61</td>
<td>0.53</td>
<td>0.46</td>
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</table>

* This value may be overestimated.

Table 4. Amount of uric acid excreted by 100 individuals

<table>
<thead>
<tr>
<th>Stage From Hatching to Matured State At Pupation* At Emergence*</th>
<th>Uric Acid g</th>
<th>Caloric Content kcal</th>
<th>N Content g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male female</td>
<td>male female</td>
<td>male female</td>
<td>male female</td>
</tr>
<tr>
<td>0.76 0.64</td>
<td>0.76 0.64</td>
<td>0.31 0.37</td>
<td>0.60 0.29</td>
</tr>
</tbody>
</table>

* These values were estimated not from the urine itself, but from the larva or the pupa bodies which were going to excrete it.

Table 5. Amount of exuviae for 100 individuals

<table>
<thead>
<tr>
<th>Stage</th>
<th>Dry Weight g</th>
<th>Caloric Content kcal</th>
<th>N Content g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Moul</td>
<td>0.005</td>
<td>0.0075</td>
<td>0.003</td>
</tr>
<tr>
<td>2nd Moul</td>
<td>0.012</td>
<td>0.0003</td>
<td>0.0014</td>
</tr>
<tr>
<td>3rd Moul</td>
<td>0.047</td>
<td>0.019</td>
<td>0.0056</td>
</tr>
<tr>
<td>4th Moul</td>
<td>0.20</td>
<td>0.94</td>
<td>0.022</td>
</tr>
<tr>
<td>At Pupation</td>
<td>0.45</td>
<td>2.2</td>
<td>0.050</td>
</tr>
<tr>
<td>At Emergence</td>
<td>1.9</td>
<td>6.1</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* Sexes are not distinguished.

Table 6. Amount of cocoon for 100 individuals

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dry Weight g</th>
<th>Caloric Content kcal</th>
<th>N Content g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>77</td>
<td>2.6</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>87</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Table 7a. Amount of imago for 100 individuals

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dry Weight g</th>
<th>Caloric Content kcal</th>
<th>N Content g</th>
<th>Nmg/g d.w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>14.0</td>
<td>94</td>
<td>1.0</td>
<td>71</td>
</tr>
<tr>
<td>Female</td>
<td>21.6</td>
<td>94</td>
<td>2.1</td>
<td>97</td>
</tr>
</tbody>
</table>

Table 7b. Amount of egg laid by 100 females

<table>
<thead>
<tr>
<th>Dry Weight g</th>
<th>Caloric Content kcal</th>
<th>N Content g</th>
<th>Nmg/g d.w</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>67</td>
<td>1.1</td>
<td>92</td>
</tr>
</tbody>
</table>

* A female produced approximately 500 eggs.

toward the end of each instar, except the last, hardly goes through during the quiescent period, there inevitably happen estimation errors in the amount of assimilated matter. We have neglected the error caused by the deficiency of the data for evaluating how much uric acid was excreted during the infant instars. We can follow the dry weights, caloric contents and nitrogen contents of the excreted uric acid, exuviae, cocoon, imago and eggs in Table 4~7.

From the tables (1, 2 and 3) we have evaluated the conversion efficiencies in terms
of dry weight, caloric content and nitrogen content defined according to Ivlev's conception (Ivlev, 1960), and PER (protein efficiency ratio) written in a text book for a nutritionist (FAO and WHO, 1965) as follows:

\[ E_{GM} = \frac{\Delta w_{i} / t}{\Delta w_{i} / t} \] where \( w_{i} \) represents the true dry weight of a larva, \( \Delta w_{i} \), the dry weight of ingested matter, and \( t \), time

\[ E_{GC} = \frac{\Delta C_{i} / t}{\Delta C_{i} / t} \] where \( C_{i} \) represents the true caloric content of a larva, and \( \Delta C_{i} \), the ingested calory

\[ E_{GN} = \frac{\Delta w_{N} / t}{\Delta w_{N} / t} \] where \( w_{N} \) represents the true nitrogen content of a larva and \( \Delta w_{N} \), the amount of the ingested nitrogen

\[ E_{NM} = \text{net conversion efficiency of assimilated dry matter (similar to } E_{GM} \] \[ E_{NC} = \text{net conversion efficiency of assimilated calory (similar to } E_{GC} \] \[ E_{NN} = \text{net conversion efficiency of assimilated nitrogen (similar to } E_{GN} \]

Increase in the true body weight (\( \Delta w_{k} \)) during the \( k \)th instar is defined as follows:

\[ \Delta w_{1k} = w_{1(k+1)} - w_{1k} + w_{ex}, \text{ when } k \leq 4; \]

\[ \Delta w_{5k} = w_{5k} - w_{5(k-5)}, \text{ when } k = 5. \]

That is to say, \( \Delta w_{1k} \) is the increment in dry weight of a larva from the start of the present instar to the start of the next instar plus the dry weight of the exuviae. Until the end of the third instar, the true dry weight of a larva is temporarily reckoned to be equal to its apparent dry weight. A similar definition is applied to the caloric or the nitrogen content of a larva.

The above-mentioned conversion efficiencies for each instar and the whole larval stage are computed in Table 8. These values in average of both sexes as to the whole larval stage are 15% (\( E_{GM} \)), 19% (\( E_{GC} \)), 45% (\( E_{GN} \)), 42% (\( E_{NM} \)), 47% (\( E_{NC} \)) and 82% (\( E_{NN} \)).

Any of the efficiencies of the second instar is distinctly high—though we cannot tell the reason—as compared with the values of the first and the third instar. Besides, \( E_{GN} \) is

**Table 8. Conversion efficiencies (%)**

<table>
<thead>
<tr>
<th></th>
<th>1st Instar</th>
<th>2nd Instar</th>
<th>3rd Instar</th>
<th>4th Instar</th>
<th>5th Instar</th>
<th>Whole Larval Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_{GM} )</td>
<td>15</td>
<td>22</td>
<td>18</td>
<td>15</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>( E_{GC} )</td>
<td>16</td>
<td>23</td>
<td>18</td>
<td>16</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>( E_{GN} )</td>
<td>37</td>
<td>54</td>
<td>44</td>
<td>37</td>
<td>42</td>
<td>50</td>
</tr>
<tr>
<td>( E_{NM} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( E_{NC} )</td>
<td>22</td>
<td>55</td>
<td>39</td>
<td>38</td>
<td>44</td>
<td>53</td>
</tr>
<tr>
<td>( E_{NN} )</td>
<td>50*</td>
<td>91</td>
<td>68</td>
<td>64</td>
<td>70</td>
<td>96</td>
</tr>
</tbody>
</table>

* These values may be underestimated because of the overestimates of assimilation rates in the 1st instar.

![Fig. 2a. Relationships of the dry body weight of silkworms (hybrid "Daizo" × Q "Japanese 106") to cumulative amount of dry weight of ingested and assimilated mulberry leaves along the growth course from hatching to pupation. Abscissa, hours from hatching; ordinate, g dry weight in logarithmic scale. Solid line, "true" body weight of 100 larvae or pupae; broken line, cumulative amount of intake for 100 individuals; dotted line, cumulative amount of assimilated matter for 100 individuals.](image)
from twice to three times as much as $E_{GM}$, and $E_{NN}$ is approximately twice as much as $E_{NM}$. The following inequalities are composed from Table 8 at all times;

$$E_{GM} \leq E_{GC} < E_{GN}, \text{ and } E_{NM} \leq E_{NC} < E_{NN}.$$  

These relationships are read more intuitively from Figs. 2a, 3 and 4, since those values are proportional to the distances in vertical direction from points on the solid lines to the corresponding ones on the broken lines or the dotted lines in the figures. The solid line in Fig. 4 is closer to the broken or dotted line than in either of the other two figures.

Theoretically, the value of $(1-E_{NC})$ means the ratio of respiratory caloric loss to assimilated calory during a period, and was 57 per cent in the male and 49 per cent in the female for the whole larval stage.

We think that the significance of conversion efficiency has a problem in respect to a silkworm, since it makes a non-protoplasmic cocoon, for which about 60 per cent of the nitrogen contained in a matured larva is allotted. Here we propose another conception named "reserve ratio" which is defined as follows:

$$R_{GM} = \frac{\text{dry weight of pupa}}{\text{sum of ingested dry matter}},$$  

$$R_{GC} = \frac{\text{caloric content of pupa}}{\text{sum of ingested calory}},$$  

$$R_{GN} = \frac{\text{nitrogen content of pupa}}{\text{sum of ingested nitrogen}},$$  

$$R_{NM} = \frac{\text{dry weight of pupa}}{\text{sum of assimilated dry matter}},$$  

$$R_{NC} = \frac{\text{caloric content of pupa}}{\text{sum of assimilated calory}},$$  

$$R_{NN} = \frac{\text{nitrogen content of pupa}}{\text{sum of assimilated nitrogen}}.$$  

Among the respective ratios, there are the
2. Experiment B

In respect to Expt. B, only comparison of the amount of intake between the two groups was made in this report. Fig. 5 represents the results. On the first day of the last instar, the cumulative amount of intake of the test group was twice as much as that of the control group. However, the difference became smaller after a day, and reached nearly nil towards the end of the instar. The total amount of the control group was almost the same as that in Expt. A. Due to shortage of mulberry trees for the test group, no result could be obtained at the end of the instar. No silkworm was found dead throughout this experiment.

The result suggests that the total intake of a silkworm in Expt. A, where a silkworm was supplied with detached leaves every six hours, is no less than the total intake of a silkworm consuming intact leaves at all times, except when a silkworm consumes autumn leaves which become dehydrated soon after detached as manifested by TAZAKI (1960).

Discussion

Many authors, for instance, SLOBOKIN (1960), IVLEV (1960), etc., have computed energy-conversion efficiency from plants to
herbivores. As for silkworm, a caloric balance sheet was made by KAWASE (1914) and HIRATSUKA (1917); their works are appreciable even in the present time. Usually, the growth efficiency is represented as "ratio of potential energy in an animal's tissues to potential energy used in its birth and growth" (quoted from MACFADYEN's textbook, 1963, p. 74). SCOTT (1965), however, insisted that the value measured by a calorimeter never implies that of "potential energy". We also think so. At all events, the ratio in terms of energy unit is of course much better than that of dry weight. And yet, we consider that the energy flow cannot tell the full story of an ecosystem solely by itself.

In parallel with the energy flow, the circulation of protoplasmic substances should be studied. In this sense, nitrogen will be regarded as a significant indicator, for it is one of the indispensable and major elements for protoplasm as DUGDALE and GOERING have suggested (1967). We support MACFADYEN's view that "...calorific content is far from being the only criterion of a satisfactory human diet. The crops which produce the highest calorific yield, for instance, and those which are most deficient in proteins..." (MACFADYEN, 1964, p. 18). Nitrogen-conversion efficiency as well as the energy conversion-efficiency has already been computed in some reports (SASA et al., 1960; CORNER et al., 1965).

Our data allow us to compose a diagram which describes the matter, energy and nitrogen flow from the mulberry to silkworms. An average mulberry farm in Tokyo district yields nearly 0.28kg/m² of dry matter edible for silkworms at the beginning of June. This diagram as exhibited in Fig. 6 is based on the following assumptions;

1) silkworms could ingest all the leaves when they were supplied just as much as they could ingest ad libitum,
2) they could always take in just picked fresh leaves,
3) they were reared in a room with the environmental conditions as in our experiments.

Expt. B ascertained that the total amount of ingested leaves in Expt. A was nearly equal to that in the case of taking in fresh leaves at all times. On the above assumption 2), 0.28kg of edible matter would be capable of maintaining a population consisting of 50 males and 50 females, if the leaves were fully consumed on assumption 1).

According to HIRATSUKA's results (1917), a silkworm population of 50 males and 50 females could store 22%, 28% and 56% of ingested dry matter (E_{GM}), calorific content (E_{GC}) and nitrogen (E_{GN}), respectively, into their bodies during their larval stage, whereas in our Expt. A, the corresponding values were 15%, 19% and 45%. Furthermore, in his experiment R_{GM}, R_{GC} and R_{GN} were 12%, 15% and 25%, respectively, either of these taking rather higher value than each of ours. The difference of the results between his experiment and ours is mainly due to that of the strains of silkworms as well as of mulberries, and also to that of environment, in particular, air temperature (22°C in his, and 24°C in ours).

Our results can be compared with similar data of other lepi-

Fig. 6. Diagram quantitatively representing dry matter, calory and nitrogen flow from the mulberry to silkworms according to each developmental stage of silkworms.

An average mulberry farm in Tokyo district yields nearly 0.28kg/m² of dry matter edible for silkworms at the beginning of June, and this value is approximately equivalent to half of the yearly production.

227
dopterus larvae by EVANS (1939 a, b). From his data of *Phalera bucephala* fed on hazel leaves (1939a), we computed its $E_{GM}$ and $E_{NM}$, 17% and 30% respectively, the percentage of $E_{GM}$ is similar to $E_{GM}$ in our experiment, though his $E_{NM}$ takes a lower value than ours. We cannot directly evaluate $E_{GN}$ from his paper. He estimated the equivalent of $E_{NN}$ to be 87 per cent by analyzing the composition of faeces. This value is similar to ours which is 82 per cent (average in both sexes—Table 8). In his paper, such respects as duration of experiment, air temperature, etc. are not mentioned, so that we should be careful on comparing our values with his.

Another paper by EVANS (1939 b) demonstrates the equivalent of $E_{NM}$ in *Phalera bucephala* fed on hornbeam leaves (33% and 50% respectively on the two days chosen by him in the 5th instar), *Malacosoma neustria* fed on willow leaves (41%) and *Aglais urticae* fed on nettle leaves (62%). $E_{NM}$ in our experiment falls in the range of these values observed by EVANS. In this paper (EVANS, 1939b), we should carefully examine his estimates, because some of them were based on daily calculation which must naturally have fluctuated owing to the daily physiological state of the larva.

$E_{GM}$ partly depends on the assimilation rate, so that $E_{NM}$ would be more significant than $E_{GM}$ on comparing the efficiency of various animals which take in different kinds of matters. For example, it can be shown from McCAY's data (1938) that a cockroach fed on mixed food of skim milk and whole ground wheat converts the ingested food into its tissue with much higher efficiency than a silkworm and phytophagous larvae of other Lepidoptera. This phenomenon would be partly due to the high digestibility, since both skim milk and wheat contain a less fraction of cellulose than the leaves ingested by phytophagous larvae.

It should be emphasized in Fig. 6 that the gross reserve ratio of nitrogen ($R_{GN}$) takes a much higher value than those of matter ($R_{GM}$) and of calory ($R_{GC}$). This fact seems to suggest that the form of the ecological pyramid represented by the nitrogen content is rather different from that done by biomass or caloric content. Our idea would be demonstrated more clearly by Fig. 7, where the height of the respective parts in a column proportional to the relative value in Fig. 6, and the abscissa corresponds to the developmental stage of a silkworm, though not perfectly.

However, there is a problem specific to Lepidoptera, particularly to the silkworm, which makes a too splendid cocoon. Considering this character, we would like to adopt the reserve ratios rather than the conversion efficiencies as standards, to discuss on the values of coefficients like conversion efficiencies, especially nitrogen-conversion efficiencies, of various animals—such as *Daphnia* (SLOBODKIN, 1959; SASA et al., 1960), Copepoda (IVLEY, 1960; CORNER, 1965), vertebrates (MAYNARD, 1954)—together with lepidopterous insects.

**Summary**

1. Experiment A was carried out with the silkworms supplied with detached mulberry leaves at every six hours.

   i) A silkworm population (hybrid $\delta$ "Daizo" × $\varphi$ "Japanese 106") of 50 males and
50 females ingested 279g dry matter of mulberry leaves (variety "ichinose") from hatching to pupation, and this amount of leaf dry matter contained 1222 kcal. heat value and 10.9g nitrogen.

ii) The silkworms of the population assimilated 102g dry matter (37% of the total ingested leaf matter, 279g) during the whole larval stage, converted 42g (15%) into their own tissue and reserved 24g (8.6%) dry matter till pupal stage.

iii) Similar values can be derived in terms of caloric content; the silkworm population assimilated 446 kcal. (37% of ingested mulberry leaves), converted 234 kcal. (19%) into their bodies throughout the larval stage and reserved 142 kcal. (11%) till the pupal stage.

iv) In terms of nitrogen content, they assimilated 6.1g nitrogen (56% of 10.9g), converted 4.9g (45%) nitrogen and reserved 2.1g (19%) till pupal stage.

v) Eggs of 6.0g in dry weight from 50 female imagines of the population corresponded to 2.1% in dry matter, 2.8% in caloric content, and 5.5% in nitrogen content.

2. From the data of Experiment B where the silkworms were reared with intact leaves, it was concluded that the total intake of a silkworm in Experiment A would be no less than that of the larva consuming intact leaves at all times.

Acknowledgements

We are indebted to Prof. Seijirô Moro Hoshi and his colleagues (Tokyo University of Agriculture and Technology) whose support enabled us to accomplish the experiments in this report. They circumstantially taught us how to rear silkworms and offered much convenience to our work. We are grateful also to Prof. Tadayoshi Tazaki and Mr. Tadahiro Usijima (Tokyo University of Agriculture and Technology) who helped us in our work with the mulberry tree and other respects. Finally, we wish to express our gratitude for the kindness of Assoc. Prof. Yûzô Kitazawa (Tokyo Metropolitan University) who gave us advice and indispensable comments to our ideas.

References

葉素含量では5.5%だった。
副実験の結果、主実験で得た総投食量の値はクノキの
木にたかせてインクの葉を常時食べさせた場合と
大して変わらないことがわたった。尚、この実験は7月
〜8月におこなった。
これまでのことから、カイコは葉素をよく体内にとど
めることが言える。葉素を基準にした場合に記
したような相対値は、他の二つはなかった時に比べ、高
い値を示している。特に一度同化された葉素が80%以上
体内に残ることに目を向けなければ、以上の点を考えに
入れると、葉素保有量をもとにして作った生態的ビラミ
ッドは低株数や熱量をもとにして作ったものと様相がち
がわる。ただ、カイコを始め、鱗翅目の昆虫の場合
に問題となるのは、それらが大きなまゆを作り、そこに
大量の窒素を投入することである。「生きている物質」
の流れを観察する立場に立ってその点を考えると、他
の動物と異なった点がはっきりと認められる。

カンレンボタ小型林分の葉落に関する2，3の考察
京都大学農学部森林生態学教室 斉藤秀樹・四手井綱英
THE LEAF LONGEVITY OF A YOUNG STAND OF CAMPTOTHeca ACUMINATA
Hideki SAITO and Tsunahide SHIDEI
Forest Ecological Institute, Faculty of Agriculture, Kyoto University, Kyoto
Synopsis
SAITO Hideki and Tsunahide Shidei (Kyoto Univ., Kyoto) The Leaf Longevity of a
The leaf longevity of five-year-old seedlings of Camptotheca acuminata (Nyssaceae) was
investigated in a sample stand at the Forest Experimental Nursery of the Kyoto University
in Kyoto. The annual height growth was vigorous, being more than 1 m/yr. In spring a
great number of leaves emerged (70% of the total number in a year), but the leaf size
was small. As time passed, the number of leaves decreased and conversely the area of the
individual leaf increased until it reached 130 cm² in September. The individual leaf longevity
was rather short; the leaves which appeared in spring lasted only one or two months.
Those which came out in summer continued to stay on the seedlings for two to three
months. None of the leaves which emerged in spring or summer could last to the end of
the vegetative season in late autumn, when the major leaf-fall occurs, when about 25 per
cent of the annual leaf mass was shed. The leaf-fall was observed through the
seasonal variation of the amount of fallen leaves seems to have a close rela-
tion to the height growth of the seedlings. From these observations, it can be concluded
that Camptotheca seedlings have a relatively shorter leaf longevity than those of deciduous
species indigenous to Japan. Further comparative studies are necessary to explain the
shortness of the leaf longevity of the Camptotheca species; to ascertain whether it is in-
herent to the species or due to rapid self-thinning owing to the rapid height growth.

葉の落葉現象は春に葉を収穫始め、秋葉の脱落をはじめとし、普通日本で
でこれが見られるのが、普通日本での落葉樹の名である。そし
てこの落葉樹の分類がそれにあたるのに相当する変化については、いままでに
をアキリン1)，イエリガ2)，カ
ンレンボタ3)の小型林分を使って、その菌の働きはとら
えられている。十分に問題にしたこれらの林分葉素の季節
変化は、おおまかにみると5月下旬から6月上旬にかけ
て一度葉が極大に達し、8月から9月にかけては一定量
を保持して9月の終わりからは急激減少の一途をたど
るようである。この葉在の季節変化を察知出来るとし
に、落葉樹林では少なくとも5〜6月頃には葉の枯

1968年7月1日受理
Contributions from JIBP-PT No. 37

死が見られるはずである。またある分類の葉を、かり
春に春の2時点で調査したとき、その葉量が同じであ
っても秋に調べた時の葉が春の調査時のそれと同一の葉
であるという保証はない。
一般に落葉樹林では春に出た葉は、初夏に一部を落
葉させ、残りを秋まで着葉していると考えられているが、
樹種によっては一生育期間内で、春に着葉している葉
と秋に着葉した葉と季節の違いによってまったく別の葉
である可能性もある。
樹木のばあい、上記のような分類においては葉の構成
状態、すなわち葉の寿命別に葉量を調べた研究はほとん
doないと断言できない。しかし葉の寿命を知ることは、林分
葉量の季節変化の内容を知り、また落葉量の季節変化な