

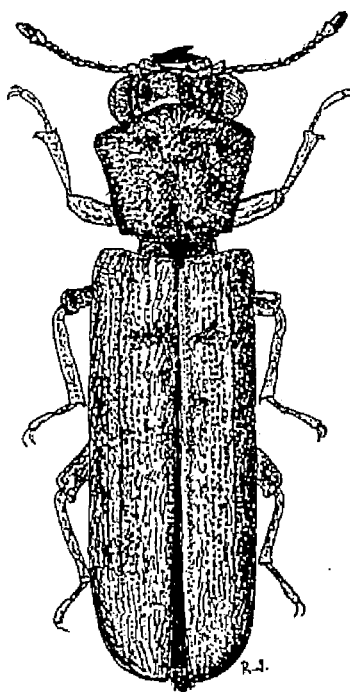
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**Mass Culture Method and Biology
of the Wood-Boring Beetle,
Lyctus brunneus (STEPHENS)**

Ryûtarô IWATA

- 1986

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Preface

Since the spring of 1978 up to present, the author has been engaged in the study of Lyctus brunneus (Stephens) (Coleoptera, Lyctidae), a very important insect pest on timber.

The study, at first, was simply aimed at establishing the culture method of this species in a mass scale so as to supply large number of insects to every kind of bioassay for its control. The study, meanwhile, came to involve the biology of the species as fundamental knowledges for the mass culture, bioassays and control of this species.

The present thesis includes the data and knowledges obtained during the course of study for these 8 years.

The author not only wishes the present study to contribute to the control of this species, but also wishes that this species would come to be one of the insects whose biology and rearing method are so well-known that they are often utilized as materials for fundamental studies on physiology, biochemistry, biophysics, and so forth.

The author wishes to express the sincerest gratitude of his to Prof. Koichi Nishimoto, the director of Wood Research Institute, Kyôto University, for his guidance during the course of this study.

The author also wishes to express his sincere gratitude to Prof. Tetsuo Koshijima, Wood Research Institute, Kyôto University, and to Prof. Hiroshi Fukami, College of Agriculture, Kyôto University, for their critical readings of the manuscript.

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Without their help, the present study would not be realized.

And, finally, the author expresses his hearty thanks to his wife Atsuko for her devotion, to whom this study is dedicated.

Ryûtarô Iwata

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1. Introduction and review

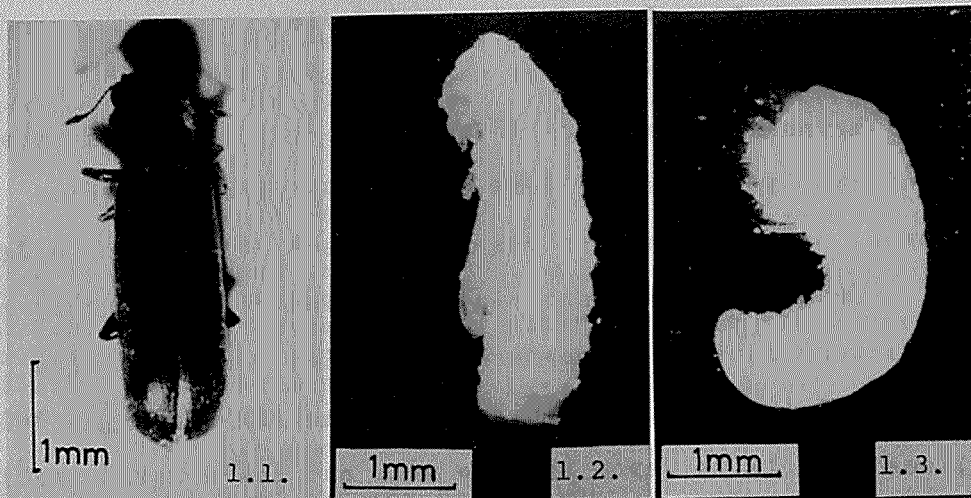
1.1. Economical importance of the species

Lyctus brunneus (Stephens) (Figs. 1.1.-1.3.), a most common species of the lyctid powder-post beetles in Japan, is also the most common, and the most serious pest insect of drywood in Japan proper. In the other industrial countries of the world, some other dry-wood-boring coleopterous species occur, such as Hylotrupes bajulus L. (Cerambycidae) and Anobium punctatum DeGeer (Anobiidae), while in Japan they do not, and the present species, L. brunneus, apart from the termites, is the unique object of intensive control within the Japanese wood industry.

It has been suggested that L. brunneus is an introduced species for Japanese fauna^{y2,iii)}, being recorded as early as in 1879 by G. Lewis¹⁵⁾, and now it is wide-spread in Japan from Hokkaido^{h2)} to Okinawa^{k16)} and Ogasawara Islands^{k19)}.

Globally, this species is a cosmopolitan and plenty of efforts have been made to control this species in various countries such as Japan, Taiwan, Philippines, Indonesia, Indochina, Australia, Fiji, India, Sri Lanka, Iran, USSR, Finland, Germany, Switzerland, Italy, France, Spain, United Kingdom, Madagascar, Zaire, Gabon, Cameroon, Nigeria, Ghana, Ivory Coast, Mozambique, Zimbabwe, South Africa, USA, Mexico, Cuba, Colombia, Paraguay, Brazil, Argentina, and so forth.

This species can be recognized as a household pest, a forest product pest, or a construction pest insect. It is very unlikely that this species give rise to a collapse of construction, but it is very likely to cause only small damages on wooden furni-



Figs. 1.1.-1.3. Lyctus powder-post beetles, Lyctus brunneus (Stephens).
 1.1.: adult.
 1.2.: pupa.
 1.3.: larva.



Figs. 1.4.-1.9. Six species of Lyctidae from Japan.
 1.4.: Lyctus linearis (Goeze).
 1.5.: L. sinensis Lesne.
 1.6.: L. brunneus (Stephens).
 1.7.: L. africanus Lesne.
 1.8.: Hinthea rugicollis (Walker).
 1.9.: Lyctoxyton dentatum (Pascoe).

ture, ceilings, floorings, and other wooden or bamboo materials within the houses. Once it occurs, the commercial value of the damaged piece decreases greatly; even if only one exit hole of the beetle occur on a piece of furniture, it will be rendered of no value, and there have been a number of wood-dealers' bankruptcies and lawsuits, to all of which this species is responsible.

Though there is little specificity in the host-wood preference of this species, only starchy portion of sapwood of certain hardwood species, and also bamboo material, are attacked. Before the World War II in Japan, hardwoods were less utilized than softwoods, and thus this species were recognized as a mere bamboo borer. However, in the postwar days the way of wood utilization in Japan has changed to attach weight to hardwood, especially to the lauan (melanti) woods, thus this species was actualized to be an important "lauan borer". Now that the damages and losses due to this species, L. brunneus, are so serious, its control is attracting attention as an emergent problem in Japan.

1.2. Taxonomy of the family Lyctidae from Japan

Lyctidae, the family to which Lyctus brunneus belongs, comprises 2 tribes, 12 genera and about 60-70 species in the world⁽⁴⁾, a bulk of which are, more or less, of dry-wood boring nature as well. Since the work of Crowson⁽⁶⁾ this family has been established and recognized to form the superfamily Bostrychoidea (or in another term, "Teredilia") together with the other families, Bostrychidae, Anobiidae and Ptinidae.

Of this family the following 6 species are distributed or established in Japan;

- Lyctus (s. str.) linearis (Goeze) (Fig. 1.4.)
- Lyctus (s. str.) sinensis Lesne (Fig. 1.5.)
- Lyctus (Xylotrogus) brunneus (Stephens) (Fig. 1.6.)
- Lyctus (Xylotrogus) africanus Lesne (Fig. 1.7.)
- Minthea rugicollis (Walker) (Fig. 1.8.)
- Lyctoxylon dentatum (Pascoe) (Fig. 1.9.)

All of them are of approximately identical nature and form, namely, brown, elongate, flattened, small beetles attacking on dried hardwoods and bamboo. However, there may be some differences or specific specificities in their biology: Lyctus sinensis, L. africanus and Minthea rugicollis can attack some other plant materials such as root tuber more readily than L. brunneus; L. africanus is diurnal rather than crepuscular like L. brunneus; L. sinensis adults emerge rather earlier in spring than L. brunneus; Lyctoxylon dentatum seems to require higher level of starch content of wood than the other species; etc. Such differences, more or less, should be taken into account in their control, and precise comparative investigations on their biology are needed. The following key would contribute the classification for the Japanese species:

---A key to Japanese Lyctidae (after Nobuchi^[4], partly modified)

- 1 Elytral punctuations and setae regularly arranged longitudinally; femur slender, clavate-shaped, not or slightly depressed from both sides. ----- 2
- (1) Elytral punctuations and setae irregularly arranged;

femur robust, long-oval, strongly depressed from both sides; length 1.5-2.0 mm. ----- Lyctoxylon dentatum

2 Ultimate segment of antenna longer than the penultimate, oval, with its apex being narrower; setae on the body sidelong, piliform. ----- genus Lyctus ----- 3

(2) Ultimate segment of antenna as long as the penultimate, almost rectangular, with its lateral side being parallel; setae on the body straight, scaly; length 2.0-3.5 mm. -----
----- Minthea rugicollis

3 Forefemur as broad as mid- and hindfemur; pronotum slightly spreading anteriorly, obviously narrower than the elytral bases. ----- Lyctus (s. str.) ----- 4

(3) Forefemur broader than mid- and hindfemur; pronotum strongly spreading anteriorly, with its anterior part slightly narrower than the elytral bases. ----- Lyctus (Xylotrogus) ----- 5

4 Median longitudinal groove on pronotum very obscure; elytra yellowish-brown with their sutural part sometimes being dark in color; ultimate segment of antenna markedly amplificate, markedly asymmetrical along the antennal axis; length 2.8-5.0 mm. -----
----- Lyctus sinensis

(4) Median longitudinal groove on pronotum obvious, deep; elytra monochromatic, reddish-brown or dark-brown; ultimate segment of antenna amplificate to some degree; length 2.0-5.5 mm. -----
----- Lyctus linearis

5 Clypeus sulcate sideways deeply; anterolateral angles of pronotum acuter; female 6th (4th) abdominal sternite furnished posteriorly with no fringe of hairs; length 2.2-8.0mm. -----

----- Lyctus brunneus
(5) Clypeus sulcate sideways slightly; anterolateral angles of pronotum blunter; female 6th (4th) abdominal sternite furnished posteriorly with a fringe of hairs; length 2.5-4.0 mm. ---

----- Lyctus africanus

1.3. Aims and scopes

The present study is aimed at presenting any kinds of fundamental biological knowledges that contribute to the control of Lyctus brunneus.

The most effective, economical and permanent way of control of Lyctus is supposed to involve the treatment of wood with chemical preservatives. For the development of new preservatives, the screening of the vast number of chemicals are needed as a rule of any case of chemicals, and also the mass culture of the insect species to be controlled is prerequisite to the screening. However, among many kinds of pest insects, it seems somewhat difficult, in general, to rear wood-boring beetles that live inside the wood during its immature stages. It is primarily because it is difficult to observe and detect the boring insects in wood superficially, and secondly because there are many troubles in handling them due to their excessive evolutionary specialization for the peculiar habitat i.e. the wood. Lyctus is not an exception. However, with the world-wide increase of the damages on wooden materials, studies on the rearing methods of Lyctus have been desired greatly.

Precise breeding methods for L. brunneus have been devised by

several workers^{h1, r1, m8, h6, i1, k15)}. Most of these works set up the breeding system using sapwood of the hardwood species, such as Quercus spp., as a natural and a classic way of breeding. By using sapwood pieces the relation between the climatic conditions and the growth rate of L. brunneus has been studied as summarized in Table 1.1. As one can see from this table, it is about 2 months or 8-9 weeks that is supposed to be the possible shortest life cycle length of L. brunneus with the best nutritional condition in wood coupled with the best climatic conditions of 25-26°C and 75% R.H.

It is noteworthy here that this species is supposed to be of tropical or subtropical origin since there is no need of "chilling" for the pupation^{k15)}. This is quite contrary to the case with the other lyctid species, L. linearis^{k15)} and L. sinensisⁱ¹³⁾, both of which cannot pupate unless they are exposed to low temperature. This is one of the reasons why it is more or less easy to breed L. brunneus, as well as L. africanus, compared to the palaearctic lyctids.

Rosel^{r1)} stated that although the sapwood of Sterculia acerifolia produced the shortest life cycle of the beetles, "its productivity varies considerably over short lengths due apparently to a varying percentage of starch". This seems to indicate that the use of wood is, in general, not the most appropriate way for mass cultures or control bioassays due to the uneven distribution of the nutrient substances in different pieces and parts of susceptible wood. Artificial diets, on the other hand, are more homogeneous and therefore more reliable for

this purpose.

The first development of a solid artificial diet for Lyctus, which was made to study the nutritional requirements of L. brunneus in Australia, was described in a very short report^{a1)}, stating "a successful development can take place on a diet in which the carbohydrate (sic) : protein ratio is 15 : 1", whereas "the normal ratio in wood is 3.3 : 1". Khalsa et al.^{k3)} used a dried dough (90 parts of wheat flour and 10 parts of yeast) for breeding L. africanus, a method developed by Ayyappa^{a7)} for breeding Sinoxylon spp. (Bostrychidae). Cymorek^{c9)} devised an artificial wood-like diet for Anobium punctatum (sawdust 32%, cellulose 9%, starch 11%, plasma protein 16%, baker's yeast 32%), which also proved to hold good for L. brunneus. Cymorek^{c10)} also used synthesized blocks (cellulose, methyl cellulose, starch, baker's yeast) for the nutritional study of this species. Nour & Hillalⁿ⁶⁾ investigated the productivity of Ayyappa's^{a7)} diet with L. africanus with the percentage of yeast varying from 5% to 15%, and concluded that there was no significance in the differences of progeny yields and life cycle durations. In a biochemical study of L. planicollis LeConte, Mauldin et al.^{m3)} used an artificial diet (wheat flour 500g, corn meal 400g, yeast extract 96g, methyl-p-hydroxybenzoate 2g, ascorbic acid 2g). Cymorek & Schmidt^{c15)} prepared wood-like biscuit (coniferous wood sawdust 30g, cellulose 10g, wheat flour 30g, yeast 25g), which we call "Cymorek's cake", for studying the adult feeding habit of several species of Lyctidae. Tscholl^{t2)} used a similar diet (sawdust 35%, cellulose 10%, starch 30%, yeast 25%), while Mori^{m8)} and

Ijima et al.ⁱ¹⁾ both cited "Wälchli's" (almost the same as Tscholl's) diet for L. brunneus (lauan sawdust 33%, starch 33%, cellulose 9%, yeast 25%). Mori^{m8)} tried to omit cellulose powder from it, and Higaki^{h6)} and Ijima et al.ⁱ¹⁾ improved its composition into 56%, 10%, 9%, 25% respectively. The latter is called "the diet of Tôkyô University of Agriculture". Ito & Hiroseⁱ⁷⁾ investigated the physical factor, i.e. the density of this diet and developed a method to obtain a high progeny yield. Besides, Ijima et al.ⁱ¹⁾ tried to develop a holidic diet (cellulose 80g, starch 33g, amino acid mixture 6g, vitamin mixture 0.12g, McCollum's salt 1g). Later, Ito & Hiroseⁱ⁹⁾ prepared new productive diets composed of cellulose, yeast and wheat flour, which we call "the diets of Sumitomo Chemical Co. Ltd." Furthermore, diverse substances, such as buckwheat flour cake^{s12, s13)}, cookies^{m8)} and dog biscuits^{g10, k14)} have been utilized for rearing L. brunneus. When Parkin^{p2)} studied the nutritional physiology of Lyctidae, he developed an artificial powdery substance, which seems, however, not suitable for mass cultures.

The problem of the diet composition is closely related to the nutritional requirements of the beetles which influence the degree of infestation and the extent of damage to wood.

Starch is the most vital ingredient in the composition of Lyctus food. This was first suggested by Mer^{m5, m6)}. Campbell^{c1)} concluded that Lyctus spp. cannot utilize wood cell wall components (cellulose, hemicelluloses and lignin). Wilson^{w2, w3)}, Parkin^{p2)} and many others repeatedly stressed the importance of starch. Soluble sugars other than starch (such as glucose,

maltose, sucrose) can also be utilized by Lyctus as the substitutive sugars^{p2, c10, c14, i5)}. The second most vital nutrient for Lyctus is proteins and/or amino acids^{p2, p3, h7, i3, c10)}, with methionine and tryptophan being pointed out to be the most important onesⁱ²⁾. The other substances in wood which are possibly required by Lyctus are steroidsⁱ¹⁾, linoleic acid and sterols^{m3)} as well as mineral nutrients^{j1)}. A bacterium-like symbiont in the gut of Lyctus linearis has been assumed to supply the host with vitamins^{k5, k6, p2)}. Almost all of these nutritional substances required are contained in the parenchyma cell content of the sapwood.

With all the studies cited here, there still remains a lot to be clarified for establishing an indefinitely efficient system of mass culture of L. brunneus, involving a new ideal artificial diet.

The present thesis firstly deals with the mass culture methods of this beetle with the artificial diet of Tôkyô Univ. of Agric.ⁱⁱ⁾ with reference to several new techniques and suggestions (2.1.).

Next to this, a new approach was presented to develop a new artificial diet (2.2.). This investigation is multi-purpose, viz. a first approach to develop the most appropriate meridic diet, a preliminary investigation of the nutritional requirements on meridic diets and a proposition of a bioassay method to determine the suitability of the diet. This method of bioassay consists of two parts: "the experiment with adults" (2.2.1.) where the rate of growth, the progeny yield and the life cycle period were determined, and "the experiment with larvae" (2.2.2.)

where the nutritional suitability of the diets was examined.

Based upon these results, a further investigation was made to establish a new original artificial diet in due consideration of not only the productivity, but also of the beetles' size or weight (2.3.). This is because the greatest problem in carrying out control tests with this species is the variations among individual beetles in their activities, adult longevity, and the number of their progeny, all of which are more or less related to their size or weight. A selected artificial diet for practical rearing thus is required to produce not only a greater number of progeny but also individuals of similar size of greater value. This ensures the homogeneity of bioassay conditions. It is important also to reduce the number of constituents in the diet formulation.

In the practical mass culture system, the beetle productivity and the beetles' size are directly important as stated above. However, a productive artificial diet requires inevitable labor in renewing culture as it has a short life as a breeding matrix, and thus, from a viewpoint of culture system maintenance, it may be somewhat dangerous to rely only upon such a diet: a long-life matrix is also needed in beetle cultures for the strain maintenance mainly during the suspension of assays. In due consideration of this, the present culture system has adopted the alternative rearing matrices: the above-stated diet as a main matrix for almost all purposes and oak (Quercus serrata) sapwood pieces enriched by Cymorek's^{C13, C14} method as a strain-reserving matrix (2.4.).

The life history of this species, L. brunneus, has been established by a number of workers, namely by Munro^{m9, m10}, Yano^{y1}), Altson^{a3, a4, a5}), Lesne^{l4}), Fisher^{f1}), Fisher (et al.)^{f2}), Parkin^{p1, p2, p4}), Beeson & Bhatia^{b4}), Pringle^{p5}), Gay^{g2}), Schmidt & Buchholz^{s6}), Kallapur^{k1}), Rosel^{r2}) and Tscholl^{t1}). For the control of this species, however, still further biological knowledges are needed despite of an abundance of literature on this matter.

First of all, the quantitative relationships among the weight values of various developmental stages are eagerly needed since the individuals obtained at the closure of the diet suitability test with larvae (2.2.2.1.) assumed various developmental stages, among which the prepupa was thought to have the maximum weight value. Thus, trials were made to set up equations presenting the quantitative relationships between weights of prepupa and the other developmental stages (3.1.1.). Relation between the weight of new adults and their body length was also set up for the diet suitability tests (2.3.1.), as well as the relationship between their total length and elytral length (3.1.2.).

As only larvae of the lyctid species cause damage to timber, the bioassay with larvae is one of the most important test methods for developing potential chemicals. In the laboratory tests of chemicals using larvae of L. brunneus, however, sufficiently satisfactory results seem to have been unobtainable^{g7}), and the same inconvenience was encountered in the test of artificial diet suitability (2.2.3.). This is presumably due to diversities of age, activity, and adaptability of the larvae

prepared for the experiments. Precise knowledges of larval development, particularly the larval instar, therefore, are indispensable for minimizing the variance or diversity among larvae.

Information so far obtained still seems very insufficient and improper to resolve this problem. Lyctid larvae show a kind of "polymetaboly", a slight change of body shape between the 1st and 2nd instars^{a4, b7, k9)}. Regardless of this Xamheu^{x1)} stated that larva of Lyctus linearis (as L. canaliculatus) molts 3 to 4 times by the end of winter though he did not describe the research method. Tscholl^{t1)} stated L. brunneus larva molts 5 to 8 times in the course of its development. Tscholl^{t2)} later classified L. brunneus larvae, reared with an artificial diet and under constant climatic condition, into 6 instars, and graphically showed leaps in body weight increase at molts though it is doubtful that molt could cause such a leap. Finally, Suzuki^{s13)} tried to investigate the larval instar number of L. brunneus, reared with buckwheat cake, and estimated the final stage to be the 4th instar although this estimation seems to include some improper points as regard to the interpretation of the data and the citation of the author's present result.

For the study of the larval instar of coleopterous species in general, grouping the dimensions of head capsules or exuvial mandibles of the larvae seems to have been a common method for this purpose, as was adopted by Suzuki^{s13)} for Lyctus and by Keizô Kojima and his co-workers for a number of Japanese cerambycid species. In some cases of them^{w1, k7, n1, k8)}, however, a difficulty

Table 1.1. Rearing records of Lyctus brunneus with sapwood pieces under the climate control in the past studies with reference to its life cycle length.

Author(s)	Temp. (°C)	R.H. (%)	Equilibrium moisture content of wood (%)	Wood species	Life cycle length	Remarks
Parkin ^{p4)}	23		15.6	<u>Quercus</u> sp.	min. 167 days	
Parkin ^{p4)}	27.5		14.2	<u>Quercus</u> sp.	min. 4.5 month	
Gay ^{g2)}	26	80	16	<u>Alstonia</u> <u>scholaris</u>	av. 113.7 days	The best case of the various conditions
Gay ^{g2)}	26	75		<u>Sterculia</u> <u>acerifolia</u>	{ av. 75.5 days min. 63 days }	
Harris & Taylor ^{h1)}	25	75	15	<u>Quercus</u> sp.	{ 11 ~ 14 weeks min. 9-10 weeks }	
Rosel ^{r1)}	26	75		<u>Eucalyptus</u> <u>obliqua</u>	2.5-3.5 months	
Rosel ^{r1)}	26	75		<u>Sterculia</u> <u>acerifolia</u>	54-63 days	
Nobuchi ⁿ⁴⁾	25	50		{ <u>Q. serrata</u> <u>Q. acutissima</u> }	ca. 6 months	

Table 1.2. Sex ratio values of L. brunneus reported in the past studies

Author(s)	Sex ratio value (n_m/n_f)	Remarks
Altson ^{as)}	1 >	in general
Parkin ^{p1)}	7/5	rearing with sapwood piece
Gay ^{g2)}	1	rearing with sapwood piece
Harris & Taylor ^{h1)}	1	rearing with sapwood piece
Ito & Hirose ⁱ⁷⁾	1.10	rearing with artificial diet

was encountered in grouping the larvae due to the individual variation, which might be mostly ascribed to the variance of moisture or nutritional conditions of food^{m1)}. Therefore, in conducting the present research on larval instars of Lyctus brunneus, which was expected to show a wide variation, the larval development had to be followed individually and closely, where pairs of exuvial mandibles, which were mostly preserved in perfect form, enabled an estimation of the larval instar. For easier inspection of the larvae, rearing method with powder-state matrix was adopted and in combination with it, a narrow-spaced container was needed because rearing with powder-state matrix in a voluminous container had resulted in failure of development. As a container a small gelatin capsule, rather than a narrow glass tube^{p2)}, seemed suitable mainly for isolation of each individual larva. Exuviae derived from a single larva could be easily collected by using this method. It has been called the "individual rearing method". As the powder material buckwheat flour, which has been already introduced by Suzuki^{s12, s13)}, preliminarily proved to be efficient, enabling larvae to develop and to pupate within the capsule, while trials with some other powder compositions, which were utilized to form solid artificial diets of L. brunneus, resulted in failure of development. Here the result of the investigation on instars, with reference to the individual rearing method using gelatin capsules and buckwheat flour, were reported, as well as the result of a preliminary rather unsuccessful investigation using wood blocks (3.2.).

In conducting the mass culture, the population density of the

insects in the culture matters greatly because it is one of the most important factors determining the growth rate and reproduction rate; an excessive crowding often causes collapse of solid diet resulting in the annihilation of the population. The density effect in the artificial diet was thus investigated from viewpoint of population ecology as a fundamental knowledge for the mass culture (3.3.).

Adult sex ratio is also an important matter for maintaining the mass culture and carrying out bioassays. Most of the past works, as summarized in Table 1.2., recorded that the value of the ratio of the male number (n_m) to the female number (n_f) was approximately equal to or slightly more than 1, and also in some other lyctid species, this value was equal to 0.97 - 1.00^{r2)}. In L. brunneus, the first male always emerged out prior to the first female emergence (protandry) according to Parkin^{p4)}, while in an American species L. planicollis, protogyny, a mode of adult population's emergence where females emerge statistically earlier than males, was observed by Kurir^{k18)} in Austria. Gay^{g2)}, however, does not seem to have been able to decide whether protandry or protogyny is involved in L. brunneus. In the present study some examples of the sex ratio values of L. brunneus are reported with reference to the problem of this matter (3.4.).

Adult longevity seems a factor that varies considerably, and minimizing its variation must be desired eagerly in any kind of bioassay with adult beetles. Here, this factor was investigated to demonstrate whether it is related to the adult sex (gender) and/or to the adult's body weight or length. The longevity test

was also carried out with filter papers treated with preservative chemicals to give a result as a model of their efficacy in relation to adult longevity or resistance (3.5.).

Further, some peculiarities are briefly noted on the ethology of adult beetles (3.6.): an aggregating behavior possibly induced by a kind of pheromone produced by females (3.6.1), a peculiar boring behavior observed with two kinds of "the diets of Sumitomo Chemical Co. Ltd." containing wheat flour¹⁹⁾ (3.6.2.), a complicated phototaxis and a sexual difference of the readiness of flight (3.6.3), all of which have never been reported till now despite of their curiosity.

Short notes are also given on the other kinds of organisms encountered mainly during the course of mass culture of L. brunneus, including knowledges on its natural enemies (3.7.).

As a conclusion of the life history and ecology, a general discussion is given to make a whole image of this species from the ecological viewpoint (3.8.).

Thirdly, a chemical study was made on the digestion as a physiological aspect of the biology of this species. During the course of diet suitability experiments, as stated in the section 2.2., starch content in the diets was found to be much more important than protein content from quantitative viewpoint. In order to extend this study, an investigation was conducted to compare the amino acid and starch compositions of the wood and of the new artificial diet with those of two kinds of feces (4.).

The fourth aspect of its biology investigated is the morphology. Review of the literature on the external morphology of L. brunneus reveals that there is no information about the fine surface structure and the fine changes in larval morphology during succeeding instars. Further, no morphological studies have ever been done with SEM on the wood-eating species of the superfamily Bostrychoidea including this species, L. brunneus. The present study reports SEM observation on the fine detail of the external morphology of all the developmental stages of L. brunneus, especially on the larval changes among instars, pupal and imaginal sex characters, and so on (5.). An additional note was given on the corporal malformations of the adults (5.6.), which were encountered during the course of the diet test (2.3.) and of the mass culture.

2. Development of mass culture methods

In this chapter, the description of the general mass culture techniques is given (2.1.), followed by the preliminary (2.2.) and succeeding (2.3.) experiments on artificial diet suitability. Finally, techniques of rearing with enriched wood blocks are briefly noted (2.4.).

2.1. Installations and general procedure of the mass culture

2.1.1. Installations and conditions^{A)}

The development ratio of L. brunneus is dependent upon the temperature and humidity, and as is shown in Table 1.1., it has the maximum value in 25-26°C and ca. 75% R.H. so far within the range where the mortality is not too high. There, what matters is that these conditions are to be maintained changelessly. Mass culture of the insect, otherwise than the small-scaled rearing in general, needs a space of more than one room, which could not be climatized without automatic control. Thus a rearing chamber was set up, as seen in Fig. 2.1.-2.2., yielding an enough space and conveniences for the mass culture, to make a total system managing the climatization of this space. All the climatization system is under automatic control, working permanently. Furthermore a ventilator and an air purifier were introduced in it as countermeasures to mold occurrence, one of the greatest obstacle of the mass culture.

The climate in the rearing chamber is desired to be 25-26°C and 75% R.H. for the quickest development. In this case, however, there may occur mold on the artificial diet surface in

the Japanese climate, causing inconvenience to rearing (see 3.7.)). Therefore the temperature and relative humidity are to be lowered down to 24.5°C and 70%.

2.1.2. Selecting the adult beetles for the culture^{H)}

Out of a lot of adults collected, some are selected to be supplied to the next new culture or to bioassays. In order to ensure mating followed by reproduction, the collected adult beetles, not sexed yet, are kept in a Petri-dish for a day or some. Though keeping them in a Petri-dish ensures their mating, there come to be some individuals that are weakened, dying or dead. In order to exclude them, a very convenient method of selecting adults was devised: small pieces of filter paper are put in a Petri-dish where adults are accommodated, and the Petri-dish, then closed, is shaken to mix the insects and the paper pieces. The paper pieces are picked up together with many active adults sticking to them, and are slightly tapped by fingers to let drop the dead corps mixed with live ones. The remainders on the paper are surely active, free from dead or weakened individuals.

They are sexed, if it is necessary, according to the sex characters^{[4, 11, p11)}, which are presented in the sections 5.5.1. and 5.5.3. It may be recommended that the beetles supplied to bioassays should be young enough: the productivity is suspected to decrease with days after they emerged out. Furthermore, old stock of beetles sometimes contains females whose ovipositor has been fully or partly extruded. It may be suspected that these

have lost their potentiality of oviposition.

In consideration with this, a small test was conducted where normal females were compared as regard to their productivity with those whose ovipositors had been extruded, with the relationship of the productivity and age (number of days after their emergence) being also investigated.

The result, as presented in Table 2.1., suggested that "new adults" are more fecund than the "old adults", and the degree of ovipositor extrusion of females is correlative with their infertility. The females whose ovipositors had been fully extruded was fertile no more. Thus females with extruded ovipositors need to be excluded in supplying adults to bioassays. However, when only a small part of females have extruded their ovipositors among population to be supplied to the cultures, they need not be excluded from labor-saving viewpoint.

The size of the adult beetle is also an important factor in selecting them for bioassays. This will be discussed later in relation to the sex difference and longevity in the sections 2.3.2. and 3.5.

2.1.3. Rearing procedure^{A, H)}

The mass culture of this insect was begun using "the diet of Tôkyô University of Agriculture"¹¹⁾. The following is a procedure of mass culture system with this kind of diet.

The artificial diet used has the following composition: lauan sawdust 56%; soluble starch 10%; cellulose powder 9%; dried yeast powder 25%. In preparing the lauan wood, only the visually

Table 2.1. The productivity of L. brunneus adult beetles with different age and condition of female ovipositor, using the standard artificial diet (2.3.).

Parent adult beetles	F ₁ Progeny
15 new males ^a vs. 15 new normal females ^a	204♂♂ 224♀♀
15 old males ^b vs. 15 old normal females ^b	122♂♂ 136♀♀
15 old males ^b vs. 15 old females ^b with their ovipositor slightly extruded ^c	73♂♂ 67♀♀
15 old males ^b vs. 15 old females ^b with their ovipositor fully extruded ^d	none

- a) Beetles had never been kept in a Petri-dish.
b) Beetles had been kept in a Petri-dish for 1-10 days.
c) Ovipositor extruded over less than half the body length.
d) Ovipositor extruded over more than the body length.

uncolored portion (sapwood) was utilized, wood species being undetermined but belonging to so-called "red lauans" (Shorea (Rubroshorea) spp.). Sawdust is selected by passing it through a sieve of No. 20-40 mesh, while as dried brewer's yeast powder "Ebios" is used. The above-mentioned composition in dry powder-state is shaken and mixed in a vinyl sack, to which 100-120% weight of deionized or distilled water is added to make dough. In the original methodⁱ¹⁾ 80-90 ml of water per 100g of powder material was required, which seems insufficient, resulting in more brittle and less solid diet. This dough is put in the wood frame 40cm long, 5cm wide and 3cm high, on which a wood plate of the same area (40cm x 5cm) and of 1cm thickness is placed to push until the diet dough being pressed down to 2cm thicknessⁱ¹⁾. The wet diet, free from the wood frame, is then cut into 3 equal pieces of the size ca. 13cm x 5cm x 2cm. These diet blocks are dried, baked and solidified in a 60-70°C electric oven-dryer for 48 hr. The drying duration needs this at least, while a duration of only one day allows mold occurrence, as reported in the section 3.7., and liability to collapse. Such a method of making diet produces about 15 blocks of diet from 1kg of powder material, one block weighing ca. 65g, thus its density being ca. 0.50, which is nearly equal to that reported by Ito & Hiroseⁱ⁷⁾ to yield a good number of progeny.

For the convenience of availability, wide-mouthed glass bottles, 900 ml volumed, 18cm high, 9cm and 6cm diametered at the median part and mouth, are used as culture vessels. A disk of filter paper, 9cm in diameter, is placed on the bottom of each

bottle. This supports the walk of beetles that cannot walk on glass surface^{h1)}. The bottle, together with the filter paper, is disinfected for the control of entomogenous mites such as Pyemotes sp., as reported in the section 3.7. The adoption of soil material such as forest loam, as recommended by Rosel, in place of a filter paper, may be troublesome in regard to the disinfection and following drying.

For the disinfection ethyl alcohol solution of beta-naphthol (ca. 2.5%)^{g3, m8)} is used, covering all the inner surface of the bottle. Bottles once used for the culture is to be washed to remove the frass dirt before disinfection. During this operation one must be careful in handling beta-naphthol solution for it is, in fact, a toxic reagent to human bodies though it was reported to be harmless to L. brunneus at this concentration. On finishing the disinfecting operation, a sheet of cotton gauze cut beforehand in 30 cm square is folded in four, put on the mouth of the vessel, inner side of which is still wet with beta-naphthol ethyl alcohol solution, and fixed with rubber band. The vessel is air-dried in the rearing chamber, with the gauze on it being also disinfected through evaporation. The alcohol solution will be evaporated within one day to leave deposited beta-naphthol layer. The invasion of parasitical mites is prevented until this layer is sublimated away.

Spraying the same disinfectant within the rearing chamber occasionally is also recommended for the mite control though an intensive caution should be exercised so as to prevent contact with and inspiration of this chemical.

About 130 g of artificial diet is put into the prepared culture vessel, into which ca. 20-50 individuals (or ca. 8-20 pairs, if sexed) of selected beetles are released to attack. In releasing the beetles, a funnel is used to introduce them directly onto the diet surface. A rough release to the vessel may spoil secure contact of the beetles with the diet. Each vessel thus prepared is then called "a culture", a unit of mass culture, and labeled with the culture number and initiation date (Fig. 2.3.).

A further means has also been taken to prevent the mites; all the culture vessels are placed in the water-filled tray, with which mite invasion is intercepted. Water in the tray is to be changed occasionally.

Under the indicated climatic condition (24.5°C, 70% R.H.) the shortest life cycle duration, comes to be a little less than 3 months with this diet, while in the case with the quickest development (25-26°C, 75% R.H.), it is about 2.5 months. Slight falls of temperature and relative humidity are assumed to contribute not only to mold but also to mite control.

After a certain period since the release of parent beetles, new beetles of the next generation begin to emerge. They are to be collected and are to be supplied to the next culture: cultures are to be renewed. The manner of "the renewal of culture" differs with different kind of rearing matrix. Specific notes are given for the cases with the other kinds of rearing matrices in the sections 2.3.4. and 2.4.

The adult insect that has finished its development and metamorphosis emerged out of the diet block, boring the exit hole



Figs. 2.1.-2.2. Rearing chamber of *L. brunneus* mass culture (In Laboratory of Wood Protection, Wood Research Institute, Kyoto University, 1970).

2.1.: Outside view.

2.2.: Inside view.



Fig. 2.3. *Lyctus brunneus* cultures.

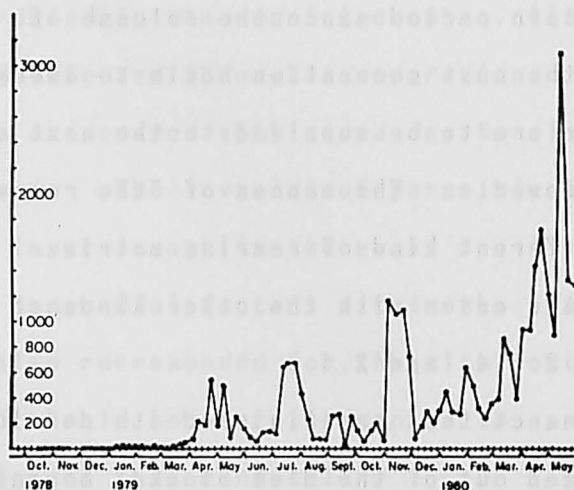


Fig. 2.4. Transition of the number of beetles weekly collected in the beginning of *L. brunneus* mass culture.

from the pupal chamber, which is made just below the diet surface. This "emergence" consists of the preceding "ecdysis" at the pupal chamber and the "escape" to the outside. The adult of this species is said to be of crepuscular nature^[2], as discussed in the section 3.6.3., preferring dark condition for "escape". Thus, when the rearing chamber is illuminated, they take refuge into the exit holes, pupal chambers or larval tunnels immediately. On the other hand, due to the nature of the species, once the adult beetle had oviposited on the diet block, the larva that has hatched from the egg is obliged to inhabit within that block throughout its life unless it is translocated to a hole on another diet piece artificially. Supposing the diet block is much crowded with larvae and is eaten in holes excessively, it becomes a brittle mass filled with a large quantity of frass. The diet of Tôkyô Univ. of Agric. has a soft property, and is all the more liable to collapse if it is eaten too heavily by the insects. There the larvae fail to get the foothold for boring, being left wholly free within the vacant space, or obliged to drop out of the diet piece, resulting in their death and annihilation. In due consideration with this, all the adult beetles that have newly emerged out need to be set apart from their original diet block and to be released onto another new block.

Therefore, in order to make the "renewal of cultures" smoother, the rearing chamber has been illuminated permanently to force the new adult beetles to remain in their pupal chambers; their "escapes" are controlled. This method enables us to control sharply the beetles' activity and ovipositions on the

same block as they have emerged from. Although this method requires "the beetle collection by force", diet collapse and annihilation can be avoided thereby. "The beetle collection by force" is carried out by breaking the circumference of the adult's exit hole with a firm pincette. A sieve of No. 20 mesh is very useful in removing the frass (feces) from the mixture of insects and diet particles.

Properly speaking it is preferable to collect the emerging beetles every day in order to make "the renewal of cultures" smoother. However, unless a particular rearer is employed full time, weekly task can still maintain the mass culture of sufficient scale^{hi)}, with permanent illumination practiced.

Just before the moment when adult beetles of the new generation begin to emerge, crunching noise caused by the inside boring activity of the larvae is slightly heard when a diet block is pressed against the ear, with the sound increasing with the increased number of the beetles of the parent generation. The detection of larval activity with this method seems effective to some extent.

Since September 1978 the mass culture of L. brunneus has been carried out in earnest, with some beetles from "the strain of Tôkyô Univ. of Agric." and some from "the strain of Sumitomo Chemical Co., Ltd." used as the parent stocks. During the early phase of the mass culture, a few lots from natural occurrences in Ôsaka Prefecture, Japan, were introduced, resulting in a formation of "the strain of Kyôto University" as a new hybrid strain.

Figure 2.4. shows the transition of the number of beetles

weekly collected in the early phase of the mass culture, serving as an outline of its progress.

Until the end of 1985 about 30 generations have been obtained in the present mass culture, with no apparent symptom of the affection of inbreeding observed.

2.1.4. Disposal of the larvae^{A)}

In the course of rearing the following phenomena may take place^[1, 17]: (i) some larvae drop out of diet blocks spontaneously when the rearing chamber is not illuminated; (ii) in the course of beetle collection by force some larvae are unavoidably laid bare out of diet piece; (iii) if the population density of the larvae is too high in the diet block, it collapses, with a number of larvae dropping out of it. Treatment of a number of such bare larvae is the greatest annoyance in the early phase of mass culture. This is because they are not able to survive unless each of them is thrown one by one into a suitable hole artificially bored on a diet block, and if the number of such larvae is too great, they need considerable labor. In order to diminish the labor of saving them, the following method was devised:

A large rearing vessel, such as a beaker, 1-3 l in volume, is prepared and disinfected as above. Blocks of the diet of Tôkyô Univ. of Agric. are divided into two or more by hands, some of which are placed directly to the filter paper at the bottom of the vessel, and small diet particles including live larvae are scattered on, around and between the blocks. Further, another part of divided diet blocks is placed on, with particles and

larvae scattered again. Additional repeats may be made to set a final situation where intact divided diet blocks are wholly surrounded by diet particles including live larvae. The larvae among the particles will writhe and wander to seek for narrower spaces to burrow, and at last some of them will be observed boring into new diet piece with acquisition of suitable body support. After these diet blocks are put apart, kept and observed, new adult beetles will emerge, opening their exit holes at different sites from their entrance holes. With this method a part of properly grown larvae are saved.

2.2. Nutritional experiments with artificial diets^{D)}

2.2.1. Experiments with adults

As a trial to obtain the information on the essentialities of the artificial diet, bioassays were made with adult beetles and a series of artificial diets.

From the mass culture with the method described in the section 2.1.3., new beetles that had emerged within 7 days were collected and used for the experiments.

The diet components were restricted as follows: red lauan (Shorea (Rubroshorea) spp.) sawdust (passing No. 20-40 mesh), cellulose powder, soluble starch and dried brewer's yeast powder "Ebios". These powder components were well mixed to prepare 14 different compositions (Table 2.2.). A sufficient amount of water, more than 100% of the weight of the powder materials was then mixed to make a dough, which was wrapped in aluminium foil, pressed into rectangular vessels and was incubated and baked in a

60°C electric oven-dryer for 48 hr to get solid or semi-solid diets.

For each composition 30-50 g of diet block was prepared. The great weight differences are due to the fact that several of the baked diets showed severe cracks or collapses, and had to be mended by adding more dough material. The mended diet blocks were not cut nor planed for fear of re-collapse.

A filter paper was placed on the bottom of a Petri-dish of 11.5 cm diameter, which had been treated with beta-naphthol (ca. 2.5% solved in ethyl alcohol) to keep off parasitical mites. A piece of diet cake was placed on filter paper in such a way that only one edge contacted the bottom. This set-up enabled the beetles to attack all 6 faces. For each diet 10 pairs of active, freshly emerged beetles were selected and released to the Petri-dish which was then sealed with a filter paper (Fig. 2.5.).

All the experimental cultures were placed in a mass culture chamber illuminated all day, as described in the section 2.1.3. When parent beetles died, they were removed and the cakes were observed whether they show any signs of larval activity, such as frass discharge. The emerging beetles were collected once a week, then dated, sexed and their body length measured with calipers between the top of the mandibles and the end of the elytra. Weighing the adult beetles proved difficult and improper because of their violent activity, vast number of them, and above all the change of the value with time, as discussed in the sections 3.1.2. and 3.2.4.

Each experiment was terminated when double the time of the

shortest life cycle had passed, for fear that the second generation (F_2) might be mixed with the first (F_1). In some cases the experiment was terminated earlier because the diet was eaten up to be reduced to powder and particles.

Results were presented with regard not only to the number of progeny (F_1 only) and the shortest life cycle period, but also to the weight values of the progeny as an index of the degree of their development. In the last item, summing and averaging males' and females' values collectively were assumed to be improper because there tended to be a sexual difference in the degree of development, as discussed in the section 3.1.2.

On the other hand, the prepupa, or the fully grown larva, is considered to be the heaviest developmental stage of the life cycle, in particular of those species of which the adults do not feed any more, as in L. brunneus⁽¹⁵⁾. Up to this stage the weight increases (except for at some ecdyses), and then it decreases due to pupation, sclerotization, energy consumption and genital activity. The body weight value of the final stage of larva, or prepupa, thus represents the result of the total feeding of the individual.

Keeping these two points in mind, the weight values obtained in this experiment were transformed into those of prepupa, with the values of male and female adults calculated separately with different formulae, and then weighted averages of both sexes were presented.

For the value transformations, the following formulae were employed:

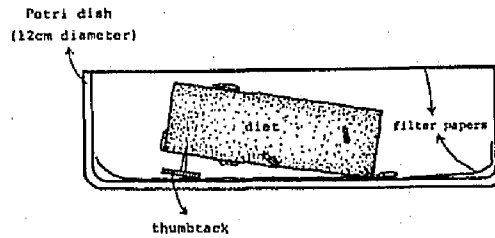


Fig. 2.5. Positioning of the diet cake for the experiment with adults.

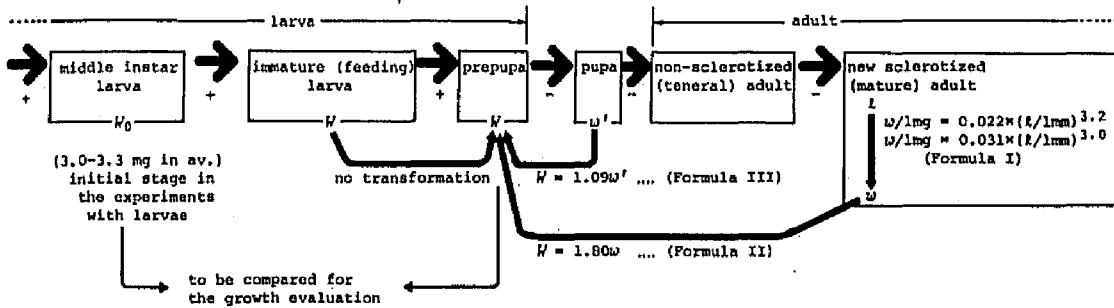


Fig. 2.6. Methods of evaluating larval growth and weight.
(+ : weight increase; - : weight decrease)

Table 2.2. Artificial diets investigated

Diet	Components (%)				Almost equal to the diet devised by:
	Red lauan wood sawdust	Cellulose powder	Soluble starch	Dried brewer's yeast powder	
A	66	10	0	24	Ijima et al. ⁱⁱ⁾ Tscholl ¹²⁾
B	56	10	10	24	
C	33	10	33	24	
D	0	10	66	24	
E	60	10	30	0	
F	45	10	45	0	
G	30	10	60	0	
H	50	0	26	24	
I	38	0	38	24	
J	26	0	50	24	
K	78	10	0	12	
L	39	10	39	12	
M	0	10	78	12	
N	0	43	33	24	

Table 2.3. Results of experiments with *Lyctus brunneus* adults on artificial diets.

Diet	Progeny yield number	Shortest life cycle period (weeks)	Weight in prepupal stage ^a W (mg)
A	0	--	---
B	153	11	3.6
C	241	11	5.1
D	95	13	4.9
E	8	37	1.45
F	7	34	1.04
G	7	28	2.5
H	353	11	5.1
I	315	11	5.8
J	298	11	6.3
K	0	--	---
L	107	11	4.6
M	45	13	5.5
N	120	14	5.8

^a) Average including males and females. For each sex the average of adult body lengths was transformed with Formulae I and II.

$$\begin{aligned} \text{Formula I : } w / 1 \text{ mg} &= 0.022 \cdot (\ell / 1 \text{ mm})^{3.2} && \text{for males} \\ w / 1 \text{ mg} &= 0.031 \cdot (\ell / 1 \text{ mm})^{3.0} && \text{for females} \end{aligned}$$

(see 3.1.2.)

$$\text{Formula II : } W = 1.80 w \quad (\text{see 3.1.1.})$$

(ℓ : body length of adult; w : weight of new, sclerotized adult;
 W : weight of prepupa)

The results of the experiments with adults are summarized in Table 2.3.

2.2.2. Experiments with larvae

The second part of this investigation is the test with "larval transfer". While the section 2.2.1. is aimed at assaying the diet suitability for the ovipositing female adults, this part is aimed at assaying the diet suitability for the feeding larvae.

The larvae employed in the experiments were taken out from the mass culture in the same condition and method as described in the sections 2.1.3. and 2.2.1. Cultures were started with the diets exposed to the parent beetles for only 7 days. Eight weeks after half the exposure time, the diets were broken into pieces to get the "middle instar larvae", as defined in the section 3.2.1.

The series of artificial diets investigated was just identical with that in the experiments with larvae, as presented in the section 2.2.1. and Table 2.2. Holes of a diameter of 2mm and about 2.5 cm deep were drilled into the diet blocks. One of the "middle instar larvae", with their average weight being 3.0-3.3 mg and S.D. value less than 1.0 mg, was put into each hole of

a diet block. Then the holes were stuffed with powder materials of the same composition as the respective diet and sealed with glued paper labels. The diet quantity for each larva was sufficient. They were also placed in the same mass culture chamber as described in the section 2.1. The emerging beetles were collected once a week, then dated, sexed and measured similarly as described in the section 2.2.1. Two series of experiments were conducted;

Series I: 30 larvae were used for each diet. The emerging beetles were collected for 9 weeks. All the remaining immature individuals were then taken out of the diet and weighed.

Series II: 10 larvae were used for each diet. The emerging beetles were collected until the last had emerged.

In Series I of this experiment, individuals obtained assumed various developmental stages. In the same manner and circumstances as described in the section 2.2.1. all the weight values obtained in this series were transformed into values of prepupa, except for the values of the immature (feeding) larvae.

For the value transformations, Formula III, as presented in the section 3.1.1., and also Formulae I and II, as presented in the sections 3.1.1. and 3.1.2., were employed:

Formula III : $W = 1.09 w'$

(w' : weight of pupa; W : weight of prepupa)

The present bioassay process of growth evaluation on diets based on these formulae is summarized in Fig. 2.6.

The results of the experiments with larvae are summarized in Table 2.4.

Table 2.4. Results of experiments with *Lyctus brunneus* larvae on artificial diets.

Diet	Series I (30 individuals) ^a								Series II (10 individuals) ^a			
	Emerging adults within 9 weeks		Remaining pupae after 9 weeks		Remaining larvae after 9 weeks		Weight (av.) of initial larvae V_0 (mg)	Weight increase $V - V_0$ V_0	Emerging adults		Weight (av.) of initial larvae V_0 (mg)	Weight increase $V - V_0$ V_0
	Number	Weight in prepupal stage V (mg) ^b	Number	Weight in prepupal stage V (mg) ^c	Number	Average weight V (mg)			Number	Weight in prepupal stage V (mg) ^b		
A	6	2.2	0	--	18	4.4	3.0	0.3	9	2.3	3.0	-0.2
B	4	3.0	0	--	23	7.8	3.0	1.4	9	7.1	3.0	1.4
C	6	5.2	0	--	19	9.6	3.0	1.8	9	10.9	3.0	2.6
D	6	3.9	0	--	19	8.6	3.1	1.4	9	8.8	3.0	1.9
E	5	2.8	0	--	21	4.6	3.1	0.4	9	3.3	3.0	0.1
F	12	3.0	0	--	17	4.7	3.3	0.2	9	3.7	3.0	0.2
G	8	3.4	0	--	18	4.4	3.2	0.3	10	3.0	3.0	0.0
H	8	5.2	0	--	22	9.6	3.3	1.5	9	9.8	3.0	2.3
I	8	5.7	0	--	18	9.6	3.3	1.5	8	9.5	3.0	2.2
J	10	5.6	0	--	18	9.6	3.3	1.5	6	8.1	3.0	1.7
K	9	2.5	0	--	12	1.9	3.3	-0.4	2	1.7	3.0	-0.4
L	11	4.0	1	10.1	17	9.4	3.3	1.2	9	9.5	3.0	2.2
M	9	4.6	0	--	18	8.2	3.3	1.1	4	8.3	3.0	1.8
N	7	4.9	0	--	19	5.8	3.3	0.7	5	5.0	3.0	0.7

^a) Including some larvae dead or lost during the experiment.

^b) Average including males and females. For each sex the average of adult body lengths was transformed with Formulae I and II.

^c) The value of pupal weight was transformed with Formula III.

2.2.3. Discussion

From the results presented in Tables 2.3. and 2.4., following information is obtained:

The results of diets A and K indicate that a full development cycle cannot be obtained if starch is lacking, although some of the transferred middle instar larvae were able to pupate with little or no weight increase. These larvae are supposed to have put on enough weight to pupate without feeding any more. "Yeast glycogen", a kind of soluble sugar, was supposed to be contained in the yeast powder. The results of A and K showed that it did not prove to be a reliable nutritional source of carbohydrates; either yeast glycogen was not utilized, or the amount was insufficient if it is utilizable.

A comparison of the results of the diets B and C indicates that C is more efficient in yielding progeny and increasing weight. This conflicts with the result by Ijima et al.ⁱⁱ⁾

The diets D and H, which were prepared without sawdust, cracked easily during baking due to their high density; they were very hard and therefore unsuitable as a practical diet. An attempt to reduce the density of these diets by using cotton instead of cellulose powder made them even harder, with little progeny and little development of insects obtained.

The absence of yeast or any other protein and vitamin source in the diets E, F and G resulted also in poor progeny and development. However, in the experiment with adults a small number of beetles completed their life cycle on these yeast-free

diets, possibly due to the nutritions in the sawdust, in contrast to those reared on the starch-free diets A and K, which failed completely. These facts suggest that the used lot of sawdust contained no or only insufficient amount of starch, yet a small amount of other nutrients, above all proteins. The apparently greater importance of starch compared with proteins in the remaining components may thus be explained by the quantitative differences of these two substances. This result is more or less similar to that reported by Cymorek⁽¹⁰⁾, who further stated that the larvae in earlier stage were able to survive the protein deficiency for a limited period, possibly by utilizing the yolk of the egg.

The absence of cellulose powder in the diets H, I and J did not affect the production of beetles negatively, but did on the contrary increase the yield. Mori⁽⁸⁾ was skeptical whether the addition of cellulose to the diets had any effects. The present investigation yet revealed that it has some effects which will be mentioned later on.

The effects of a reduction of the amount of yeast was not clearly noticeable from the comparison of the diet series {A, B, C, D} and {K, L, M}. However, it is remarkable that the progeny yield of the diet L was relatively small.

Contrary to the results of the diets H, I and J, the diet N could produce a certain progeny yield and development though poorer than H, I and J. Thus, it can be said that when sawdust is not available, it may be substituted by cellulose powder in making diets: either cellulose or sawdust, one will suffice for

the purpose. Furthermore, the cellulose powder and the sawdust, containing no or insufficient nutritional substances, are considered to play the same role in the composition of a diet. They are not only simple matrices but also components contributing to lowering the density and to increasing the porosity and roughness of the diet. This is also obvious from the results of the diets D and M, where the other two fine powder materials amount to as much as 90% and thus the diets lose their surface roughness and porosity. In these two diets the progeny yields in the experiment with adults were low in spite of the weight increases in the experiment with larvae. This indicates that these two diets are comparatively suitable for larval development, but not so suitable for oviposition, being not easily accepted by the ovipositing females. The present method of bioassay distinguished clearly between the diet suitabilities for the oviposition and for the larval development. The present result does not seem to be incompatible with a report by Cymorek⁽¹¹⁾, who only showed a delayed larval development with increased density of wood. Ito & Hirose⁽¹⁷⁾ reported on the influence of the diet density on the progeny yield. However, the quantitative information on the larval development, such as their weights, was not dealt with by them.

Khalsa et al.⁽¹³⁾, Cymorek⁽¹⁰⁾ and Ijima et al.⁽¹¹⁾ emphasized the fact that oviposition of Lyctus took place in artificial diets. It is, however, not surprising since all the diets devised by them have many small cracks, pores and/or depressions that are utilized as oviposition sites. It is noteworthy that L.

brunneus, compared to other lyctids, prefers wide and large oviposition sites⁽¹⁰⁾; this "species specificity" in ovipositing behavior should be taken into consideration. This fact may be responsible for the difference in the suitability of the diets D and M for larval development and for oviposition.

As the first approach to develop the most suitable meridic diet, the present investigation singled out C, H, I and J to be the best four diets, followed by B, L and N. This investigation will be succeeded by the section 2.3.

It should be mentioned in addition that the larval development was hindered to a certain extent in the experiments with larvae. In Series II several individuals emerged very late; it took as much as 9-10 months for the beetles to emerge even under the mass culture condition. This phenomenon cannot be attributed obviously to a nutritional deficiency because it occurred even in "good diets", such as in H. Cummins & Wilson⁽⁷⁾ also reported such an inconvenience in the result of the laboratory tests of chemicals with larval transfer method. The cause of this phenomenon is not clear although it is perhaps related to the different ability of the individuals to adjust themselves to a new diet or to the physical and geometrical variation of the bored holes on a diet block. In general, larvae of lyctids and bostrychids suffer more from transfer than certain cerambycid and anobiid species.

2.3. Development of new artificial diets^{E,H)}

2.3.1. Materials and methods

As the final investigation on the artificial diet, tests were carried out with a new series of diets and adult beetles.

The adult beetles employed in this investigation were reared, collected and selected with the same conditions, methods and diet as in the sections 2.1. and 2.2.1., with only males of more than 4.5mm length and females of more than 5.0mm length selected so as to eliminate weak individuals.

A series of artificial diets, as presented in Table 2.5., was prepared, which were made from wheat flour, buckwheat flour (powder from the "middle layer"), as well as the four raw materials utilized in the section 2.2.1. These diets had been supposed to be well accepted by and suitable for Lyctus to some extent, including those already dealt with in the section 2.2. (H, I, J, N, B, C), the buckwheat flour cake^{s12,s13} (W) and so-called "the diets of Sumitomo Chemical Co. Ltd."ⁱ⁹ (X, Y). Compared to the diets O, H, P, I, Q, J, the diets R, S, N, T, U, V, respectively, are those in which wood sawdust, which proved to be a physically contributing ingredient, is replaced entirely with cellulose powder.

Diets were prepared in the manner similar to but slightly modified from that in the section 2.2.1.; 100 g of the raw material mixture was stirred thoroughly and mixed in a dry powder state. To this distilled water was added in an appropriate quantity for each diet as shown in Table 2.5. Such a variety of water quantities was aimed at preventing cracking or collapse in diet blocks, as encountered in the section 2.2.1. The kneaded dough was then solidified in a 7cm x 3.5cm frame to make two or

Table 2.5. Artificial diets further investigated

Diet	Composition (%)					Water (%)	Average density (g/cm ³)	Almost equal to the diet devised by:
	Lauan wood sawdust	Cellulose powder	Soluble starch	Dried brewer's yeast powder	Other constituent (flour)			
O	66	--	10	24	--	110	0.59	
H	50	--	26	24	--	100	0.69	
P	43	--	33	24	--	100	0.76	
I	38	--	38	24	--	90	0.79	
Q	33	--	43	24	--	80	0.78	
J	26	--	50	24	--	70	0.86	
R	--	66	10	24	--	130	0.54	
S	--	50	26	24	--	120	0.65	
N	--	43	33	24	--	110	0.69	
T	--	38	38	24	--	100	0.72	
U	--	33	43	24	--	100	0.76	
V	--	26	50	24	--	90	0.83	
B	56	10	10	24	--	110	0.56	Ijima et al. ⁽¹⁾
C	33	10	33	24	--	100	0.72	Tscholl ⁽²⁾
W	--	--	--	--	Buckwheat 100	80	0.73	Suzuki ^(12,13)
X	--	50	--	10	Wheat 40	120	0.64	Ito & Hirose ⁽⁹⁾
Y	--	50	--	20	Wheat 30	120	0.66	Ito & Hirose ⁽⁹⁾

three blocks. They were incubated and baked in a 60°C electric oven-dryer for 48 hr, except for the diet W (buckwheat cake), which was wrapped in paper and incubated for only 24 hr. As shown in Table 2.5., each kind of diet block had its own density or, in another term, specific gravity, which depended on its composition and the quantity of water added to it. Four replications were made for each diet composition. Because all the present diets are supposed to be "good" to some extent, experiments with larval transfer, as performed in the section 2.2.2., were not made, and only the "experiments with adults" were performed, examining the result of only the first progeny generation (F_1).

For each experimental replication, a circular filter paper was placed and glued firmly with polyvinyl acetate resin adhesive on the bottom of a Petri-dish of 11.5 cm in diameter. After the adhesive solidified, the dish was disinfected with beta-naphthol in the same manner as described in the sections 2.1.3. and 2.2.1. About 100 g of diet blocks was placed on a dish in the same manner as before so as to expose all six faces of each block. To this 10 pairs of beetles were released.

All of the experimental cultures were placed in a mass culture chamber (24.5°C, 70% R.H.), as described in the section 2.1.1., with very dim illumination all day. Each culture was closed when double the shortest duration of one generation (double the time between the initial release of the parent beetles and the first emergence of their progeny) had passed, for fear that the first and second progeny generations might be mixed.

The progeny from each replication, dead and alive, was collected at the time of culture closure; weekly beetle-collecting, as performed in the section 2.2.1., was avoided in this investigation because it might affect or injure some of the other individuals in the course of development.

In order to assess the weight, namely the degree of development, of the progeny, adult body length was measured as its parameter, in the same manner as in the section 2.2.1. In this case, dead beetles among the progeny had to be treated to make them supple because a dried dead beetle, as it is, becomes broken easily when handled. Therefore, the collected progeny from each culture, dead and alive, was wholly soaked in so-called "Barber's fluid"^{vi)} (benzene 5.5%, ethyl acetate 14.8%, ethanol 39.3% and water 40.4%), specially devised for softening beetle specimens, by which dead beetles became as flexible as live beetles.

All the beetles were sexed, counted, and their body length measured with slide calipers. The bodies of some individuals, which had been dead at the time of collection, were broken, with head and pronotum separated from the rest. For such cases, the following formula, which is presented in the section 3.1.2., was utilized so as to estimate the whole body length of the broken specimens.

$$l = 1.39 \, l'$$

(l : body length of adult; l' : elytral length of adult)

Out of four replications for each diet, one replication with the smallest number of progeny was abandoned and the other three

replications were presented as the results.

2.3.2. Results

The results obtained are shown in Table 2.6., while the analyses of variance of the shortest duration of one generation, progeny number, and progeny body length are given in Tables 2.7., 2.8. and 2.9., respectively. These values then were treated statistically as shown graphically in Figs. 2.7., 2.8. and 2.9. respectively, together with their 95% confidence limits.

The average of the shortest duration of one generation registered in three replications seem rather uniform, falling between 71 and 90 days. The diet C and W had the shortest values of all the diets, whereas the diets R had the longest. According to the analysis of variance of the shortest life cycle duration (Table 2.7.), the variance ratio among the diets did not exceed the F value in a 5% risk, indicating that there was no significant difference regarding the shortest duration of one generation among the diets. Figure 2.7. also indicates a uniformity of values, with the possible exception of the diet R which showed a value that was a little higher. The last column in Table 2.6. presents body length differences between males and females, with the average 0.29 mm, which will designate a measurable value of 0.3 mm as the standardized difference of body length limits for bioassays.

As for the progeny body length, highly significant differences among both diets and sexes were shown in Table 2.9. The latter means that female individuals are statistically larger-sized than

Table 2.6. Results of the progeny of Lyctus brunneus reared with artificial diets
(3 replications in each diet)

Diet	Average of the shortest duration of one generation (days)	Males (♂)		Females (♀)		♂ + ♀ Total progeny number	Body length difference between ♀ and ♂ (mm)
		Average of body length (mm)	Total progeny number	Average of body length (mm)	Total progeny number		
O	83	4.58	225	4.84	287	512	0.26
H	77	4.69	369	4.99	399	768	0.30
P	77	4.78	339	5.09	399	738	0.31
I	79	4.84	299	5.18	282	581	0.34
Q	73	4.77	446	5.10	467	913	0.33
J	77	4.79	548	5.06	599	1147	0.27
R	90	4.50	200	4.71	223	423	0.21
S	78	4.91	470	5.11	462	932	0.20
N	75	4.72	633	4.98	647	1280	0.26
T	74	4.68	648	4.98	613	1261	0.30
U	75	4.66	646	4.97	642	1288	0.31
V	78	4.74	539	5.13	606	1145	0.39
B	79	4.49	313	4.75	394	707	0.26
C	71	4.82	354	5.20	322	676	0.38
W	72	3.92	434	4.16	441	875	0.24
X	80	4.64	660	4.96	645	1305	0.32
Y	84	4.72	652	4.98	640	1292	0.26
Averages...						932	0.29

Table 2.7. Analysis of variance of the shortest duration of one generation

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio (F)
Diet	1038.71	16	64.919	1.11968 ^a
Error	1971.33	34	57.980	
Total	3010.04	50		

$$a) F_{(16,34)(5\%)} = 1.95 > 1.12 = F_{\text{diet}}$$

Table 2.8.. Analysis of variance of the progeny number

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio (F)
Diet	243231.5	16	15202.0	10.30 ^a
Sex (gender)	841.7	1	841.7	
Error	124003.1	84	1476.2	0.57 ^b
Total	368076.3	101		

$$a) F_{(16,84)(0.1\%)} < 3.32 < F_{\text{diet}}$$

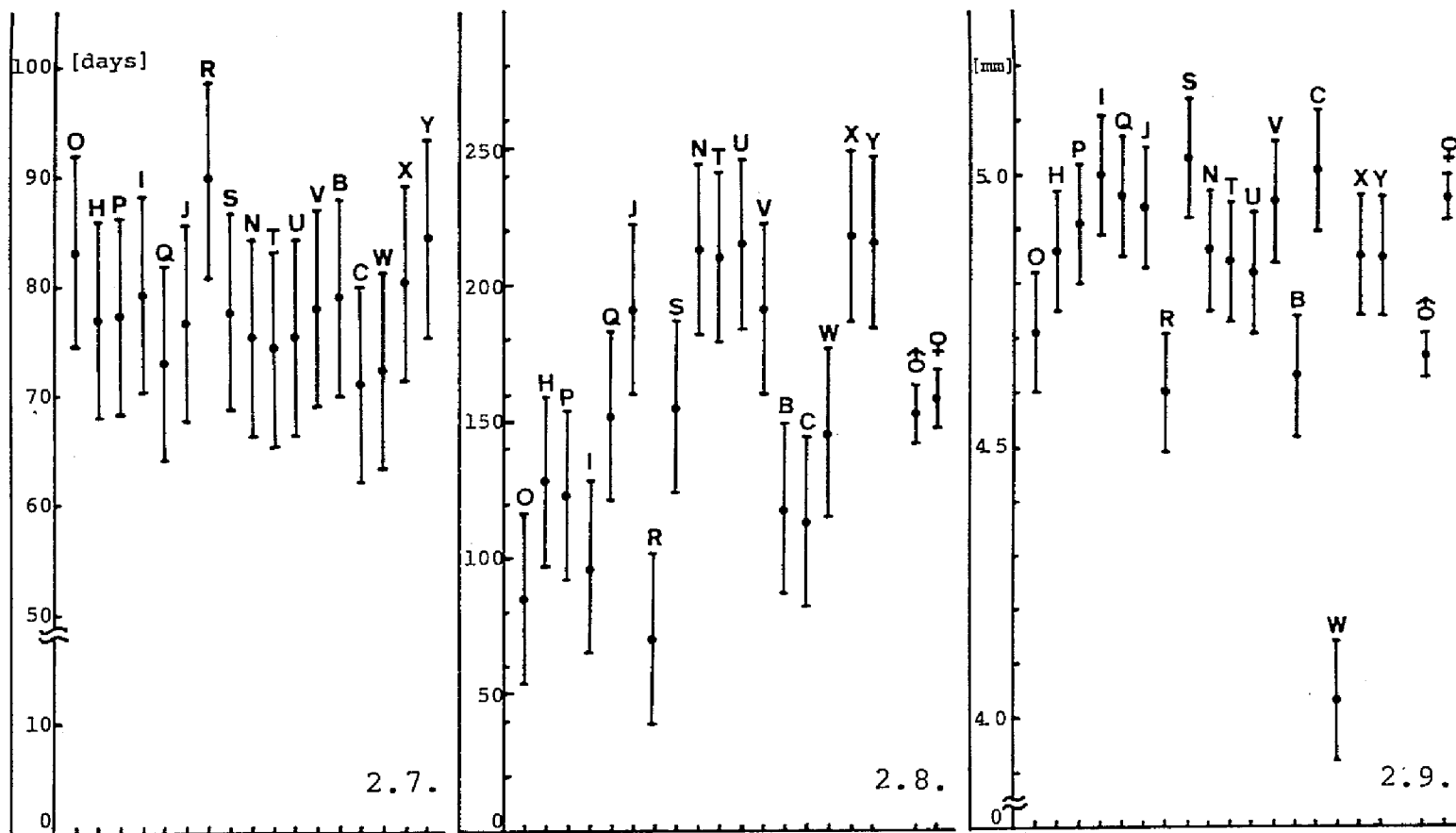
$$b) F_{(1,84)(5\%)} = 3.95 > F_{\text{SEX}}$$

Table 2.9. Analysis of variance of the progeny body length

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio (F)
Diet	5.39	16	0.34	20.1 ^a
Sex (gender)	2.10	1	2.10	
Error	1.41	84	0.0168	125.1 ^b
Total	8.90	101		

$$a) F_{(16,84)(0.1\%)} < 3.32 << F_{\text{diet}}$$

$$b) F_{(1,84)(0.1\%)} < 11.97 << F_{\text{SEX}}$$



Figs. 2.7.-2.9. Average values (dots) and their 95% confidence limits of the results of rearing with artificial diets.
 2.7.: The shortest duration of one generation (days).
 2.8.: Progeny number.
 2.9.: Progeny body length (mm).

males, as discussed in the section 3.1.2., although size is no guide in distinguishing the sexes¹⁶⁾.

Table 2.6. and Fig. 2.8. show that among the 17 diets, the following seven formed a group that produced more progeny than the average of 932 individuals with 3 replications collectively: J, N, T, U, V, X and Y. In each of the two series of six diets, namely {O, H, P, I, Q, J} and {R, S, N, T, U, V}, progeny number seems to increase roughly as the specific gravity of the diet increases. This agrees with the report by Ito & Hirose¹⁷⁾.

As for the progeny number, the variance ratio among the diets greatly exceeds the F value in even the 0.1% risk, as shown in Table 2.8., indicating a high significant difference among the diets, whereas the variance ratio between the sexes indicates that there is no significant difference between the male and female numbers; the final sex ratio is nearly 1 : 1 in every diet.

Standard deviation (S.D.) of the body lengths of the populations varied to some extent; in males the maximum S.D. value encountered in this investigation was 0.50 mm in a replication of the diet H, with 80 individuals of the progeny, and a replication of B, with 99 individuals, also; the minimum 0.23 mm in a replication of C, with 166 individuals, whereas in females the maximum S.D. value was 0.53 mm in a replication of O, with 72 individuals, the minimum 0.29 mm in a replication of W, with 206 individuals. Contrary to Suzuki's¹²⁾ report, it seemed difficult to detect a relationship between the standard deviation of the progeny body length and the progeny number as well as between the

former and the kind of diet. From a practical viewpoint, a standard diet is desired to produce adult beetles with their body length as uniform as possible. However, as the S.D. value cannot be expected to be reduced with any of the diets, the average body length becomes important in selecting diets.

Although it is difficult to draw a definite line, 7 diets, P, I, Q, J, S, V and C may form a group that produces well-developed (large-sized) individuals (Fig. 2.9.). The diets R, B (that of Tôkyô University of Agriculture) and W (buckwheat cake) clearly are unable to produce well-developed individuals. The value of the diet W reported here agrees well with a value "4.0 mm", as reported by Suzuki⁵¹²⁾. The differences of the maximum and minimum average body length data of the three replications of each diet were small enough; in males the maximum difference between them was 0.42 mm in the diet X, in females, 0.44 mm in X also.

2.3.3. Discussion

As two common elements between the set of diets {J, N, T, U, V, X, Y} with the more progeny than the average and the set {P, I, Q, J, S, V, C} with comparatively larger-sized progeny, the diets J and V are designated as the ideal diets, where the identical percentages of the nutritional ingredients, namely soluble starch (50%) and brewer's yeast powder (24%), are contained. The quantity of water added in making diet dough differed between these two: in the diet J 70% water is required, whereas in V it is 90%. This is due to the difference of fineness between lauan wood sawdust, passing a No. 28 mesh, and cellulose powder.

All of the diets investigated in this study apparently contained sufficient quantities of nutritional ingredients required by the species, and yet variations in the progeny number and body length were displayed to some extent. This seems, at present, to be due to the variation of physical properties, such as density, and/or the nutritional balance. The latter will be discussed further in the chapter 4. in relationship to Lyctus development.

The content of starch as well as that of some other nutrients in certain artificial diets has been thought to be too high and thus unnatural⁽¹⁸⁾. This idea, however, seems to be of questionable validity because, in fact, there exist some natural materials which are often and heavily attacked by lyctid beetles because of their very high starch content. An appropriate example may be bamboo, whose starch content, according to Joseph⁽²⁾, can amount to as much as 19% as in Bambusa arundinacea. Cymorek⁽¹⁰⁾ reviewed the non-wood-feeding habits of lyctids, such as on dried potato, wheat grains, and so forth. All of them are thought to have high starch contents when compared to hardwood sapwood. These facts suggest that lyctids in the nature are ready to utilize these materials as food, and hence the high starch content of the diet series in this investigation does not seem "unnatural" for lyctids.

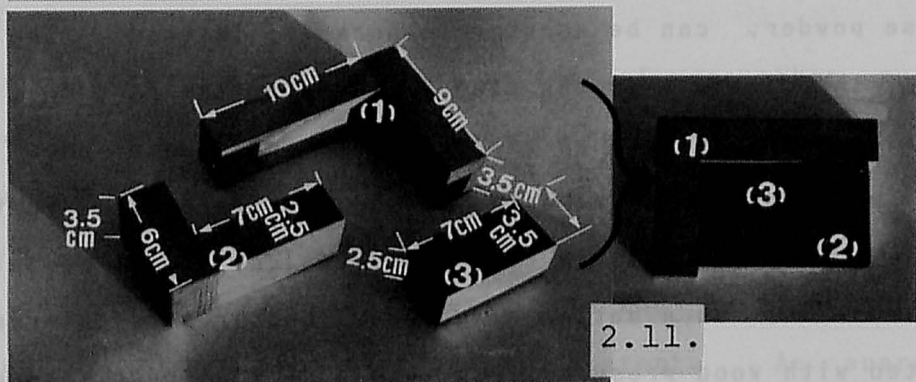
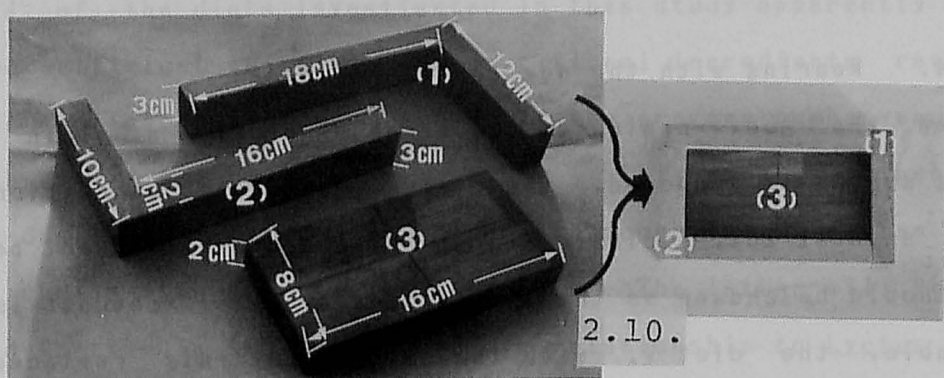
It is shown to be difficult to reduce the S.D. value of the progeny body lengths with all the diets. Such a variation is inevitable in general though the longevity is dependent upon the body length greatly, as shown in the section 3.5.

2.3.4. Rearing with the new artificial diets

With the above-nominated two kinds of diets, a plain diet making system is proposed: the diet J, with the composition of lauau sawdust 26%, soluble starch 50% and brewer's yeast powder 24%, should be chosen as the standard diet, and if sawdust is not available, the diet V, with the sawdust entirely replaced by cellulose powder, can be adopted temporarily instead. The priority of the diet J over the diet V is due to the fact that the diets without wood sawdust were observed to produce more malformed individuals, as reported in the section 5.6. Furthermore, in practical tests in wood preservation, a diet containing wood sawdust is, as a matter of course, better as a material to be treated with wood preservatives than a diet without sawdust.

Here we call the diet J "the standard diet", and the diet V "the second standard diet", while J and V collectively "the diets of Kyôto University".

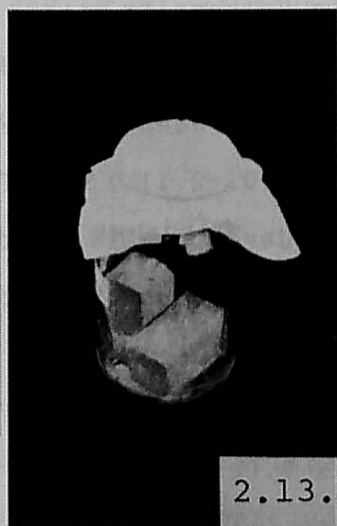
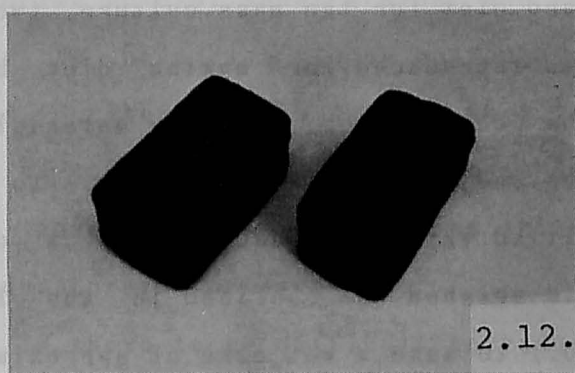
In utilizing this standard diet for the mass culture, two sets of wooden frames have been introduced for making diet blocks (Figs. 2.10.-2.11.). The composition of 3 raw materials in dried powder-state is shaken and mixed in a vinyl sack, to which 70% of deionized or distilled water is added to make a dough. Just 400 g of this dough is weighed and confined in the larger wooden frame set (Fig. 2.10.) to make a wet cake of approximately 16.0cm x 8.0cm x 2.4cm in size, which is cut with a thin wire or a knife into 4 equal pieces, each being approximately 8.0cm x 4.0cm x 2.4cm in size and weighing about 100 g. When the



Figs. 2.10.-2.11. Wooden frames prepared for making the diets of Kyôto University. (1) and (2): side frames, (3): pressing plate.

2.10.: Larger set yielding 4 blocks.

2.11.: Smaller set yielding 1 block.



Figs. 2.12.-2.13. The standard artificial diet blocks and a culture of *L. brunneus* using them.

smaller wooden frame set (Fig. 2.11.) is used, only 100 g of dough is weighed and confined to yield only one block. These blocks are then put up on a large heat-proof tray with a sheet of paper on its bottom, and this tray together with the diet blocks is put into a 55-60°C electric oven-dryer for 48 hr to dry, bake and solidify them all. The diet blocks are then climatized in the mass culture condition (24.5°C, 70% R.H.) for more than 2 weeks to get an air-dried state with appropriate moisture content for the insects. Such an air-dried diet block weighs about 55-60 g, resulting in ca. 0.7-0.8 g/cm³ of density (Fig. 2.12.). Two pieces of diet blocks are used per one culture vessel of the same kind (Fig. 2.13.) with the same treatment and manner as described in the section 2.1.3. The number of parent beetles released to one culture will be designated later in the section 3.3.2.

This standard diet has been utilized in the present mass culture instead of "the diet of Tôkyô Univ. of Agric." since its establishment in May, 1982 up to present (1986), and has been confirmed to have the ability to produce progeny in the present mass culture system over several generations.

This artificial diet has proved efficient also in rearing some other lyctid species, such as L. sinensis, L. africanus and Lyctoxylon dentatum, and a bostrychid species, Dinoderus minutus (Fabricius).

2.4. Rearing with nutritionally enriched woods^{H)}

The alternative rearing matrix adopted for another purpose in

the mass culture system is the wood treated with the following method, the outline of which has been given by Cymorek^{613,614}. This treatment of nutritional enrichment yields the progeny rather surely, compared to untreated wood.

A large glass vessel for vacuum treatment is prepared, to the glass lid of which a separatory funnel was attached. Sapwood blocks of an oak species, Quercus serrata Murray, each measuring approximately 7-11 cm (longitudinal) x 3-6 cm x 1-1.5 cm, stored in a 5°C refrigerator for 1-5 years, are put into the vessel, which is then depressurized by suction-pump down to ca. 7mb for a period of 15 min. After switching off the motor of the suction-pump, the nutrient solution, 15% w/w maltose and 3% w/w peptone dissolved in distilled water, is poured into the vessel using a separatory funnel under the low pressure to dip wood blocks wholly in the solution and to impregnate the blocks with the solution. After the vacuum released, the wood blocks are left untouched in the solution for a period of more than 1hr⁶¹⁴. (Although a preliminary trial using lauan veneers to substitute soluble starch for maltose resulted in a comparable yield of progeny, dissolving the starch required much time and labor, especially in alkalizing the solution with NaOH before the troublesome dissolution of starch, followed by neutralization with HCl.) The wet wood blocks, impregnated with maltose and pepton solution, are taken out from the vessel and dried in a 50-60°C electric oven-dryer for 24 hr, and then climatized in the mass culture condition (24.5°C, 70% R.H.) for more than 3 weeks to adjust the moisture content.

About 100-150 g of these wood blocks are used per one culture vessel as the alternative rearing matrix in the same manner as described in the section 2.1.3. The progeny beetle collection by force is performed, if necessary, by striking or knocking the wood block against another or some other hard material, by which new adult beetles still remaining in their pupal chamber slip out by themselves¹¹⁾. Attention should be paid if the block is heavily infested because in such a case the block is very liable to break.

This rearing matrix, in comparison to the standard diet, has some different characteristics: the duration of beetle yield (period from the first progeny emergence to the ceasing) is much longer though the progeny is less abundant and their bodies are smaller. The greatest merit of this matrix is that the urgency of beetle collection is mitigated to some extent especially in the use during suspension of assays: only a little labor is to be required in maintaining and preserving the strain.

Since the introduction of this rearing matrix, the present mass culture system has come to include three kinds of rearing matrices mainly used:

The sawdust-containing artificial diet of Kyôto University, namely the standard diet, or the diet J in Tables 2.2. and 2.5., serves for general use, especially for the intensive multiplication of the population, and for supplying well-developed individuals for any kind of bioassay. The cellulose-containing artificial diet of Kyôto University, namely the second standard diet, or the diet V in Table 2.5., serves only for temporary use

when wood sawdust is not available. The enriched wood blocks serves only for secondary but not for temporary use, especially for strain maintenance. During suspension of assays, using only the last kind of matrix is recommended.

2.5. Summary

The summarized results and conclusions obtained in the investigations on the mass culture method of L. brunneus are:

(1) The automatic control of the temperature and the relative humidity in the rearing chamber is essential for the mass culture of this species, and it is recommended to set the climatic conditions to be 24.5°C and 70% R.H. in due consideration of the mold occurrence.

(2) Productivity of emerging adult beetles decreases with age, and the females whose ovipositors had been fully extruded need to be excluded from adults supplied for new cultures and bioassays.

(3) Cultures have to be renewed by collecting the new adult beetles and providing them with new diet blocks.

(4) The activity of newly emerging adult beetles on the diet blocks can be controlled by illuminating the rearing chamber throughout the day.

(5) A number of larvae exposed out of diet blocks can be saved to some extent by scattering them together with diet particles among diet blocks.

(6) There is a duality of suitability of an artificial diet, i.e. the suitability for larval development, as the nutritional factor, and the suitability for oviposition, as the physical

factor. And the diets that are too hard and not porous enough proved to be rather unacceptable for ovipositing females even if they are rich in nutrients.

(7) Quantitatively starch is the most important nutritional substance compared to the protein and/or amino acids.

(8) Cellulose powder in addition to sawdust is not necessary as a diet component, while wood sawdust may entirely be replaced by cellulose powder.

(9) The shortest duration of one generation hardly varied under mass culture condition among the diets that were supposed to be effective. The progeny number varied considerably among these diets, while the sex ratio for every diet was near 1 : 1. On the other hand, the progeny body-length, as a parameter of the degree of development, varies among the diets as well as between the sexes.

(10) The standard artificial diet was designated to have the composition of lauan sawdust (or cellulose powder) 26%, soluble starch 50% and brewer's yeast powder 24%. Rearing with this diet was very successful not only of this species, but also of the other lyctid species.

(11) Oak wood treated with a nutritional solution of 15% maltose and 3% peptone is also utilized as an alternative rearing matrix principally for the purpose of culture and strain maintenance.

3. Life history and ecology

In this chapter, some quantitative relations among the developmental stages and some others (3.1.), larval development and instar number (3.2.), density effect from the viewpoint of population ecology (3.3.), sex ratio from the viewpoint of rearing (3.4.), adult longevity from the viewpoint of control bioassay (3.5.), ethology of adult beetles (3.6.), relations with other organisms including natural enemies (3.7.) are investigated and/or discussed.

3.1. Quantitative relations

3.1.1. Relations between the weights of prepupa and the other stages^{D)}

As discussed in the section 2.2.1., prepupa is just the heaviest stage throughout the life cycle of this species, presenting the total feeding of its life. In order to understand the pattern of body weight change in the life cycle of this species, the following attempt was made: several pupae and prepupae were collected from the mass culture with the diet of Tôkyô Univ. of Agric., as described in the section 2.1.3., and their weight changes with metamorphoses were recorded. The ratio values were calculated between the stages and were averaged respectively to set up the weight relation between newly sclerotized adult and prepupa, as well as that between pupa and prepupa.

As for the relation between the weights of newly sclerotized adult (w) and prepupa (W), the value of the ratio W / w averaged 1.80, with the S.D. being 0.20, as 9 individuals had been ex-

examin ed. Thus, the following equation could be set up:

$$W = 1.80 w$$

This equation was successfully utilized as "Formula II" in the sections 2.2.1. and 2.2.2. for the evaluation of the degree of development of both male and female adults, which had to be assessed collectively.

As for the relation between the weights of pupa (w') and prepupa (W), the value of the ratio W / w' averaged 1.09, with the S.D. being 0.03, as 9 individuals had been examined. Thus, the following equation could be set up:

$$W = 1.09 w'$$

This equation was utilized as "Formula III" in the section 2.2.2. for the evaluation of the degree of development of pupa, which had to be assessed together with the individuals of the other stages.

These equations contribute greatly to the establishment of the pattern of body weight change throughout the life cycle of the species, as is discussed in the section 3.2.4.

3.1.2. Relations among several parameters of adults^{D,E)}

Establishment of the relation between the body length and body weight of the adult of this species is eagerly needed since the body weight of adult is highly changeable not only in living but also in dead condition, while the body length is unchangeable.

A number of freshly sclerotized adults that had emerged less than 7 days before (Group A) and 7 - 14 days before (Group B) were collected from the mass culture with the diet of Tôkyô Univ.

of Agric. They were sexed, weighed in mg and measured for the body length in mm. Logarithms of both values were treated with the method of least squares to set up the relation between their body length and weight assuming a linear relation.

The relation between the length (ℓ) and the weight (w) of newly sclerotized adults (Group A) is shown in Fig. 3.1. The exponents in the equations, i.e. the inclination of the straight lines, proved to be nearly 3 as had been expected. The equations obtained are:

$$\text{Group A: males: } w/\text{mg} = 0.022 \times (\ell/\text{mm})^{3.2} \quad (r=0.95)$$

$$\text{females: } w/\text{mg} = 0.032 \times (\ell/\text{mm})^{3.0} \quad (r=0.99)$$

$$\text{total: } w/\text{mg} = 0.024 \times (\ell/\text{mm})^{3.1} \quad (r=0.97)$$

$$\text{Group B: males: } w/\text{mg} = 0.0182 \times (\ell/\text{mm})^{3.1} \quad (r=0.97)$$

$$\text{females: } w/\text{mg} = 0.052 \times (\ell/\text{mm})^{2.6} \quad (r=0.95)$$

$$\text{total: } w/\text{mg} = 0.021 \times (\ell/\text{mm})^{3.1} \quad (r=0.94)$$

In some coleopterous species, such as those of Cerambycidae, the body construction of males and females differs considerably; the male is more slender than the female; a female that has the same length as a male is assumed to be heavier therefore. Furthermore, in most cases the female has statistically greater values of both size and weight. Parkin¹⁴⁾ also gave a similar statement with this species.

In due consideration of this remarkable sexual difference, though it does not serve as a definite sex character, equations for males and females were established separately and also Group A was adopted as a model of fresh population:

$$\text{Males: } w / \text{mg} = 0.022 \times (\ell / \text{mm})^{3.2}$$

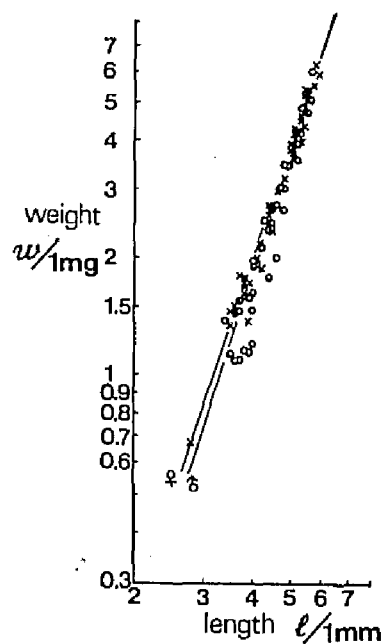


Fig. 3.1. Length-weight relation of new adults of L. brunneus.

$$\delta(o): \quad w/_{mg} = 0.022 \times (\ell / 1_{mm})^{3.2} \quad , \quad (r = 0.95)$$

$$\varphi(x): \quad w/_{mg} = 0.031 \times (\ell / 1_{mm})^{3.0} \quad , \quad (r = 0.99)$$

$$\delta(o) \text{ and } \varphi(x): \quad w/_{mg} = 0.024 \times (\ell / 1_{mm})^{3.1} \quad , \quad (r = 0.97)$$

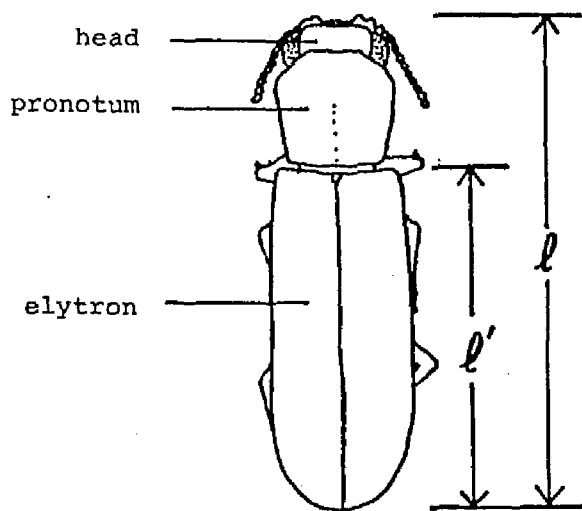


Fig. 3.2. Elytral length and body length of L. brunneus adult.

$$\text{Females: } w / 1\text{mg} = 0.031 \times (\ell / 1\text{mm})^{3.0}$$

A set of these two equations was available as "Formula I" for the evaluation of the degree of development of both male and female adults in the sections 2.2.1. and 2.2.2. According to Parkin^[4] the moisture content of beetle's body varies with the moisture content of wood. Consequently, these equations should be applied only to the cases with the same condition as the measurement for them were performed in, namely 24.5°C and 70% R.H.

Another equation established is that showing the relation between adult body length (ℓ) and elytral length (ℓ') (Fig. 3.2.). Dead adult specimens were prepared using "Barber's fluid"^[1], as was once used in the section 2.3.1., so as to make them supple for the convenience of measurement. After the whole body length (ℓ) of each specimen was measured, its head plus pronotum was removed to measure the length of elytra (ℓ'). The value of the ratio r ($=\ell/\ell'$), averaging 1.39, as calculated from the data of 50 specimens, with the S.D. being 0.05, gives the following equation:

$$\ell = 1.39 \ell'$$

This equation well resembles that for another species, L. planicollis, as presented by Kurir^[18]:

$$\ell = 1.4530 \ell' + 0.0942 \quad \text{or} \quad 1/r = 0.6829$$

That equation was available for the estimation of whole body length of adults in the examination of the progeny with several imperfect specimens as in the section 2.3.1.

3.2. Larval development and instars

3.2.1. Nomenclature of the larval developmental stages^{B)}

It appears from the previous work that larval form of Lyctus brunneus changes earlier on, for Altson's^{a4)} description of the 1st instar larva differs markedly from his own description of the 2nd instar and also from the form described as a "larva" by Munro^{m9)} and Pringle^{p5)}. This difference seems to be designated to be a "polymetaboly", rather than a "hypermetamorphosis"^{G12)}. On the other hand, there is little essential difference between 2nd instar and subsequent instars. Such a morphological uniformity of larva of the 2nd and the subsequent instars makes it difficult to investigate the instar number and to distinguish one instar from another.

For the convenience of designation of larvae of various stages, a tentative nomenclature was devised, including two tentative terms, "middle instar larva" and "mature larva". Figures 3.3.-3.7. present the 4 main larval phases in the development of L. brunneus with this nomenclature, showing the polymetaboly as well. This will be utilized in many kinds of investigations to categorize and designate the various larval stages. The correspondence of the "middle instar larva" or "mature larva" to a larva with a certain instar number will be discussed in the section 3.2.4.

3.2.2. Experiment with wood blocks^{F)}

In order to observe the transition of larvae, an attempt was made to rear some larvae with oak sapwood by transfer.

Test blocks, measuring ca. 10 cm (longitudinal) x ca. 4 cm x 1.0-1.5 cm, were cut from the sapwood of an oak species, Quercus serrata. Holes of ca. 2.0-2.5 mm diameter and ca. 2.0 cm depth were bored on the selected blocks as shown in Fig. 3.8. Larvae of various developmental stages were taken out from the mass culture, as described in the sections 2.1.1. and 2.1.3., and each was accommodated in a hole of wood blocks, with no stuffing material used. The holes were sealed with cellophane adhesive tapes, to which a small circle of transparent vinyl sheet was attached to avoid sticking of the larvae on the tape (Fig. 3.9.).

The test blocks were kept in the mass culture chamber, with the condition of 24.5°C and 70% R.H. Larvae and their exuviae were examined mostly at 2-5 days' interval, and each insect was weighed with the chemical balance.

Several examples of weight changes of the larvae and the later developmental stages are presented in Fig. 3.10. This wood block method was rather unsuccessful in rearing larvae as demonstrated by their weight decrease. There might be lack of support for boring and the poor nutritional condition of the wood substrate, which led to the subsequent starvation and death of the larvae. However, the individuals Nos. 8 and 20 were found exceptionally to succeed in pupation.

The result that most of the larvae molted supernumerarily presented a very interesting aspect. The maximum number of molts was 10 in individual No. 18. The larva No. 14, the heaviest one, molted 8 times until its death.

As a rule with almost all the prepupae exposed and left free

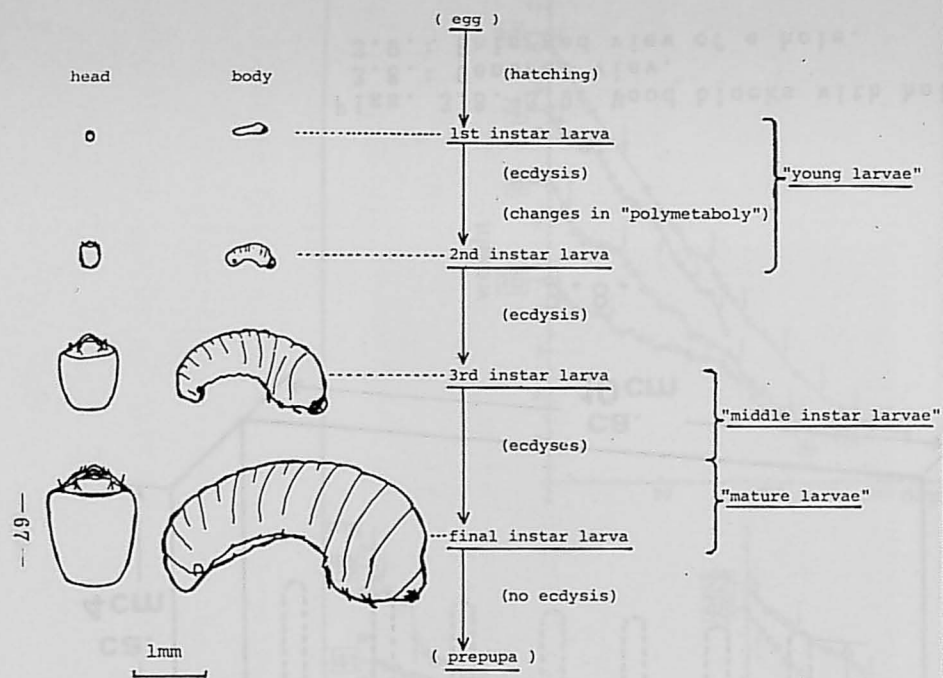
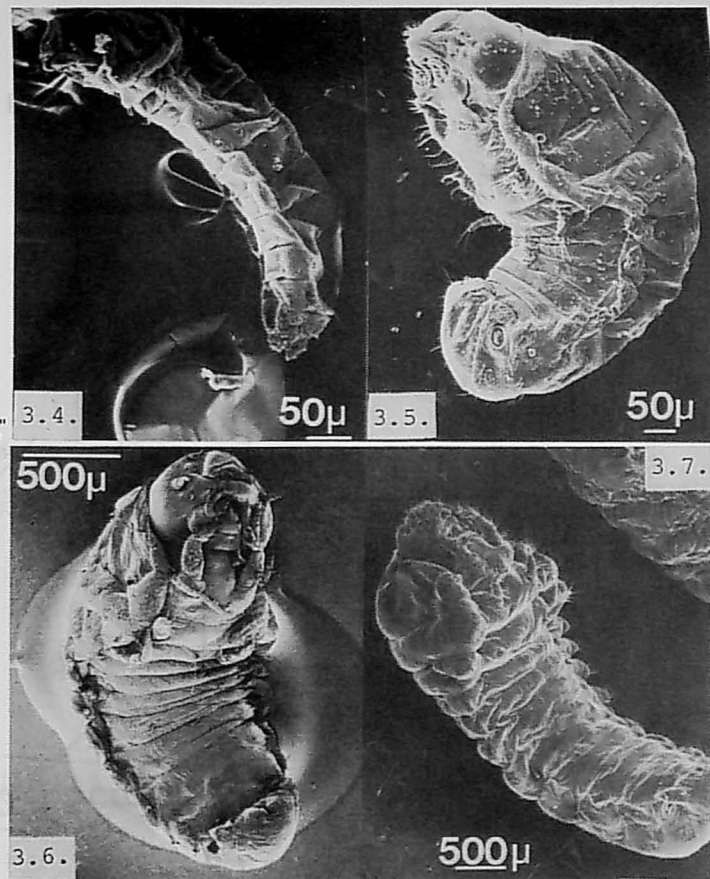
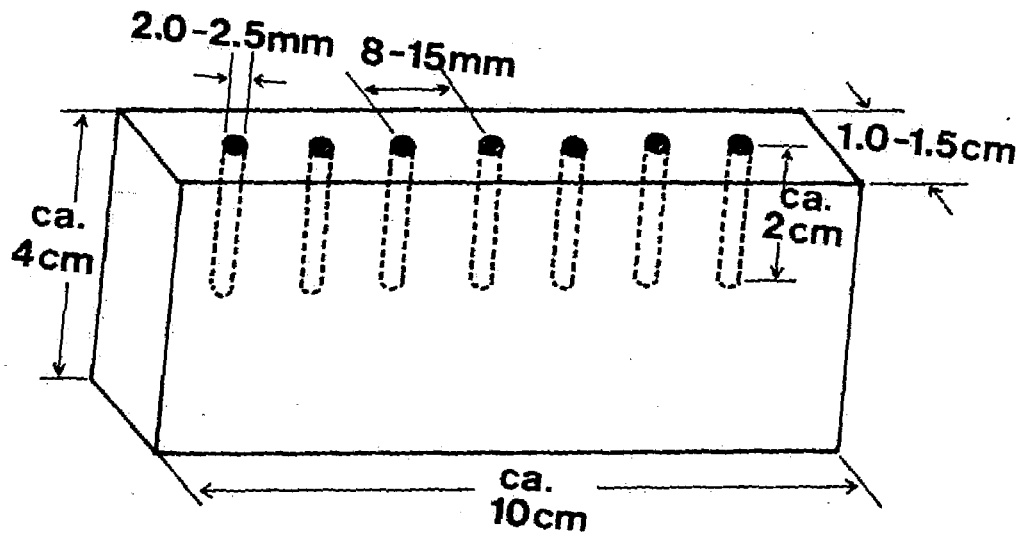


Fig. 3.3. The four main phases of the larvae of *Lyctus brunneus* designated with a tentative nomenclature.

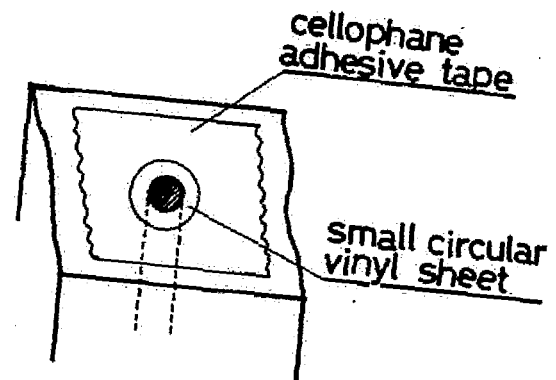


Figs. 3.4.-3.7. SEM photographs of the four larval phases shown in Fig. 3.3.

- 3.4.: 1st instar larva.
- 3.5.: 2nd instar larva.
- 3.6.: Middle instar larva.
- 3.7.: Mature larva.



3.8.



3.9.

Figs. 3.8.-3.9. Wood blocks with holes accommodating the larvae.
3.8.: General view.
3.9.: Enlarged view of a hole.

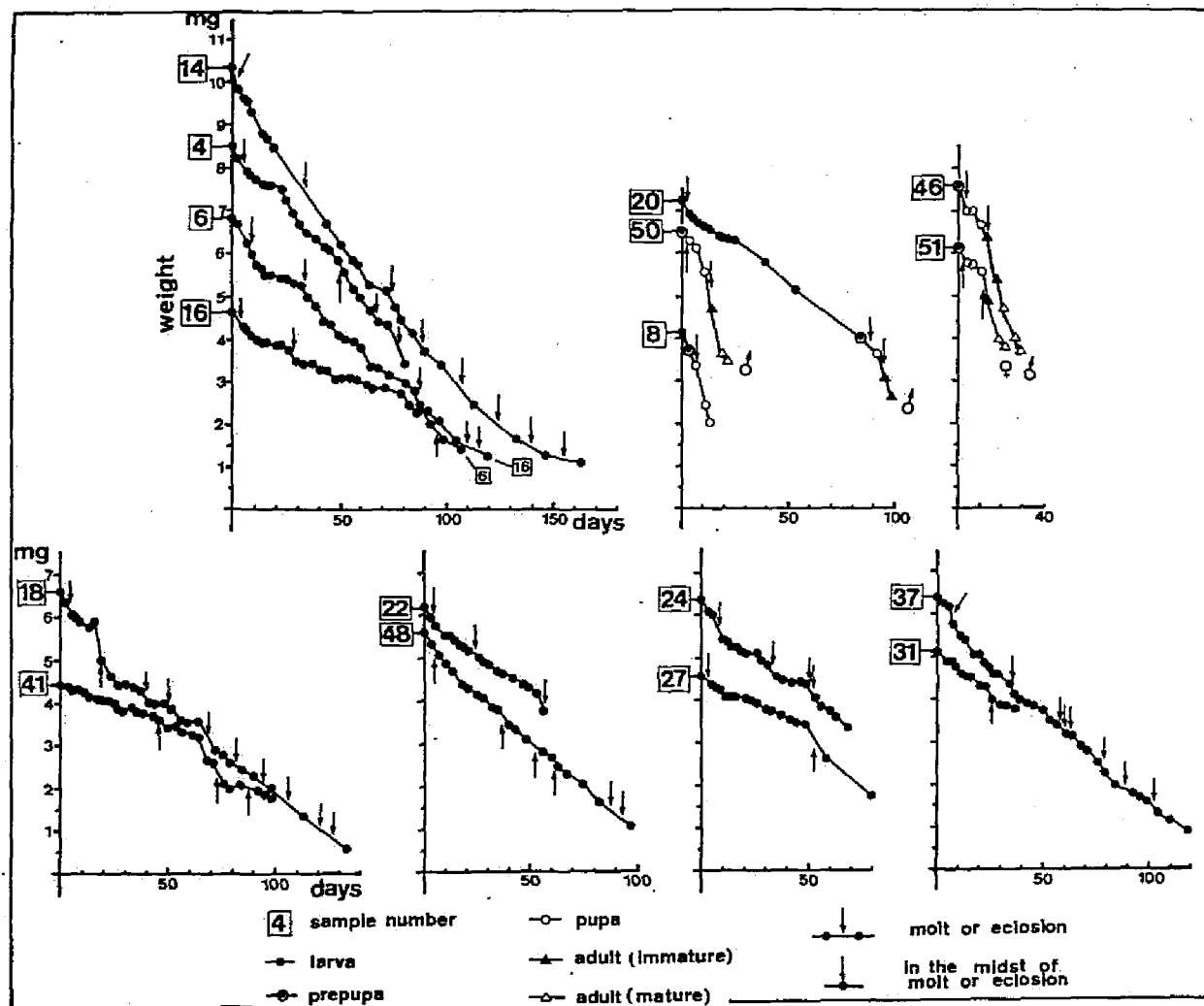
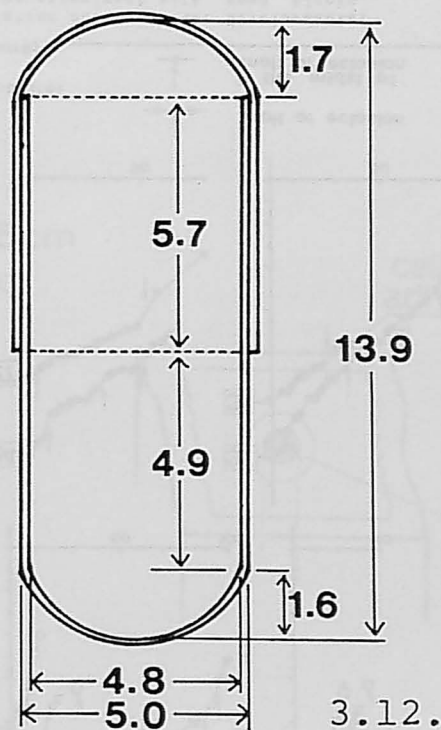
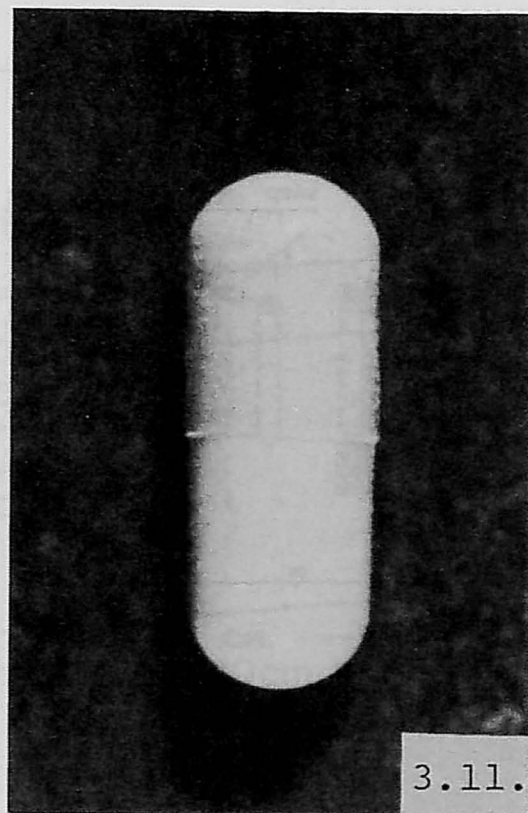


Fig. 3.10. Weight changes of larvae and the later developmental stages of *Lyctus brunneus* in the experiment with wood blocks. Abscissa represents the time in days after the introduction of the larvae.



Figs. 3.11.-3.12. Gelatin capsule used in the "individual rearing method".

3.11.: Photograph of a capsule in rearing situation, stuffed with buckwheat flour.

3.12.: Schematic diagram of a capsule with minimum volume of content, dimensions in mm.

in a Petri-dish, experiments with prepupae, as seen in the individuals Nos. 48, 50 and 51, yielded successful pupation with a steep decrease in weight in the course of the metamorphosis. The pupal duration, as seen in Fig. 3.10., was 10-12 days in the present condition.

3.2.3. Experiment with gelatin capsule and buckwheat flour^{F)}

The second part of this investigation following the test with wood blocks is an attempt with the "individual rearing method" using a gelatin capsule and buckwheat flour.

A gelatin capsule used as a container in the "individual rearing method" is shown in Figs. 3.11.-3.12.

Buckwheat (Fagopyrum) flour lot used was identical with that described in the section 2.3.1. The powder lot was passed through a No. 200 mesh (passing particles of less than 150 μ m diameter) and was sufficiently homogenized. About 0.19 g of flour could be stuffed in the capsule (ca. 0.21 cm³ in volume), thus resulting in ca. 0.90 g/cm³ of density of the powder.

Parent beetles were obtained from the mass culture with the standard artificial diet, as described in the sections 2.1.1. and 2.3.4. Young larvae were collected by means of Bletchly's^{b5)} "veneer technique"; several radial sections of 200 μ m thick veneers of red lauan (Shorea (Rubroshorea) spp.), 1.5 x 1.5 cm in size, were piled, with the grain of all veneers running in the same direction, and were stuck together by sandwiching them between two glass plates, followed by drying at 90°C for 24 hr. The pile of two or more square veneers were placed in Petri-

dishes onto which parent beetles were released to attack. In consideration of the developmental periods of eggs⁸²⁾ and 1st instar larvae⁸⁴⁾, the piles of veneers were opened up to collect the 1st instar larvae 12-13 days after the parent beetles were released. In the piles of veneers early 2nd instar larvae were also found at that time, resembling to the 1st instar larvae in size. They were excluded by distinguishing them from the 1st instar ones by the curled body shape, as shown in the section 3.2.1., and the absence of the "egg-tooth" on the 9th abdominal segment, as shown in the section 5.2.5. These 1st instar larvae, weighing less than 0.1 mg, were carefully removed with a specially devised pincette made of an animal hair, and a single larva was confined into each capsule stuffed densely with buckwheat flour.

Every capsule containing buckwheat flour and a single larva was mounted in a depression of a styro-foam piece in a mass culture chamber. It was occasionally opened up and its content was sieved through a No. 200 mesh. The remainder on the mesh, as well as the powder passing the mesh, was carefully examined under binoculars to detect all the larval exuviae, especially the exuvial mandibles. In several cases the smallest exuvial mandibles detected did not appear to be those of the molt from 1st to 2nd instar because they were not so minute as were in most cases. In such cases the exuviae from 1st to 2nd instar were regarded as lost.

At every inspection insects were weighed with the chemical balance, and all the buckwheat flour was renewed for the larvae.

When a larva had metamorphosed to be a prepupa or a pupa, no powder was supplied to it because of their ability to metamorphose without sustain, as shown in the section 3.2.2.

Of the 45 1st instar larvae employed, 20 individuals succeeded in pupation, 6 were victimized to SEM observation; as reported in the section 5.2., and other 19 individuals died in the form of larva; about half of the larvae reared with this method succeeded in pupation.

Table 3.1. summarizes the results with regard to the instar number and larval duration, where the larval duration was regarded as being as long as the period from the introduction of 1st instar larvae to the capsule up to the estimated pupation time. The pupation time was estimated from the information on the pupal duration, which had been estimated to be 10 - 12 days in the section 3.2.2.

Figure 3.13. presents the metamorphoses and weight changes in several examples. The number of larval instars between hatching and pupation generally ranged from 4 to 7 under the present condition, with an exception of the individual No. 118 having at least 11 instars. There seems to be a tendency of positive correlation between the instar number and the larval duration. Sexual difference of larval instar number was not clear insofar as the present results showed. In general, the variation of the larval instar number among individuals was observed.

3.2.4. Discussion^{F,H)}

From the results in Table 3.1. it may be safely said that most

Table 3.1. Numbers of the larvae of Lyctus brunneus that succeeded in pupation in the "individual rearing method" with various instar numbers and various larval durations (males / females).

Instar number	Larval duration				Total
	40-60 days	60-100 days	100-140 days	More than 140 days	
4	5 / 1	0 / 1	1 / 0	0 / 0	6 / 2
5	0 / 0	1 / 2	0 / 4	0 / 0	1 / 6
6	0 / 0	2 / 0	0 / 2	0 / 0	2 / 2
7	0 / 0	1 / 0	0 / 0	0 / 0	1 / 0
More than 8	0 / 0	0 / 0	0 / 0	0 / 0 ^a	0 / 0
Total	5 / 1	4 / 3	1 / 6	0 / 0	10 / 10

^{a)} The individual No. 118 in Fig. 3.13. (sex unknown) is possibly included here.

of L. brunneus larvae reared with the present method pass through 4-6 instars in the course of development. However, there was an exceptional case where the larva continued its growth up to the 11th instar as represented by the individual No. 118 in Fig. 3.13. Such an exception is not ascribed to the variations of environmental or nutritional factors because the larvae were kept under identical and constant conditions. Only hereditary or endocrinological factor would be its cause. Larval instar number tended to increase when the life cycle is prolonged, as is inferred from Table 3.1. The cause of this variation in life cycle duration is not known yet.

By general inference the larvae designated as "middle instar" in the sections 2.2.2., 3.2.1. and 5.2. are thought to correspond to the 3rd and 4th instar larvae if the final instar is 5th or 6th, or to the 3rd if the final is 4th.

Sexual difference in the larval instar number was not clearly observed in this species though Kreyenberg⁽¹⁹⁾ reported this in Dermestes in the order Coleoptera.

As seen in Fig. 3.10., if the larvae are placed in starving and/or exposed situation, they show "supernumerary molts" in response to the unfavorable conditions. Judging from the other results in Fig. 3.13., most of the larvae employed here (Fig. 3.10.) undoubtedly had developed into 4th or later instar(s) since they were heavier than 5mg at the beginning of the test. The larva with the greatest number (10) of molts (No. 18 in Fig. 3.10.) thus proved to have died at the time of at least the 14th instar. Supernumerary molts seem to occur more frequently and

irregularly toward the death of the larva, as often occur in many other insect species. In contrast, the individual No. 118 in Fig. 3.13. has shown that supernumerary molts may occur even under a nutritionally rich condition.

The larva of this species does not seem able to become the "prepupa", the final phase of the final instar larva, unless it is in "boring situation". On the other hand, prepupa can metamorphose very readily, as is seen in the cases with the individuals Nos. 46, 50 and 51 in Fig. 3.10. The "boring situation" or, in another term, the "tightness" seems prerequisite to "prepupation", i.e. the change into the prepupal form. Contrary to the case with this species, an anobiid, Lasioderma serricorne (Fabricius), belonging to Bostrychoidea as well, often makes a cocoon at pupation¹³⁾, which may ensure the "tightness" for the larva to pupate and the subsequent sound emergence of the adult. This seems more advanced manner of pupation in comparison with Lyctus.

There were two exceptional cases with successful pupation of the larvae: the individual No. 8 in Fig. 3.10., though not in the prepupal form, might be a full-grown larva in the course of "prepupation", and No. 20 seems a rare case where the larva happened to achieve the "boring situation".

As was assumed and verified in the sections 2.2.1. and 3.1.1., the body weight of this species throughout the whole life cycle has its maximum in the stage of the full-grown larva or the prepupa. Molting is a factor of decreasing the body weight in this species, as well as pupation, adult sclerotization, energy

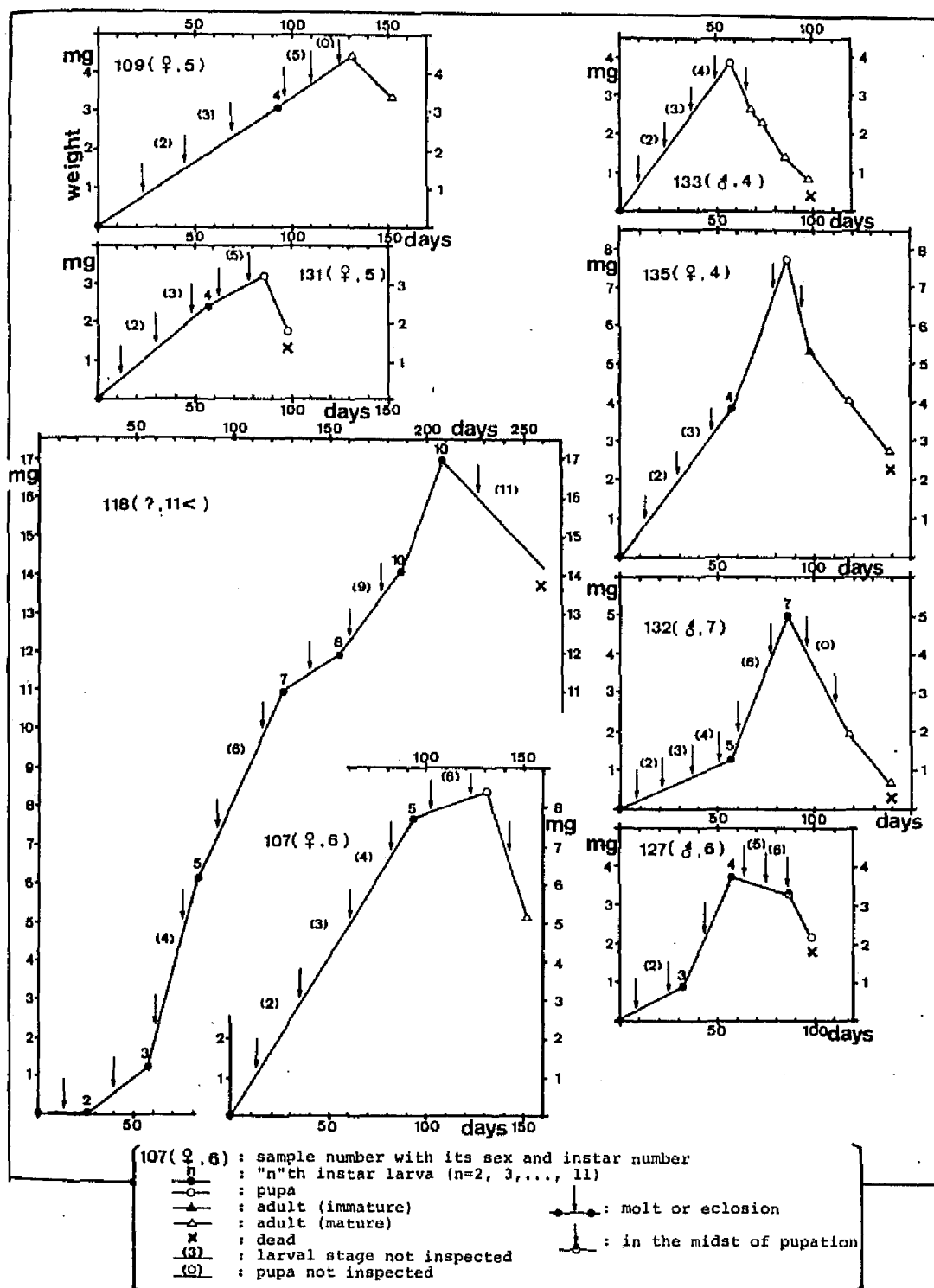


Fig. 3.13. Several examples of the weight changes and metamorphoses of *Lyctus brunneus* reared with a capsule and buckwheat flour ("individual rearing method"). Abscissa represents the time in days after the collection and introduction of the 1st instar larvae.

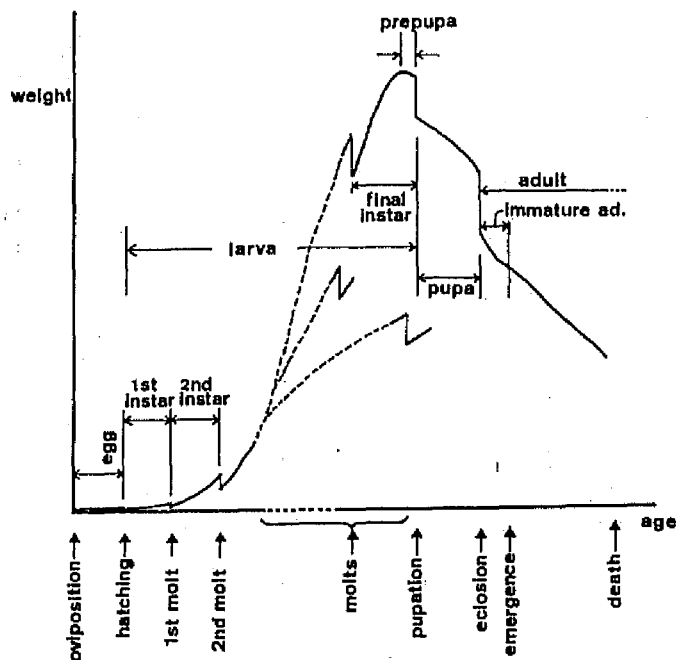


Fig. 3.14. Schematic diagram showing the weight change of *Lyctus brunneus* throughout the life cycle.

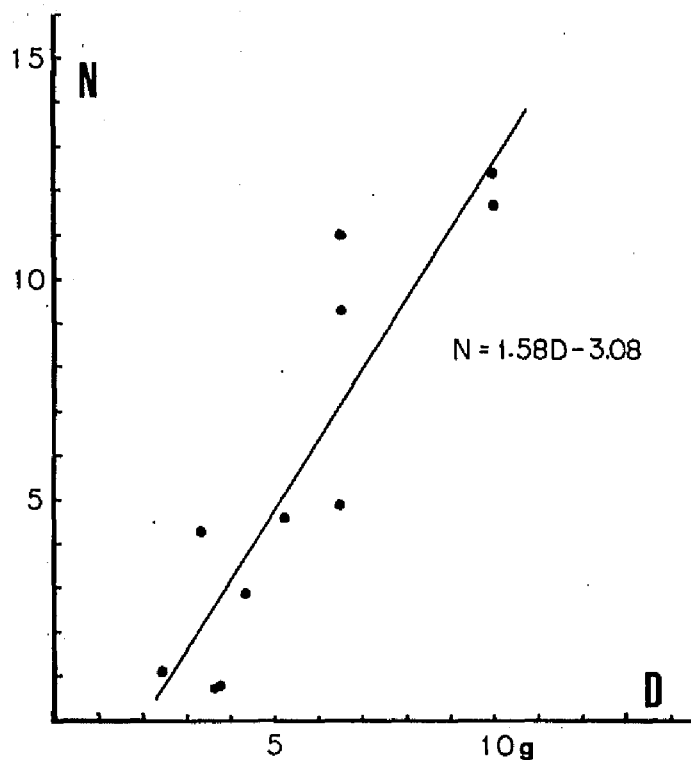


Fig. 3.15. Graph showing the density effect in the rearing of *Lyctus brunneus* with the diet of T6ky8 Univ. of Agric. Ordinate represents the progeny (F_1) number per one ovipositing female (N), while abscissa represents the weight of diet supplied per one ovipositing female (D) in gram.

consumption and genital activities. Results presented in Figs. 3.10. and 3.13. are compatible with these facts: larval molts cause weight decreases for a short time in the course of development, and the body weight always diminishes monotonously after the prepupal stage, as represented by the individuals Nos. 8, 20, 46, 50 and 51 (Fig. 3.10.).

From the information obtained here and in the section 3.1.1. the gross weight change of this species throughout its life cycle is summarized schematically in Fig. 3.14., where the broken curves mean wide variations of molt number and growth rate according to the nutritional and environmental conditions. Under the condition of lower temperature and/or lower R.H., the broken part of the curve may become less steep and longer, resulting in prolongation of life cycle duration^{g², k¹⁴)}. On the other hand, under the poorer nutritional condition, the curve may become less steep, longer and more variable in length with greater and more variable number of moltings taking place, resulting in prolongation of life cycle duration as well.

Information on the growth rate of this species, despite of an abundance of literature on the life history of this species, still seems insufficient. A trials was made to estimate the "developmental zero" temperature and the "effective accumulative temperature" of L. brunneus from the data given by Gay^{g²)} and Kühne^{k¹⁴)}. Only very rough estimations were obtained from them that the former is approximately 13°C, and the latter approximately 1000-1400 days·°K, with the latter possibly varying with varying R.H. and nutritional condition. Further investigations

are needed to confirm these values and their relations to other factors.

As was observed in other Coleoptera species^{k13,17)}, shifting of molt number may well occur in this species when the temperature changes though the shifting of molt number in the present data seems to be related only to the variable life cycle duration at the same temperature. The hereditary, physiological, ecological, physical and some other factors may also influence the larval growth and instar number to some extent.

3.3. Density effect in the artificial diet^{A,H)}

3.3.1. Materials and methods

The population density of progeny larvae of L. brunneus is thought to be in reciprocal proportion to the weight of diet supplied to one parent female adult supposing that average number of eggs deposited by a female is invariable with different proportion of diet quantity to female adult number. In the present investigation, as the parameter of the larval population density in the diet block, the diet quantity per one parent female beetle was adopted instead because the population density in the diet block is not easily assessed without breaking the block. The progeny yield number, as well as their body lengths, was thus investigated in relation to the weight of artificial diet supplied to beetles.

Investigations were conducted in the mass culture chamber, which is described in the section 2.1.1., with two series of experiments presented:

Series I comprised the 11 data extracted from the rearing results in the early phase of mass culture using the diet of Tôkyô Univ. of Agric., as is described in the section 2.1.3. As this kind of diet enabled the progeny beetles to be collected "by force" due to its brittle nature, all the newly emerged adult beetles (F_1 only) were collected thoroughly, where sound development of some immature individuals might be disturbed to some degree.

Series II was also carried out in the mass culture chamber, but differed from Series I in that the standard artificial diet, as developed in the section 2.3.4., was used. Parent beetles were also reared with the standard diet, among which males of more than 4.7 mm body length and females of more than 5.0 mm were selected, where the difference of 0.3 mm was based on the result in the section 2.3.2. Two subseries were included in Series II: Subseries IIa comprised 10 cultures, with ca. 116g of diet supplied to different number of parent beetles, ranging from 2 to 30 pairs; Subseries IIb also comprised 10 cultures, with different quantity of diet, weighing ca. 58 - 580 g, supplied to 10 pairs of parent beetles. In the cultures with less than two blocks of the standard diet, i.e. 110 - 120 g, Petri-dishes of 11.5 cm diameter were used, while in the cultures with more diet, other kinds of glass vessels were used.

All the progeny beetles (F_1 only), dead or alive, were collected at the time of culture closure, as defined in the section 2.3.1., and were sexed to separate males and females according to the sex characters not only on the ventral anal portion^(4, p1, p1, k15),

as shown in the section 5.5.3., but also on the mandibular pair^(4,62), as shown in the section 5.5.1. Their body lengths were measured using the same treating fluid, calipers and the equation as in the section 2.3.1.

3.3.2. Results and discussion

In Series I, the number of progeny (F_1 only) per one ovipositing female (N) was calculated and plotted with the data of the weight of diet supplied per one ovipositing female (D) (Fig. 3.15.). The correlation coefficient between these two values amounted to 0.90, showing a marked population density effect on the progeny yield. The relationship of N and D , given by the method of least squares is the following:

$$N = 1.58 D - 3.08$$

This equation indicates that if $N = 2$ then $D = 3.22$, suggesting that if less than 3.22 g of the diet of Tôkyô Univ. of Agric. is supplied per one ovipositing female beetle in the culture, normal population maintenance is not to be secured, with the diet blocks sometimes wholly collapsed and broken into pieces to yield a lot of larvae dropping out, as described in the section 2.1.4.

In Series II, the number of progeny (F_1 only) per one parent female beetle (male: N_m ; female: N_f ; total: N), sex ratio value (N_m/N_f), the average of body lengths (male: l_m ; female: l_f) and the total biomass per one parent beetle (B) were calculated. For the total biomass value (B), the following equation was utilized according to the equations presented in the section

3.1.2.:

$$B = B_m + B_f$$

$$= \{N_m \times 0.022\text{mg} \times (\ell_m / 1\text{mm})^{3.2}\} + \{N_f \times 0.031\text{mg} \times (\ell_f / 1\text{mm})^{3.0}\}$$

These data were plotted with those of the weight of diet supplied per one parent female beetle (D). The results are shown in Figs. 3.16. ($N_m - D$), 3.17. ($N_f - D$), 3.18. ($N - D$), 3.19. ($N_m/N_f - D$), 3.20. ($\ell_m - D$), 3.21. ($\ell_f - D$) and 3.22. ($B - D$), with the correlation coefficients of them summarized in Table 3.2.

The general relationship between the animal population densities of the parent generation (n_i) and of the F_1 progeny generation (n_{i+1}) has been expressed by the following equation⁽⁴⁾:

$$n_{i+1} = n_i [1 / \{ g + (1-g)n_i/K \} - \sigma] ,$$

where g is a species-specific constant relating the reproductive rate ($0 \leq g \leq 1$), σ is also a species-specific constant relating the degree of generation overlapping ($\sigma = 1$ in L. brunneus as there is no overlapping), and $K = \lim_{i \rightarrow \infty} n_i$.

Then, the factors, D and N , which are dealt with in the present study are expressed as follows:

$$D = 2 / n_i ,$$

$$N = 2 (n_{i+1} / n_i) .$$

Thus the above equation is transformed by these as:

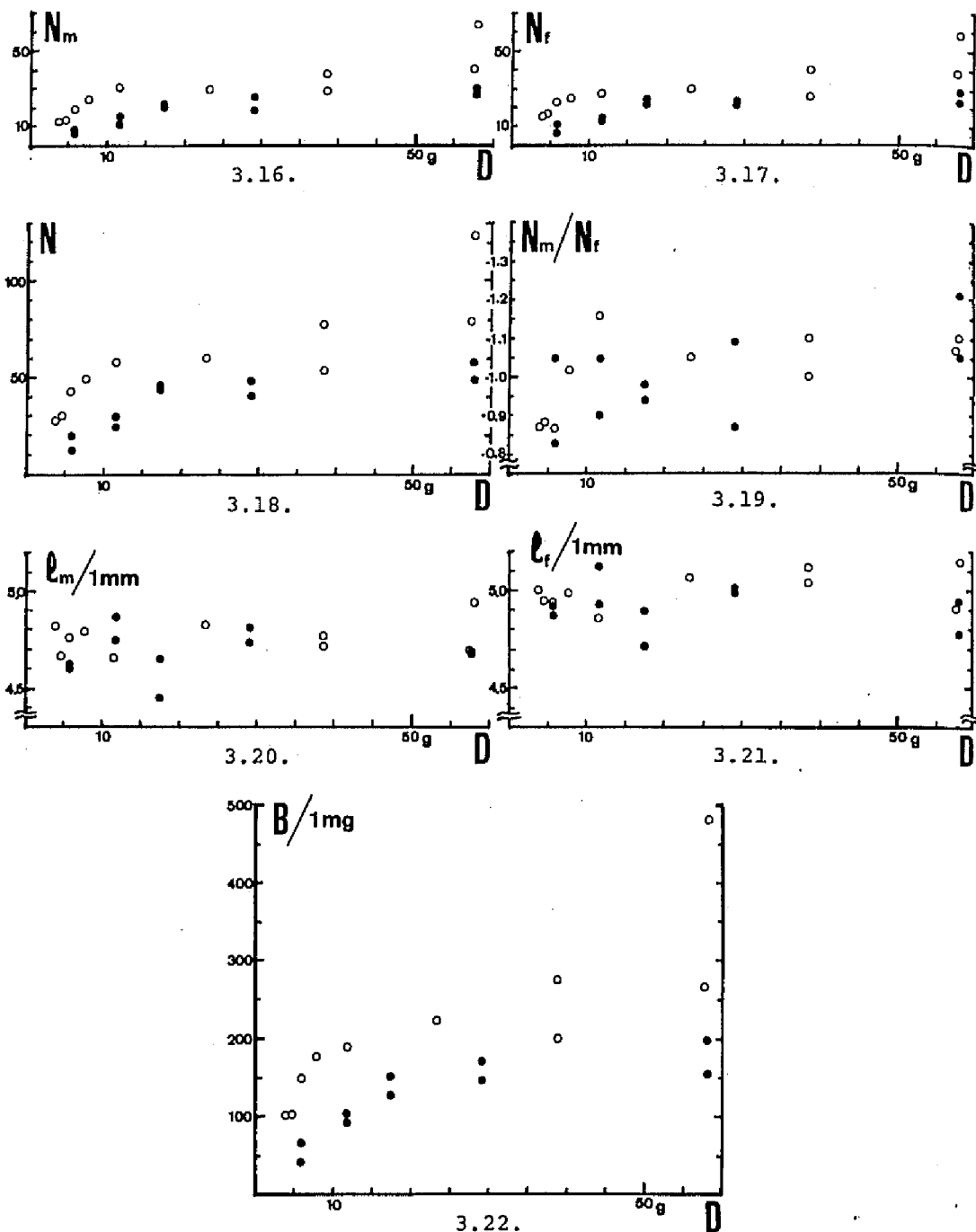
$$N / 2 = 1 / \{ g + 2(1-g)/KD \} - 1 .$$

This leads to the following relation between N and D :

$$\{ N - 2(1/g - 1) \} \{ D + 2(1/g - 1)/K \} = - 4(1-g) / Kg^2 ,$$

where $2(1/g - 1) \geq 0$, $2(1/g - 1)/K \geq 0$, $4(1-g) / Kg^2 \geq 0$.

The result presented in Fig. 3.18. seems to agree very well



Figs. 3.16.-3.22. Relations between the weight of diet supplied per one parent female beetle of *L. brunneus* (D) and below-mentioned parameters of her F_1 progeny in the rearing with the standard artificial diet.

(open circles: Subseries IIa; closed circles: Subseries IIb)

3.16.: Number of male progeny (N_m).

3.17.: Number of female progeny (N_f).

3.18.: Number of progeny ($N = N_m + N_f$).

3.19.: Sex ratio value (N_m/N_f).

3.20.: Average of males' body length ($\ell_m/1mm$).

3.21.: Average of females' body length ($\ell_f/1mm$).

3.22.: Total biomass ($B/1mg$).

Table 3.2. Correlation coefficients between the weight of diet supplied per one parent female beetle (D) and some parameters of her F_1 progeny in the rearing of L. brunneus with the standard diet.

Parameter	Correlation coefficient		
	Subseries IIa	Subseries IIb	IIa+IIb
Number of progeny males (N_m)	0.857	0.852	0.741
per one parent females (N_f)	0.854	0.760	0.704
female beetle total ($N = N_m + N_f$)	0.857	0.817	0.726
Sex ratio value (N_m / N_f)	0.577	0.596	0.582
Average of males ($l_m / 1mm$)	0.293	0.096	0.173
body length females ($l_f / 1mm$)	0.450	-0.189	0.106
Total biomass per one parent female beetle ($B / 1mg$)	0.835	0.817	0.694

with this formula. Although the value N has its upper limit with increased diet quantity (D) ($\lim_{D \rightarrow \infty} N = 2(1/g - 1)$), and thus Figs. 3.16.-3.18. and 3.22. are not expected to show linear relation, the correlation coefficients (Table 3.2.) may indicate their positive correlation: increased population density affected the progeny yield. Such a result seems very orthodox as seen in many insect species, among which Callosobruchus chinensis (L.) (Bruchidae) is involved in a classic report by Utida¹¹⁾. It cannot be safely said that the sex ratio value was increased by the increased diet quantity though there seems a slight tendency of increasing (Fig. 3.19.). Contrary to the result with the bruchid by Utida¹¹⁾, Table 3.2. also shows that the body length, and consequently the body weight as well, of both sexes was not affected at all by the increased diet quantity (Figs. 3.20.-3.21.), but, on the contrary, the total biomass was affected by it (Fig. 3.22.).

In the correlation coefficients with positive higher values ($N_m - D$, $N_f - D$, $N - D$ and $B - D$), the value of Subseries IIa always exceeded that of Subseries IIb, followed by that of the two subseries calculated together. This seems primarily due to the difference of age of parent beetles between the two subseries, as was shown in the section 2.1.2., and secondarily due to the fact that Subseries IIb was arranged so that the different quantity of diet was supplied to equal number of parent beetles, where there came to be a variation of "mean crowding" ($\frac{*}{m}$)^{16, 110)}. Further investigation is needed in which the progeny yield result of each diet block, as a definite "quadrat", is presented to give

the value and effects of mean crowding.

The value N is related to the number of eggs laid by one female beetle. Gay^{g2)} reported the numbers 221, 0 and 78 as its maximum, minimum and average respectively, and Ito & Hiroseⁱ⁵⁾ reported 117 and 0 as its maximum and minimum, while Parkin^{p1)} reported 70 as an average of the total number of eggs producible by a L. brunneus female. It is not all of them that contribute to the progeny yield. Emerging progeny per one female beetle has been reported to amount to 20 - 40 by Harris & Taylor^{h1)} or approximately 20 by Parkin^{p1)}, both being from the rearing with sapwood. Further, as much as 110 beetles were recorded by the author as the maximum progeny yield from one female beetle with the diet of Tôkyô Univ. of Agric. In general, however, the number of eggs laid, or conceived, by one female insect is thought to be greatly dependent upon her body weight, and consequently upon her body length. Further, the ratio of the number of progeny emergence to the initial number of eggs laid, or conceived, is to be greatly reduced from 100% to a smaller value due to the function of environmental resistances. Therefore it is not proper to confuse the number of progeny emergence with the number of eggs laid (or conceived), as did Ijima et al.ⁱ¹⁾.

Present investigation has shown that when the more diet is supplied to the parent beetles, the more progeny is yielded. Possible decrease of progeny yield due to the oversupply of diet, which prevents the parent beetles from coupling by undercrowding, has not been detected here.

For the practical mass culture of this species, about 10 - 15g of the standard diet supply may be appropriate for one new parent female beetle, namely about 8 - 12 pairs, or 20 - 30 unsexed adult beetles, should be released to one culture which contains 110 - 120 g of the standard artificial diet blocks, while 40 or more adult beetles are recommended to release to one culture if they are not new as the productivity may be somewhat reduced, as was shown in the section 2.1.2.

3.4. Adult sex ratio^{A,H)}

Adult sex ratio seems one of the most complicated problem in the life history and ecology of this species.

The sex ratio value, as expressed by males' number / females' number, was determined on a L. brunneus population naturally bred on a lauan non-structural building timber in Suita, Ôsaka Prefecture, Japan, in 1979, to be 0.90 with 73♂♂ and 81♀♀.

On the other hand, sex ratio value may be fluctuated considerably especially with comparatively small number of individuals examined. Table 3.3. shows the transition of the sex ratio value in the early phase of mass culture using the diet of Tôkyô Univ. of Agric., with the beetle numbers in Fig. 2.4. for the section 2.1.3. summed up for each 4 weeks during the period from the beginning of rearing up to the point when the sexing of the beetles was abolished. There, in the first half of data females were observed to have been outnumbering considerably, as Altson⁸⁵⁾ once stated.

This example (Table 3.3.), at first, led to a conclusion that

Table 3.3. Transition of the sex ratio value in the early phase of L. brunneus mass culture using the diet of Tôkyô Univ. of Agric.

Period	Number of males (n_m)	Number of females (n_f)	Sex ratio value (n_m/n_f)
15/Oct./1978 - 6/Jan./1979 (12 weeks)	7	19	0.37
7/Jan./1979 - 3/Feb.	41	144	0.29
4/Feb. - 3/Mar.	35	95	0.37
4/Mar. - 31/Mar.	72	81	0.89
1/Apr. - 28/Apr.	517	534	0.97
29/Apr. - 26/May	503	544	0.92
27/May - 23/June	231	237	0.97
Total	1406	1654	0.85

the sex ratio value in the early days of rearing might be remarkably low in general. At present, however, this result, as well as Altson's statement²⁵⁾, is believed to be attributed to a fluctuation of value especially in the case with comparatively small number of individuals examined.

Thirdly, the result of the diet suitability test, as shown in the section 2.3.2. and in Table 2.6., shows that sex ratio values of the progenies with 17 kinds of artificial diets ranged between 0.78 and 1.10, and the total average, calculated from 777536 and 806899 as a whole, was 0.96.

Fourthly, the result of the density effect investigation, as shown in the section 3.3.2. and in Fig. 3.19., shows that there seems to be little or a very slight tendency of increase of the sex ratio value with increased diet quantity: the sex ratio value increases very little or slightly in the progeny with decreased population density of their larval stage. There, the minimum value was 0.83 in the culture with 5.81 g of diet per one parent female, and the maximum was 1.21 in the culture with 57.9 g of diet per one parent female.

In the last place, the mode of emergence in the rearing with the standard artificial diet under mass culture condition, as described in the section 2.1.1., was observed for the first 3 weeks of emergence to know which has the priority, male or female, or how the sex ratio value changes. The result, as shown in Table 3.4., suggests that there seems to be a tendency of "protandry". The cumulative sex ratio value, at first, tends to exceed 1.00 greatly, but converges to 1.00 in the end.

Table 3.4. Record of emergence and the transition of sex ratio value of the progeny beetles of *L. brunneus* in the mass culture using the standard diet. Records of 6 cultures are given in each 4 days for the first 3 weeks of emergence, and then the final records are given for the whole F₁ generation (males' number / females' number). Cumulative sex ratio values are also given in the parentheses.

Period	Cultures ^a						Total
	1	2	3	4	5	6	
18 - 19/Dec./1985	1/ 1 (1.00)	5/ 3 (1.67)	1/ 1 (1.00)	1/ 1 (1.00)	1/ 1 (1.00)	1/ 0 (--)	10/ 7 (1.43)
20 - 23/Dec.	0/ 0 (1.00)	2/ 0 (2.33)	4/ 3 (1.25)	1/ 0 (2.00)	1/ 0 (2.00)	4/ 3 (1.67)	12/ 6 (1.69)
24 - 27/Dec.	19/ 2 (6.67)	10/ 4 (2.43)	19/ 2 (4.00)	4/ 1 (3.00)	22/ 6 (3.43)	32/ 19 (1.68)	106/ 34 (2.72)
28 - 31/Dec.	41/ 30 (1.85)	43/ 17 (2.50)	27/ 22 (1.82)	35/ 12 (2.93)	28/ 18 (2.08)	22/ 12 (1.74)	196/ 111 (2.05)
1 - 4/Jan./1986	36/ 42 (1.29)	39/ 40 (1.55)	49/ 28 (1.79)	27/ 31 (1.51)	30/ 16 (2.00)	26/ 20 (1.57)	207/ 177 (1.59)
5 - 8/Jan.	41/ 23 (1.41)	30/ 50 (1.13)	36/ 64 (1.13)	22/ 20 (1.38)	27/ 40 (1.35)	33/ 30 (1.40)	189/ 227 (1.28)
9/Jan. - 4/Mar. (55 days)	111/161 (0.96)	106/112 (1.04)	96/116 (0.98)	79/128 (0.88)	98/ 90 (1.21)	81/109 (1.03)	571/ 716 (1.01)
Total	249/259	235/226	232/236	169/193	207/171	199/193	1291/1278

^a) Cultures were started on 1/Oct./1985.

As is known from Table 1.2., the statement that females outnumbered males was given only by Altson³⁵⁾, while different or contrary statements were given by Parkin¹⁾ and Ito & Hirose¹⁷⁾.

Kurir¹⁸⁾ reported an example of "protogyny", a mode of adult population's emergence where females emerge statistically earlier than males, in L. planicollis. Parkin¹⁴⁾, on the other hand, suggested the contrary case, as designated as "protandry", in L. brunneus. Some coleopterous species of the other families, such as Cerambycidae, are known to exhibit the protandry in the wild condition.

The last of the present examples (Table 3.4.) suggests that the protandry is very likely to be the case with the rearing of L. brunneus in the mass culture condition.

The final cumulative sex ratio value of a generation of L. brunneus may be regarded as nearly 1.00 though there might be some physical (such as temperature), biological (such as natural enemies) and hereditary factors that influence the value of sex ratio directly or indirectly, which thus might vary to some extent with a definite center value 1.00.

3.5. Adult longevity^{H)}

3.5.1. Materials and methods

Adult longevity of L. brunneus needs to be studied further from the viewpoint of bioassay methodology. In this investigation the following three factors were examined whether they have any relations to the adult longevity.

I) Body length (ℓ)

Ia) $3.5\text{mm} \leq l < 4.0\text{mm}$

Ib) $4.0\text{mm} \leq l < 4.5\text{mm}$

Ic) $4.5\text{mm} \leq l < 5.0\text{mm}$

II) Sex (gender)

IIa) male

IIb) female

III) Insecticide treatment of filter paper disks

IIIa) untreated

IIIb) treated with chlordane 0.5% alcoholic solution

IIIc) treated with chlordane 5% alcoholic solution

IIId) treated with fenitrothion 0.05% alcoholic solution

IIIe) treated with fenitrothion 0.5% alcoholic solution

(chlordane: 1,2,4,5,6,7,8,8a-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane; fenitrothion (= Sumithion): dimethyl 4-nitro-m-tolyl phosphorothionate)

In regard to the tests with these chemicals (IIIb-IIIe), the resistance of adult beetles could be regarded as equivalent to the longevity in the untreated level (IIIa). These two chemicals were adopted in the present test as they have already been investigated and compared for the effects on L. brunneus adults by Ito & Hirose^{16, 18)} to yield results showing that fenitrothion had an acute action as a contact insecticide compared to chlordane; the present test was based upon their results.

Disks of filter paper, No. 40 "ashless" of Whatman Ltd., 9.0 cm diametered, 0.2 mm thick, weighing 0.6 g, were dipped into above chemical solutions for 20 sec, then air-dried. No treatment was done for "untreated" filter paper.

Newly emerged adult beetles were supplied from the mass culture using the diet of Tôkyô Univ. of Agric., as described in the sections 2.1.1. and 2.1.3. All the collected beetles were sexed according to the sex character on the ventral anal portion^(4, p1, r1), as shown in the section 5.5.3., and measured with calipers to categorize them into 3 degrees of body length range.

Each of the treated or untreated filter paper disks was placed in a Petri-dish of the same diameter, onto which 10 beetles, either males or females of approximately the same size, were released, and were confined in a space restricted by a glass cylinder, 3.6 cm diametered, consequently involving 10.2 cm² area, and 4.0 cm high, the top of which was closed with a 4cm x 4cm glass plate. Three replications were prepared for each combination of the 3 factors. All the Petri-dish were kept in the mass culture chamber, as described in the section 2.1.1. A view of this test is shown in Fig. 3.23.

The beetles were observed after 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12hr, and then after 1, 2, 4, 8,... days to record their conditions in 6 succeeding states (Table 3.5.), which have been originally proposed by Sato & Suwanai⁽⁸⁴⁾ for the insecticidal assay with larvae of noctuid moth, with the definitions of the states partly modified for the present species, L. brunneus.

3.5.2. Results and discussion

From the data obtained, the times when 5 beetles out of 10 came to be found in the states I, II, III, IV and V (Table 3.5.) ($T_{I,50}$, $T_{II,50}$, $T_{III,50}$, $T_{IV,50}$ and $T_{V,50}$, respectively) were read in

each replication of each experimental combination. Then, for the convenience of reducing the variances among replications, all the values ($T_{I,50}$, $T_{II,50}$, ...) were transformed into $\log_{10}(T_{I,50}/1hr)$, $\log_{10}(T_{II,50}/1hr)$, etc. The logarithmic values of all the data were shown in Tables 3.6.-3.10., respectively.

Among the data in these 5 tables the data in the last one (Table 3.10.) was submitted to the analysis of variance as the original value of this ($T_{V,50}$) corresponds to a well-known parameter "LT₅₀". Though a certain relation had been known between the body length and the sex, as shown in the section 3.1.2., the interaction between these two factors was disregarded in the present statistic analysis as they were artificially separated when the adult beetles were supplied to the test. The result of analysis is shown in Table 3.11.

Apart from a matter of course that there was a very highly significant variance among the treatments, with fenitrothion having acute action compared to chlordane, this table indicates that the body length was also a significant factor of the adult longevity or the resistance to chemicals in a 0.1% risk as well. This leads to a conclusion that a larger-sized adult beetle of this species is more resistant to chemicals and/or is more longevous, as is the case with most insects and animals in general. The sex, however, was not shown by this table to be a significant factor of longevity or resistance even in a 5% risk.

By way of caution, further analyses of variance were made within each level of body length and treatment with the data presented in Table 3.10. to detect the sexual difference. Out

Table 3.5. Six succeeding states of adults in the adult longevity test of L. brunneus (after Sato & Suwanai⁵⁴) ; partly modified).

Code	Definition	Time period required until 50% of the test beetles came to be found in the state
0	Completely normal in appearance	--
I	Slightly abnormal in walking	T _{I,50}
II	Disordered in walking	T _{II,50}
III	Knocked down, writhing	T _{III,50}
IV	Knocked down, only showing reaction to a stimulus	T _{IV,50}
V	Dead, showing no reaction to a stimulus	T _{V,50} (LT ₅₀)

Table 3.6. Logarithmic values of T_{I,50}/1hr (see Table 3.5.) in the adult longevity test of L. brunneus.

Body length	3.5 - 4.0 mm		4.0 - 4.5 mm		4.5 - 5.0 mm	
Sex	♂	♀	♂	♀	♂	♀
Untreated	2.3	2.3	2.3	2.6	2.6	2.6
	2.2	2.3	2.2	2.5	2.3	2.5
	2.2	1.7	2.3	2.5	2.5	2.5
Chlordane 0.5%	1.4	1.4	1.4	1.4	1.7	1.7
	0.9	0.9	1.4	0.9	1.4	1.4
	1.4	1.4	1.4	1.4	1.4	1.4
Chlordane 5%	0.6	0.6	0.6	0.6	0.6	0.9
	0.6	0.6	0.6	0.6	0.9	0.9
	0.8	0.6	0.8	0.8	0.8	0.9
Fenitrothion 0.05%	0.6	0.6	0.6	0.9	0.6	0.9
	0.6	0.9	0.6	0.6	0.6	1.4
	0.8	0.8	1.1	0.9	1.1	1.1
Fenitrothion 0.5%	0.2	0.2	0.0	0.2	0.0	0.6
	0.2	0.2	0.2	0.2	0.2	0.3
	0.3	0.3	0.5	0.3	0.5	0.8

Table 3.7. Logarithmic values of $T_{m,50}$ /1hr (see Table 3.5.) in the adult longevity test of *L. brunneus*.

Body length Sex	3.5 - 4.0 mm		4.0 - 4.5 mm		4.5 - 5.0 mm	
	♂	♀	♂	♀	♂	♀
Untreated	2.3	2.6	2.3	2.6	2.6	2.6
	2.2	2.3	2.3	2.5	2.5	2.6
	2.2	2.2	2.3	2.5	2.5	2.5
Chlordane 0.5%	1.4	1.4	1.4	1.7	1.7	1.7
	1.4	1.4	1.4	1.4	1.4	1.7
	1.4	1.4	1.7	1.4	1.4	1.4
Chlordane 5%	0.6	0.6	0.6	0.9	0.9	0.9
	0.6	0.9	0.9	0.9	0.9	0.9
	0.8	0.8	0.8	0.8	0.9	1.4
Fenitrothion 0.05%	0.6	0.6	0.9	0.9	0.6	0.9
	0.6	0.9	0.6	0.9	0.6	1.4
	0.8	0.8	1.4	0.9	1.4	1.4
Fenitrothion 0.5%	0.2	0.2	0.0	0.2	0.2	0.6
	0.2	0.2	0.2	0.3	0.2	0.3
	0.3	0.3	0.5	0.5	0.5	0.8

Table 3.8. Logarithmic values of $T_{m,50}$ /1hr (see Table 3.5.) in the adult longevity test of *L. brunneus*.

Body length Sex	3.5 - 4.0 mm		4.0 - 4.5 mm		4.5 - 5.0 mm	
	♂	♀	♂	♀	♂	♀
Untreated	2.3	2.6	2.3	2.6	2.6	2.6
	2.2	2.5	2.3	2.5	2.5	2.6
	2.3	2.2	2.5	2.5	2.5	2.5
Chlordane 0.5%	1.7	1.4	1.7	1.9	1.9	1.9
	1.4	1.4	1.4	1.4	1.7	1.7
	1.4	1.4	1.7	1.4	1.4	1.4
Chlordane 5%	0.9	0.6	0.6	0.9	0.9	1.4
	0.9	0.9	0.9	0.9	1.4	1.4
	0.8	0.8	0.9	0.9	0.9	1.4
Fenitrothion 0.05%	0.9	0.6	0.9	0.9	0.9	0.9
	0.6	0.9	0.9	0.9	0.6	1.4
	0.9	0.8	1.4	0.9	1.4	1.4
Fenitrothion 0.5%	0.2	0.2	0.0	0.3	0.2	0.6
	0.2	0.2	0.2	0.3	0.3	0.6
	0.3	0.5	0.5	0.5	0.6	0.8

Table 3.9. Logarithmic values of $T_{V,50}$ /1hr (see Table 3.5.) in the adult longevity test of L. brunneus.

Body length Sex	3.5 - 4.0 mm		4.0 - 4.5 mm		4.5 - 5.0 mm	
	♂	♀	♂	♀	♂	♀
Untreated	2.3	2.6	2.3	2.6	2.6	2.6
	2.2	2.5	2.3	2.5	2.5	2.6
	2.3	2.2	2.5	2.6	2.5	2.6
Chlordane 0.5%	1.7	1.7	1.7	2.0	2.0	2.0
	2.0	1.7	1.7	1.7	2.0	2.0
	1.7	1.7	2.0	1.7	2.0	2.0
Chlordane 5%	1.7	1.4	1.4	1.7	1.7	1.7
	1.4	1.4	1.7	1.7	1.7	1.7
	1.4	1.4	1.7	1.7	1.7	1.7
Fenitrothion 0.05%	0.9	0.9	0.9	0.9	0.9	0.9
	0.9	0.9	0.9	0.9	0.6	1.4
	0.9	0.9	1.4	1.4	1.4	1.4
Fenitrothion 0.5%	0.3	0.6	0.2	0.3	0.3	0.6
	0.2	0.3	0.3	0.3	0.3	0.6
	0.5	0.5	0.6	0.5	0.6	0.8

Table 3.10. Logarithmic values of $T_{V,50}$ /1hr (see Table 3.5.) in the adult longevity test of L. brunneus.

Body length Sex	3.5 - 4.0 mm		4.0 - 4.5 mm		4.5 - 5.0 mm	
	♂	♀	♂	♀	♂	♀
Untreated	2.3	2.6	2.3	2.6	2.6	2.6
	2.2	2.5	2.5	2.5	2.5	2.6
	2.3	2.2	2.5	2.6	2.5	2.6
Chlordane 0.5%	1.7	1.7	1.7	2.3	2.0	2.0
	2.0	1.7	2.0	1.7	2.0	2.0
	1.7	1.7	2.0	1.7	2.0	2.0
Chlordane 5%	1.7	1.7	1.4	1.7	1.7	1.9
	1.7	1.7	1.7	1.7	2.0	2.0
	1.7	1.7	2.0	1.7	2.0	2.0
Fenitrothion 0.05%	0.9	0.9	0.9	0.9	0.9	1.4
	0.9	0.9	0.9	0.9	0.9	1.4
	1.1	1.1	1.7	1.4	1.7	2.0
Fenitrothion 0.5%	0.6	0.6	0.2	0.6	0.6	0.6
	0.3	0.3	0.3	0.3	0.3	0.6
	0.9	1.1	0.9	0.9	1.1	2.0

Table 3.11. Analysis of variance of the value $\log_{10}(T_{V.50} / \text{1hr})$ shown in Table 3.10.

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio (F)
Treatment	34.57	4	8.64	123.4 ^a
Body length	1.18	2	0.59	8.4 ^b
Sex (gender)	0.16	1	0.16	2.3 ^c
Error	5.43	82	0.07	
Total	41.34	89		

- a) $F_{(4,82)(0.1\%)} < 5.31 < 123.4 = F_{\text{treatment}}$
b) $F_{(2,82)(0.1\%)} < 7.77 < 8.4 = F_{\text{body length}}$
c) $F_{(1,82)(5\%)} > 3.92 > 2.3 = F_{\text{sex}}$

Table 3.12. Analysis of variance of the value $\log_{10}(T_{V.50} / \text{1hr})$ for the "untreated" in Table 3.10.

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio (F)
Body length	0.1477	2	0.0739	7.32 ^a
Sex (gender)	0.0671	1	0.0671	6.64 ^b
Error	0.1412	14	0.0101	
Total	0.356	17		

- a) $F_{(2,14)(1\%)} = 6.51 < 7.32 = F_{\text{body length}}$
b) $F_{(1,14)(5\%)} = 4.60 < 6.64 = F_{\text{sex}}$

of 8 analyses of variance carried out, i.e. those within each of the 3 levels of body length (Ia-Ic), followed by those within each of the 5 levels of treatment (IIIa-IIIe), only the analysis with the data of the "untreated" (IIIa) (Table 3.12.) demonstrated the significant difference between the sexes in only a 5% risk, showing a statistic superiority of females in the "longevity" as distinguished from the "resistance to chemicals". It is somewhat noteworthy in Table 3.12. that the difference among the body length levels was significant in only a 1% risk, while in Table 3.11. it was in a 0.1% risk.

The present test has shown that in the practical tests with chemicals to be contacted to the adults of this species, the body weight, or the body length, of the beetles supplied may not be varied too much so as to reduce the variance of LT_{50} value, and also females and males must be supplied separately at least to the untreated part of the test.

At the closure of each test with female beetles, as compared to males, the filter paper was observed to have been more heavily damaged on its surface by gnawing. Such a behavior has been described and studied by Fisher (et al.)⁽²⁾, Parkin⁽²⁾, Cymorek & Schmidt⁽¹⁵⁾, Ito & Hirose⁽⁵⁾, Ito⁽⁴⁾ and others, with a conclusion that females of this species gnaw wood more heavily than males, but they never feed on wood particles they have gnawed off. Further study may be needed whether adult longevity is influenced or not by this behavior followed by a possible feeding in due consideration of the observation described in the section 3.6.2.

The values of adult longevity of this species have been re-

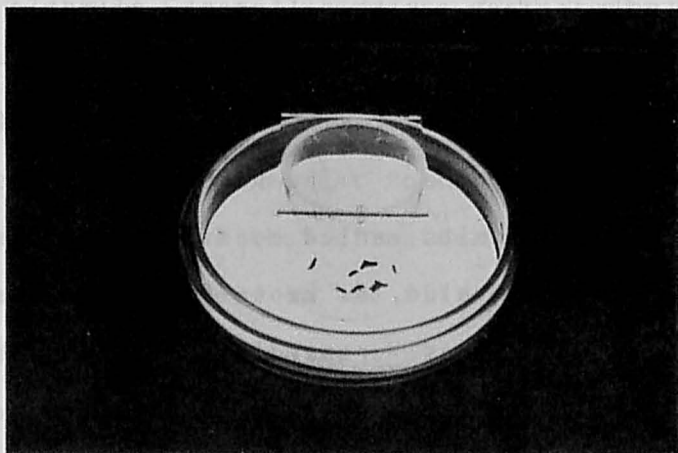


Fig. 3.23. A view of adult longevity test. Ten adult beetles, either males or females of the same body length range, were confined in a spece made of a filter paper disk, treated or untreated, at bottom, a glass cylinder and a glass plate at top.

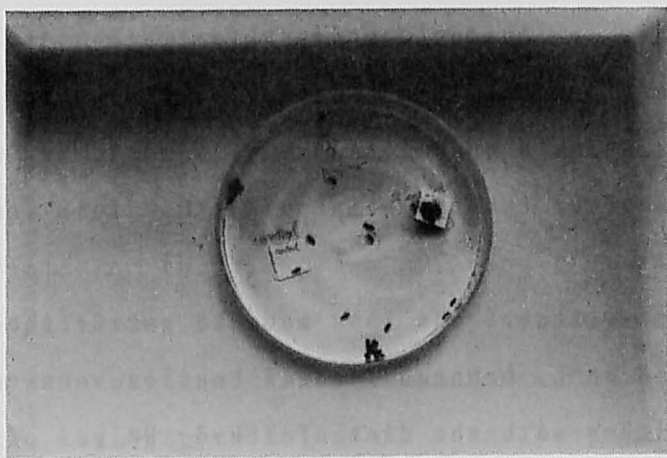


Fig. 3.24. A view of the bioassay with L. brunneus adults on the attractiveness of filter paper square fragments (1.5 x 1.5 cm) exposed to males or females.

ported repeatedly^{a5, f2, p1, g2)} with different climatic conditions. The information for this value is summarized as: 1-58 days in males and 12-84 days in females with a tendency of decreasing with increased temperature. The present test also has shown that the longevity of adults varied considerably, namely 1 day to 1 month, and the median value, as known directly from $T_{v,50}$ value, was 6-16 days in both sexes, regardless of the number of days from the emergence up to the supply to the test.

3.6. Ethological peculiarities of adults

3.6.1. Aggregating behavior^{H)}

Although some of the bostrychids^{k4)} and anobiids^{c5, k20)} have been reported to possess their own sex or aggregation pheromones, no example of such pheromones has been presented in the related family Lyctidae. In due consideration of the ecology and life history of these 3 "Teredilia" families, lyctid species seem very likely to possess such pheromones, which could be, if any, of great interest from the viewpoint of methodology for control and occurrence survey.

A preliminary attempt was thus made to verify the existence of such pheromones in L. brunneus: adult beetles were collected from the mass culture with the diet of Tôkyô Univ. of Agric., as described in the sections 2.1.1. and 2.1.3., and were sexed to separate males and females in one Petri-dish and another. A sheet of filter paper was cut into a number of 1.5 x 1.5 cm square fragments, a few of which were put into the Petri-dish containing 100-150 adults of each of the sexes. Each Petri-dish

was placed inclined for ca. 4 days to contact the beetles to the filter paper square fragments.

Male-exposed and female-exposed filter paper square fragments were supplied to the choice test to compare their attractiveness with each other or with that of untreated filter paper square fragments: they were placed on the filter-paper-glued bottom of a 11.5 cm-diametered Petri-dish so that one edge of the Petri-dish, the center of one paper fragment, the center of the Petri-dish, the center of another paper fragment and the opposite edge of the Petri-dish were ranged linear at almost regular intervals. The area ratio of one paper fragment to the bottom of Petri-dish was calculated to be 2.2% ($= 1.5^2 / \{(11.5/2)^2 \times 3.14\}$), indicating the distribution of only ca. 2 adults in this small area if 100 are randomly distributed. The surviving beetles once used for the filter paper treatment were again used for the assay: they were released carefully onto the center of the Petri-dish.

The numbers of adults aggregating by clinging to and hiding beneath both of the paper fragments were counted for each at 5 min intervals for the first one hr, and then at 30 min intervals for the following 3 hr. All the experiments were carried out in the mass culture chamber, as described in the section 2.1.1., with the Petri-dish turned round occasionally so as to eliminate the effect of illumination. A view of this experiment is shown in Fig. 3.24. The experimental combinations are as follows:

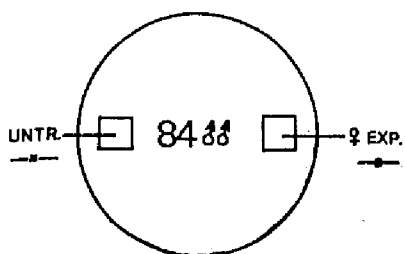
- Ia) Untreated and female-exposed filter papers; males
(Fig. 3.25.)

- Ib) Untreated and male-exposed filter papers; females
(Fig. 3.26.)
- IIa) Male- and female-exposed filter papers; males
(Fig. 3.27.)
- IIb) Female- and male-exposed filter papers; females
(Fig. 3.28.)
- IIIa) A pair of untreated filter papers; males (as a control)
- IIIb) A pair of untreated filter papers; females (as a control)
- IVa) Untreated and male-exposed filter papers; males
(Fig. 3.29.)
- IVb) Untreated and female-exposed filter papers; females
(Fig. 3.30.)

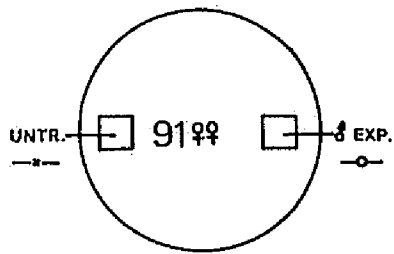
The experiment I has shown that males were attracted by the female-exposed paper fragment intensely (Fig. 3.25.) and females were attracted by the male-exposed paper fragment to lesser extent (Fig. 3.26.). In this experiment (Ia), some males clinging to the female-exposed paper fragment were observed to attempt mating intensely with the other male individuals, mounting each other and extruding the aedeagus.

The experiment II has shown that both males (Fig. 3.27.) and females (Fig. 3.28.) were attracted by the female-exposed paper fragments more intensely than by the male-exposed paper fragments.

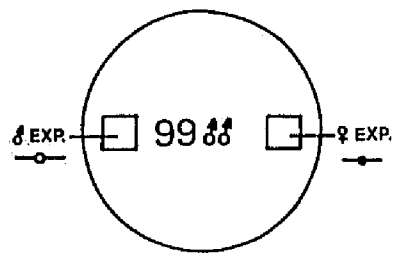
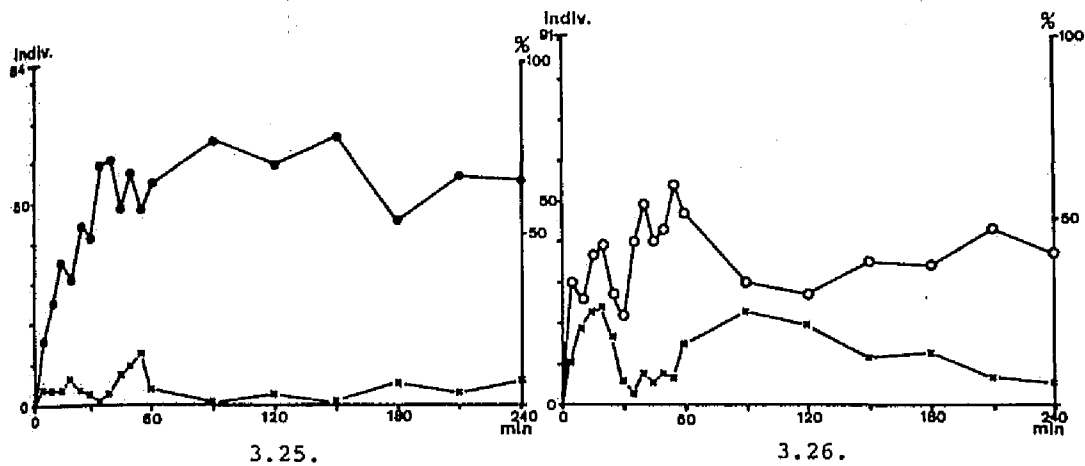
The experiment III, as the control, with 92 males (IIIa) and 110 females (IIIb) employed, has shown that both of the two untreated paper fragments gathered only less than 5 males or females, indicating that there were no other factors than the



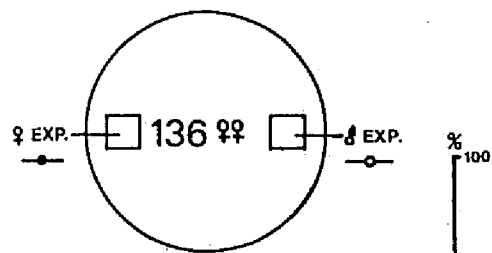
I-a



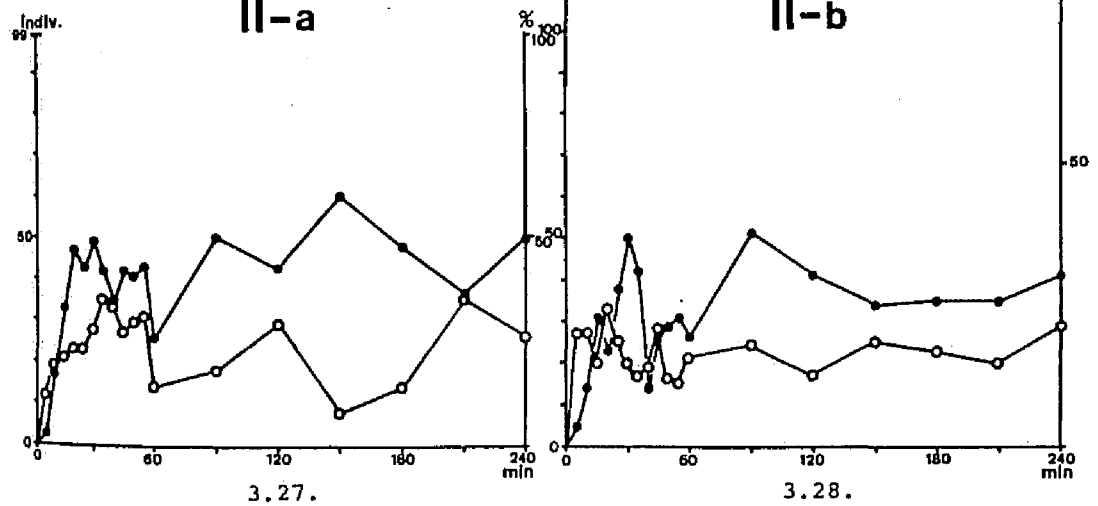
I-b

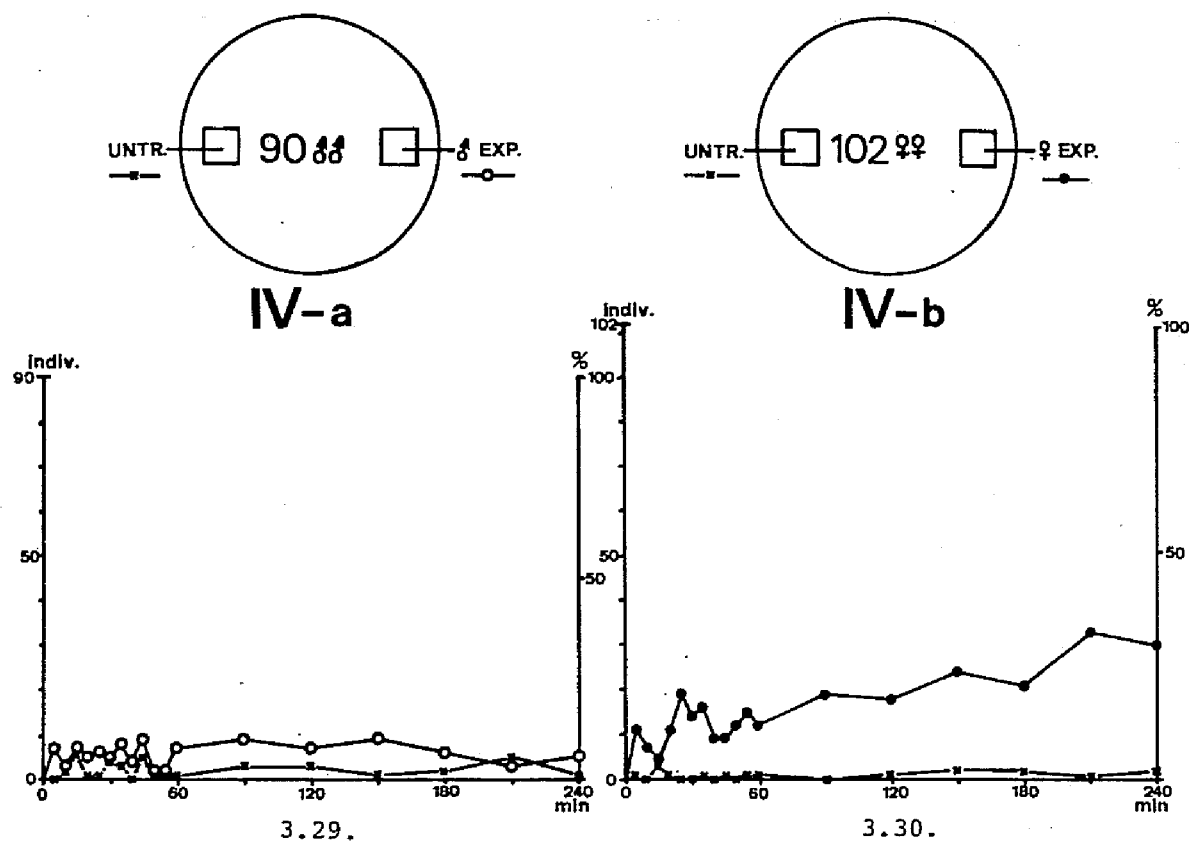


II-a



II-b





Figs. 3.25.-3.30. Results of bioassays to compare the attractiveness of male- and female-exposed filter paper square fragments with each other or with that of untreated filter paper square fragments (see text).

- 3.25.: Experiment Ia.
- 3.26.: Experiment Ib.
- 3.27.: Experiment IIa.
- 3.28.: Experiment IIb.
- 3.29.: Experiment IVa.
- 3.30.: Experiment IVb.

chemical condition of the papers that relate to the differences of numbers of aggregating adults.

The experiment IV has shown that males were indifferent to the presence of male-exposed paper fragment (Fig. 3.29.), while females were attracted by the female-exposed paper fragment to some degree (Fig. 3.30.).

The differences of the numbers of attracted or aggregating adults between two paper fragments were quite significant in a 1% risk in all the experiments from 60 min to 240 min after, except in the experiments IIIa and IIIb, which showed no significant difference at all.

From these results, it can be safely said that males, and also females themselves to lesser extent, are intensely attracted by females' odor. The activity of male-exposed paper fragments to females (Ib; IIa; IIb) may be ascribed either to the activity of males' odor itself or to the transfer of females' odor to males before the separation of the sexes. However, it is very likely that females produce a kind of aggregation pheromone that attracts both females themselves and males.

Pheromones that are produced by male and/or female adults and attract both male and female adults have been found out in many coleopterous species. Either this type of pheromone should be designated as "sex pheromone" or "aggregation pheromone" is a question at issue. Particularly in the present species, L. brunneus, the significance of the aggregation of both sexes is thought to consist only in the increased chance of mating.

Further bioassays are eagerly needed to reconfirm whether the

above-reported activity of the paper fragments is due to such a pheromone.

3.6.2. Boring behavior^{E)}

In the experiment for the artificial diet selection, as reported in the section 2.3., an interesting behavior was observed in the adult beetles released onto the diets X and Y, i.e. the diets of Sumitomo Chemical Co. Ltd. In two of the three replications of the diet Y, adult beetles bored into the blocks, and in two of the three of the diet X they bored more intensively and deeply as if they were bostrychid beetles, a related group with the adult boring habit. Furthermore, these beetles survived rather longer, showing such an abnormal behavior; the most longevous male individual on the diet X lasted for 7 weeks after the release to the diet. This record is noteworthy when compared to the data presented by Gay⁸²⁾: the maximum male adult longevity was 33 days in 26°C and 75% R.H.

Such a behavior has never been observed before in L. brunneus. A possible cause for this is the peculiarity in the use of wheat flour in these two diets. A further investigation is needed to determine whether such a phenomenon is general with diets containing wheat flour and also to examine whether such a boring behavior increases or not the adult longevity.

3.6.3. Phototaxis and flight^{H)}

As has been often stated on the life history of L. brunneus, e.g. by Fisher (et al.)^{f2)}, etc., the adult beetles of this

species are of crepuscular nature and able to fly very well. They always take refuge in their exit holes , crevices of wood, etc. if they feel light. On the other hand, very strangely, they are attracted to light when they are flying and they are easily caught by means of light traps^{hi)}.

When they were flying, a very strange or a rather possibly reasonable behavioral pattern was observed many time in the present mass culture chamber: beetle, if it has begun to fly, always makes its way toward the fluorescent lamp of the chamber, but when it has just reached to the lamp, it is always observed to turn aside as if it wanted to keep away from the lamp: it is always oriented to the lamp light, but always avoids to reach it. Such an inconsistent combination of behaviors may be elucidated to be due to the duality of behavior, namely the flight with rough orientation by light and avoidance of light as a general behavior.

As the escapes with flight have been observed more frequently in females than males, the readiness of flight of both sexes was compared as follows: From the mass culture with the diet of Tôkyô Univ. of Agric., as described in the sections 2.1.1. and 2.1.3., 3 groups of 8 new adult beetle pairs were taken out and each group was put into an open 9 cm-diametered Petri-dish, which was then put into the center of a 20 cm-diametered glass vessel closed with a glass plate cover, 20 x 20 cm in size. All of these 3 large glass vessels were placed in a mass culture chamber under dark condition. Twelve days later, the beetles that were found outside the smaller Petri-dish were counted. As the

adults of this species are not able to climb on glass surface, they were regarded as those that had flown out.

The result (Table 3.13.) simply indicates that females fly more readily than males.

3.7. Relations with the other organisms^{A,H)}

There are some organisms that do harm to L. brunneus population in the mass culture; to begin with, the mold^{h6)} which grows on the artificial diet of Tôkyô Univ. of Agric. As stated in the sections 2.1.1. and 2.1.3., if the duration of drying of the wet diet dough is not sufficient, and/or if the relative humidity in the rearing chamber is a little higher than is designated (70%), it often occurs on the dried diet surface. If mold once occurs on the diet surface, the progeny yield of this insect decreases considerably.

The Lyctus development and occurrence has been reported to be possibly related (?) to fungal existence^{f1, f2)}, or to be either promoted or repressed, according to the case, by blue staining fungi in wood^{c10)}. On the other hand, mold occurrence has been reported to be often harmful to lyctid cultures^{k15)}, probably due to some toxic substances produced by mold. Mold control is simply dependent on the baking duration of diet blocks and the relative humidity of the mass culture chamber, as already stated in the sections 2.1.1. and 2.1.3.

The second to be mentioned as a very harmful enemy to lyctids is an exoparasitical mite species, Pyemotes sp. (Heterostigmata, Pyemotidae). Some of the mites encountered in the present mass

Table 3.13. Numbers of *L. brunneus* beetles that were found inside and outside the 9cm-diametered Petri-dish after the test period of 12 days.

Test replication	Number of beetles supplied		Number of beetles found inside (n_{in})		Number of beetles found outside (n_{out})		$\frac{n_{out}}{n_{in} + n_{out}} \times 100\%$	
	males	females	males	females	males	females	males	females
1	8	8	4 live + 2 dead	1 live + 4 dead	2 live + 0 dead	3 live + 0 dead	25%	38%
2	8	8	4 live + 3 dead	2 live + 1 dead	1 live + 0 dead	4 live + 1 dead	13%	63%
3	8	8	4 live + 3 dead	1 live + 1 dead	1 live + 0 dead	3 live + 3 dead	13%	75%
Total	24	24	12 live + 8 dead	4 live + 6 dead	4 live + 0 dead	10 live + 4 dead	17%	58%

culture were identified to be this species by Dr. Kazuyoshi Kurosa. This species has been called Pyemotes ventricosus (Newport) or Pediculoides ventricosus Newport, and has been reported not only to be parasitical on many kinds of insects^{g3, h5)}, including lyctid beetles^{h1, k15)}, but also to cause itch on human skin as reviewed by Sasa^{s2, s3)}. The nomenclature of this mite species is now open to question among acarologists.

Despite of some efforts to control this parasite, as described in the sections 2.1.1. and 2.1.3., it has often been found walking on a Petri-dish containing Lyctus larvae, pupae and/or adults for test uses. It is very easily controlled by spraying alcoholic solution of beta-naphthol occasionally. Mite occurrence is assumed to be somewhat related to mold occurrence^{k15)}.

Another kind of mite, belonging to Mesostigmata, has also been found from Lyctus cultures, which, however, does not seem harmful to Lyctus.

A species of book lice, Liposcelis sp. (?) (Psocoptera, Liposcelidae) (Fig. 3.31.), has been observed frequently occurring on filter papers and Lyctus frass in the cultures. Although Kühne^{k15)} claimed cautions against this insect as a possible predator of Lyctus eggs, there does not seem to have been any apparent damage on Lyctus population. Frequent removals of frass will suffice for its control.

Before undertaking the mass culture in earnest, Tarsostenus univittatus (Rossi) (Coleoptera, Cleridae) had once occurred in large numbers in the course of Lyctus beetle collection from the infested timbers in 1978. However, no further occurrence has

been encountered since the isolated mass culture was established. This clerid beetle is well-known as a lyctid predator both in larval and imaginal stages^{b6, s11, b2, k11, m8, k15}). Manual elimination of this species, if any, in the renewal of Lyctus cultures and firm coverings of culture bottles with gauze, as stated in the section 2.1.3., will suffice for its control.

The second coleopterous species to be mentioned is a very well-known insect pest on flour and stored foods, Tribolium castaneum (Herbst) (Coleoptera, Tenebrionidae), and its allies, namely Tribolium spp. This group of tenebrionids has been quoted in relation to lyctids in different aspects:

In consideration of the resemblance of gross characteristics to Lyctus, but regardless to the fact that there is little phylogenetic relationship, Tribolium castaneum had been utilized for bioassays with chemicals for Lyctus control as the "substitute insect" in place of L. brunneus^{i3, m7}) until the mass culture of L. brunneus came to be successful in Japan. Further, Tribolium has been often confused with Lyctus mainly by non-entomologists. Nobuchiⁿ⁵), being apprehensive of this, gave distinguishing points of Tribolium from Lyctus.

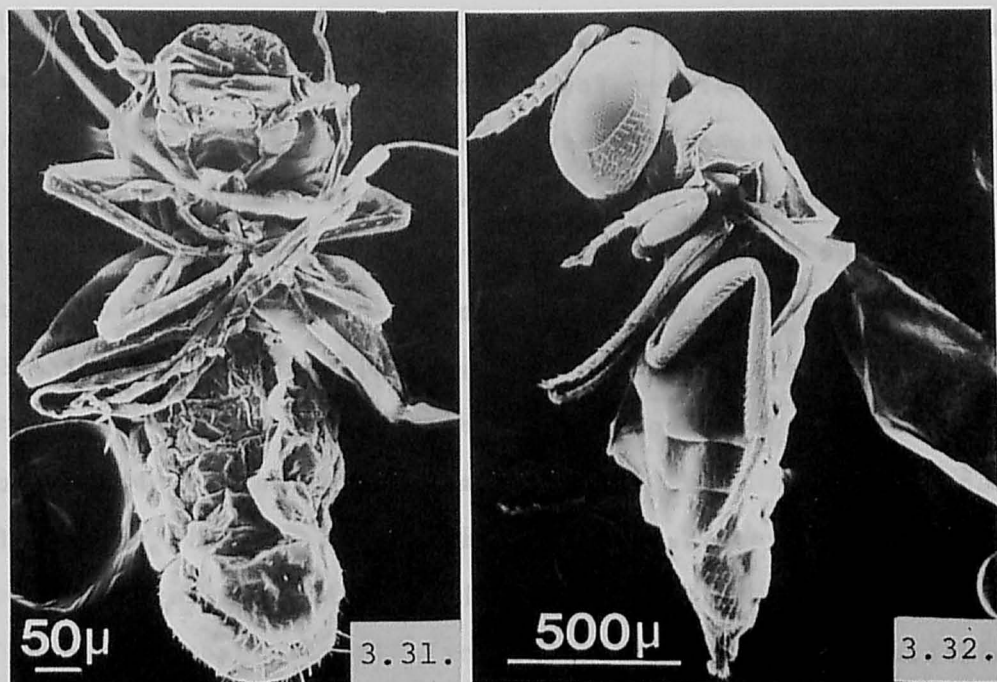
The most interesting aspect of the relation of Tribolium to lyctids is that there are some cases where the former was or might be found together with the latter in woods that are heavily infested by the latter^{h3, m2, i12}). As Tribolium is known to be highly omnivorous^{l2}), the ecological relation between these two coleopterous taxa is assumed that the former is a facultative predator of the latter and/or the former feeds on the latter's

frass to utilize the remaining nutrients. The following are the unpublished examples further indicating the direct relation of Tribolium to Lyctus:

In the course of collection of L. brunneus larvae for mass culture from a heavily infested hardwood timber as a part of furniture piece sent from Toyonaka, Ōsaka Pref., Nov./1978, 3 adults of T. castaneum were found together with L. brunneus larvae in a tunnel mixed with Lyctus frass; several adults of T. castaneum were collected again from a hardwood timber, found in Sakurai, Nara Pref., July/1985, which had been heavily infested by a kind of insect, which, however, was not found out in situ but most likely to be either L. brunneus or L. sinensis judging from the circumstances.

The ecological relation of Tribolium to Lyctus needs to be studied further from the viewpoint of ecology and control of the both insects.

From the culture with the diet of Tōkyō Univ. of Agric., as introduced in the section 2.1.3., several adults of Stegobium paniceum (L.) (Coleoptera, Anobiidae), identified by Mr. Masahiro Sakai, a pest insect on dried plant materials, were found together with L. brunneus adults in spring, 1979. In 1985 the same species was again found occurring indoors in large numbers on blocks of the standard artificial diet, as described in the section 2.3.4. This anobiid species is harmful not to Lyctus but to stored diet blocks for Lyctus. Manual elimination of the adults of this anobiid beetle, if any, and/or elimination of diet blocks infested by this anobiid species, which can be distin-



Figs. 3.31.-3.32. SEM photographs of two insect species in ecological relations to *L. brunneus*.

3.31.: *Liposcelis* sp. (Psocoptera, Liposcelidae), a possible egg predator.

3.32.: Pteromalidae sp. (Hymenoptera), a parasitical wasp.

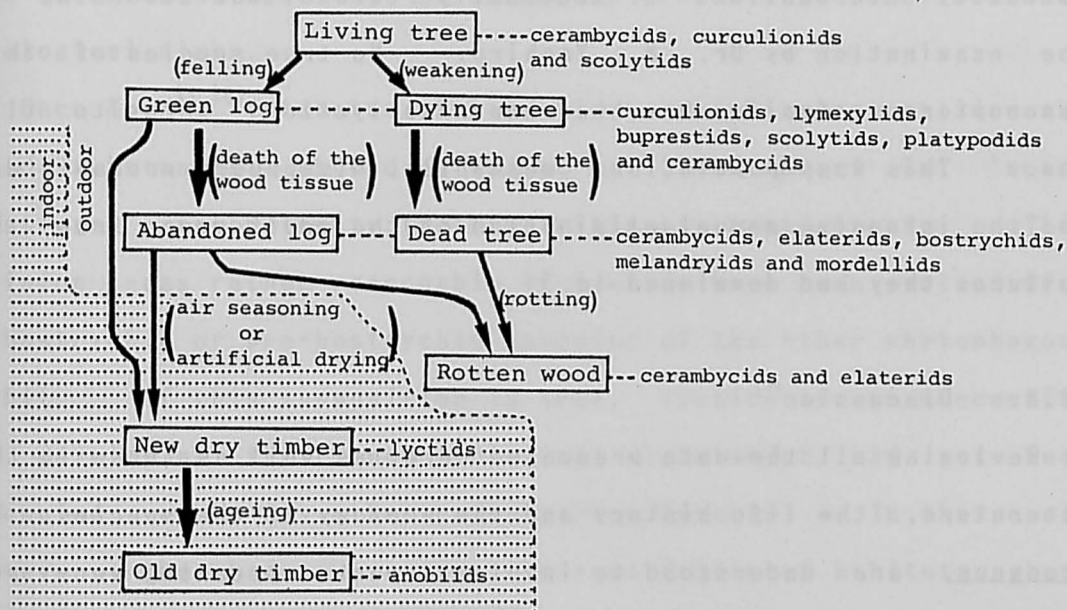


Fig. 3.33. Succession of wood-boring Coleoptera in Japan proper with the succeeding phases of wood.

guished from uninfested blocks by the issue of the frass peculiar to anobiids, will suffice for its control.

Although there are many coleopterous species that act as predators of Lyctus, parasitical wasps must be mentioned as the most important enemies of Lyctus.

Along with the above-stated occurrence of a clerid beetle, Tarsostenus univittatus, in the Lyctus-infested timbers in 1978, a kind of minute wasp also occurred in large numbers. The number of emerged wasps at that time was so great that the Lyctus beetle collection was unavoidably abandoned. This unidentified wasp proved to belong to the family Braconidae according to the examination by Dr. Naoya Yashiro.

In the course of mass culture, another unidentified wasp species (Fig. 3.32.) occurred in the culture with the standard artificial diet in 1982 - 1984. This proved to belong to the subfamily Pteromalinae of the family Pteromalidae according to the examination by Dr. N. Yashiro. No true species of this hymenopterous family has been known as lyctids' parasite till now. This wasp population, meanwhile, disappears probably due to the intensive manual elimination of the adult wasps from the cultures they had developed in.

3.8. Discussion^{H)}

Reviewing all the data presented here, as well as those in the literature, the life history and the ecology of the species, L. brunneus, are understood to involve aspects appearing very peculiar and very common among the order Coleoptera.

Its feeding habit may be the most peculiar aspect among many items of its life history and ecology, with its resistance to dryness being worthy of special mention as a generic or a familial character among the wood-boring Coleoptera. Figure 3.33. will serve as an aid for understanding its peculiar "ecological niche". Most of the coleopterous families known as wood-borers in Japan are also mentioned in this diagram to show the faunal succession of wood-boring Coleoptera with the succeeding phases of wood. This figure also suggests the succession of the "Teredilia" group in wood utilized by men: bostrychids principally occur in cut wood before or during the course of air-seasoning up to air-seasoned state; lyctids occur rather exclusively in newly seasoned hardwood with the moisture content below the "fiber saturation point" (ca. 28%); anobiids occur rather exclusively in much aged seasoned wood. There, the lyctids (above all the present species, L. brunneus) proved to monopolize their ecological niche.

Once Koch^{k5, k6}, stated that lyctids, which cannot utilize the wood substances, should be called not "wood-eater" but "wood-destroyer" according to their nutritional requirements. This opinion seems rather reasonable if lyctids have been evolved from a bostrychid or pre-bostrychid ancestor of the other phytophagous habit. If this supposition is true, lyctids appear successful in developing their new ecological niche which had not been occupied yet by any other coleopterous insects. In that case, however, they had to overcome the restriction of habitats where their development takes place in accordance with their nutri-

tional requirements. This habitat specialization is remarkable in L. brunneus compared to the other lyctids^{c10)}.

Because new dead wood in air-dried state as the habitat of lyctids, especially of L. brunneus, does not seem to abound in the wild, next comes the problem of their wild occurrences. Although some other lyctids, such as L. sinensis^{a2, i11)}, L. villosus Lesne^{s7)}, L. pubescens^{c8, n2)}, Trogoxylon auriculatum Lesne^{b3, b4)} and Minthea rugicollis^{b8)}, have been reported to occur in the open or in the wild forest, L. brunneus has not been reported thus at all. From the viewpoint of control, a field survey is needed to know whether the wild occurrence of L. brunneus takes place.

That the adults of L. brunneus gnaw but do not feed wood is another peculiar ethological character not of the family nor of the genus but of the species^{c15)}. The superphylogenetic grouping of the family Lyctidae, according to the adult gnawing and feeding habit, the degree of restriction of the oviposition sites, the intestinal structure and fauna, and also the biogeographic range of distribution, as proposed by Cymorek & Schmidt^{c15)}, needs to be studied further to detect the causalities among these items.

Most of the other characteristics of the life history and ecology of L. brunneus do not appear to be peculiar aspects. The sexual difference of the body shape; the larval development; the larval instar number and its variation with different conditions; the population density effect on the progeny yield; the adult sex ratio and its change; the adult longevity and its variation with different sexes and body lengths; the possible

presence of aggregation pheromone; all of them do not appear uncommon.

With the increase of the ecological knowledges of this species, new methods of its control will be devised further.

3.9. Summary

The summarized results and conclusions newly obtained in the investigation on the life history and ecology of L. brunneus are:

(1) Prepupa is the stage with the greatest body weight value in the life history of this species. Quantitative relations among the weights of prepupa, pupa and new adult were set up.

(2) For the convenience of the bioassays, relation between the adult body length and the body weight and that between the adult body length and the elytral length were established.

(3) A rearing method with buckwheat flour and a small gelatin capsule enabled a single 1st instar larva to develop. It is also applicable to demonstrate the larval instar number by examining the number of exuviae.

(4) Most of the larvae reared with gelatin capsules passed through 4-6 instars under mass culture condition though a few exceptionally had much more instars. In general, larval instar number varied among individuals, and tended to increase with the prolonged larval period.

(5) When larvae were in starving condition and/or in want of support for boring action, they showed supernumerary molts and weight decrease, resulting in death. Prepupa could pupate readily even without the support. The pupal duration in the mass

culture condition was ca. 10-12 days.

(6) The population density effect was remarkable on the number and the biomass of emerging beetles, and was hardly remarkable on their sex ratio and body length.

(7) Providing one new parent female beetle with about 10-15g of the standard artificial diet is appropriate for the practical mass culture.

(8) Although protandry is the mode of adult emergence of this species, the final cumulative sex ratio is always nearly 1 : 1.

(9) Adult longevity and resistance to chemicals were shown to be positively correlated to the adult body length, while only adult longevity was shown to be statistically different between the sexes though it was very variable among individuals.

(10) Existence of a possible aggregation pheromone produced by females was suggested by the aggregating behavior of both sexes.

(11) Adult beetles were observed to bore into diet blocks containing wheat flour.

(12) Adult beetles showed two kinds of quite different reactions in relation to light: they may fly by means of orientation by light, while they hate light as they are of rather crepuscular nature.

(13) Female beetles fly more readily than male beetles.

(14) Some other organisms that occur in the diet cultures of L. brunneus, such as mold, mites, book lice, clerid beetles, anobiid beetles and parasitical wasps, are easily controlled by intensive cautions. Among them a pteromalid wasp is new as a member of lyctids' enemies. Ecological relation of Lyctus and

Tribolium (Tenebrionidae) is discussed as well.

4. Chemical analyses of the food nutrients^{B, G)}

In this chapter, two kinds of L. brunneus foods (natural oak sapwood and the standard artificial diet, as described in the section 2.3.4.) and feces of this species taken out from the cultures with these two foods are analyzed chemically, as well as the whole bodies of adult beetles to give the nutritional and physiological aspect of the biology of this species. SEM observation of the feces was also made.

4.1. Materials and methods

The insects and materials subjected to the present chemical analyses are as follows, with their code names headed:

D: Intact standard artificial diet, composed of air-dried 26% lauan (Shorea spp.) wood sawdust, 50% soluble starch (Nakarai Chemicals, Ltd.), and 24% brewer's yeast powder "Ebios". These values correspond to 27.4% lauan wood sawdust, 47.7% soluble starch, and 24.9% brewer's yeast powder in an absolutely dried state.

DF: Frass (feces) of the insects taken out from the culture with the standard artificial diet.

BA: Whole bodies of 504 adult beetles of L. brunneus, supplied from the present mass culture with diets, as described in the sections 2.1.1., 2.1.3. and 2.3.4.

Q: Intact wood particles remaining in the heavily infested sapwood of an oak species, Quercus serrata, used for insect culture. Oak was cut about 5 years before and the wood was stored at 5°C before exposure to insect attack.

QF: Frass (feces) of the insects taken out from the culture with the above oak wood.

In the amino acid analyses, all the samples were extracted with organic solvent prior to the analyses. About 1g of the sample BA was defatted with acetone at -10°C for 72 hr. The sample Q was smashed and passed through a coarse sieve, while the sample D was originally supplied in a powder state. Since the samples DF and QF were obtained in a very fine powder state, these materials were directly passed through a No. 100 mesh sieve. The four samples, D, Q, DF and QF were then extracted with ethyl alcohol - benzene (1:2, v/v) for 6-8 hr. Each extractive-free sample was divided into 2 parts: one was used for the general amino acid analysis and the other for tryptophan analysis.

The conditions of acid hydrolysis in the general amino acid analysis were essentially identical with those of Ijima *et al.*¹²⁾. Briefly, for general amino acid analysis, each sample was hydrolyzed with 6N hydrochloric acid for 24 hr at 105°C . The hydrolyzed solution was passed through Amberlite CG 120 column (NH_4^+ form), which was then exhaustively washed with distilled water to remove components other than amino acids. Amino acids were then recovered by elution with 2N aq. ammonium hydroxide solution.

For tryptophan analysis, each sample was hydrolyzed with saturated barium hydroxide solution for 48 hr at 105°C under nitrogen¹¹⁾. The hydrolyzed solution was neutralized with sulfuric acid, and the precipitated barium sulfate was removed by filtration. Tryptophan was recovered from this neutralized solution

by passing it through Amberlite CG 120 column as described above. The amino acid content and composition were analyzed with Hitachi 835 Amino Acid Analyzer after the total amino acid content was adjusted to be in the range of 1 - 5 μ M/ml.

For the starch analyses, the sample Q was slightly ball-milled for 10 min, as well as the sample D, to liberate amyloplasts from the parenchyma cells. The samples DF and QF were passed through No. 100 mesh sieve to remove intact portions.

Starch was extracted from each powdered sample with perchloric acid in the same way as described in the standardized method of AOAC (14th ed.) except in that phosphowolframic acid was used as deproteinization reagent instead of uranyl acetate. The amount of the solubilized starch was determined by the method of Humphreys & Kelly^{h8)} based on the color reaction with iodine using potato starch, Nakarai Chemicals, Ltd., as the standard.

The weight ratios of amylose to amylopectin in the starch were also determined by the iodine colorimetric method^{m4)} using amylose and amylopectin, Sigma Chemical Company, as the standards.

In addition, the sample QF were subjected to SEM observation in the same manner as described in the section 5.1.

4.2. Results and discussion

The amino acid compositions of the foods (D; Q) and feces (DF; QF) together with the whole bodies of adult beetles (BA) of L. brunneus are summarized in Table 4.1. The results showed that acidic amino acids, such as aspartic acid and glutamic acid, and amino acids with hydroxyl group, such as serine and threonine in

Table 4.1. Amino acid compositions (μ moles/g)

Amino acid	Sample (see text)				
	D	DF	Q	QF	BA
Lys	12.1	22.5	1.9	1.3	172.7
His	trace	0.7	0.2	0.1	19.9
Try	3.1	1.6	0.1	trace	7.5
Arg	15.4	10.6	3.6	4.0	74.5
Asp	63.4	2.2	0.2	0.1	4.4
Thr	23.9	0.8	0.2	0.1	1.0
Ser	35.1	2.1	0.3	0.2	4.9
Glu	78.6	5.6	1.5	0.5	14.9
Pro	33.0	8.3	trace	0.2	16.6
Gly	13.4	25.8	1.0	0.7	97.9
Ala	57.5	36.2	0.8	0.4	86.5
1/2 Cys	2.7	1.9	0.5	0.2	7.8
Val	44.0	23.1	0.1	0.2	85.3
Met	trace	6.0	0.3	0.1	15.1
Ileu	30.1	14.9	1.6	0.6	123.1
Leu	45.3	34.0	4.8	2.5	211.8
Tyr	9.3	10.4	0.9	0.6	101.6
Phe	16.6	19.1	2.7	1.6	160.7
Total amino acid contents (%) ^a	6.13	2.84	0.30	0.20	16.53

^a) The value is expressed as a percentage of the dry weight of each sample.

Table 4.2. Starch contents in the foods and feces based on the dry weight of each sample

Sample (see text)	D	DF	Q	QF
Starch content (%)	47.7	14.2	3.22 $\pm$ 1.17	0.91 $\pm$ 0.11
Ratio of amylose to amylopectin	2:98	14:86	14:86	17:83
Amylose content (%)	1.0	2.0	0.43 $\pm$ 0.14	0.15 $\pm$ 0.01
Amylopectin content (%)	46.7	12.2	2.79 $\pm$ 1.03	0.76 $\pm$ 0.10

the artificial diet were highly utilized. When the results of the samples D - DF and the samples Q - QF were compared, utilization of amino acids were found to vary considerably and no importance of methionine and tryptophan was noted in contrast to the results of Ijima et al.^{[2)} Since the lauan wood sawdust added to the artificial diet seems to contain relatively very small amount of protein as shown in the oak wood (Q), yeast powder in the diet must contribute the major source of protein in the artificial diet.

The relative amounts of some amino acids such as lysine, glycine, tyrosine and phenylalanine in the samples D - DF were observed to increase after the digestion by Lyctus. This might be due to the actions by the reagents used, the metabolic actions by the insect and/or those by the symbiotic microorganisms present in the gut of the insect^{k5, k6)}. However, the total amounts of amino acids are of interest since they clearly show the utilization of amino acids. The newly presented data of the amino acid composition of the whole bodies of adult beetles (BA) will be expected to be useful in making an adequate holidic diet for biochemical studies.

In the next place, we pay attention to the starchy polysaccharide content and composition in the foods and feces. Previously starch contents in sapwoods of various wood species have already been reported, the values being widely varied from 0.2% to 8.7%^{[2)}, and a comparison has been made between the values of wood and Lyctus frass. However, the comparison of starchy polysaccharide analyses of diet and feces from diet culture has not

been carried out yet.

Table 4.2. summarizes the results of the starch contents and the ratios of amylose to amylopectin in the foods and feces. The starch content of oak wood (Q) (3.22%) was in good agreement with the minimum value (3%) of those observed in the sapwoods which could be infested by Lyctus¹²⁾. A small amount of starch was found to remain in the feces, indicating that this insect could not always utilize starch in the diet completely.

The ratio of amylose and amylopectin, as well as their amounts, are also shown in Table 4.2. A remarkably low amylose content in the artificial diet (D) may be ascribed to the fact that a partially hydrolyzed water-soluble starch was utilized as the starch component. The starch in oak wood (Q) also comprised amylopectin for the most part. The ratios of amylose to amylopectin in the feces were slightly higher than those in the intact foods. These results indicate the preferential utilization of amylopectin by Lyctus. This seems a kind of physiological adaptation of the insect to the food nutrition as an environment.

Changes of the nutrient contents in the foods by Lyctus digestion, as calculated from the results shown in Tables 4.1. and 4.2. are summarized in Table 4.3. According to this table, more than 70% of starch originally present in the foods proved to be utilized. On the other hand, although as much as 67% of amino acids in the artificial diet was utilized, only 33% of amino acids in oak wood could be utilized. The ratios of the consumed starch and amino acids in the diet (D - DF) and wood (Q - QF) were 9.9:1 and 23.3:1, respectively. This low utilization

Table 4.3. Changes of the nutrient content in the foods by *Lyctus* digestion

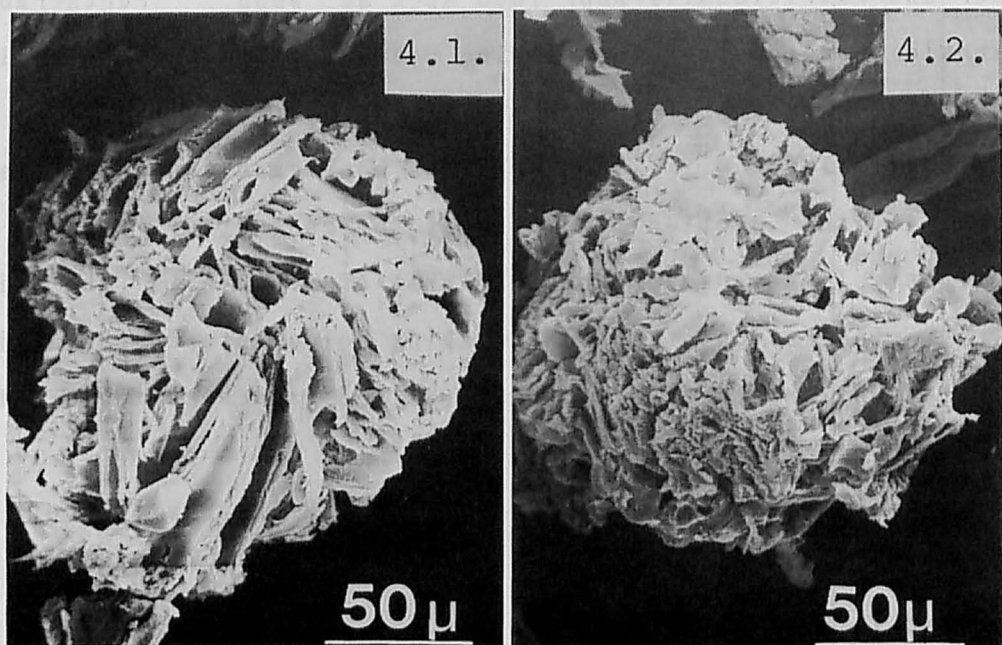
		Diet digestion (Comparison of D and DF)	Wood digestion (Comparison of Q and QF)
Composition of food (D or Q) (%)	Starch (Amylose) (Amylopectin)	47.7 (1.0) (46.7)	3.22 (0.43) (2.79)
	Amino acids	6.0	0.30
	The other	46.3	96.48
		(D)	(Q)
Composition of frass (feces) ^a (DF or QF) (%)	Starch (Amylose) (Amylopectin)	{14.2} 8.0 (1.1) (6.9)	{ 0.91} 0.89 (0.15) (0.74)
	Amino acids	{ 3.5} 2.0	{ 0.20} 0.20
	The other	{82.3} 46.3	{98.89} 96.48
		(DF)	(QF)
Difference between food and frass (%) (Quantity consumed (g) per 100g of food)	Starch	39.7	2.33
	Amino a.	4.0	0.10
Utilization ratio (%) (Ratio of quantity consumed to the initial content in food)	Starch	83	72
	Amino a.	67	33
Ratio of consumed quantity of starch to that of amino acids		9.9 : 1	23.3 : 1

^a) Composition values of frass in parentheses { } were transformed so that the values of "the other" are fixed to those of foods.

ratio of amino acids, and therefore the high utilization ratio of starch in wood, would be reasonable since a higher amount of energy transformed from starch would be required in masticating a harder and nutritionally poor food such as wood. Furthermore, it should be noted that the samples D and Q showed rather similar values of the ratios of starch to amino acids, namely 8.0 : 1 for D and 10.7 : 1 for Q. These results confirmed the above observation in the section 2.2.3. that the content of starch was quantitatively more important than that of protein. The similarity in the ratios of starch to amino acids between the artificial diet (D) and the oak wood (Q) may indicate a suitable nutrient balance of the diet.

In the last place, as the result of SEM observation of the larval frass grains from wood culture (QF), two examples are presented in Figs. 4.1.-4.2.

Larval frass has been observed by Fisher (*et al.*)⁽²⁾, Schmidt⁽⁵⁵⁾ and Schmidt & Buchholz⁽⁸⁶⁾. Further observation with SEM has revealed a new aspect of its structure, namely that within these grains intact wood tissue and cell walls can be seen. Frass is assumed to consist of two kinds of particles, the excreta and the intact wood shreds. At present the latter cannot be eliminated for the preparation of the former. However, if the grains in these photographs are the real frass, the presence of intact wood tissue and cell wall not only agrees greatly with the well-known fact that this species is unable to digest wood cell wall components, but also suggests the mode of digestion occurring in the gut: wood particles are not defibrated when digested by Lyctus.



Figs. 4.1.-4.2.: Frass grains of *L. brunneus* from infested *Quercus serrata* wood.

The diameter of the grains in Figs. 4.1.-4.2., being about 0.15 mm, is comparable with that in L. planicollis, measuring 0.08 mm⁸⁹⁾.

4.3. Summary

The summarized results and conclusions newly obtained in the present chemical analyses of food nutrients and in the SEM observation of the feces are:

(1) The degrees of amino acid utilization by L. brunneus varied considerably among amino acids, with acidic amino acids and those with hydroxyl group being rather preferentially utilized.

(2) Amylopectin portion of starch seemed a better nutrient for L. brunneus than amylose, and the oak wood starch comprised the former for the most part.

(3) The value of the ratio of consumed quantity of starch to that of amino acids in the oak sapwood was higher than that in the standard artificial diet, indicating a higher requirement of energy in wood feeding than in diet feeding.

(4) SEM observation of the feces (frass) from wood cultures revealed that the grains of frass contain wood tissue and wood cell walls in an intact condition, reconfirming the fact that wood cell wall components are not utilized by this species.

5. External morphology and surface structure

In this chapter, the SEM observation of the external morphology and the surface structures of all the developmental stages of L. brunneus is reported, with an additional note given on the malformations on the corporal segmentations of adult beetles.

5.1. Observation method^{B,C,F)}

Almost all the specimens of Lyctus brunneus in various developmental stages were taken out from the mass culture with the diet of Tôkyô Univ. of Agric., as described in the sections 2.1.1. and 2.1.3.

In the observation of the larvae, several instar-known ones that had been reared by the "individual rearing method", as described in the section 3.2.3., were selected for the observation, as well as the other larval specimens directly from the diet cultures. The latter were designated with the nomenclature presented in the section 3.2.1. and Figs. 3.3.-3.7.

Some of these specimens in various developmental stages were dried with a critical point dryer, Type HCP-1, Hitachi Ltd., before coating with gold, but as drying was found to distort pupal wing form, etc., the specimens were later usually coated undried. Coat thickness was always less than 500 Å. And also, some of the live adults were put into SEM neither dried nor coated with gold.

Eggs were prepared by means of Bletchly's "veneer technique"^{b5)}, as described in the section 3.2.3. Several days after the release of parent beetles the piles of veneers were opened up

to look for eggs, which were coated with gold in situ.

All the SEM specimens were mounted on the SEM stubs and were examined with a Hitachi Scanning Electron Microscope, Type S-500.

On the other hand, observations of malformed adult specimens, as reported in the section 5.6., were carried out not by SEM but by the binocular microscope.

5.2. The larva^{B,F)}

5.2.1. The head capsule

The head of the larva (Figs. 5.1.-5.5.) is normally deeply recessed into the prothorax, and only the mouth parts and their immediate surrounding are exposed in facial view (Figs. 5.1.-5.2.). Figures 5.3.-5.5. are views of the excised head to show those features which are hidden, in particular the grooves (gr) and the epicranial suture (es).

A pair of these grooves appears to be V-shaped and has been observed and illustrated by Altson²⁴⁾ and Munro^{mg)} as "frontal sutures". However, it is apparent that these grooves are not the real frontal sutures because they do not form the Y-shape together with the epicranial suture. The real ones (fs), though not clearly observed in Figs. 5.3.-5.4., run almost parallel with the frontoclypeal suture (fcs). Pringle^{ps)} did not recognize these grooves in the "last larval stage", and possibly they are a feature of younger larvae.

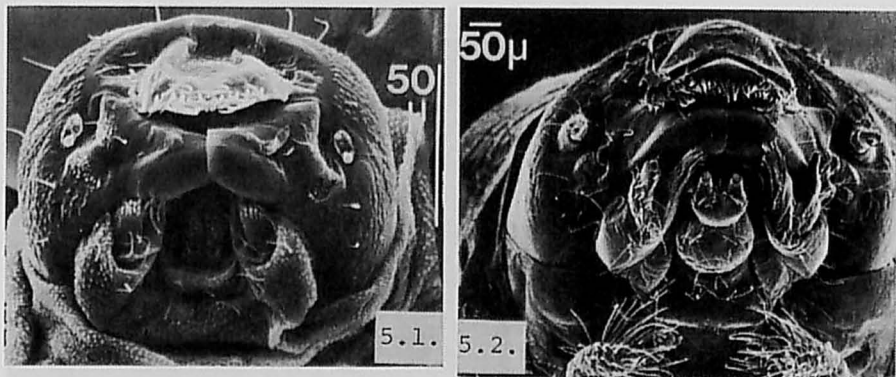
The epicranial suture (es) seems ridge-like over the frons and vertex, but becomes slightly sunken near the posterior margin of the epicranium (Fig. 5.5.).

5.2.2. The mouth parts

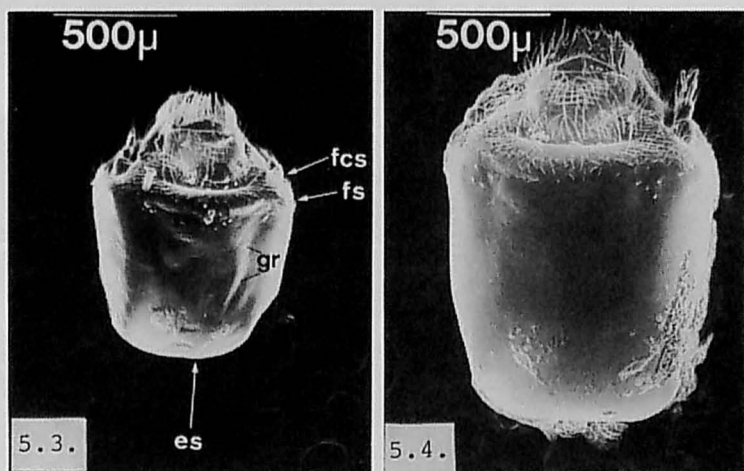
The protective dorsal flap of the labrum is seen in Figs. 5.1.-5.4., while the mandibles are shown in detail in Figs. 5.6.-5.11. & 5.13.-5.14. Of particular interest are the photographs of the sharp cutting edge (ce) (Figs. 5.7.-5.11.) and of the cutting surface (Figs. 5.8.-5.11.), where a small groove (gr) can be seen running from the inside edge. It is supposed that this groove channels the masticated wood particles backward into the pharynx.

The inner surface of a tunnel in wood bored by a larva was also observed with SEM (Fig. 5.12.). In this photograph numerous gouge marks are seen carved parallelly and regularly. The width of these marks is ca. 30-40 μ m, and when this width is compared with Figs. 5.9.-5.11., this tunnel appears to be bored by a middle instar larva (Fig. 5.10.).

The structure of the whole mandible has been described by several authors, and three main "appendages" or "projecting structures" are recognized (Figs. 5.7.-5.8.). One is the "pseudomola" (pm) (Fig. 5.13.), first described in this species by Altson^{a4)}. It has been reported that it owns "brush-like setae" on its posterior side, and its medial surface is serrate^{p5)}. The SEM revealed that the brush-like setae and the serration are of the same nature, the serration being the insertion points of setae (Fig. 5.14.). Altson^{a4)} ascribed a crushing function to the pseudomolae, under the name of "crushing organs", in L. brunneus, as did Kojima^{k9, k10)} in L. linearis. However, this



Figs. 5.1.-5.2. Facial views of larval heads.
 5.1.: 1st instar larva.
 5.2.: Middle instar larva.



Figs. 5.3.-5.4. Dorsal views of larval heads dissected out.
 5.3.: Middle instar larva.
 5.4.: Mature larva, some contaminations attached.

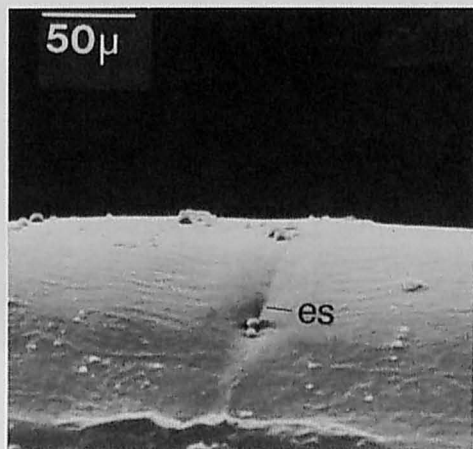
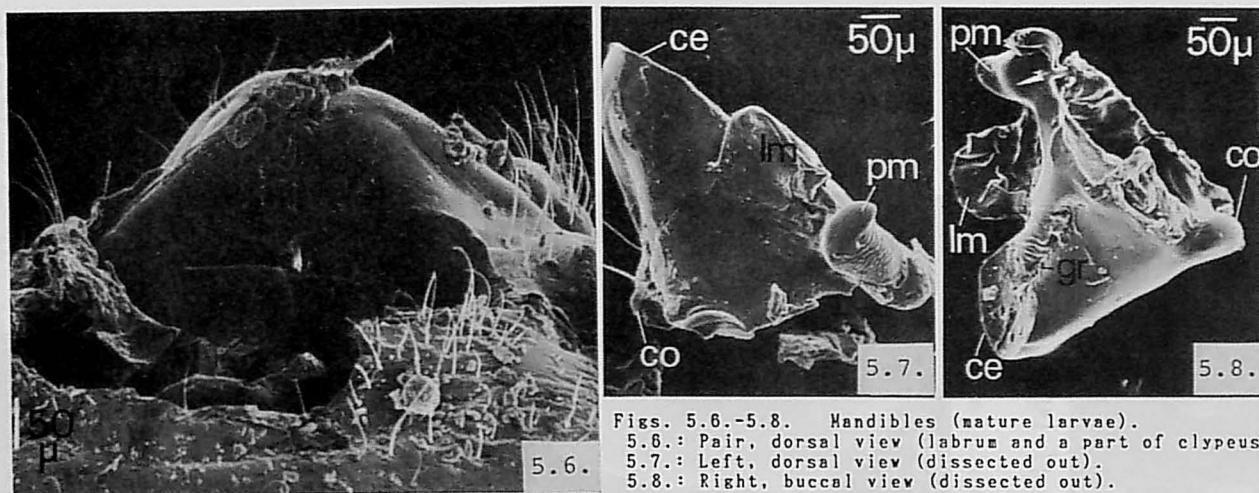
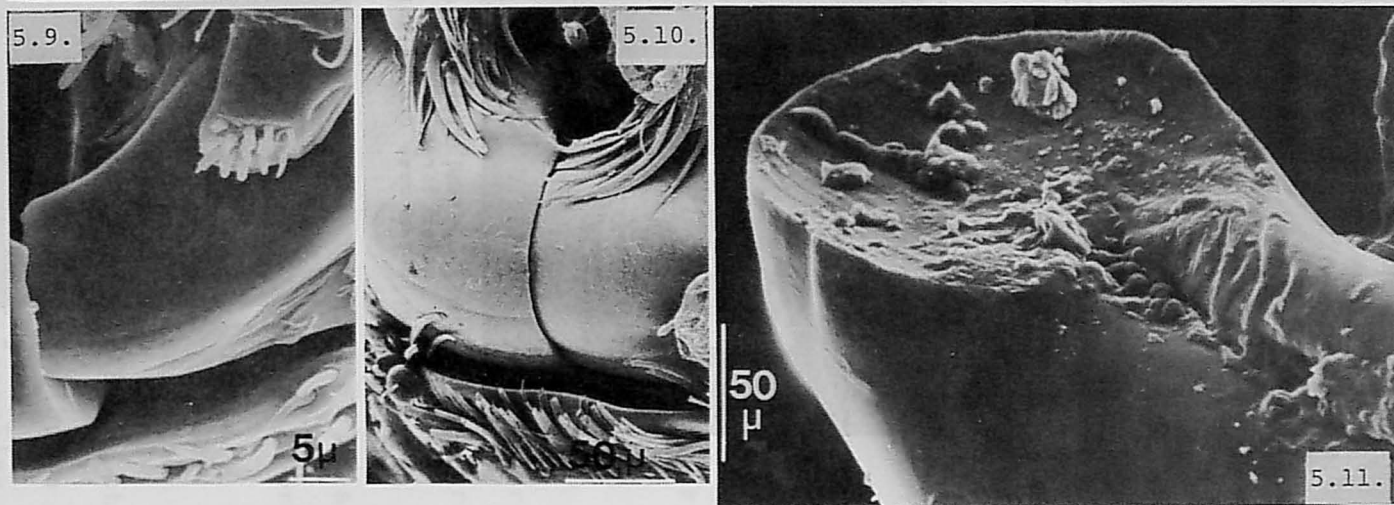


Fig. 5.5. Epicranial suture in hindmost area (mature larva, head dissected out).



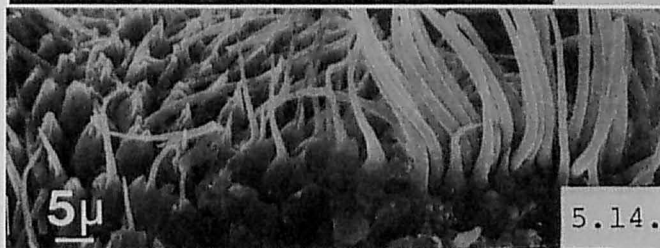
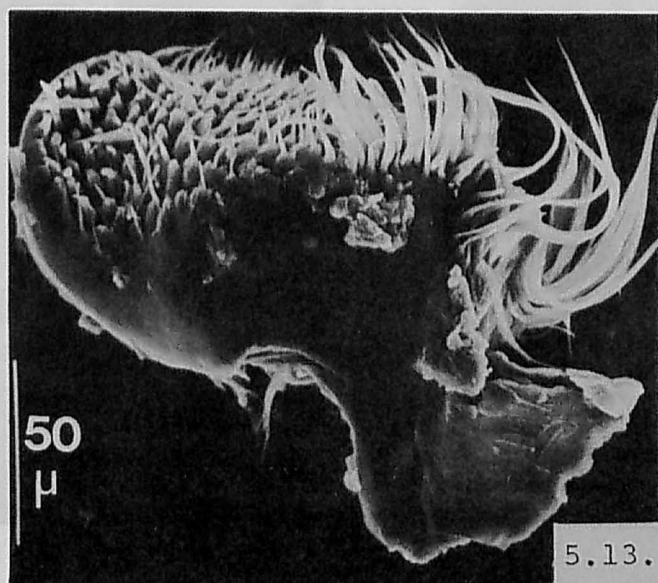
Figs. 5.6.-5.8. Mandibles (mature larvae).
 5.6.: Pair, dorsal view (labrum and a part of clypeus removed).
 5.7.: Left, dorsal view (dissected out).
 5.8.: Right, buccal view (dissected out).



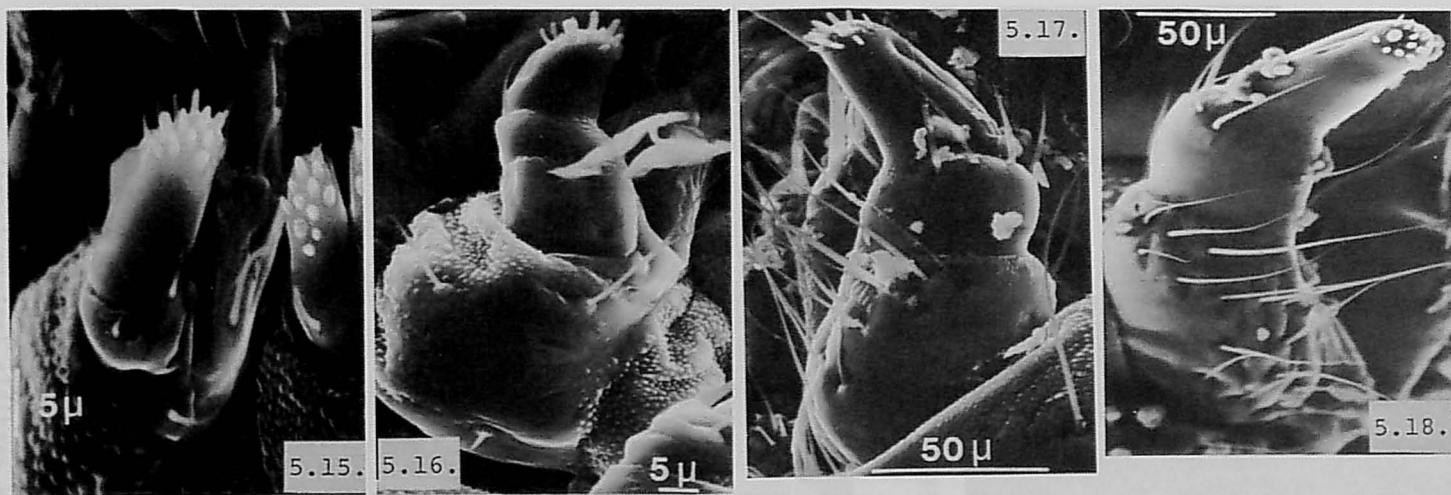
Figs. 5.9.-5.11. Cutting edges of mandibles.
 5.9.: 2nd instar larva.
 5.10.: Middle instar larva.
 5.11.: Mature larva (mandibles dissected out, showing inside groove).



Fig. 5.12. Surface of a tunnel in *Quercus serrata* wood bored by a larva of *L. brunneus*.



Figs. 5.13.-5.14. "Pseudomola" of the mandible (mature larva).
 5.13.: General view.
 5.14.: Surface structure.



Figs. 5.15.-5.18. Maxillae.
5.15.: 1st instar larva.
5.16.: 2nd instar larva.
5.17.: 4th instar larva.
5.18.: 5th instar larva.



Figs. 5.19.-5.21. Maxillary palpi and the sensory papillae.
5.19.: Palpus (2nd instar larva).
5.20.: Palpus (middle instar larva).
5.21.: Papillae (middle instar larva. some contaminations attached).

does not appear likely from the present study, for it is doubtful that these setae on the serration could stand it; brushing or conveying function is more likely. Schmidt & Buchholz⁸⁶⁾ are of the same opinion since they observed that the pseudomolae contacted each other neither in open nor in close condition of the mandibular pair.

The second projecting structure is the "lacinia mandibulae" (lm) seen in Figs. 5.6.-5.8., which, in life, is a smooth, transparent, sac-like projection between the cutting edge and the pseudomola. It is very broadly attached to the dorsal side of the mandible (Fig. 5.7.), and the pair meet and slightly overlap above the mandible bodies (Fig. 5.6.). Whether this contact has something to do with their function, as discussed by Kojima^{k9, k10)} and by Schmidt & Buchholz⁸⁶⁾, is uncertain.

When in situ, the pseudomolae and laciniae mandibulae are enclosed in a vertical oral cavity which is formed by the clypeus, labrum and mandible bodies.

The third projecting structure of the mandible is the condyle (co) (Figs. 5.7.-5.8.), on whose insertion into the head the whole mandible hinges.

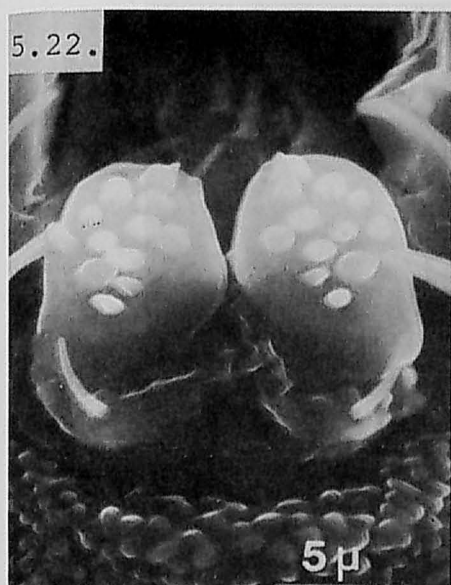
The maxillae are seen in position in Figs. 5.1.-5.2., lying just below the mandibles, while its change in the course of larval development is shown in Figs. 5.15.-5.18. The maxillary palpus (Figs. 5.19.-5.20.) has many papillae (Fig. 5.21.) supposedly with a sensory function. It was evident from the SEM work that the form of the palpus changes with age, being shorter and rather cubic in younger larvae (Figs. 5.15.-5.16. & 5.19.),

becoming longer and more cylindrical in later instars (Figs. 5.17.-5.18. & 5.20.). Altson⁴⁴⁾ thought it to be telescopic although it is doubtful that it could indeed be retracted in this way. The maxilla possesses a brush-like structure, which may be called the "mala" (Figs. 5.2. & 5.10.), probably with a brushing function. This structure is yet undeveloped in younger larvae (Figs. 5.1. & 5.15.).

The labium lies between the maxillae (Figs. 5.1.-5.2.), and Figs. 5.22.-5.23. show a magnified view of the whole structure. The labial palpi resemble the maxillary palpi not only in their form, but also in that they change their shape with age. Sensory papillae are clustered round their tips also (Fig. 5.24.). Between two labial palpi lies the ligula, furnished with its setae (Fig. 5.23.).

5.2.3. The antennae and the other cephalic structure

The larval antenna (Figs. 5.25.-5.30.) consists of 3 segments. In the larvae older than 2nd instar, the whole antenna is observed to be situated on the antacoria (basal articulating membrane), the 2nd and the 3rd segments being fused to form a chair shape (Figs. 5.25. & 5.27.-5.30.). The antenna is furnished with two major projections, some other minor organs and a large complex pit, all of which are probably sensory organs; the "1st projection" on the 2nd segment, parallel to the 3rd segment, and the others on the 3rd segment. In 1st instar larva, the articulations are obscure, while in 2nd instar larva the 1st segment is unrecognizable. The comparison of the past and the present



Figs. 5.22.-5.23. Change of labium in the course of larval development.

5.22.: 1st instar larva.

5.23.: 4th instar larva.

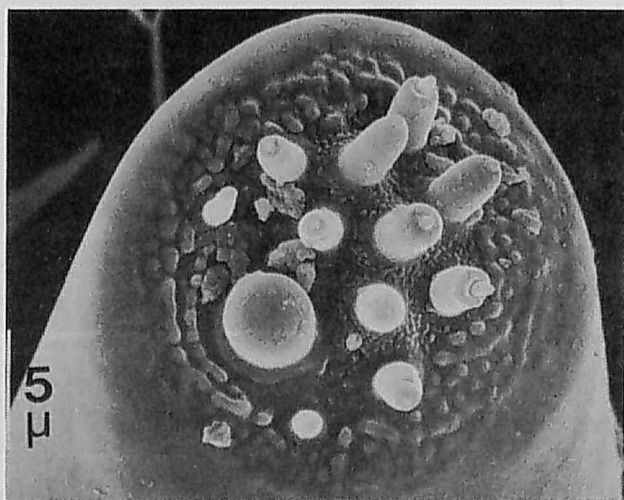
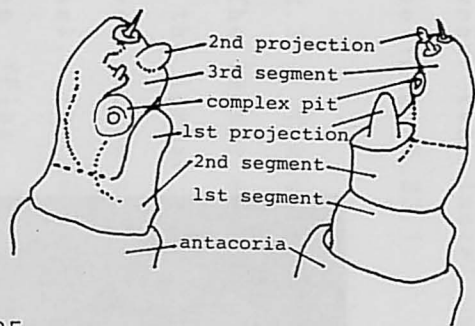
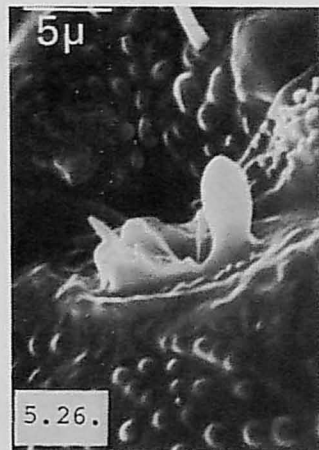


Fig. 5.24. Labial palpus and its sensory papillae (middle instar larva).



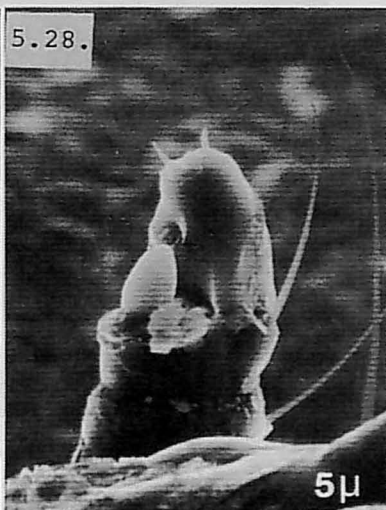
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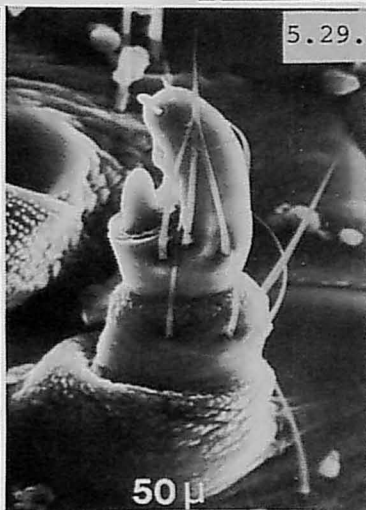
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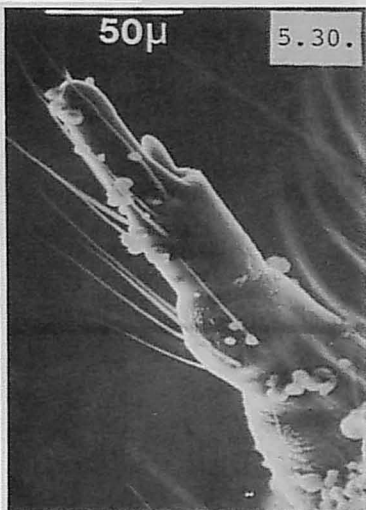
5.27.



5.28.



5.29.



5.30.

Figs. 5.25.-5.30. Antennae.
 5.25.: Schematic diagram.
 5.26.: 1st instar larva.
 5.27.: 2nd instar larva.
 5.28.: 3rd instar larva.
 5.29.: 4th instar larva.
 5.30.: 5th instar larva.

Table 5.1. Comparison of the nomenclature of the larval antennal parts of Lyctus (see also Fig. 5.25.)

Present study (<u>L. brunneus</u>)	Antacoria	1st segment	2nd segment	3rd segment	1st projection	2nd projection
Altson (1922b) (<u>L. brunneus</u>) (1st and 2nd instar)	----	Basal joint		Apical joint 1	Apical joint 2	Fleshy protuberance
Lesne (1924) (<u>L. brunneus</u>) (Aged larva)	----	1er article	2e article	3e article	Appendice du 2e article	----
Kojima (1932) (<u>L. linearis</u>)	(1st instar)	----	kurze Basis (1. Glied)		2. Glied	Nebenglied Fortsatz
	(mature)	1. Glied	2. Glied	3. Glied		4. Glied ----
Gardner (1933) (<u>Lyctus</u> in general)	Basal con- necting membrane	Segment 1	Segment 2	Segment 3	Accessory appendage	----
Pringle (1938) (<u>L. brunneus</u>)	Basal segment	2nd segment	3rd segment		4th segment	Sensory papilla
Kangas (1947) (<u>L. planicollis</u>)	(1)	"Fühler viergliedrig" (2) (3)			(4) ----	----

nomenclature of the larval antennal parts is summarized in Table 5.1.

The larval antenna is transformed markedly in the course of development, the most obvious change being in the length of the "1st projection" relatively to the 3rd segment; the former decreases with each succeeding instar. In the antenna of 1st instar larva the 1st projection is most developed relatively to the whole structure (Fig. 5.26.), as noted and accurately illustrated by Altson^{a4)}. And in the mature larva, it is reduced to a shorter projection (Fig. 5.30.). The overall length of the antenna compared with its diameter also increases with age of the larva.

Figure 5.31. presents a high magnification of the tip of the 3rd segment of the antenna, revealing its array of sensory papilla and setae. Another supposed sensory device is clearly visible in Figs. 5.27.-5.28., comprising a complex pit on the side of the 3rd segment.

When Dugès^{d1)} described the larva of L. planicollis, he noted that the frons "bears on each side a smooth brown 'mamelon', which is round and free anteriorly and cornered posteriorly on its adherent side", and that "immediately outside from this organ is found a short antenna". Dugès^{d1)} assumed this to be "a simple eye". Altson^{a4)} confirmed the presence of larval eyes, describing it as "a pair of rudimentary eyes composed of pigmented spots and situated below and posterior to the antenna". He found them to be "present in all larval stages of L. brunneus, being "most clearly defined in the 1st and 2nd instars", and

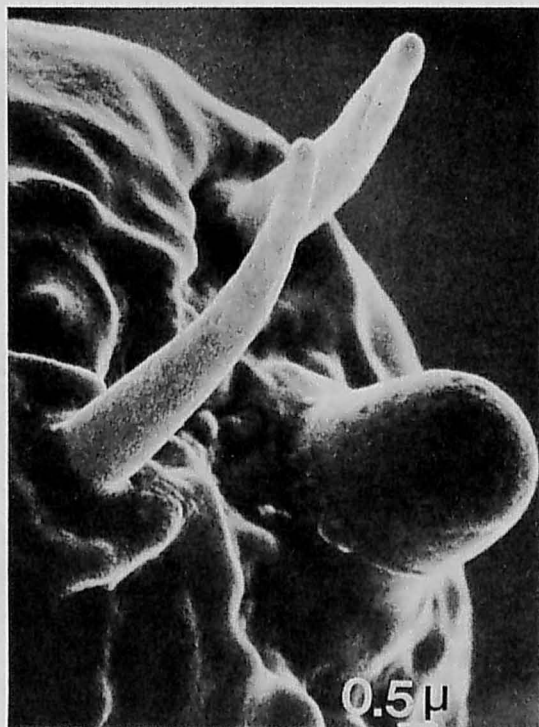
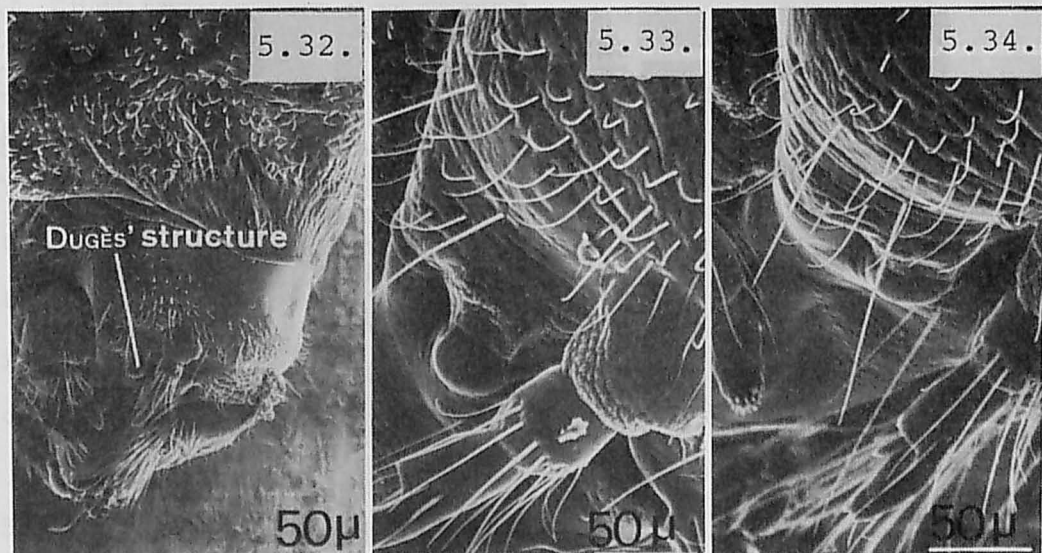
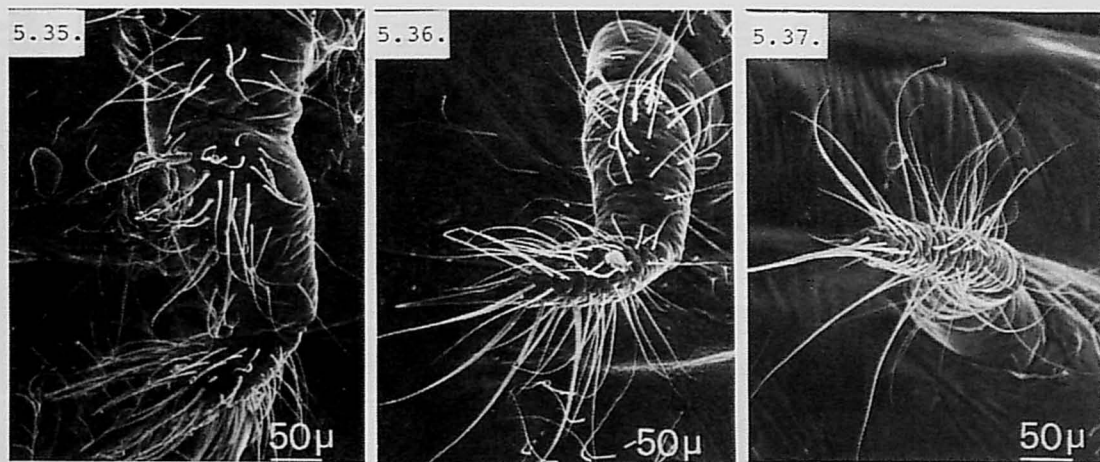


Fig. 5.31. The tip of the 3rd segment of the antenna furnished with sensory organs (middle instar larva).



Figs. 5.32.-5.34. "Dugès' structure" (middle instar larva).
 5.32.: Its situation on the gena.
 5.33.-5.34.: Views from different angles.

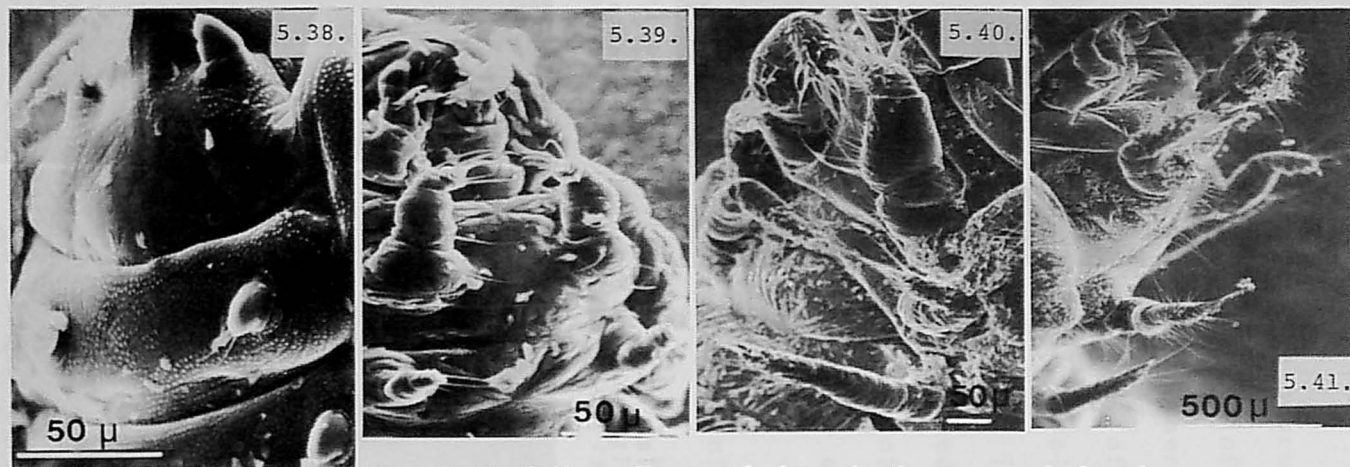


Figs. 5.35.-5.37. Legs (mature larva).

5.35.: Right fore leg.

5.36.: Right mid leg.

5.37.: Right hind leg.



Figs. 5.38.-5.41. Change of legs in the course of larval development.

5.38.: 1st instar larva.

5.39.: 2nd instar larva.

5.40.: 4th instar larva.

5.41.: 5th instar larva.

ascribed a light sensitive role to them, to warn the larva of its approach to the wood surface^{83, 84}). However, it is greatly noteworthy that there is a difference between the positions of Altson's eye in the young larva of L. brunneus and Dugès' structure in L. planicollis.

In the present investigation it was found that Dugès' description of them in L. planicollis was identical with their form in L. brunneus. From an ontogenetic point of view, what Altson observed is supposed to be the real eyes because they are clearly defined in the young larvae, while Dugès' structure does not become less remarkable in later instars contrarily, and consequently did not prove to be a rudimentary eye. On the other hand, Altson's rudimentary eyes are not detected in situ by SEM. This indicates that these eyes are completely embedded beneath the integument and thus have no surface structure. Further, Lesne¹⁴) found no ocelli in the "aged larva".

Figures 5.32.-5.34. show Dugès' structure ("mamelon") in a L. brunneus larva, situated just below the antenna near the insertion of the mandibular condyle. Due to the monochromatic nature of SEM it was first thought to be part of the strongly rounded structure of the condyle insertion, but this mamelon was optically observed to be sclerotized to some extent, and is now supposed to be a kind of muscular or integumentary protuberance.

5.2.4. The thorax

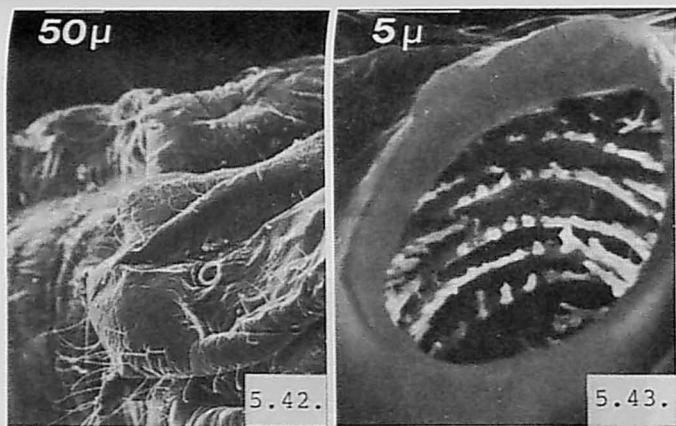
Each thoracic segment bear a pair of legs (Figs. 5.35.-5.37.), which, in life, show "continuous rhythmic motion" together with

maxillary palpi and mandibles to convey scattered wood shreds backward⁸⁶⁾. The fore legs are distinctly stouter than the others^{14, 81)}. Each leg lacks any articulate tarsus and terminates in a single claw of distinctive shape. Kurir¹⁷⁾ described the changes of the segmentations of the fore leg (2 to 3) and the mid and hind legs (1 to 2) between the 2nd instar and mature larvae of L. planicollis. The legs of L. brunneus larvae also changes with age: in the 1st instar they are merely papilla-like structures, while in older stages they become more slender, furnished with longer and more distinct claw and larger number of setae, and composed of more segments than in younger stages. Their morphological changes are clearly seen in a series of photographs of succeeding instars (Figs. 5.38.-5.41.).

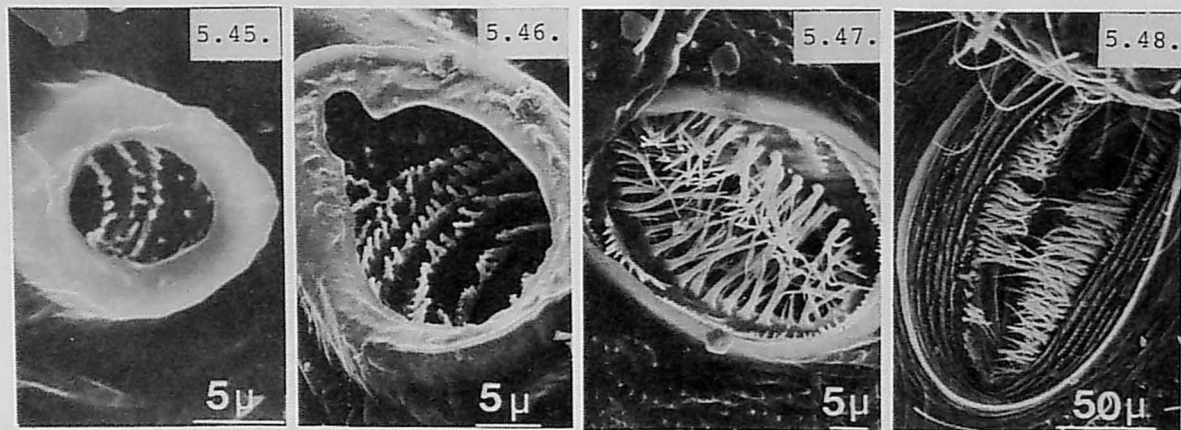
Only one pair of spiracles are found on the thorax (Figs. 5.42.-5.43.), each being found on the lateral side of the prothorax as described by Munro^{m9)}, Altson^{a4)} and others.

5.2.5. The abdomen

The abdomen consists of 10 segments, with spiracles present on the lateral side of urotergites of the 1st to 8th segments, as has been described by Munro^{m9)}, Altson^{a4)}, and others. This arrangement of 9 pairs (together with the thoracic spiracles) is the commonest found among the Insecta. It is a distinguishing feature of lyctid larvae that the 8th abdominal, i.e. the posteriormost, pair are very large relatively to others, and are easily visible by the naked eye^{m9, p5)} (Fig. 5.44.). The others vary in diameter to some extent, and Gardner⁸¹⁾ pointed out that



Figs. 5.42.-5.43.: Thoracic spiracle (2nd instar larva). Fig. 5.44. The abdominal 6th-8th segments and their spiracles (2nd instar larva).
 5.42.: Its situation on the pronotum.
 5.43.: Spiracle with undeveloped spines.



Figs. 5.45.-5.48. Abdominal spiracles.
 5.45.: The 7th segment (2nd instar larva).
 5.46.: The 7th segment (middle instar larva).
 5.47.: The 8th segment (2nd instar larva).
 5.48.: The 8th segment (mature larva).

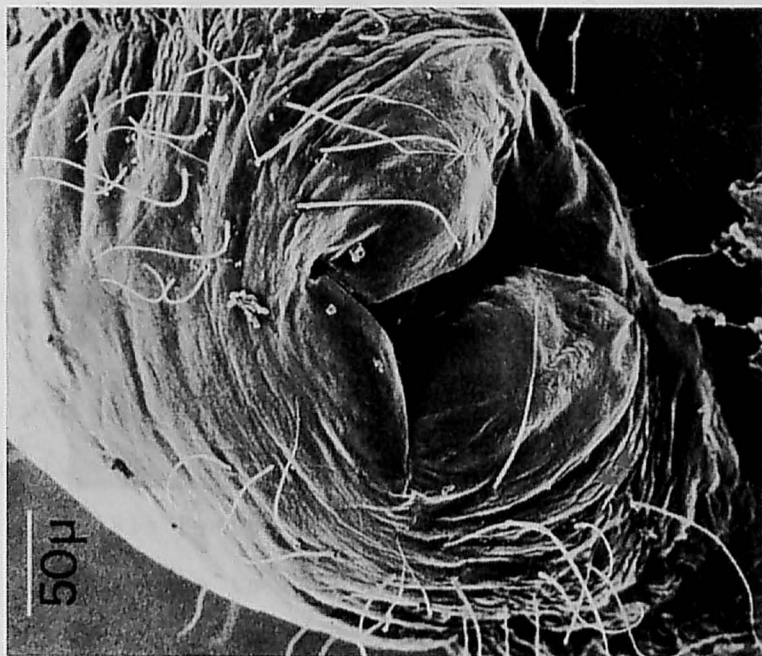


Fig. 5.49. Anus (middle instar larva).

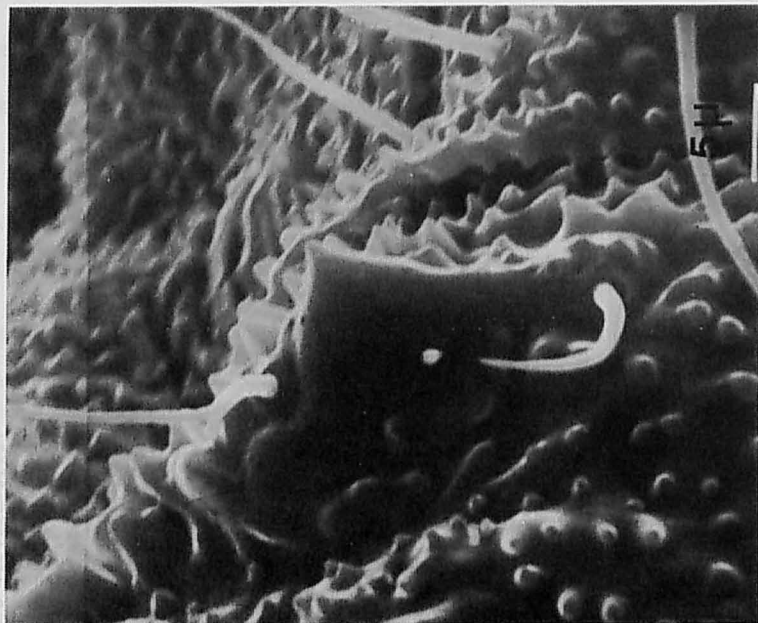


Fig. 5.50. Chitinous process in the 9th abdominal segment (1st instar larva).

the 5th abdominal spiracle is the smallest. Typical measurements of the spiracle diameters in a mature larva are: the 8th abdominal 200 μ m, the thoracic 100 μ m, the rest ca. 60 μ m. Further, variation of spiracular form is found with instar, particularly in the number of spines found within them; younger larvae (Figs. 5.45. & 5.47.) have fewer spines along the interior ridges than older larvae (Figs. 5.46. & 5.48). Since fragments of wood were frequently observed caught in the spiracles by these outward facing spines, they clearly serve a protective role, preventing the ingress of potentially obstructing material.

Another obvious structure of the abdomen to note is the anus, a feature which Pringle^{p5)} described as a "longitudinal slit". However, seen from the photograph (Fig. 5.49.), it proved to be more complex than this, with an arrangement of interlocking folds.

The last abdominal structure to note is the "chitinous process" on the 9th abdominal segment of 1st instar larva (Fig. 5.50.), clearly described by Kojima^{k9)} in L. linearis. Since it disappears in later instars (Fig. 5.49), it may serve as an "egg-tooth" for breaking open the chorion at hatching, as Kojima^{k9)} pointed out. Altson^{a4)} described "several large setae which act as 'hatching spines'". The relation between these setae and the above-mentioned "chitinous process" is uncertain.

5.3. The prepupa^{B)}

This stage is defined as the final phase of the final instar larva.

As the description of the prepupal stage of Lyctidae, only that of L. planicollis has been illustrated before by Wright^{W4)}. The prepupa of L. brunneus is essentially the same, being characterized by a straight body shape when compared to the feeding larva, and being less active when taken out.

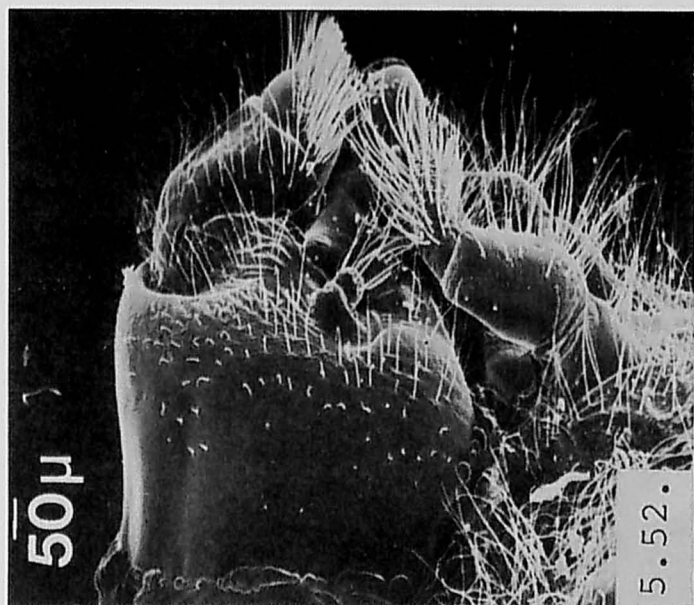
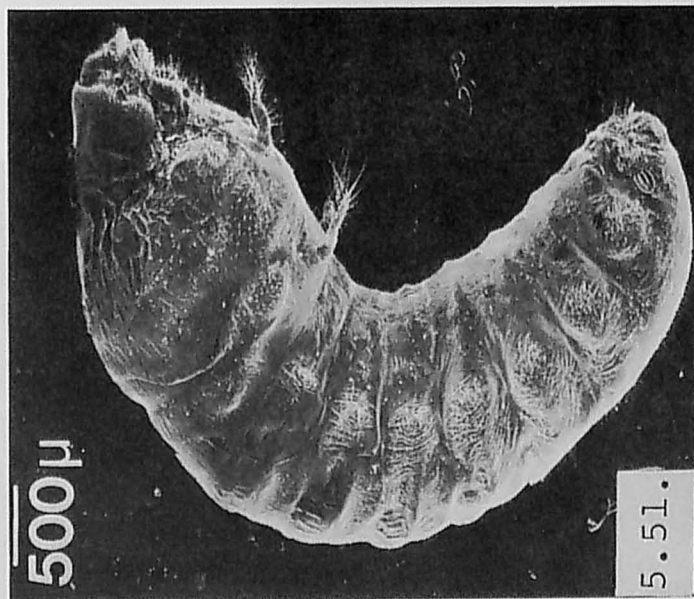
The SEM revealed that the detailed structure was identical with the mature larva, as might be expected (Figs. 5.51.-5.52.). Unfortunately it proved impossible to observe the transition from the mature larva to the prepupa, as full-grown larvae could not enter pupation or rather "prepupation" when removed from the diet or wood to an open dish, as stated in the section 3.2.4. Furthermore, it seemed very difficult to prepare the prepupa for SEM observation probably due to its pharate condition.

5.4. The pupa^{B)}

The whole body of the pupa is shown in Fig. 5.53. Till now Altson^{ab)} described and illustrated only the apex of the pupal abdomen in L. brunneus, and Pringle^{p5)} prepared a more general description. Hickin's illustration^{h4)} of it is not an accurate representation.

The pupal head (Fig. 5.54.) possesses developed antennae and eyes, the latter being sulcate longitudinally.

The mouth parts (Fig. 5.55.) show a transitional form between those of the larva and adult. The differences from those of the larva are as follows: i) Labrum curvate medianly and heart-shaped; ii) Mandibles thinner; cutting edge blunter; iii) Maxillary palpi more distinctly separate from stipes; iv) Prementum



Figs. 5.51.-5.52. Pupa.
5.51.: General view.
5.52.: Head.

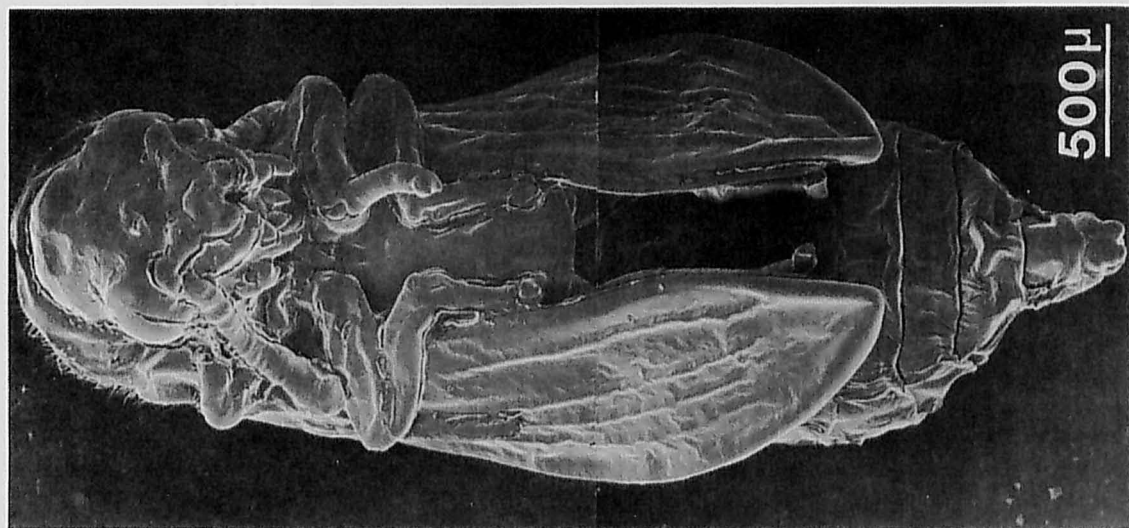


Fig. 5.53. Pupa (♀).

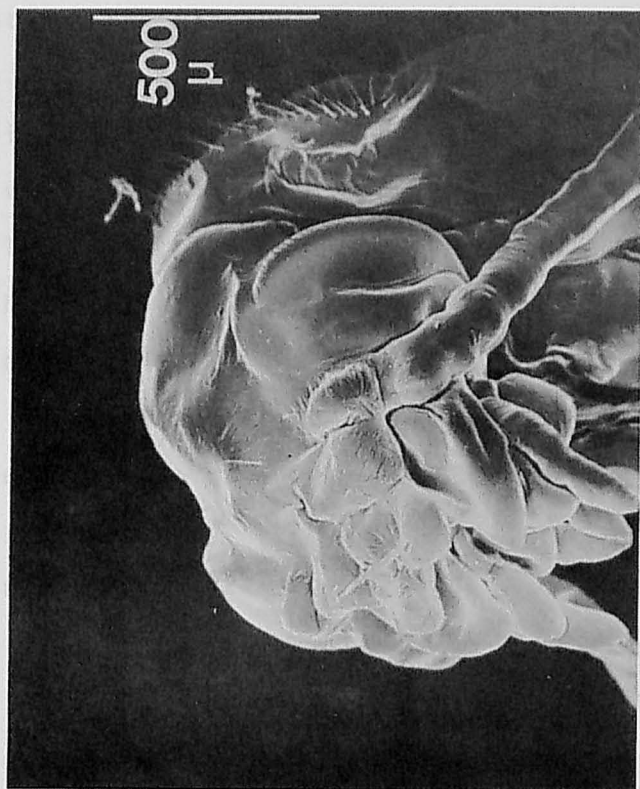


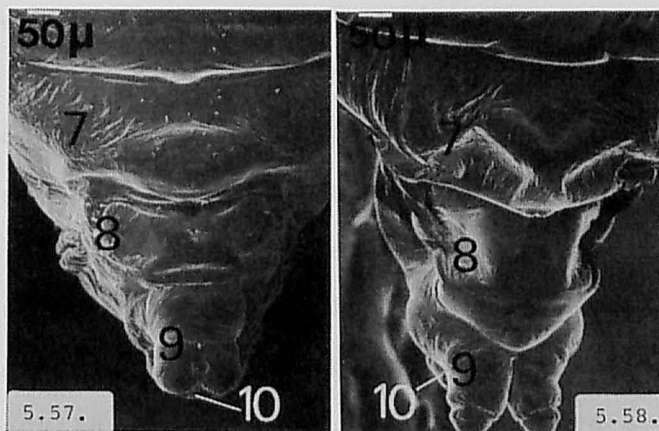
Fig. 5.54. Pupal head (♀).



Fig. 5.55. Pupal mouth parts (♀).



Fig. 5.56. End of pupal left fore leg (♂).



Figs. 5.57.-5.58. Ventral anal portion of pupae. 7, 8, 9 and 10 represent the 7th, 8th, 9th and 10th sternites respectively (see Table 5.2.).

5.57.: ♂.

5.58.: ♀.

Table 5.2. Sex characters in the last 4 sternites of L. brunneus pupa (see also Figs. 5.57.-5.58.)

Sternite	Male	Female
7th (5th)	Slightly elevated posteriorly	Conspicuously elevated in Y-shape
8th (6th)	Plane	Downward-arrow-shaped, extended on the next sternite
9th (7th)	Plane, with the distal portion divided in two parts, arranged parallel	Not plane, with the distal portion divided in two parts, each turning outside
10th (8th)	Ventrally hardly visible	Ventrally never visible

divided perpendicularly into two parts, from which the labial palpi rise, basally separate and distally occasionally contacted.

The pupal prothorax possesses "an anterior, transverse ridge fringed with setae"^{p5)} (Figs. 5.53.-5.54.).

The thoracic legs are free from the body, with the fore and mid legs exposed, while the hind legs are covered with the elytra, which curve round ventrally, so that only the distal part of the hind leg can be seen protruding from under the elytron^{p5)} (Fig. 5.53.). The end of each leg bears two swellings (Fig. 5.56.) which will become the hook-shaped claw of the adult.

The outline of the pupal abdomen is essentially the same as that of the adult beetle: the 1st and 2nd sternites are not visible, and the real 3rd, 4th,... sternites correspond to the visible 1st, 2nd,... sternites respectively²⁸⁾. Here, the terminology "3rd(1st) sternite", and so on is used in conformity with the description of adults in the section 5.5.3.

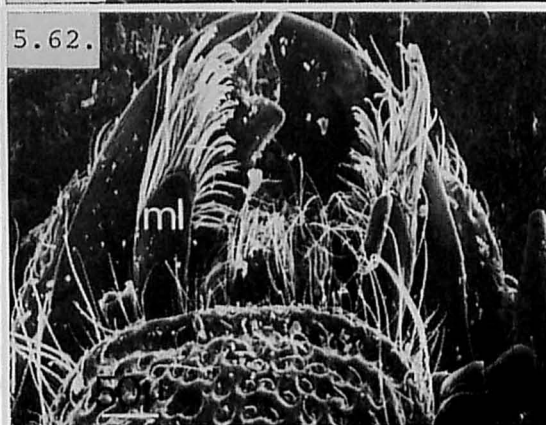
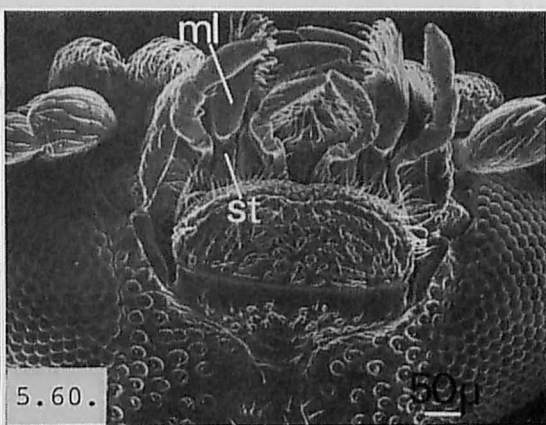
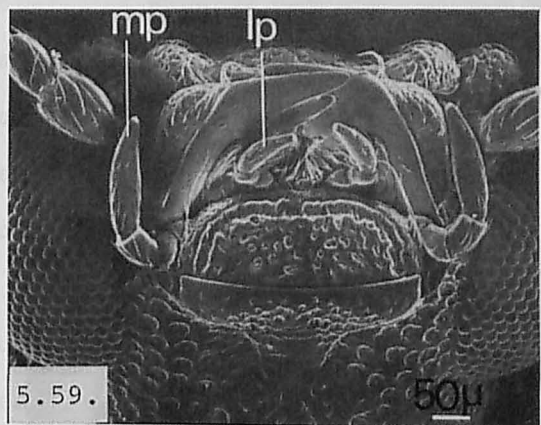
The 7th(5th) - 10th(8th) sternites show conspicuous sex characters; these are shown and described in Figs. 5.57.-5.58. and Table 5.2. The characters of the 7th(5th) sternite in both sexes correspond to those in adults, as presented in the section 5.5.3. These pupal sex characters, as observed optically, are somewhat difficult to detect because they are all in pupal white color. However, as observed with SEM (Figs. 5.57.-5.58.), they appear more conspicuous in the pupae than in adults. This is because, in adults, the 9th and 10th segments are differentiated into the genital organs which are hidden inside.

5.5. The adult^{c)}

5.5.1. The head and its appendages

The head structure of the adult is not so well differentiated as to note peculiar features compared with those of some other coleopterous species. Figures 5.59.-5.62. show its ventral side, showing eyes and mouth parts. The pair of mandibles (mb) (Figs. 5.61.-5.62.) is asymmetrical; the right always comes forth and is distinctly sulcate on its ventral surface, thus being bidentate, while the left is more variable in shape in ventral view. A certain sex character on the mandible is discernible; in the males the outer edge of each mandible forms a blunt obtuse angle, while in the females it is simple^{[4, 62)}. The maxillary palpi (mp) and labial palpi (lp) are similar to each other in form though the former are longer than the latter, with the different segmentation (Figs. 5.59.-5.60.). The similarities extend to their tips, both having depressions at their extremes, crowded with many sensory papillae (Figs. 5.63.-5.64.). The maxillary stipes (st) possesses a markedly developed mala (ml) that bears brush-like setae, probably composed of the galea and/or the lacinia (Fig. 5.60.).

Among the family Lyctidae, particularly in the genus Lyctus, it is common to find that the mentum exhibits a secondary sex character, i.e. a fringe of setae markedly longer in the males than in the females, a character that was first noted in L. planicollis by Lesne^{[3)}. Parkin^{[1)}, however, thought that L. brunneus was exceptional in not having this difference between the sexes, and even Lesne^{[4)} himself did not mention this char-



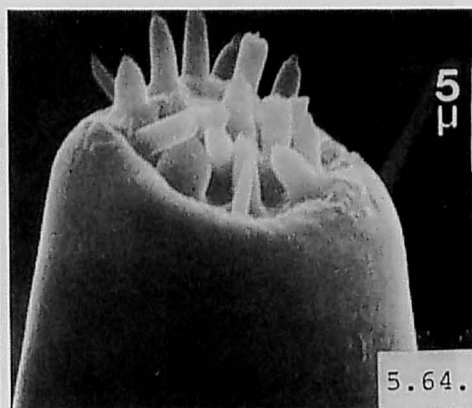
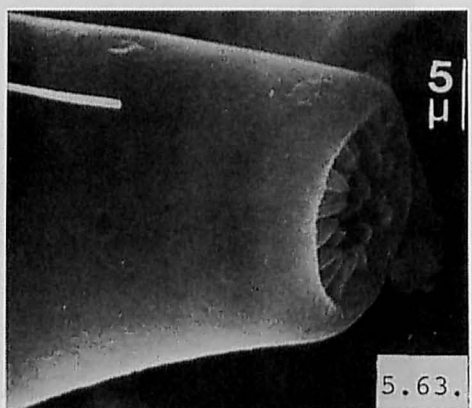
Figs. 5.59.-5.62. Ventral side of the adult heads.

5.59.: ♂.

5.60.: ♀.

5.61.: ♂, palpi removed.

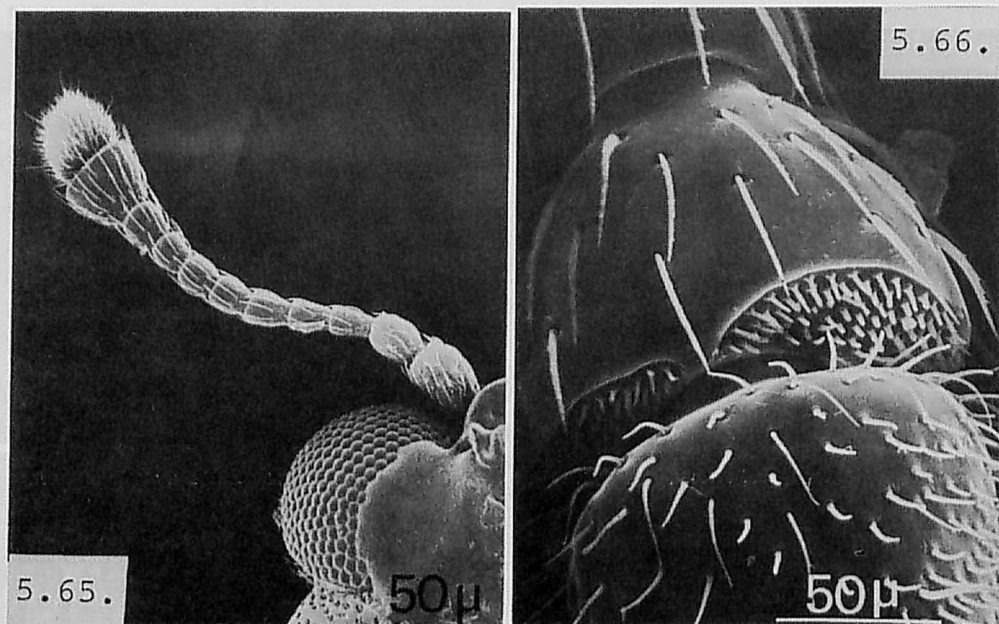
5.62.: ♀, palpi removed.



Figs. 5.63.-5.64. Apices of the adult maxillary and labial palpi (♂).

5.63.: Right maxillary palpus.

5.64.: Right labial palpus.



Figs. 5.65.-5.66. Adult left antenna (♀).
 5.65.: Whole view.
 5.66.: Sensory organ (?) on the penultimate segment.

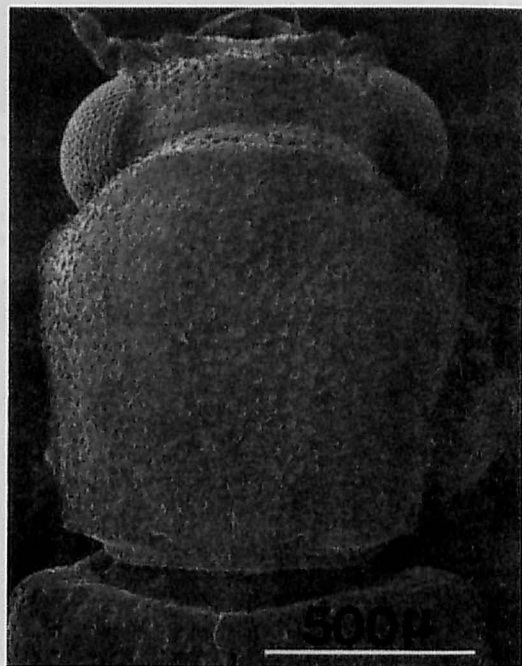
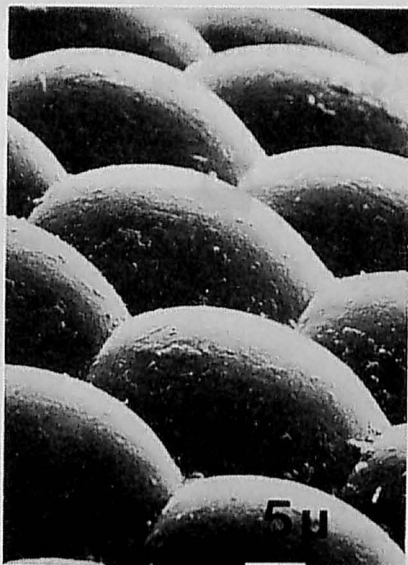


Fig. 5.67. Adult left compound eye (♀). Fig. 5.68. Adult pronotum (♀).

acter in redescribing L. brunneus. With SEM the setae on the lateral edges of the mentum (me) (Figs. 5.59.-5.62.) are observed to be variable among individuals of L. brunneus though their length is apt to be reduced in females. These long setae in both sexes sometimes fall off due to some accidents.

Lesne⁽⁴⁾ and Chûjô⁽²⁾ described the sex character in the protuberances on the each lateral side of epistoma. However, as seen in Figs. 5.59.-5.60., there seems no definite difference between two sexes in this part.

The antennae are identical in males and females, consisting of visible 11 segments, the two terminal segments being enlarged to form a distinct club (Fig. 5.65.). The penultimate segment has a characteristic structure, with the flat surface of the distal end facing the base of the ultimate segment, and being crowded with short papillae, as is clearly seen in Fig. 5.66. This aspect suggests that the sensory function of this plane probably depends upon the club being deformed by touching some object so that the ultimate segment contacts the plane. The shape of the club, which is composed of the terminal two segments, is a specific character in classifying the family Lyctidae.

The very well developed compound eye (Fig. 5.65.) consists of many hexagonal ommatidia (Fig. 5.67.) and its large size and conspicuousness are the characters of Lyctidae.

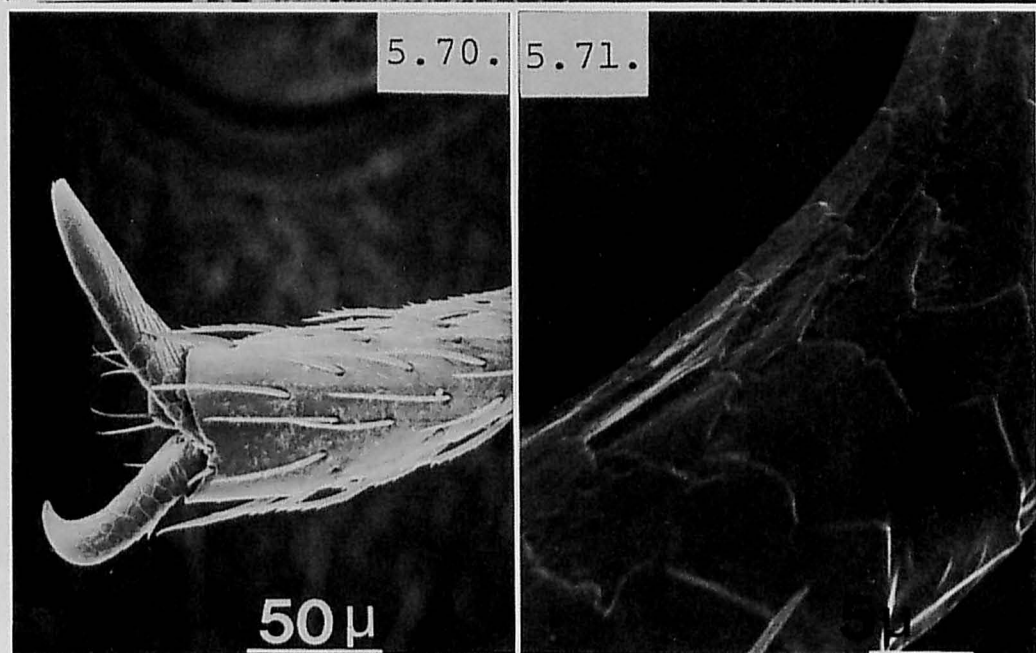
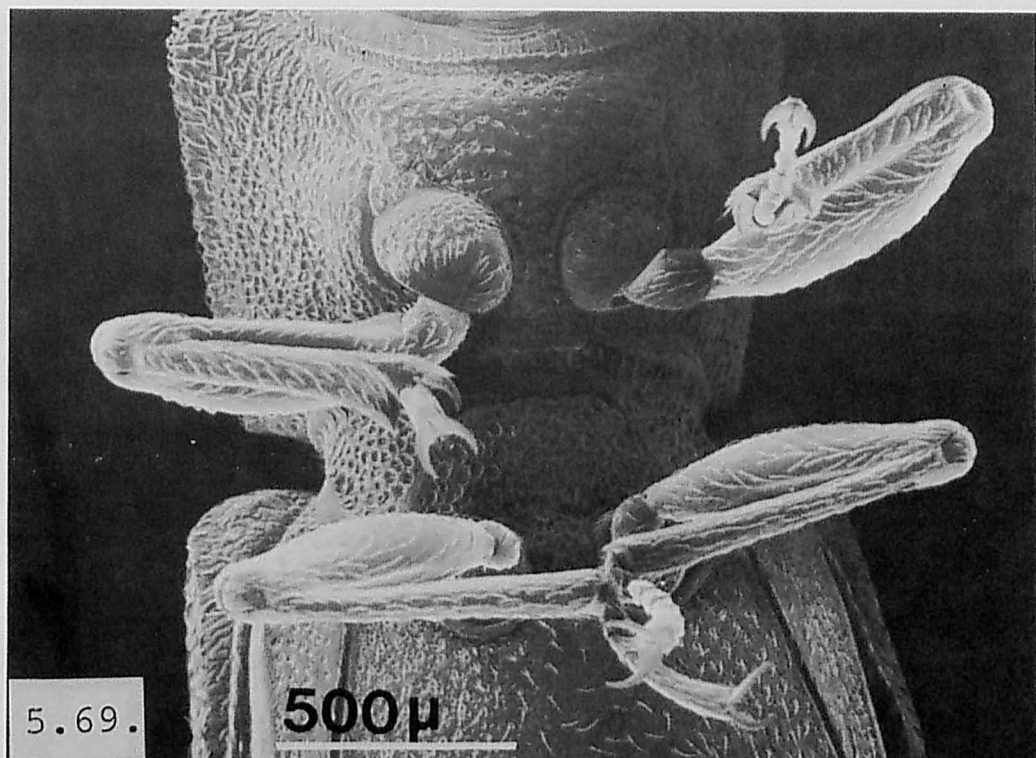
5.5.2. The thorax and its appendages

The shape of the pronotum (Fig. 5.68.) is a specific character in classifying the family Lyctidae, as was used by Kraus (et

all)^{k12)}. Mori^{m8)} stated that the pronotum has one shallow but rather broad groove running lengthwise on the median line, and there is some individual variation according to geographic source; in the European specimens, the groove has two anteriorly diverging lateral branches, and thus appears Y-shaped rather distinctly, while in Japanese specimens this Y-shape is indistinct. The specimens examined in this study mostly belonged to the typical "Japanese type", while the specimens described by Gerberg^{g4)} obviously belonged to the "European type". Lesne^{l4)} and Chûjô^{c2)} also described the existence of the two forms as an individual variation. Greater number of specimens will need to be examined, from a variety of sources, to decide whether this is indeed a geographic variation or not.

The legs of L. brunneus adult (Fig. 5.69.) show little specialization though the tibial spur on the fore leg is prominent^{g4)}, and the femur of the fore leg is thickened compared to the other legs^{c2)}. The aspect of the apical claw (Fig. 5.70.) explains why this beetle is unable to climb vertical glass surface: the leg lacks the adhesive pad. The photograph at high magnification (Fig. 5.71.) shows the scalified surface structure of the apical claw.

The surface of each elytron is thickly furnished with punctations and setae. Figure 5.72. shows that the punctation consists of quite deep, flat-bottomed pit, while the setae arise from shallow depressions among the pits. These punctations and setae were utilized as the specific characters within the family Lyctidae by Hickin^{h4)} and Santoro^{s1)}, and the latter classified the



Figs. 5.69.-5.71. Adult legs (♀).

5.69.: Fore and mid legs.

5.70.: Tarsal claw of the right fore leg.

5.71.: Tarsal claw of the left fore leg, magnified.

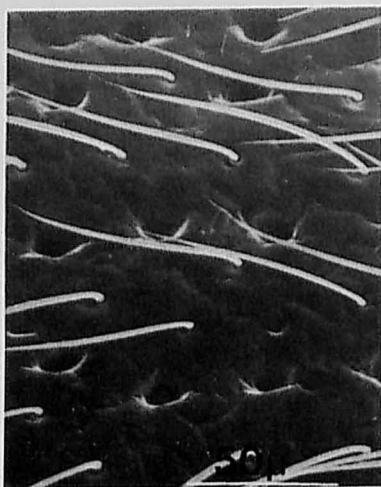
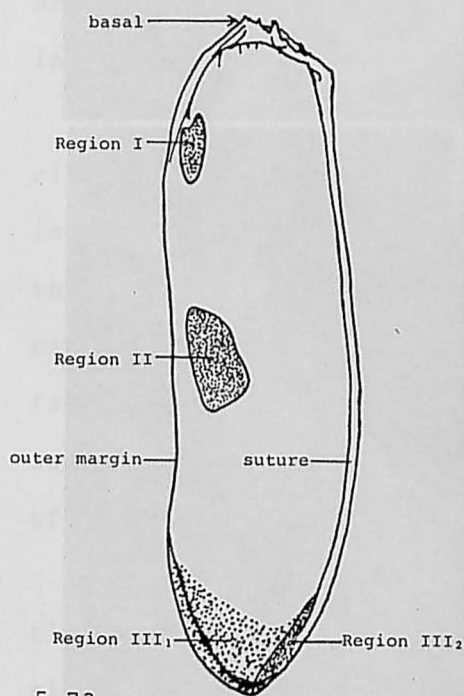


Fig. 5.72. Surface of the median portion of the left elytron (♂).



5.73.



5.74.



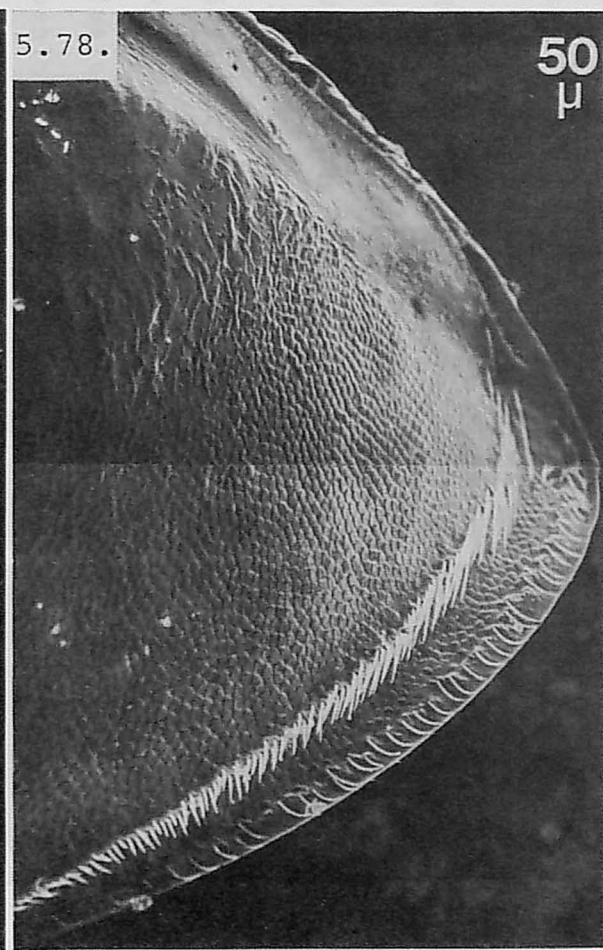
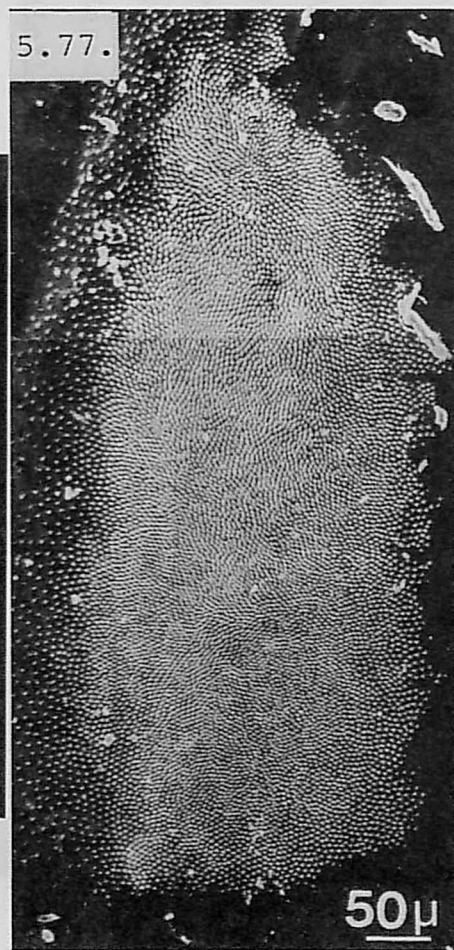
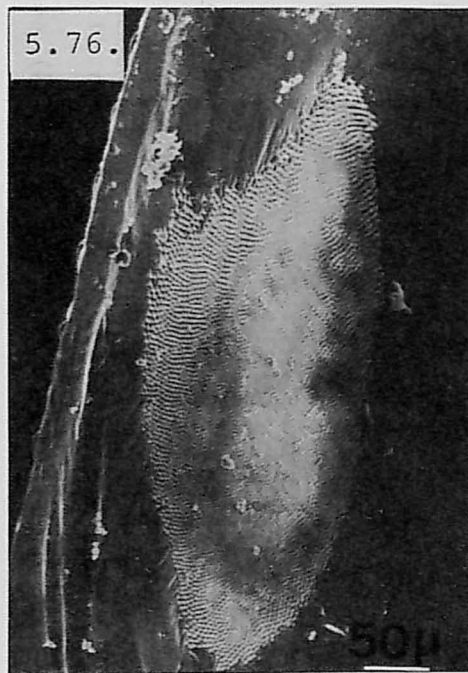
5.75.

Figs. 5.73.-5.75. Underside of the right elytra.

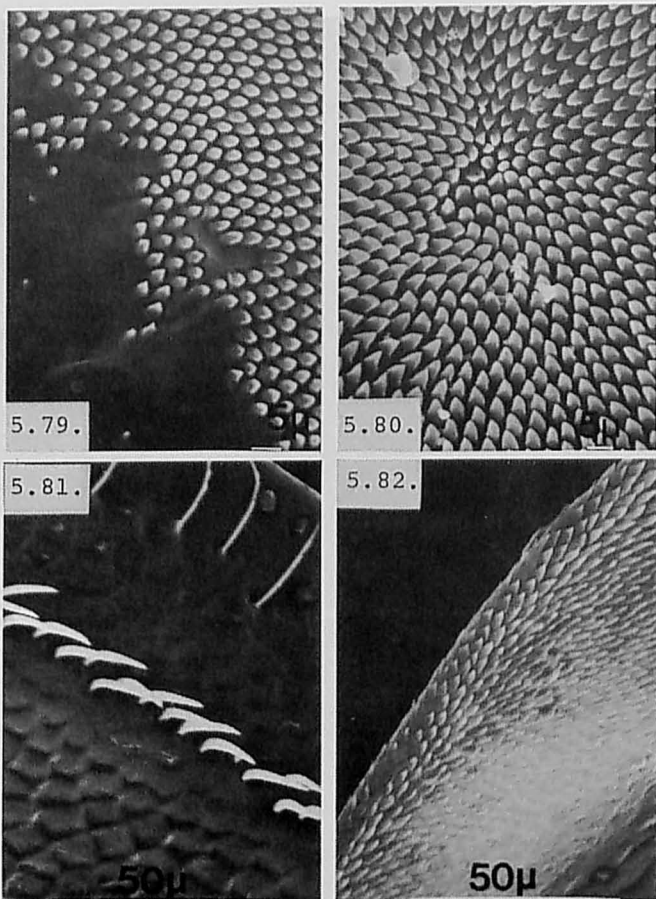
5.73.: Schematic diagram.

5.74.: ♂.

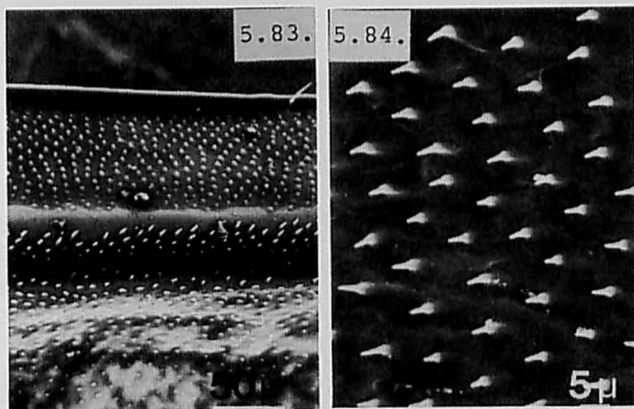
5.75.: ♀.



Figs. 5.76.-5.78. Rasp-like patches on the underside of the right elytron (♀).
5.76.: Region I.
5.77.: Region II.
5.78.: Region III.



Figs. 5.79.-5.82. Rasp-like patches on the underside of the right elytra, magnified.
 5.79.: Edge of Region II (δ).
 5.80.: Anterior vortex of Region II (δ).
 5.81.: Region III₁, elytral margin (δ).
 5.82.: Region III₂, elytral suture (δ).



Figs. 5.83.-5.84. Adult wings (δ).
 5.83.: Anterior margin of the right wing.
 5.84.: Discoidal area of the left wing.

elytron of L. brunneus as having the "stomata-shaped" rather than the "crater-shaped" punctations.

The elytral lusters, supposedly different in two sexes, as described by Lesne⁽⁴⁾ and Chûjô⁽²⁾, the males having more lustrous elytral surface than females, did not appear to be a reliable and definite sex character in the present study.

The undersides of the elytra in both sexes are shown in Figs. 5.73.-5.75. It possesses 3 regions of particular interest, which can be distinguished optically in having relatively low luster; the first one named "Region I" is situated a little behind the basal joint laterally, and the second one named "Region II" is situated mediolaterally. The SEM revealed each of these two regions to be a patch of fine chitinous "teeth", somewhat like a rasp (Figs. 5.76.-5.77. & 5.79.-5.80.). The posterior portion of the elytron, which was named "Region III", possesses a complex surface structure (Fig. 5.78.). The same kinds of surface structures on the underside of elytra were also found in some other species of Teredilia, viz. L. sinensis, Minthea rugicollis (Lyctidae), Dinoderus minutus (Bostrychidae) and Stegobium paniceum (Anobiidae). In L. brunneus, Region I is observed to be like an up-ended polished rice-grain in shape (Fig. 5.76.), and Region II to consist of an area more than twice of that of the former (Fig. 5.77.). The teeth of the anterior portion of Region II are arranged in a vortex pattern (Fig. 5.80.). Region III consists of two peculiar areas, the first (III₁) with scale-like or testudinal pattern away from the elytral suture (Fig. 5.81.), and the second (III₂) with dentate form

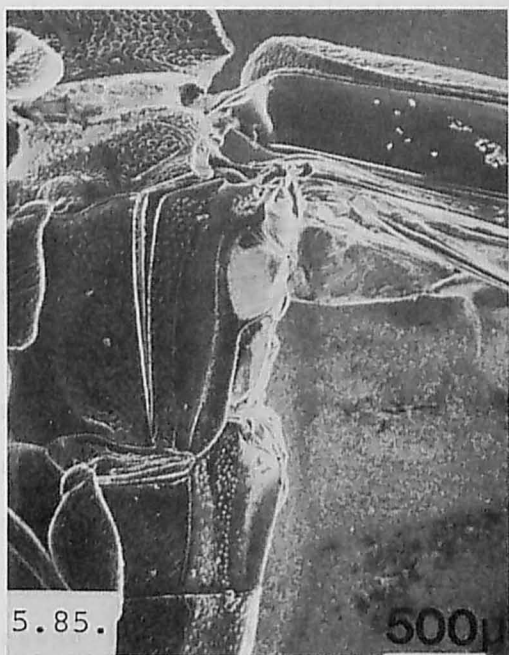
like Regions I and II near the elytral suture (Fig. 5.82.). Further, the elytron possesses a row of spines and that of setae on its underside near the lateral edge of Region III₁, as seen in Fig. 5.78. and at greater magnification in Fig. 5.81. The counterparts of these 3 regions are also found on the metathorax, the 3rd(1st) abdominal sternite and the 7th abdominal tergite, as described in the following of this section and in the section 5.5.3. The function of these 6 regions, or rather 12 regions in total as they are symmetrically arranged on the body, will be discussed in the section 5.7.

The wing of this species, which was once illustrated by Hickin^{h4)}, is furnished with very numerous spinulae all over the surface (Figs. 5.83.-5.84.).

Figure 5.85. shows the lateral side of metathorax, in which the metaepisternum seems exactly opposite to Region I of the underside of the elytron. And it also possesses a region of the rasp-like surface structure (Fig. 5.86.), which is similar to Region I. It is proposed to name it "Inferior Region I" as it comes just beneath Region I.

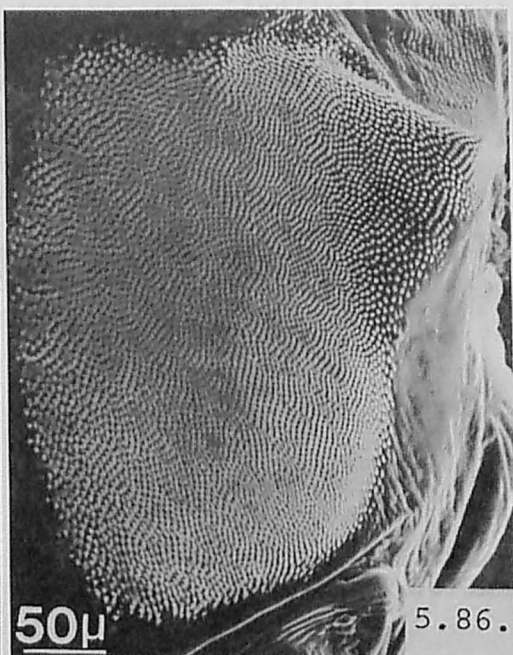
5.5.3. The abdomen

In the adults and pupae of many coleopterous species only five of the ten abdominal sternites are visible; the 1st and 2nd are reduced or hidden under the metasternum, the 8th has been differentiated to be the pygidial plate and the 9th and 10th to be the genital structures normally held within the abdomen. Thus the 1st "visible" sternite corresponds to be 3rd "real" sternite^{k12, a6)}



5.85.

500 μ



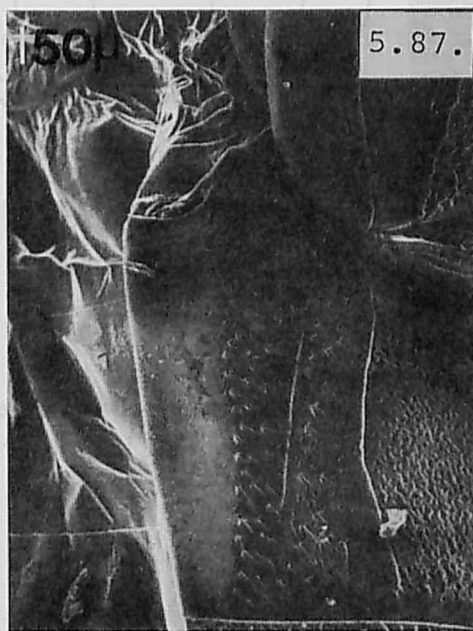
5.86.

50 μ

Figs. 5.85.-5.86. Lateral side of the adult metathorax (♀).

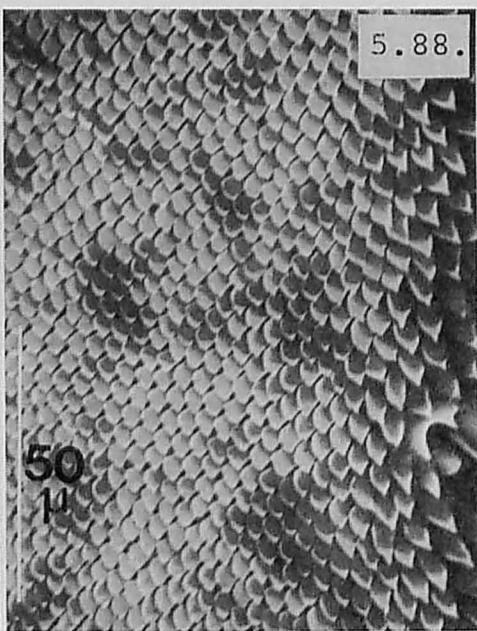
5.85.: Whole view.

5.86.: Rasp-like patch (Inferior Region I) on the metaepisternum.



5.87.

50 μ



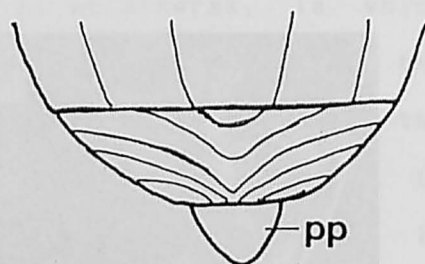
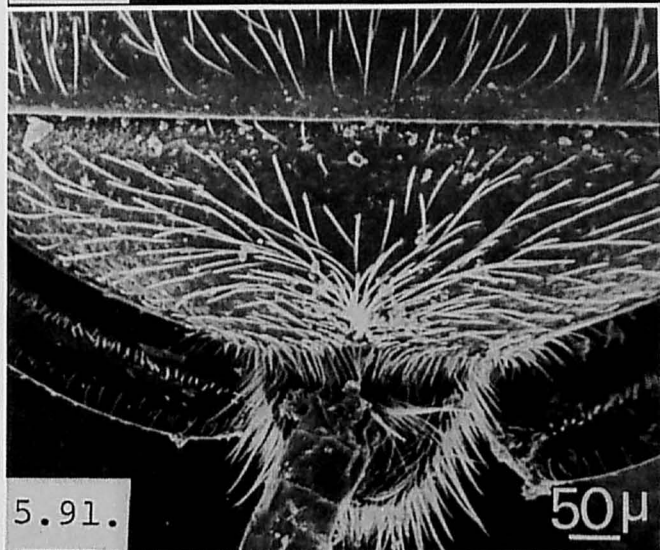
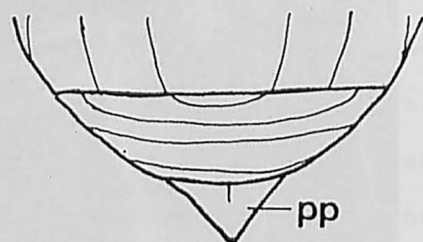
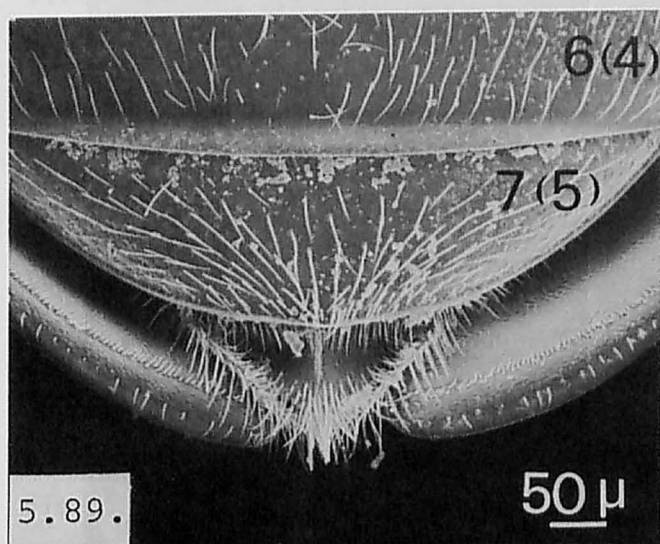
5.88.

50 μ

Figs. 5.87.-5.88. Lateral view of the adult abdominal 3rd(1st) sternite (♂).

5.87.: Rasp-like patch (Inferior Region II).

5.88.: Ditto, magnified.



Figs. 5.89.-5.92. Sex characters in the ventral anal portion of adults.

5.89.: ♂.

5.90.: ♂, schematic diagram with contours.

5.91.: ♀, with ovipositor extruded.

5.92.: ♀, schematic diagram with contours.

In the present investigation the terminology "3rd(1st) sternite", and so on is used.

On the side of the 3rd(1st) abdominal sternite, another rasp-like patch was found (Figs. 5.87.-5.88.), which was observed to be opposite to Region II, and following the convention established above (5.5.2.) it is called "Inferior Region II".

The shape of the 8th(6th) sternite, i.e. the pygidial plate (pp), is a sex character in this species^{a6, l4)}, as well as the shape of the 7th(5th) sternite accompanied by the arrangement of setae upon its surface^{l4, p1, r1, k15)}. The shape of the pygidial plate (Figs. 5.89.-5.92.) is only of limited use for sexing beetles because it normally retreats out of sight. On the other hand, the 7th(5th) sternite possesses the most reliable sex character in the adults of this species in conformity with that in the pupae, as described in the section 5.4.:

In the males (Figs. 5.89.-5.90.), this sternite has a relatively flat surface, bearing only a few weakly convergent setae. In the females (Figs. 5.91.-5.92.), on the other hand, the setae upon this sternite are much denser than in the males, arranged in a strongly convergent pattern toward the middle of the posterior margin. This is so marked that under low-powered optical microscope it often has an appearance of a "pencil" of setae on this sternite.

Each of the 1st to 7th abdominal tergites possesses a pair of spiracles (Fig. 5.93.) which are positioned laterally. The 1st is the largest of all the spiracles^{a6)}.

A pair of rasp-like patches was found out on the lateral parts

of the 7th tergite (Figs. 5.94.-5.95.), just facing Region III on the elytra. Following the convention above (see 5.5.2.), this is called "Inferior Region III".

All of the patches on the body, i.e. Inferior Regions I, II and III, also appear to be identical in both of the sexes.

In the last place, the morphology of the adult external genitalia is discussed as one of the most interesting aspects of the insect morphology.

The female external genitalia, i.e. the ovipositor, is highly differentiated, and made up by adaption of the 9th and 10th abdominal segments. According to Altson⁸⁶⁾, the ovipositor is stowed in the female body through a halfway evagination. After the oviposition, the ovipositor is often left extruded, and its full length amounts as long as the female's body. As was discussed in the section 2.1.2., once the ovipositor is fully extruded, the female loses its potentiality to oviposit. The ovipositor after the oviposition (Fig. 5.96.) often shows some false articulations, the appearance of which is due to telescoping like a sleeve of the clothes (Fig. 5.97.). The "apex" of the ovipositor is shown at high magnification in Figs. 5.98.-5.99. It consists, after the nomenclature by Altson⁸⁶⁾, of the 10th uromere, i.e. the double-jointed vaginal palps (vp), and a part of 9th uromere, i.e. basal piece (bp). It differs from the preceding portion of the ovipositor in having much smoother surface and in bearing several erect setae. The pair of vaginal palps homologizes with the pair of parameres of the male in having sensory pits as well⁸⁶⁾. The whole ovipositor is evident-

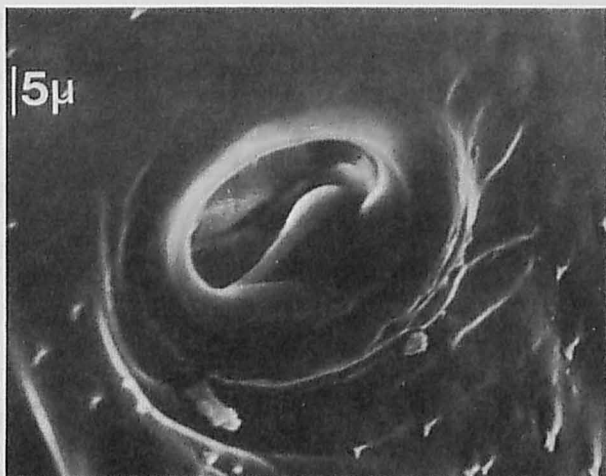
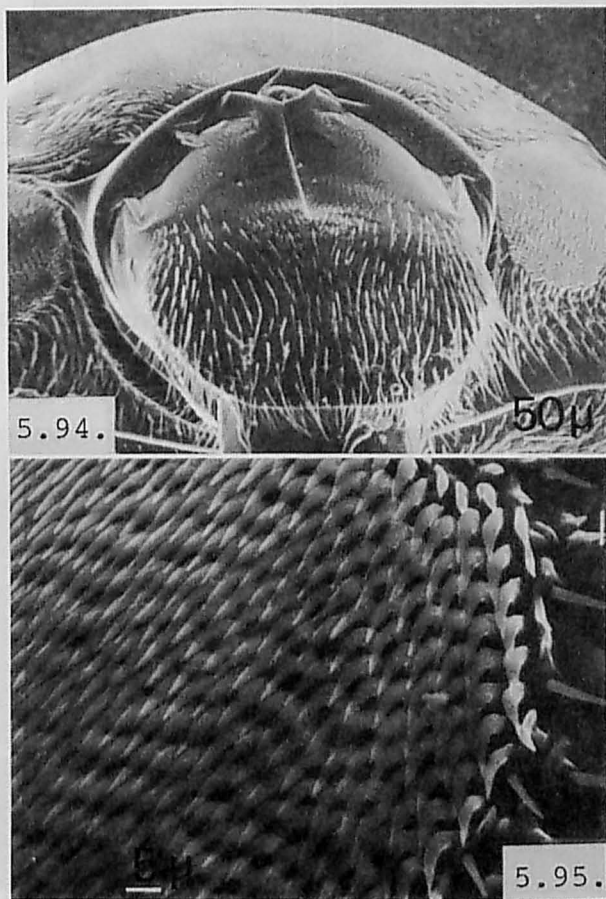


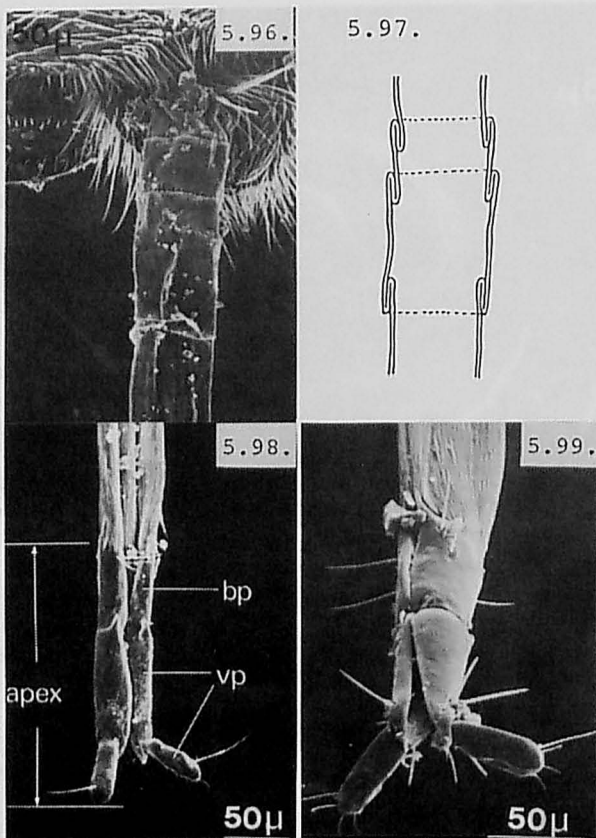
Fig. 5.93. Right spiracle of the adult 3rd abdominal segment (♀).



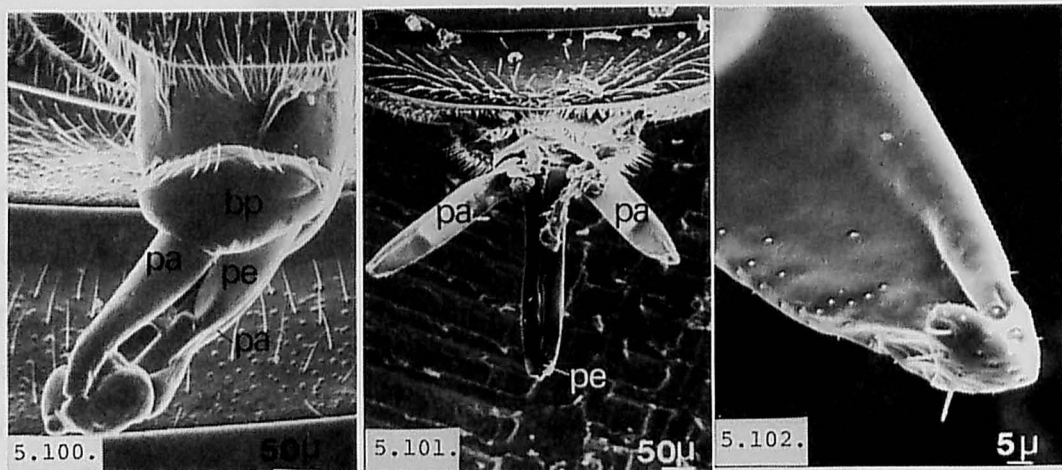
Figs. 5.94.-5.95. View of the adult anal portion (♂).

5.94.: 7th tergite and dorsal pygidial plate.

5.95.: Rasp-like patch of the 7th tergite (Inferior Region III).



Figs. 5.96.-5.99. Ovipositors.
 5.96.: False articulations after the oviposition.
 5.97.: Schematic diagram of telescoping for Fig. 5.96.
 5.98.: Dorsal view of the apex.
 5.99.: Ventral view of the apex.



Figs. 5.100.-5.102. Aedeagus.
 5.100.: Extruded sideways *in situ*.
 5.101.: Extruded and developed *in situ*.
 5.102.: Apex of the left paramere.

ly highly adapted for oviposition into wood vessels, being "of a suitably ingenious nature"⁽¹²⁾ for this purpose. The diameter of the ovipositor is about 90 μ m (Fig. 5.96.), which corresponds with the minimum vessel size of wood utilized for oviposition⁽¹⁾.

The male external genitalia (aedeagus) of this species (Figs. 5.100.-5.102.) has been described and illustrated by Altson⁽⁸⁾ and Cymorek⁽⁸⁾. It consists of a penis (pe), a pair of parameres (pa), and a basal piece (bp). Gerberg⁽⁴⁾ stated "differences between genera and species are subtle" with regard to the male external genitalia of the family Lyctidae. The basal piece is observed by SEM to possess two unidentified peculiar processes on each of its sides (Fig. 5.100.), and the apex of a paramere to bear a number of papillae situated within depressions, which were regarded as "sensory pits" by Altson⁽⁸⁾ (Fig. 5.102.).

5.6. Malformations of adults^{E,H)}

During the course of the present study of L. brunneus several types of corporal malformations have been encountered. The main source of them was the experimental cultures with the artificial diets containing no wood sawdust, as described in the section 2.3., but some have been found also from the routine mass culture. Most of the malformations encountered existed on the ventral abdominal segmentations. Such malformations are rarely but occasionally found in the course of sexing the adults according to the most reliable sex character on the ventral anal portion, which is described in the section 5.5.3. Malformations on the thoracic segmentations have also been found as well.

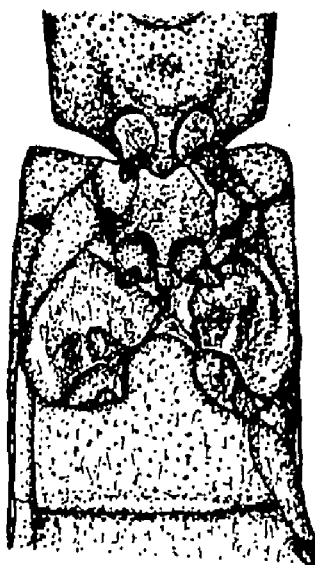
The typical examples of the malformations on the thoracic and abdominal segmentations are shown in Figs. 5.103.-5.104. and Figs. 5.105.-5.114. respectively, where the teratological diagnoses after Balazuc^{b1)} and the terminology of adult abdominal sternites, proposed in the section 5.5.3., are involved.

These malformations are classified as follows: (i) those induced by the failure of the median agglutination of the germs of the sternites or tergites (Figs. 5.103.-5.104. & 5.107.-5.108.); (ii) those induced by the erroneous median agglutination(s) between adjoining sternites (Figs. 5.105.-5.106.); (iii) those induced by excessive agglutination(s) (Fig. 5.110.); (iv) intermediate type between (i) and (iii) (Fig. 5.109.); (v) those induced by the lack or trouble of one of the two sternite germs (Figs. 5.112.-5.113.); (vi) unaccountable types (Figs. 5.111. & 5.114.). At present, it may be safely said that Balazuc's teratological diagnoses^{b1)} do not always contribute to the explanation and classification of the mechanisms in the formation of them: another diagnostic system needs to be proposed for the corporal malformations of Coleoptera in general.

As already stated in the section 2.3.4., the diets without sawdust, i.e. the diets R, S, N, T, U and W (buckwheat cake) in Table 2.5., tended to produce more malformed adults. An inference drawn from this fact is that some substance in wood itself might play a role in lowering the frequency of malformation and thus in maintaining a sound culture of the population. Overcrowding in the larval stage might be another possible cause for the malformations supposing these malformed characters had been



5.103.

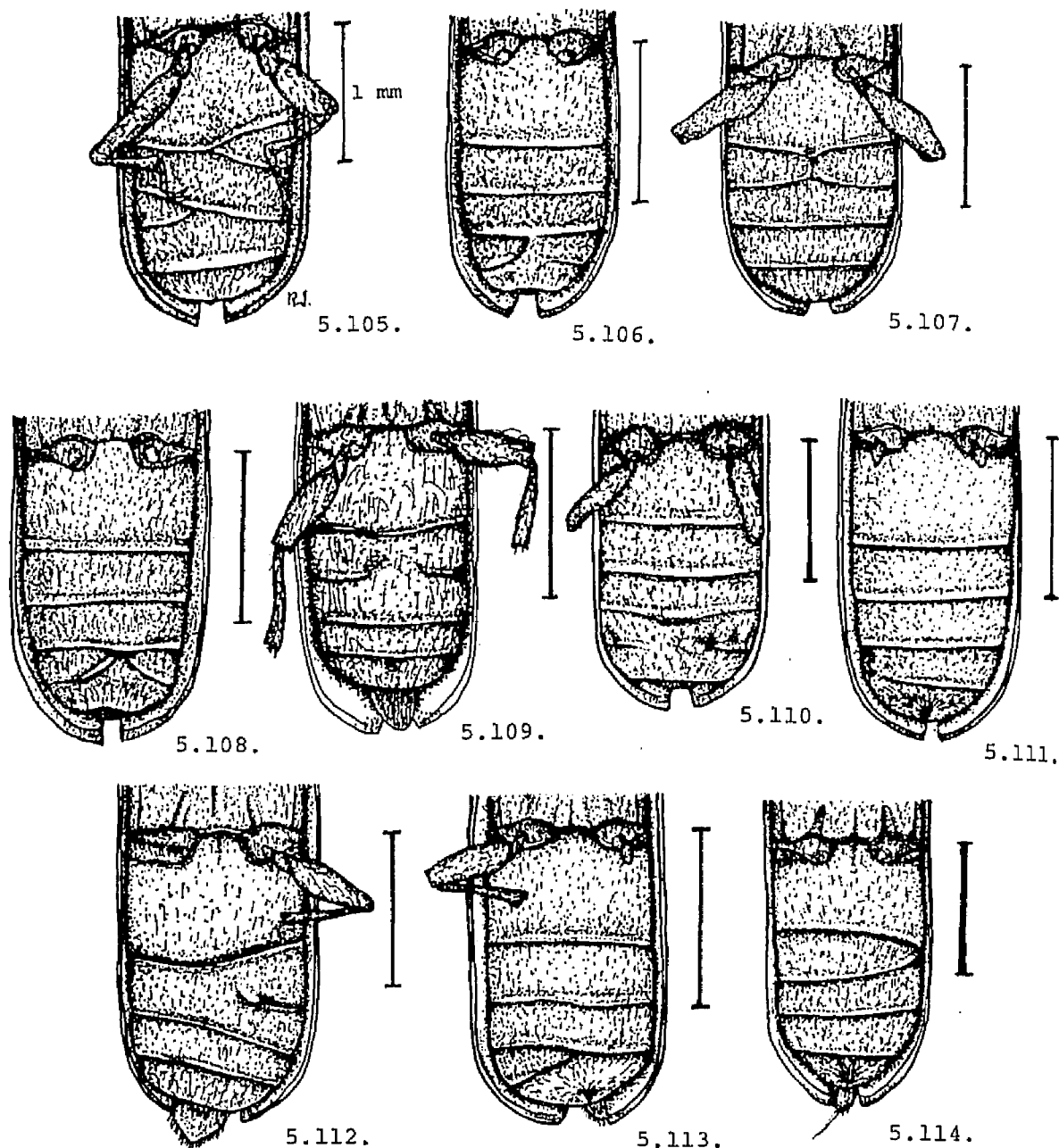


5.104.

Figs. 5.103.-5.104. Malformations on the thoracic segmentations in L. brunneus.

5.103.: Pronotum hemiatrophy (♀) (compare with Fig. 5.68.).

5.104.: Metasternoschisis (♂).



Figs. 5.105.-5.114. Malformations on the abdominal segmentations in *L. brunneus*.

5.105.: Monocyclic levogyrate helicomery on the 4th(2nd)-5th(3rd) sternites (♂).

5.106.: Monocyclic dextrogyrate helicomery (?) or symphysomery (?) on the 6th(4th)-7th(5th) sternites (♂).

5.107.: Hypogastroschisis on the 4th(2nd) sternite (♂).

5.108.: Hypogastroschisis on the 6th(4th) sternite (♂).

5.109.: Symphysomery (?) on the 4th(2nd)-5th(3rd) sternites or hypogastroschisis (?) on the 4th(2nd) sternite (♂).

5.110.: Symphysomery on the 6th(4th)-7th(5th) sternites (♂).

5.111.: Slight hemimery on the 6th(4th)-7th(5th) sternites (♀).

5.112.: Hemimery on the 4th(2nd)-5th(3rd) sternites (♂).

5.113.: Hemiatrophy on the 6th(4th) sternite (♂).

5.114.: Hemiatrophy on the 4th(2nd) sternite (♀).

acquired in larval or pupal stage.

The appearance frequency of helicomery, a form of corporal malformations (Figs. 5.105.-5.106.), was estimated by Balazuc^{b1)} at one specimen per 2000 individuals in Tenebrio molitor L. (Tenebrionidae), while he cited the other authors' results, where the ratios were 1/300 - 1/400 and 1/1000. In the present cases with L. brunneus, the appearance frequencies of the abdominal malformations in general are estimated as follows:

Eight malformed specimens were found only from the experimental cultures with the 9 kinds of diets containing no wood sawdust described in Table 2.5., which collectively produced 9801 progeny individuals (Table 2.6.), thus the ratio being ca. 1/1200. On the other hand, in the special rearing cultures with buckwheat flour cakes in the mass culture condition, 3 malformed specimens were found out from 333 specimens obtained as a progeny, thus the rate being ca. 1/100.

According to Balazuc^{b1)}, the adult individuals of T. molitor with helicomery were vivid enough in spite of their morphological abnormality. Also in the present case with L. brunneus all the malformed specimens collected alive were vivid enough, some of which were let copulate to produce normal progeny up to the second generation (F_2), except for a few examples of malformed individuals found among their progeny. These malformations are not supposed to be hereditary.

5.7. The egg^{c)}

Most eggs are laid into the starchy hardwood sapwood or into

the starchy bamboo. In the case with hardwood, vessels are chosen for oviposition site. Figure 5.115. shows two eggs deposited in a red lauan vessel. Such multiple laying is not uncommon in Lyctus, as noted by Altson^{a5)} and Fisher (et al.)^{f2)}, with up to eight eggs being laid in a single vessel. Measurements from these photographs of eggs show the diameter of ca. 150 μ m, which has been noted by Clarke^{c3, c4)}. This value is comparable to 90 μ m, the diameter of the ovipositor stated in the section 5.5.3.

The curious feature of the eggs of Lyctus spp. is the anterior "strand" or "process" (Figs. 5.116.-5.117.), first noted by Snyder^{s10)} in L. planicollis, by Altson^{a5)} in L. brunneus, and by Parkin^{p1)} in L. linearis, etc. Later, Rosel^{r2)} examined eggs of 5 species of Lyctidae and segregated them into two types, those with and those without such a strand, with L. brunneus belonging to the former. Its function is obscure, or rather it does not seem to be of any use. The shape of the strand tip varies considerably; some are club-shaped, and the others are rod-shaped, representing its plasticity at the time of laying.

The other feature of the egg hitherto noted is the "striations" or "striae" around the base of the egg process^{a5, p1)}. Altson^{a5)} elucidated the formations of the striations and the strand in relation to the structure of the ovipositor. The SEM revealed the fact that the striations are due to wrinkling of the outer membrane, i.e. the chorion, and that they merge into the strand (Fig. 5.118.).

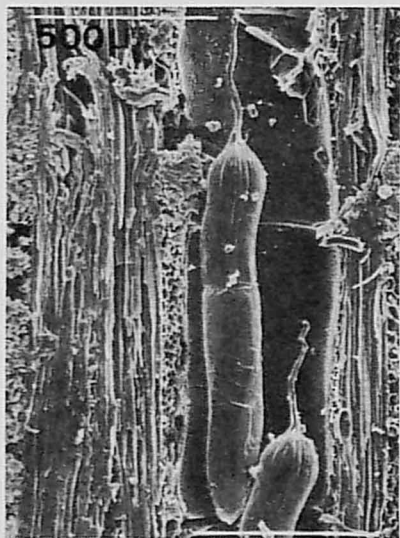
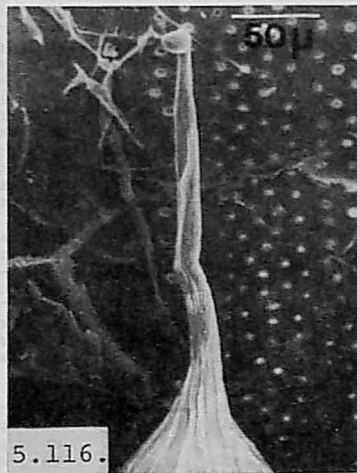


Fig. 5.115. Eggs laid in a lauan wood vessel.



Figs. 5.116.-5.117. Strand of the eggs and its variation.

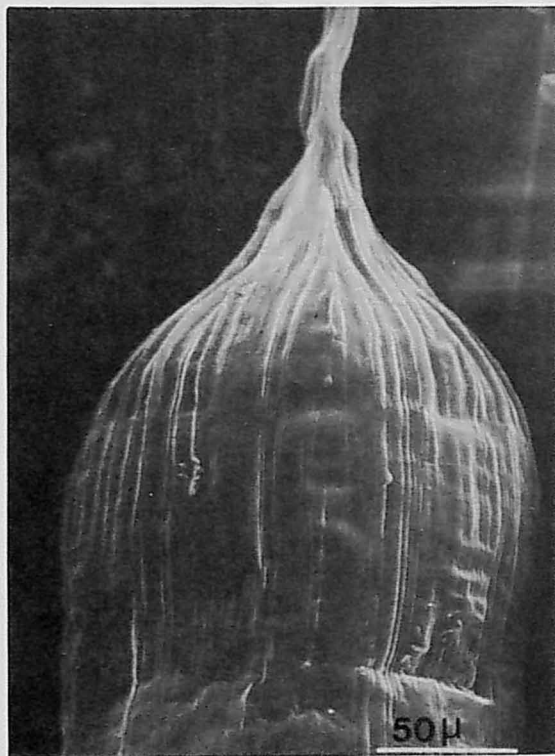


Fig. 5.118. Striations on the anterior portion of the egg.

5.8. Discussion^{C,F,H)}

The larva of L. brunneus naturally shows several morphological changes with succeeding instars: namely, the changes of the vertex groove of the head capsule (Figs. 5.3.-5.4.), the maxillae (Figs. 5.15.-5.18.), the labium (Figs. 5.22.-5.23.), the antennae (Figs. 5.26.-5.30.), the legs (Figs. 5.38.-5.41.) and the abdominal spiracles (Figs. 5.45.-5.48.), as shown by scanning electron microscopy (SEM) in the section 5.2. The maxillary and labial palpi become longer and more cylindrical in later instars, and the antennae and legs become comparatively longer and more slender in later stages; development of the appendices in general is observed.

The SEM observation thus showed several morphological changes in the course of larval development though they were not conspicuous enough between succeeding instars, especially in the later stages, to distinguish the larvae according to their instars. A very distinct feature of the possible final instar larva on its legs (Fig. 5.41.) may be utilized in separating it from the penultimate stage, which is approximately identical with the middle instar larva defined in the section 3.2.1. Utilizing these morphological characteristics in selecting a certain stage or instar for bioassays from a lot of larvae requires some kinds of optical devices with which to observe the fine morphology of living larvae without affecting them.

Several larval structures and organs whose significances are still unknown, such as the huge spiracles of the 8th abdominal segment, the "1st projection" of the antennae relatively huge in

the younger stages, etc., need to be studied further as they are conspicuous enough to be suspected of having some significant and peculiar functions.

The transformation from full-grown larva to prepupa without molt needs to be studied further especially with regard to the changes of the body shape and of the body weight.

The sex characters and sexual differences abounds in adult of L. brunneus in comparison with its relatively simple appearance. As discussed and verified in the sections 3.1.2. and 2.3.2., males are statistically smaller than females both in size and weight, the difference of mean body length being calculated to be 0.29 mm (Table 2.6). Further, males have also been shown to be more slender than females (Fig. 3.1.). Adults, as shown in the sections 5.5., exhibit some definite sex characters on the mandibles (Figs. 5.61.-5.62.), the 7th(5th) sternite and the pygidium (Figs. 5.89.-5.92.). It should be noted that all of these characters are not familial nor generic, but specific. There are also some sexual morphological differences or tendencies, though no definite sex characters, on the lateral sides of epistoma, the setae on the mentum and the elytral luster.

The 6 patches of the peculiar surface structures on the body and the undersides of elytra of adults (Figs. 5.73.-5.82., 5.85.-5.88. & 5.94.-5.95.) are one of the most spectacular aspects of the fine morphology of the species L. brunneus. Such rasp-like surface structures are suggestive of a stridulatory function as found in Scolytidae by Rudinsky & Michael¹³⁾, etc. and in Curculionidae by Selander & Jansson⁵⁸⁾, etc., where the male

communicates with the female using this device. However, none of these 6 regions show sexual dimorphism, denying the possibility of communicating function between the sexes. It is also possible that these structures are vestigial, with their practical function for stridulation being wholly reduced in this species. Another possibility of their function is that they may be the device for folding wings between the elytra and the tergites, as has been found in some other coleopterous species. When an adult beetle, laid supine, tries in vain to turn prone by raising the abdomen and flapping the wings, its abdomen always shows a continuous up-and-down motion accompanied by stowing the wings between the elytra and the tergites. The rasp-like 6 regions are also assumed to be closely related to this motion in stowing wings.

5.9. Summary

The summarized results and conclusions obtained in the investigation on the external morphology and surface structures are:

(1) Some morphological changes according to the larval instar were observed by SEM in the epicranium, fine structure of the mouth parts, antennae, legs and abdominal spiracles. The appendices were observed in general to develop and become more slender in the course of larval development.

(2) Simple eyes were not detected by SEM in the larval stage.

(3) Pupal sex characters in the anal portion are more conspicuous than in the adults.

(4) Three rasp-like patches on the undersides of elytra and

their counterparts on the body were found by SEM. Their function was supposed either to produce sound signals or to serve as a device for folding wings.

(5) Some other features of the external morphology and the surface structures of larvae, pupae, adults and eggs of L. brunneus were reviewed and scrutinized by SEM as well.

(6) Several types of adult malformations on the thoracic and abdominal segmentations were described and discussed for their cause, classification and appearance ratio.

Abstract

The present thesis deals with the mass culture method and the biology of Lyctus brunneus (Stephens) (Coleoptera, Lyctidae), the most important dry-wood boring insect in Japan, yielding the fundamental knowledges for the control bioassays.

For the mass culture of this species, a rearing chamber has been utilized with the climatic conditions maintained to be 24.5°C and 70% R.H. After the preliminary investigation on the essentialities of the solid artificial diet, the standard artificial diet was designated, with the composition of 26% lauan sawdust (or cellulose powder), 50% soluble starch and 24% brewer's yeast powder, which ensures the quicker development and produces progeny of greater number and of larger size than the other kinds of diet.

The weight changes in the course of metamorphoses and development was followed, with the larval instar number determined to be 4 - 6 by means of the newly devised "individual rearing method". The final sex ratio was determined to be 1 : 1, the density effect was detected on the progeny number and biomass, the ethology of adult beetles in relation to the presence of aggregation pheromone and to some other factors was investigated and/or described, and the relations to the other organisms, especially to the natural enemies, were discussed as well.

The chemical analyses of the foods and feces especially on the balance of required quantities of the essential nutrients, namely, starch (mainly the amylopectin portion) and protein (and/or amino acids), clarified the difference between wood feeding and diet feeding.

By means of SEM, the external morphology and surface structures of all the developmental stages were studied to yield detailed information including the changes of fine morphology with succeeding larval instars, pupal sex characters, peculiar surface structures on the adult body and on the underside of elytra, and so on. Additional note was also given on the malformations on the corporal segmentations of adult beetles.

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