BULLETIN OF THE ALLYN MUSEUM

Published by
THE ALLYN MUSEUM OF ENTOMOLOGY
Sarasota, Florida

Number 14 31 December 1973

BUTTERFLY ULTRASTRUCTURE

1. Sound Production and Associated Abdominal Structures in Pupae of Lycaenidae and Riodinidae

John C. Downey

Biology Department, University of Northern Iowa, Cedar Falls, Iowa,
and
Research Associate, Allyn Museum of Entomology

and

Arthur C. Allyn

Director, Allyn Museum of Entomology

The relatively few, and widely scattered, published accounts of pupal sound production in the families Lycaenidae and Riodinidae have been summarized by Downey (1966). In that report, and a subsequent note on stridulation in Netherlands lycaenids (Downey, 1967) sound organs were described and their occurrence noted in slightly over 100 species belonging to the two families. All observations were based on slides of the pupal integument examined with light microscopy.

Since that time, several important contributions on life histories, morphology and behavior have helped elucidate our understanding of sound in this stage of development. Most of these have been field studies, but at least three include laboratory verifications of behavior or sound production, and one involved electron microscopy of larval organs.

A chronological arrangement of authors and the areas of their studies include: Elfferich (1966), field and laboratory data on behavior of life histories of Netherlands lycaenids, including ant colony rearings; Ross (1966), detailed account of field ecology and ethology of *Anatole rossi* Clench, a myrmecophilous metalmark; Malicky (1970), field and laboratory studies on larval organs and myrmecophily, including electron microscope analysis of ant relational organs; Clark and Dickson (1971), detailed accounts of life histories of African lycaenids including excellent illustrations; Common and Waterhouse (1972), detailed field summaries of life histories of Australian species; and Hoegh-Guldberg (1972), analysis of sound and laboratory experiments with six lycaenids from Denmark.

While other individuals have contributed to our knowledge with individual species, the above authors must be consulted in attempts to understand and explain aspects of this phenomena, or the life histories and ethology of the species on which it is based. In the discussions which follow, specific works will be referred to or should be apparent in context. The number of species known to be sound producers was not increased markedly, in spite of this expansion of knowledge.

It is our purpose to present details of analysis of the pupal sound organs and associated abdominal structures in fifty-nine additional species and one subspecies, not previously known as capable of stridulation. These records together with new observations on fifteen earlier reported species, are augmented by observations made by scanning electron microscopy, which adds a new depth of clarity to an understanding of pupal ultrastructure. It is hoped that these studies, while expanding our knowledge of sound producing species by over 50 percent, might lay the ground work for continued studies of the ultrastructure of immature lepidoptera.

SOUNDS PRODUCED

We have heard the sounds produced by approximately 15 Nearctic, one Neotropical and five Palearctic species. All of these noises, plus those noted in thirty or more species reported by other observers, are very slight "creaking" or "chirping" sounds. Oftentimes, the low amplitude of the noise makes it hard to detect, as do the many ambient noises in field and laboratory. Sounds are best heard by placing one to several pupae in a vial or some other container which reflect the noises. When held close to the ear, shaking or thumping the vial will usually disturb the pupae and cause them to produce the intermittent sound.

While Downey (1966) had indicated differences in both the intensity and frequency of the sounds produced by two species, the experiments of Hoegh-Guldberg (1972) convincingly demonstrated a wide but uniform frequency spectrum for the sound emitted by a single species. Further, analysis of oscillography indicated that the pulse-trains of sound were produced by a movement of the same teeth of the file against the same irregularities in the stridulating plate. Basically a file containing numerous teeth is rubbed rapidly across an anterior stridulating plate, and the resulting vibrations produce a sound which might be simulated by pulling the teeth of a hair-comb across a resistant, non-yielding surface. Just as the speed of movement and/or the nature of the teeth in the comb as well as the nature of the surface against which a comb is rubbed, result in different sounds, so slight differences in pupal morphology and movement (behavior), may cause differences in the quality and quantity of noise produce. Hoegh-Guldberg (*loc. cit.*) also found differences in the pupal sounds (of several species) just prior to adult emergence from noises made earlier in pupal development.

Further details of the structure of the stridulating organs, their general and comparative nature, together with implications of origin and function are presented elsewhere (Downey, 1966) and will not be duplicated here. However, some additional facts, corrections of data, and new evolutionary implications will be discussed in appropriate sections below.

METHODS

Pupae were examined by both standard light and scanning electron microscopy. A JSM-U3 was the instrument used for the SEM studies. Specimens were prepared for the latter by mounting on a JEOLCO specimen holder by means of a conductive lacquer adhesive (Dotite paint, type D-550) prior to coating with 40/60 gold-palladium. They were not previously treated with carbon. To ascertain feasibility, uncoated pupae were scanned with low accelerating voltage (5kV) but resolution was not adequate. Particular pupal abdomens had to be grounded along the margins of their sclerites when the accumulated charge could not be conducted adequately across intersegmental membranes. Some species (and specimens) particularly *Glaucopsyche, Philotes,* and related genera, had a thin outer layer[1] which resisted adequate coating, and the resultant charging impaired detailed observations and precluded photographs from particular areas of these pupae.

During the present study we were psychologically as well as optically aided by the SEM. Both the three dimensional effect and all-angles view, as well as the magnification made possible a refreshingly new approach to the sound producing structures. The specimens were left in their normal, albeit coated, condition, and observations were sufficiently different from the fragmented integuments of prepared slides, that we were able to re-evaluate the function as well as the structure of the organs observed. Further, with observations up to 10,000 diameters possible on a TV scanner, not only could both of us see and discuss each specimen, but we were able to view objects from several directions, in many cases, even from inside the pupal integument. In other words, all observations could not only be made jointly, but varied positions, angles and views permitted functional interpretations which might otherwise have gone unheeded.

With each specimen we had to evaluate whether or not sound producing organs were present, but further, whether intersegmental movements would bring the organs into contact.

Very little information was located which deals with microstructures of pupae. We have also been handicapped by being unable to find published information on how the pupal covering is formed inside the last larval skin. This ignorance permitted only conservative interpretations of structures, possible homologies, and features which might be effected by individual variations and/or artifacts of preparation.

At least the following facts indicate some justification for concern about types of variation. Downey (1966: 139) noted a degree of variation between individuals involving tubercles of the stridulating plate. Also, variation in the clustering, and in the number of structures per unit area was noted in individuals of *Lycaena thoe*. Lawrence and Downey (1967) reported that the scar of the honey gland could be present or absent in *Everes comyntas*. Their observations were made with reflected light under a dissecting microscope.

In addition to the above natural variability, artifacts of preparation need also to be considered: differences between observations made with a light microscope and a scanning electron microscope, between a cast pupal skin and an intact integument, between living and dead specimens, between pelts preserved dry, or in various fixatives, or particularly those treated with potassium hydroxide.

To better interpret our results, and as an aid in future observations, particularly SEM work, observations on one to several individuals of the same species were conducted. These were selected because of the method of preservation, and included many sibling pupae. Not only the sound structures were studied and compared as a check on condition of the ultrastructures, but the spiracles and the adjacent clusters of delicate setae and sensillae were also observed.

The results were rather surprising. The dry untreated cast pupal skin was just as useful as the dried, untreated intact skin. Further, both were as good or better than any liquid form of preservation with the possible exception of alcohol (70 to 95%). Since all specimens have to be dried, or are dried in the vacuum process preparatory to viewing with the scan scope, any advantage of alcohol preservation over the non-preserved cast skin could not be determined. Pupae preserved in cytological fixatives such as Bouin's solution (either hot or cold), or in larvae preservatives such as KAAD (subsequently stored in 95% alcohol or not) were very unsatisfactory for SEM work. Delicate structures, such as spiracles, appeared to be coated with a precipitate, and much detritus and "residue" covered the body

[1] It is possible that this material might be related to the molt fluid residues noted by Slifer *et al.* (1959: 159-60) in grasshoppers. She describes what was thought to be "pollen grains," smooth ovoid bodies trapped in depressions of the cuticle. Later studies on lab material, freshly molted in a dust free environment, confirmed that molt fluids dry down on exposed areas to form scales and flakes, which resembled the pollen bodies. We also were struck by the amount of "pollen" on our samples and consider that much of it may have been due to these residues. Such detritus is visible in figures 55 and 69.

surface. Further, some features such as the stridulatory plate and teeth, seemed to be dissolved or eroded away in many areas. We have concluded, and recommend for continued studies of this type that the pupae, or cast skins thereof, be saved dry in vials or containers along with tissue to prevent damage by physical contact while stored.

RESULTS

STRIDULATORY AREA

Table **1** is a list of species in which actual or potential pupal sound producing organs were discovered during the present study. Species examined include representatives from every major biographical realm (Australian, Ethiopian, Nearctic, Neotropical, Oriental and Palaearctic). In addition, new data are also included for the following 15 species whose pupae are known to be capable of noise production: (Riodinidae) *Anatole rossi* Clench, *Apodemia mormo* (Feld. and Feld.), *Lephelisca wrighti* (Holl.); (Lycaenidae) *Aricia artaxerxes* F., *Atlides halesus* (Cram.),

Table 1. New Records of Species with Sound-Producing Organs in Pupae

RIODINIDAE

Calephelis nemesis (Edw.)
C. perditalis (B. & McD.)
C. rawsoni McAlpine
C. virginiensis (G-M.)
Caria ino G. & S.
Hamearis lucina L.
Lymnas pixe (Bdv.)

LYCAENIDAE

Agrodiaetus damon Schiff. & Den.
Callophrys augustinus (Westw.)
C. dumetorum (Bdv.)
C. eryphon (Bdv.)
C. hadros Cook & Wat.
C. iroides (Bdv.)
C. liparops (Le Conte)
C. miserabilis (Clench)
C. niphon (Hbn.)
C. siva (Edw.)
C. xami (Reak.)
Calycopis cercrops (Fabr.)
Chilades laius (Cram.)
Chlorostrymon simaethis sarita (Skin.)
Curetis acuta paracuta de Nice.
Euristrymon ontario autolycus (Edw.)
Glaucopsyche alexis Poda
G. lygdamus (Dbldy.)
Hemiargus ceraunus zacheinus (Bll. & Dru.)
H. isola alce Edw.
Hypochrysops ignita (Leach)
Iolaus glaucus jordanus Stgr.
Jamides bochus (Stoll.)
J. celeno (Cram.)
Leptotes cassius striatus (Edw.)
L. cassius theonus (Lucas)
Lycaeides idas L.
Lycaena gorgon (Bdv.)
L. helle Schiff.
Lysandra bellargus Rott.
Maculinea nausithous Bergstr.
Meleageria meleager Esp.
Nacaduba beroe gythion Fruh.
N. biocellata (Feld.)
N. pactolus cela W. & L.
Nordmania ilicis Esp.
Phaeostrymon alcestis oslari (Dyar)
Philotes mohave Wats. & W.P. Comst.
P. rita (B. & McD.)
P. speciosa (H.Edw.)
Phasis thero (L.)
Plebicula amanda Schiff.
P. dorylas Schiff.
Poecilmitis thysbe (L.)
Prosotas dubiosa indica (Evans)
P. nora auletes (W. & L.)
Satyrium calanus falacer (Godt.)
Scolitantides orion Pallas
Spindasis vulcanus (Fabr.)
Strymon bazochii (Godt.)
S. columella istapa (Reak.)
S. dryope (Edw.)
Tmolus azia (Hew.)

Callophrys gryneus (Hbn.), *Feniseca tarquinius* (Fabr.), *Habrodais grunus* (Bdv.), *Lycaena dispar* Haw., *L. helloides* (Bdv.), *L. phlaeas* (Linn.), *L. thoe* Guer., *Ogyris genoveva gela* Waterh., *Polyommatus icarus* Rott., and *Zizina otis labradus* (Godt.).

For convenience in the following discussions, only the trivial epithet of the scientific name will be used for the species indicated above. Generic assignments and the author of each species is given either in Table **1** or the preceding paragraph. In references to other species, the full species citation will be used.

The stridulatory devices involved in sound production were all located on the intersegmental membrances of the movable abdominal segments; *4/5, 5/6* and *6/7*. The anterior component consists of a roughened stridulatory plate (the Schrillplate of Prell, 1913). In its normal enfolded position (see Figs. **6** through **17**) the stridulating plate provides a grating surface for the posterior component, a file containing numerous teeth. Both surfaces, either gradually or abruptly (depending upon the nature of the surface and the species involved) become more membranous toward the apex of the intersegmental fold, where they lose their structural identity and form a continuous membrane. The latter is a smooth, structureless surface, although appearing plicate in many slide preparations due to artifacts, located between the two opposing surfaces.

The following data on the stridulating area supplements that presented by Downey (1966). An attempt has been made to present only new and additional findings.

STRIDULATING PLATE

Position: All species in Table **1** possessing stridulatory structures had stridulating plates on the posterior end of segment 5 (intersegmental region 5/6). The only exception was *vulcanus,* and those with tooth-cast systems (see below) whose sound production is debatable. In addition, all Riodinidae and a few Lycaenidae such as *grunus,* (Fig. **17**), had the plate on segment 4 as well. In *Spindasis vulcanus,* the sound organs appeared to be limited to the 4/5 area. Some species had, in addition to the organs on 5/6, evidence of the structures on the other movable intersegmental 6/7 (i.e. *bochus*), as did those with the tooth-cast systems.

All the stridulating plates occurred on the dorsal surface of the pupae, most often extending laterally to the region of the spiracles. In *helloides* and *celeno* for example, the plate ended at the spiracular line (an imaginary straight line connecting the midpoints of adjacent spiracles); in *phlaeas* the plate extended just beyond the spiracular line. In all Riodinidae, the stridulating plate on both segment four and five extended completely around the body.

The degree of sclerotization of the stridulating plate was compared to that found in the exposed portion of the same segment. This was only possible with light microscopy, as sclerotization is not directly observable with SEM (some extremely thin membranes do appear semi-transparent in the electron beam however). While many factors may effect this, including digestion and resorption of the integument prior to adult eclosion, any KOH treatment, clearing etc., it was felt that comparing the two areas on the specimen might lessen the subjectivity.

It was noticed that all species with grainy tuberculate plates such as shown in Figs. **6** through **11**, had a degree of sclerotization that was the same as, or slightly less than, that found in exposed sclerites on the same segment. However, those species with a ridge-type stridulating surface, such as shown in Figs. **24** through **29**, had plates that were more heavily sclerotized than other regions on the same segment. This would include all the hair-streaks and their relatives, plus other lycaenids such as *simaethis.* One explanation for this difference is that the granular surface is not as thick as the ridged surface, and one sees less transmitted light through the latter type plate. In this case, however, the valleys between the ridges should appear lighter, since they would be in thinner regions which would allow more light to penetrate. Indeed, this is the case with some species such as *niphon;*

in other cases such as *miserabilis* and *xami*, the entire plate is quite dark compared to other areas of the pupae. This condition is also visible to the naked eye in cast pupal skins of hair-streaks. If they are held so that the inside surface of the abdominal segments is visible, the stridulating plate of the 5/6 intersegmental region shows as a distinct dark band, which circles the dorsum to the circumspiracular area. This is in marked contrast to the other membranes which lack any such sclerotized plate.

Nature of grating surface: The stridulating plate appears to overlay the integument and associated structures. Fig. **33** illustrates this condition in *Lycaena dispar*. Note that a papilla protrudes up and through the materials which form both the stridulating plate, and the file. These organs ordinarily occur at the junction of ribs (see below), part of the regular surface sculpturing, which is buried except for these prominences. Other surface features cannot be seen in this intersegmental sound-producing region. This condition may also be noted on several other figures (**7**, **12**, **28**) where the ribs which overlay the surface of the pupae, are themselves covered and disappear as they encounter the stridulating plate. On the enlargement of *helloides* in Fig. **53,** not only the ribbing, but the junctions of several and associated papillae are still discernible as they become buried by the material that forms the sound organs in these intersegmental areas.

In *rossi* (Fig. **51**), the interface between the highly granular plate and the polygonal surface sculpturing is marked by an arrow. This region of the plate is cephalad of the dorsal lip of the intersegmental cleft, and would not contact other surfaces at this point.

Whether the sound organs are a tooth-cast system (Fig. **12**) (see below) or granular (Fig. **7**) or of transverse ridges (Fig. **37**) the surface sculpturing is obliterated by the material forming the stridulating device. Even on the file side, the teeth appear to be embedded in a matrix which overlies the external sculpturing. This may be noted in Figs. **28** and **37**.

The "cap-like" nature of the plate, on top of the underlying lip of the sclerite is also implied from the differences we observed the the color of the plate and the surrounding material.

A fractured specimen of *dryope* in which some of the plate material had been differentially exposed from underlying material enabled us to estimate the thickness of this "coat" at .003 mm.

Of the several types of grating surfaces observed, two were most common and will be discussed under each category: granular surfaces and ridges.

a) *granular surface:* This roughened surface consists of tubercles, small domed-shoped protuberances whose apices are not sufficiently sharp that they could be confused with teeth, nor sufficiently flat that they could be mistaken for flat-topped plates. Generally such a surface consists of pimple-like structures varying in size from tiny ill-defined roughenings to rather large well-formed knobs.

This type of plate occurs in all riodinid genera examined (*Anatole, Apodemia, Calephelis, Caria, Hamearis, Lephelisca* and *Lymnas*) and in many of lycaenids, including all *Lycaena* and most plebejines. In all species where stridulatory devices were on two or more membranes they had granular plates.

This grainy or tuberculate type of stridulating plate is illustrated on the upper part of several figures (**6** to **11**, **13**, **15**, **17** and **32**). In addition, comparative close-ups of the granular nature may be noted in Figs. **19**, **21** and **23**. The grains may be relatively rounded (**19**, **23**) and in the genera *Aricia, Lycaena* and *Caria,* or be quite pointed (**21**) and almost tooth-like as in *Apodemia*. There is a tendency, repeated in several unrelated genera, for the grains to be aligned in a transverse direction. We think this is mostly due to the transverse nature of the ridges on which they occur, no doubt an aftermath of the semiflexibility of the stridulating plate in a transverse rather than a longitudinal, direction. From two to four tubercles may align themselves on the "ridges" between the crease-induced valleys, as may be observed in Fig. **41**.

The size and number of tubercles not only varies between species (the larger grains tending to be found in the largest pupae) but individuals may vary from area to area along the length of the plate. The roughest surface is middorsally on the stridulating plate, with fewer and lower (smaller) grains along the plate toward the lateral margins, and toward the membranous areas. Fig. **41** shows how the tubercles disappear toward the membrane of the intersegmental cleft.

b) *ridged surface:* Ridges on the stridulating plate usually have a pronounced crest on the dorsal exposed lip of the plate (note Figs. **27, 28, 29, 39**) and two or more sloping surfaces. Note the figures of *halesus* (**24, 40**) in which this type of plate is very pronounced. In this and most species, the ridges usually run in a longitudinal direction along the main axis of the body of the pupa, but in a dorsal to ventral position on the intersegmental cleft *in situ.* These ridges may be single or they may branch, like mountain ridges whose crests run in roughly parallel directions. They may end abruptly (see Fig. **40**, fourth ridge from the left in the photo) or gradually disappear into the membrane toward the inner area of the plate.

Examples of various ridge conditions may be seen in photos **24** to **29, 31, 34** and **37** to **40.** Sometimes the ridges appear to be transected with transverse folds which may increase their efficiency as a grating surface. These surfaces might be compared to the roughened appearance of the cast of a waffle, except that the latter would have greater symmetry to its surface than is observed in the plate of *dorylas,* for example (Fig. **36**).

Not all ridges are of relatively simple character with corresponding ease in categorizing. Casts of teeth (see below) occur on and between ridges of *dryope* (Fig. **38**) and irregular "humped" ribs are found on *dubiosa.* There are also granular or nodular tendencies on the ridges of *columella* (Fig. **37**) though these are not detectable at this low magnification. In *glaucus,* the heavy ridges on the dorsal side of the posterior of segment 5, give way to hexagonal cobblestones, a distinct "warty" type of plate which continues past the stigmatal line.

c) *tooth-cast surface:* In many intersegmental regions, the teeth on the anterior margins of each sclerite have apparently made casts on the opposing intersegmental membrane of the posterior margin of the adjacent anterior segment. No doubt some of these casts are in relatively soft membranes in less sclerotized areas which have hardened in later pupal development or on death of the specimen. Under SEM observations, the pliability of surface structures cannot be determined, and we are left to speculate whether the teeth, rubbed across their own casts in the opposing areas, can or may produce sound. These areas, which we have elected to call tooth-cast areas or systems, regardless of how they may have originated or whether they produce sound, are found in such diverse genera as *Callophrys, Habrodais, Lycaena* and *Nacaduba,* all of which are also equipped with regular stridulating devices. Example of tooth-casts may be seen in Figs. **12, 14, 16** and **30.**

Tooth-cast systems are found in many intersegmental areas from 2/3 to 7/8. Interestingly, they are also found between segments 5 and 6 in many species whose stridulating plate is most apparent and functional in the same cleft. In such cases the tooth-cast areas are lateral to the well-formed stridulating areas, usually lateral to the spiracular line. They are not found in the Riodinidae in clefts 4/5 or 5/6, since in this family well-formed tuberculate stridulating plates circle the entire pupae in these areas. They are found between other segments on riodinids however.

As will be noted below, the teeth in the intersegmental area are helpful during the time the pupa is shedding the larval skin. Later, in conjunction with the casts on the membrane opposite, they help lock the segments together. Any movement still possible, as in the 5/6 area would, of course, bring the teeth in contact with other surfaces and produce stridulating noises. This function must have an advantage, or the teeth would not have increased in numbers rather markedly in the stridulating areas.

Primarily because of their widespread occurrence in species which are known to produce sound with other organs, and in intersegmental regions lacking motility (and hence sound producing potential) we have come to the conclusion that the tooth-cast system does not function as an efficient and/or significant sound organ in pupae. It is possible that in relatively non-motile areas, these devices fitting tightly together aid solidarity in the pupae; in more flexible regions, friction of tooth against the cast could produce some sound. In our opinion, however, they function in later stages of pupal development, primarily as an additional locking device.

In eight of the species on Table **1**, we located only the tooth-cast system, and no other sound producing organs. Downey (1966: 131) reported three of these (*lygdamus, mohave, rita*) as lacking sound structures, but he was not familiar with the tooth-cast system. The latter is not readily apparent in prepared slides. Sparse, but apparent, tooth-cast formations were located during this study on the intersegmental 5/6 region of each of these species, and on congeneric species including *alexis* and *speciosa*. In addition, they were the only possible sound organs noted on *orion, thero* and *acuta*. We have had an opportunity to listen for the sounds in living pupae of three of these (*lygdamus, mohave* and *speciosa*, only single observations with the latter however) and were unable to hear any sounds. However, we still do not discount the possibility of missing the subtle noises, or that other subjective factors (ultrasonics, condition of pupae, type of stimulus) may have prevented this. Since all these species have the tooth-cast system, and since it is still only assumed that noise is not produced by this mechanism, we have left the eight species on Table **1.**

Location of similar structures and behavioral potential in two species of *Glaucopsyche,* three species of *Philotes,* and in *Scolitantides,* all taxonomically related groups, is not unexpected. It does afford some evolutionary insights however on pupal versus adult structures and comparative rates of evolution in the two stages. Such taxonomic and evolutionary data are presently being investigated. Inferences were also supplied by two subspecies of *Leptotes cassius (striatus* and *theonus)* whose pupal sound organs were essentially identical.

FILE

The posterior subdivision of the stridulating area is termed the file and its most conspicuous feature is the presence of sharply pointed teeth (see Figs. **18, 20, 22**). These always project in a posterior direction, so that their functional position in the intersegmental region is with the sharp apex of the tooth pointing upward, toward the exterior surface.

Some teeth were encountered on every species examined. Most occurred on the outer surface of the body where they might be large and conspicuous (*nora)* or relatively small but occurring on every segment (*dorylas*). The external teeth averaged .0007 mm. in length and sometimes were arranged in bands (*thero).* Fig. **35** shows the arrangement of teeth in irregular longitudinal rows approximately .007 mm. wide in *glaucus.* The latter had such rows on both the anterior and posterior edges of segments 6, 7 and 8.

It was originally thought that the external arrangement of the teeth facilitated shedding of the last larval skin. While this may still be the case, it is weakened by their relatively small size, coupled with their continued occurrence on species such as the riodinids and hairstreaks, which have long setae. The latter might prevent the teeth from ever making contact with the skin to perform this function. However, they do occur in definite rows in some species, and in irregular but definite position on other sclerites, and these facts alone suggest some function as yet unknown.

The shape of the teeth in the intersegmental region may vary from needle-like (Fig. **22**) in *ino* to relatively blunt as in *liparops* and *miserabilis.* The base of the tooth also must effect its prominence and its sound qualities, as the teeth vibrate against the stridulating plate. Compare for example, the tooth bases in photos **18, 20** and **22**. The pick-like nature of the teeth in *mormo* differs markedly from the rounded protuberances in *artaxerxes,* where the surface of the tooth itself may not contact the stridulating plate. One can notice this gradual transition such that in some species like *lucina,* the roughened ridges and tooth bases become much more important in sound production, than the relatively few teeth. It is important to note however, that in all such cases, teeth are still in evidence, which leads us to suspect that in these butterflies, teeth were derived first in an evolutionary sequence, to any other type of file.

While it may generally be assumed that the larger pupae bear the largest teeth, there is variation in this character, particularly when comparing tooth size with the size of the tubercles on the stridulating plate. This might be done in three species by comparing Figs. **18** with **19, 20** with **21** and **22** with **23.** As with the latter figures (**22-23**) in *ino,* tooth size is relatively small in *amphidamas* compared to the size of the tubercles in the same species.

Teeth occur on the lateral margins of most intersegmental membranes, and they extend further laterad and ventrad than corresponding stridulatory plates. They are most often shorter than the ribs or other outer sculpturing which may occur in these relatively tightly closed clefts, so that it is doubted that any contact is made with other surfaces which might cause vibrations and make sound. Nonetheless, it is important to note their distribution in these areas, and their relatively more widespread occurrence that corresponding stridulating plates. They usually fit so snugly against the opposing membranes in these intersegmental clefts, that casts are formed. As indicated above, we surmise that casts were made prior to the hardening of the pupal integument.

As can be noted while looking at the figures, there is a tendency for the observer to attempt to align the teeth on the file; to see them as though they were on transverse or longitudinal lines. These apparent alignments are subject to individual and species variation. However, it would appear that this tendency may be real, and the *damon* and *xami* for example, with the teeth arranged in longitudinal rows, may differ from *falacer* and *ilicis,* where the teeth occur in tolerably regular oblique rows. Staggered spacing of teeth is found in *biocellata* so that every other tooth is in an anterior-posterior line. The tendency for teeth to pair has been noted in some species such as *laius.* Note also this tendency in *ino* on Fig. **22.**

There is a superficial resemblance in dorsal view between the pupae of the Nearctic *Feniseca tarquinius,* the Palearctic *Curetis acuta,* and *Iolaus glaucus jordanus*. Part of this is due to the relative flattening of the ventral surface and the associated angulate lateral margins of the abdomen. The resemblance is particularly noticeable in abdominal segments nine and ten, which in the three species could be roughly compared in shape to the hoof of a horse. If the stridulatory devices had any functional relationship to pupal shape, one might expect convergence in abdominal segment shape between the three species to be reflected by some convergence in noise producing structures. This is certainly not the case with these species, whose intersegmental stridulating devices show no similarity: the plate of *tarquinius* has tuberculate reticulations; *glaucus* has longitudinal ridges dorsally on 5/6 and hexagonal protuberances (warts) laterally; *acuta* has a tooth-cast system only, and presumably is incapable of sound production.

While superficial convergence is usually explained in terms of adaptations to similar habitats rather than similar heredities, it may also be purely "accidental" and have no evolutionary implications. We are assuming the latter explanation for the similarity in pupal shape noted above, but we infer genetic explanations and infer taxonomic relationships in many instances of similarity in both abdominal shape and stridulatory devices. For example, the distinctive longitudinal ridges on the plates of the Strymoniti is characteristic and predictable for the pupae of any species included in the group even though the tribe was formed on adult characters. It seems clear that this is because of genetic similarity, rather than evolutionary convergence.

OTHER ABDOMINAL STRUCTURES

General Sculpturing

The surface sculpturing of a typical lycaenid may be noted in several figures;

for example, **46, 52** and **54**. It appears as a reticulation of fine elevated lines or ribs, with small papillae at their intersections, and sometimes in the polygon-shaped spaces between. The pattern of the polygons is somewhat larger than that Hinton (1970: 42) has located in some area of every insect he has examined. He considers this the primitive or basic pattern of microstructure in insects.

The appearance of the ribs forming the polygons varies considerably between prepared slides and under SEM. Under transmitted light, the ribs do not appear elevated but appear as a fine network of parallel lines, resemble tiny canals running in irregular fashion across the non-sculptured surface. The photos in Tutt (1905-1906: Vol. 1) taken from slide preparations of pupal skins illustrate the "canal" appearance of the ribs in several species. At their junctions are doughnut-shaped tubercles. An SEM view is near the actual condition of the surface, as the reticulations are solid, elevated structures, demonstrated in the cross sectional view in Fig. **56**. An enlarged papillae at a rib junction is shown in Fig. **55**. The lips of this structure vary in height from species to species (and less markedly, even within one individual) but there is a tendency for the lips around the central area to be broken by two or three cross furroughs into radial divisions, giving the structure a resemblance to the mouth area of an ascarid worm, with dorsal and ventral lips. The central core is occupied by a blunt peg, on which six dimples (pores?) may be seen. These organs resemble, and may function, as sensilla basiconica in early pupal development. However, in several cases, we noted what appeared to be an effluvial overflow from this organ, which completely filled the cavity, overflowed and obscured the lips and spread down to the pupal surface. The analogy of a lava flow from a crater, which solidified in the act, portrays and accurate appearance of such papillae, and while infrequently encountered leads us to suspect these might have been the glands out of which the molting fluids poured in forming the pupal integument beneath the last larval skin. This opinion might have been reinforced had we been able to find openings or canals in the elevated ribs between the papillae. While the canals themselves might have been completely filled and obliterated with the material which subsequently hardened to form the pupal skin, now suggestion of this was found.

It seems likely that the elevated ribs act as strengthening struts and braces on the hardened outer surface of the pupae. They vary slightly from area to area. For example, on the wings, and appendages of the ventral surface, they tend to form a series of sinuous anastomosing transverse lines. Individual elevations may join other ribs, or may gradually lose their elevation and become obsolescent on the general surface.

Not all surfaces are of this type as described above. Some species have a very regular honey-comb like network (see Fig. **36**) or be variolate (Figs. **24** or **66**) or tuberculate (Fig. **64**).

While the network of ribs may extend over the lip of the enfolded intersegmental regions (see Fig. **31**), it is usual that they disappear in the more membranous intersegmental areas. Further, they are overlaid by other material in the region of the stridulating plate as was described above. The posterior segments often contain greater amounts of ribs, tubercles, teeth, setae, and other surface structures than the first abdominal segments.

Integumental sculpturing is usually disrupted in the circumspiraclar regions. Sensilla of several kinds usually occur in these regions and the warty, tuberculate nature of the region often gives it a different appearance, even in color, than surrounding areas.

Small pores or canals were commonly located on abdominal segments. One such pair of small openings about 0.4 mm. dorsomesad of each spiracle was spotted by one of us (Allyn) early in the study. Almost every species and every abdominal segment had some detectable opening in nearly the same vicinity, and its highly predictable occurrence demanded some sort of designation. They occur in pairs, usually more anterior in the segment than the spiracles, and always dorsad of the spiracular line. Once a pair is located, the small openings can be sought and identified on every segment in relatively the same position. Outer sculpturing sometimes makes them obscure, but even in small specimens such as *speciosa*, where surface features are quite reticulate, the openings are apparent. Their function is unknown, though we presume they may be associated with fluids involved in the molting process.

Abdominal Sensilla and Associated Structures

The cuticular surface contains an assortment of outgrowths and processes, mostly involved with surface sculpturing with no visible modifications on the inner side of the pupal skin. Other structures, such as the various types of sensillae, do have an inner structure which marks their position, and perhaps origin and function. Setae are the most common and noticeable armature of the pupal skin. They are hair-like or bristle-like, but commonly assume other forms. Most setae are sense organs (sensilla trichodea) of one or more kinds having characteristic structural differences in both cuticular and cellular parts. They originate independently from ribs or other skin reticulations or sculpturing.

As may be noted on the photos of setal types, Figs. **60** to **77**, each setae has at its base an elevated cone-like chalaza. In the case of short peg-like setae as in *vulcanus* (Fig. **66**) the chalaza may be one-quarter to one-third the height of the entire structure. In other species such as *thoe* (Fig. **60**) or *dispar* (Fig. **61**) the chalaza has a low profile, and might be more properly designated a pinaculum. This well-defined sclerotized setal base is most often of smooth surface, but, depending on the extent of surface sculpturing elsewhere on the pupal integument, may have crenulations, grains and tubercles involved on or around it as in *glaucus,* Fig. **64.**

The setal socket or alveolus is always apparent in these pupae and is usually a rather narrow space surrounding the external base of the setae as it emerges from the edges of the chalaza. Some setal types however, have a large alveolar space surrounding them (see Figs. **64** and **72**). The setal or articular membrane which externally closes the trichopore at the base of the setal socket cannot be seen from the outside, but may be noted when the socket is observed from inside the pupal integument. Each setal socket has a ridged "button" of material on the underside of the cast skin which projects below the surface about the thickness of the integument itself at that point.

Only one type of seta was apparent on *pixe*; a small mushroom-shaped structure .05 mm high (Fig. **72**). The membrane-thin umbrella-shaped top of the setae possessed a single opening in the center, directly above the stalk. These occurred sporadically on each abdominal segment, more commonly in the circumspiracular and posterior regions. They were also located on large, fleshy verrucae (Fig. **59**). Each verruca bears numerous deep circular or elliptical shaped cavities in which sensillae of various types are apparent. If the verruca is tall, its sensilla occur mainly near the apex (Fig. **58**), but if the verruca has a lower profile (Fig. **59**) these coeloconic sense organs may cover most of the surface. The mushroom-shaped setae are only one of several sensillar types, and are most common on the shorter, conical verrucae.

Coeloconic sense organs occur elsewhere than on verrucae. Fig. **70** shows such a structure the top of whose cavity is level with the surface. The sense organs located at the bottom of the cavity resemble those in Fig. **69**, except for the greater branching. Nearly identical sense organs to the latter were found on *wrighti* (Fig. **71**), which is taxonomically quite remote from *acuta*.

Dethier (1963: 25) states that it is dangerous as well as unprofitable to attempt to link a particular structure with a particular function since sensilla of one apparent type may serve different functions in different species, and may have more than one type of receptor associated with them. We concur in this opinion, although we made every attempt to correlate types of setae with both taxonomic groups, with known behavioral conditions (such as myrmecophily and sound production) and particular locations on the body. Since the present study is a first attempt at comparative ultra-structure in these families, much remains to be done at both the functional and behavioral level.

Our experiences with the various setal types parallel very closely those of T. A. Chapman. He first observed trumpet-shaped hairs on the pupal skin of *phlaeas*, which he described in 1905 (see Figs. **60, 61**). His curiosity lead him to observe setae on other species, and not only to describe them in series of papers (1905, 1905a, 1905b, 1906, 1906a, 1906b, 1906c, 1907, 1913) but to make attempts at setal homology. As we scanned each new abdomen, and encountered the different setae, it proved relatively easy to categorize them by similarity of shape; trumpet-shape (**60,61 73), hydroid-type (62, 63, 68, 77**), asparagus-like (**66, 67, 75**), tulip-head (**64**), and floral-cluster (**69, 71**). This preliminary association with familiar objects enabled us to communicate about the types, and perhaps psychologically made us ready to homolgize prematurely. Further, success at predicting setal types by shape and location on the body, gave us greater assurance that we were starting to recognize relationships. However, the preliminary nature of our observations, and the lack of cytological work on the setae involved, indicates a degree of caution.

One of Tutt's criteria for setal homology (Tutt, 1905: 452) was their point of origin on the pupal case. Both Tutt and Chapman thought that trumpet hairs and other setae were homologues as much by their placement on the interspaces between the elevated ribs as by any similarity in shape. This caused Chapman (1907a) to relate cremastral hooks with certain setal types because they seemed to originate on the elevated ribs.

60% of the specimens examined with SEM had two or more setal types on the abdomen; 50% had two types, 10% had three types. The remaining 40% were charaterized by having only one type of abdominal seta. In most individuals with two types, one kind was of general occurrence on the segments, and the other was usually associated with the spiracular region of segment six, or occasionally also, circumspiracular on segment seven. The latter hairs were of usually of a "hydroid" nature (Figs. **62** and **68** are good examples) with numerous long tapered and pointed hairs on the distal 1/2 to 1/3 of the setal stalk. The photos exhibit several differences which occur in these hydroid setae including, length, width, cross section of stalk, number of warts, spicules and hair-like processes. It seems clear that segment six of the abdomen, particularly in the spiracular region, must be carefully studied in terms of setal types. Chapman (1906) apparently also recognized that different spiracles on the abdomen had different setae in association. He published (*op. cit.*) a photograph of the spiracle on segment six and described both the "spiculate" setae and the trumpet hairs adjacent. He also spoke of a "variant" setae intermediate between both types described as "... clearly a hair that was not quite sure it ought not to have been a trumpet-hair." The presence of more than one setal type in more than 60% of these pupae rules out Scudder's taxonomic system based on the dermal appendages. However, Scudder (1889: 797-8) was cognizant of setal variability within the family and while erroneous mostly because of insufficient observations, used it to distinguish three groups or "tribes" (Theclidi, Lycaenidi, Chrysophanidi), He certainly pointed to a fertile area of research, and we are amazed that few authors besides Tutt and Chapman, took up the challenge.

While we have emphasized the tendency for some types of setae to cluster near the spiracles, particularly on segment six, generalizations on positioning on other segments is not as clear. We have noticed the setae on the dorsal body regions tend to be longer than those in lateral areas. For example, in *Jamides bochus*, the long, tapering, spiculate setae are about .045 mm laterally and .070 mm dorsally. *Hamearis lucina*, a riodinid with only one setal type on the abdomen, likewise has longer setae on the dorsal and on the posterior surface than elsewhere on the body.

Members of a single genus may show setal differences. While trumpet-hairs are found in all members of the genus *Lycaena*, they are the only setae noted in *phlaeas* and *helloides* while *dispar* and *thoe* contain, in addition, the hydroid-hairs on abdominal segment six. So few of the latter setae occur on some species however, that care must be taken that their "loss" might be due to fixation, rather than evolution.

Although we have not noticed setal color changes, such has been reported, Tutt (1905:391) states that in *phlaeas* the trumpet-hairs are conspicuous and brilliant at the outset of pupation. Later, they take on the same color as the integument and "require considerable magnification and search to discover."

Scars

Poulton (1890) noted in several moths the persistence of larval structures into the pupal stage, mostly as scars. Not only were these scars the remnants of solid chitinous larval structures such as the dorsal horn on the eighth abdominal segment in Sphingidae, but included indications of soft elevations, and even characteristic larval colors. Scars of larval prolegs, or their crochets, not infrequently persist on the ventral surface of larger pupae; though none were observed in our studies. Persistance has been explained by Poulton (*loc. cit.*) on the basis of hypodermal cells common to both the larvae and pupae. Not all larval structures remain in evidence on the pupal integument, however, and their loss makes arguments omitting adaptive significance rather difficult.

A great number of larvae of Lycaenidae and Riodinidae possess setae, tubercles and warts which are often conspicuous. Some of these structures may be retained in the pupal integument as scars, but their detection is made difficult by surface sculpturing.

Figs. **42** through **50** are SEM photos of prominent scars to be associated with many lycaenid and riodinid pupae. These include the prominent larval honey gland on the dorsomesal area of the seventh abdominal segment, and a pair of eversible tentacles, found latero-caudad of the spiracle on segment eight. The latter spiracle is functional in the larvae, but is often closed and/or vestigial (?) in the pupae.

Attention is drawn to some scars by a relatively smooth surface and a general lack of surface sculpturing normal to other areas of the same segment. This may be noted in Figs. **43, 46** and **47**. Crenulations and invaginations at these sites likewise reveals the former oriface of larval glands. The throat of the honey gland of *biocellata* is shown in Figs. **43** and **45**. The tuberculate nature of the throat, which is lacking in other areas of the same pupae, is suggestive of tubercles found in stridulating plates and other more mobile portions of sclerotized plates. Interestingly, the stridulatory plate in the latter species consists of ridges rather than grains, however.

The honey gland scar may consist of a large slit (Fig. **47**) or several indentations (Fig. **46**). Often, the lateral parts of the transverse scar appear to be deeper, or particularly indented in such a way as to make them more prominent that the central areas.

The tentacle scars may also vary in appearance from slightly more than a slit (Fig. **50**) to having uplifted margins or lips (Fig. **49**) to a prominent irregularity in the surface sculpturing (Fig. **48**).

Not all pupae examined had these scars. As expected from the lack of these organs in the larvae, all species in *Lycaena* lacked them as did *pixe, acuta* and *glaucus*. The lack of a tentacle scar in *acuta* was unexpected; extremely rough surface sculpturing in *Iolaus* may have obscured any evidence of these organs. The pupa of *Phasis thero* lacked evidence of a larval honey gland, but did have tentacle scars in abdominal segment eight. Of interest here is that Malicky could find no evidence of honey glands in the living larvae sent to him by Dickson (see Clark and Dickson, 1971: 200), nor could we in the pupae from the same source. However, Dickson has noted these organs, and the ants attending them, and it is possible there is some variability in this character in *Phasis* species.

A number of unrelated species possessed the honey gland scars, but tentacle scars could not be detected for one of several reasons (scar not evident, larvae lack them, individual specimen was atypical, etc.); these include *Anatole rossi, Strymon* *bazochii, Jamides bochus, Nacaduba pactolus,* and *Philotes speciosa.* Over sixty percent of the pupae examined had one or the other of these ant relational scars evident.

Cremasteral Area

The cremasteral hooks on the posterior of the abdomen look very similar in size and shape in all specimens examined. As long ago as 1907, Chapman (1907a) called attention to the fact that certain lycaenids, *Callophrys rubi* L. in particular, apparently never make attachment by the cremaster, even though the functional capability remains. He noted, however, that ordinary hairs are often mixed with these anchoring devices, which would" . . . much embarrass, if not prevent, their proper functions" (*loc. cit.,* p. 221). We have noticed that most nearctic species have engaged their creamastral hooks to the molt pad below the specimen. It is to this silken pad, or the elongate setae on the posterior regions of the pupae, to which the larval skin may attach after it is shed. It was suggested above, that in some species where the larval skin is not completely removed as an abdominal cover, the cremaster may be attached inside the larval skin, and the last pair of larval prolegs cling to the silken pad.

There are no cremastral hooks in some species such as *Thestor basutus* (Wall.), which pupates unattached in ants nests (Clark and Dickson, 1971: 262). The number of cremastral hooks, their position and placement on the body would appear to have potential taxonomic usefulness in the riodinids and lycaenids.

MOVEMENT

Primitively the pupal abdomen consists of ten segments, the first four of which are only visible in dorsal or lateral views. In some genera, the fourth segment, its caudal portions, or its intersegmental fold, may also be seen below the wings on the ventral surface. Other segments are usually visible from any aspect. In most moths, and those pupae considered primitive, some movement is possible between the 4/5, 5/6, and 6/7 segments, and these are said to be the movable segments. Expansion and contraction of the abdomen, and the side to side movements thus made possible, enable some pupae to work their way out of the ground, or in the case of moths, out of a cocoon.

Some butterfly families contain members whose obtect pupae are still capable of a marked degree of abdominal flection. The Papilionidae are a good example. A fresh cast pupal skin of *Papilio cresphontes* Cram. can be flexed dorsoventrally through an arc which is approximately twice as long as the fourth segment is wide (one arc in both directions). Similar lateral movements are possible and the entire abdomen can be extended the approximate body width at the level of abdominal segment four. All of these movements are made possible by rather plastic intersegmental membranes 4/5, 5/6 and 6/7. Of course, the upright position of the *Papilio* pupae, attached as it is by caudal cremastral hooks, and with a silken girdle which might offer some restrictions, means that the body would never be arched to the extent possible in the cast pupal skin. It does indicate, however, that pupae with apparently fairly hard pupal coverings, may still be adapted for limited movements through abdominal flection, even though they are attached.

Of some interest is that *Papilio* pupae are often in exposed situations and thus could be more vulnerable to attacks by predators and parasites. It is our contention that this movement has protective value (see below) which opinion is substantiated in the case of *Troides amphrysus* (Cramer) an oriental papilio which de Nicéville claims (Marshall and de Nicéville, 1882) makes noise by flection of abdominal segments "sufficiently loud to scare its enemies." Hinton (1970a; 104 pl. 4) has an SEM photo of the stridulatory file (between 4/5 segment) of *Troides "acercus"* (=*aeacus* [Fldr.]?). The ridges of the file fit into corresponding grooves on the stridulatory plate and noise is produced by a collison of the ridges as they are pulled out of the grooves. Hinton draws an analogy to the noise produced by unzipping a zipper fastner. He claims the sound is "impure" here compared with the usual stridulatory devices. We fail to see how this type of noise should be placed in a different category as Hinton suggests, since the teeth in the "usual" type of organ also make noise upon collison with the ridges.

The Lycaenidae have been considered to have advanced forms of obtect pupae supposedly because of even greater loss of motion that found in other families. However, not all motion is lost even in these supposedly rigid pupae, and retention of even limited flexibility is assumed to a reflection of functional need as it was in *Papilio* and *Troides* above.

Abdominal Flection During Pupation

Precise discrimination of instars is of considerable importance to many studies, as pointed out by Hinton (1946). In most life history studies, a particular instar is measured from the time the exuvia is shed; in physiological studies, however, it must be calculated from the time the old cuticula is loosened from the epidermis (see Downey, 1966: 144, for a brief dicussion). Suffice to say that some care must be taken in considering the onset of pupation. It is not uncommon to consider that the larvae molt for the last time as the pupal case makes its external appearance for the first time. Of course most histogenesis has occurred prior to this time, and "larvae" normally become quiescent for a day or two prior to any apparent external morphological changes. This stage has been called a "pre-pupa" by some; it is perhaps more appropriate to use Hinton's term pharate (= covered) to designate the phase of an instar which is enclosed within the cuticle of a previous instar. The pharate pupal stage then, precedes its uncovered stage, and we call attention to the fact that it is pupal movement we are discussing in dehiscence from the larval skin.

During the process of shedding the last larval skin, the abdominal segments, particularly intersegmental cleft 5/6 and caudad, are very flexible. It is the plasticity of the intersegmental membranes which permits this movement and the contortions involved in moving the larval skin toward the posterior end and off the body. The teeth and setae on all segments project caudally, and no doubt contact the larval skin during each peristaltic wave in the posterior direction. Externally, the skin appears to move slowly, almost gliding, toward the rear, but the skin itself covers the contortions of the responsible pupal abdominal segments. Pupal movement can be compared to that observed in the bellows of an accordian where not only a simple extension is possible, but commonly the top (or bottom) of the bellows moves through a greater arc than the corresponding position on the other side.

As soon as the larval skin has been pushed from the pupae, and the latter has affixed its cremastral hooks to the silken strands of the larval mat below, the abdominal segments are pulled together in their final pupal positions. In this condition most of the intersegmental membranes are not visible externally, and are tucked in a tightly-folded position with the internal apex of the fold reflexed strongly in an anterior direction. Within a short while the entire pupal integument becomes more rigid, and even the membranes become stiff.

Many riodinids (i.e. *Apodemia*) and lycaenids (i.e. *Durbania*) have pupal stages which remain attached inside the discarded larval skin. Clark and Dickson (1971) note in *Deloneura sheppardi* Stevenson, that the old larval skin with its numerous setae is only partially shed during pupation and encloses much of the abdominal portion of the pupa. We are left to speculate if there are advantages to those lycaenid species such as *Deloneura*, which are atypical from most species in this regard. The carnivorous *Liphyra brassolis* Westwood, which pupates inside its last larval skin in nests of its host ant *Oecophylla*, requires the protective larval covering over the pupa. The latter is relatively thin-skinned (Common and Waterhouse, 1972) and must be thus protected from ant attack. Does a partially shed skin afford some protection for other species? Are the larval setae on the incompletely cast skin of any value to the pupae, or are any of the pupal setae covered by the larval skin functional? Are additional sounds produced by subtle movements of the pupae inside the dry skin? Is the trait a result of the inability to completely shed the exuviae, or to reaffix the cremastral hooks to the larval mat below? All of these questions are in need of clarification and require detailed knowledge of the biology of individual taxa before generalizations can be made.

As implied with *Liphyra* above, the site of pupation may strongly influence, or be a factor in understanding pupal morphology. The following examples demonstrate a range in variability of lycaenid pupation sites: 1) *On Plants:* in buds or on leaves, *Zizula hylax* (F); in a shelter constructed of leaves, *Anthene talboti* Stempffer; in crevices on stems or trunks, *Spindasis natalensis* (Westw.); in hollow stems of food plant, *Phasis thero* (L); in seed pods, *Deudorix diocles* (Hew.); in galls, *Spindasis ella* (Hew.); in communal webs spun by larvae, *Jalmenus evagoras* (Don.); at base of food plant, *Poecilmitis adonis* Pennington; 2) *Off Plants:* in soil, *Lampides boeticus* (Linn.); in ant pens near base of host plant, *Anatole rossi* Clench; in ant nests, *Phasis dicksoni* Gabriel, *Lepidochrysops trimeni* (Beth.-Bak.); and under rocks, *Pseudalmenus chlorinda chloris* Water. & Ly.

While the above is not meant to be exhaustive, it may be noted that physical (temperature, moisture, light) as well as biological factors (associated host plants, ants, predators and parasites) of these various pupation sites could cause correspondingly greater or lesser adaptive pressures on pupal form. As demonstrated in *Troides* pupae above, both movement and sound production are products of this adaptability.

Abdominal Flection During Adult Eclosion

While flexibility in posterior segments in the early stages of pupal development is an adaptive asset (to remove the larval skin), any posterior extension or flexion of the pupal integument during adult dehiscence would be a liability. The adult body, ecluding from the pupal case, must push against posterior segments in order to rupture through the anterior end. Attachment of a cremaster, or cremastral hooks affixed to a larval pad, would help in establishing such fulcrum, but even in an unattached lycaenid pupae, such as the African *Thestor basutus* (Wall.), relatively inflexible abdominal segments must enable the adult to push through the anterior dorsomesal rupture line.

We have watched this process in many Lycaenidae, and in *Leptotes* for example, there is relatively little movement which can be detected between abdominal segments during adult eclosion. Prior to rupturing the skin, some pulsing movements indicated slight changes in volume or positioning of the adult abdomen inside the pupal case, but these movements were extremely slight in any one intersegmental area. In no case would such movements reflect even ten percent of the flections possible during the earlier pupal period when the larval skin was being shed.

Once the skin is ruptured and the head, antennae and legs withdrawn from their pupal coverings, the legs are used to purchase leverage in order to pull the rest of the body out of the pupal case. Here again, a firmly anchored posterior tip would aid the adult in crawling from the pupal integument.

Movement Associated With Sound Production

As indicated above, the teeth on the file, and the roughened surface of the stridulating plate are brought into contact during slight but rapid dorsoventral movements of the abdomen. In most species observed, this movement is so slight as to be only visible on the dorsomesal area of the intersegmental cleft in which the structures occur. It could be described as a rapid trembling, or vibratory pulsing. In fact, it is the vibration of the integument which makes the sound as the teeth in the file receive the impact of friction against the irregular gratings of the stridulatory plate. The speed with which the surfaces contact, as well as the resonance of the vibrating integument, appear to effect the pitch of the sounds emitted. Hoegh-Guldberg (1972) demonstrated two types of sound in one species which he attributed to noises produced in different intersegmental regions.

Movement is made possible through contraction of longitudinal muscles in the dorsolateral regions of the abdomen. These were first described by Prell (1913) in *Thecla quercus* (Linn.) and subsequently noted by Strawn (1964) and Downey (1966). However, much remains to be learned about the muscles involved. In the pupal stage, the muscles attach by tonofibrillae to the membrane of the 5/6 intersegmental cleft. It is not clear if these "muscle" bands belong to the pupal stage, or represent larval structures carried over into the adult stage, as does occur with some muscle tissue (Hinton, 1948a: 399). Further, our recent studies indicate that it is the pharate adult muscle system which causes movement particularly in the latter periods of time prior to adult emergence.

The onset of the pharate adult period may be coincident with the elaboration and final hardening of an external pupal case, or at a later time, perhaps at the end of a pupal dispause. Whenever this occurs, it seems clear that scales projecting from the posterior margins of each adult abdominal segment, fit snugly into a blind pocket lined on its inside by the intersegmental membranes and on its outside, by the inner wall of the pupal case. The infolded intersegmental membranes are flexed forward toward the anterior end (which can be readily observed inside the cast pupal skin of any lycaenid) and a shallow pocket occurs above each membrane. As each adult segment is thus "locked" by scales projecting into these pupal folds, any movement of the adult abdomen would ordinarily cause corresponding movements of the pupal case. Since the latter has become semiriged losing much of its intersegmental plasticity as the pupal case hardened, only slight "trembling" movements in the more plastic areas of the pupal integument result. This is usually dorsally in the 4/5 and 5/6 intersegmental areas.

The membranous area of cast pupal skins usually contains many adult scales, detritus, and other evidence of the intimate contact of the adult surface and these inner pupal pockets.

Since we have heard pupal noises in Nearctic species, and Hoegh-Guldberg (1972) in Palearctic species, right up to the time of adult eclosion, we might assume that these "pupal" movements responsible for the noise are in reality those of the pharate adult. We might further assume that this is of fairly universal occurrence in sound producing species throughout the world. Thus, while the sound-producing organs are limited to the pupal integument, the instigating sensory structures and muscles (or body fluids) occur in the adult.

It is highly probable that the abdominal muscles observed in the pupae by the workers above, actually functioned prior to the development of the adult integument inside the pupal skin. Thus they may be involved in any movement and resulting sounds early in the pupal period which the adult system takes over at a later time. Further work with each species may clarify the muscles involved, the stage to which they belong, and the continuity of the noise producing ability. It is distinctly possible that, if different muscles are involved, with different origins and insertions, different sounds might be produced by the two sets. While Hoegh-Guldberg (*loc. cit.*) could not distinguish differences early and late in the pupal period (except just prior to emergence), he probably was only dealing with one "set" of muscles in the species observed, and further, gave no evidence to indicate he might expect different functional involvement.

Hinton (1948) has also reported the very interesting case of a functional sound organ formed by two stages of the life cycle: a file on the caterpillar inside a cocoon, and a scraper on the inner wall of the cocoon. In most cases of pupal sound, we assume that the movements are produced by either larval or adult muscles (or body fluids) which may in fact, be the same for the two stages. At any rate, internal movement causes grating on the pupal integument, and it is the latter which is our chief concern herein.

DISCUSSION

Both the egg and pupal stage of development appear to have divorced themselves to a great extent from the physical and biological factors of the environment. In terms of functional morphology their needs are relatively simple, unlike the larval and adult stage, and exclude such items as food, extensive locomotion, mates and /or social interplay. Their external needs may be reduced to those of respiration, moisture and protection. Internally, both stages have problems of the need for living within, and escaping from, their protective enclosures, largely solved in conjunction with structures which persist to the next stage of the life cycle. This interdependence of one life cycle stage upon another for part of these needs and requirements is well known. For example, the need for protection and shelter of the egg (as well as the physiological requirements of the larvae for food) are related to where the adult female places the egg; the pupation site is likewise physically dependent on larval selection, and should this go awry, the limited adaptive features available to the pupae may not be sufficient for survival.

The shell of the egg and the hardened integument of the pupae both function in a protective way, and both coverings are aided in that task by variables such as thickness, texture, sculpturing and color. Respiratory and moisture requirements must also be handled through these coverings, which seemingly would make more difficult the evolution of simple protective investitures. Secretions, sounds and scents are avenues of external defense known in other organisms which may be available as deterrents to predators and parasites. As will be discussed later, we surmise that the pupae of these butterflies makes use of both sounds and secretion in combating the biological pressures of their environment.

Attention might also be called to the shifts in location of organelles responsive to the environment from one stage to another. The head of both caterpillar and adult contains the sites of many environmental receptors, the most obvious, of course, relating to sight and food procurement. In the pupae, where head and thorax are undergoing extensive physiological changes, the abdomen assumes a proportionately much more active role in environmental perception. This is manifest in the greater number of minute appendages and ultrastructures on the pupal abdomen, whose function, while mostly unknown, is presumably present only in this life stage, and in that particular body region. The sound producing organs belong in this category.

The evolution of pupal sound-producing organs is intimately associated with other structures and functions. They may also be an extension or "back-up" system for existing structures and functions. For example, there seemingly is a direct relationship between the thickness and hardness of the integument and its protective function against small invertebrates. At what point the continued evolution of a thicker and harder covering might become detrimental (say for adult emergence) is a moot point, but we might surmise, *a posteriori*, that an evolutionary balance must be reached which would maximize the protective function of the integument, without compromising the other needs of the organism. If, for example, noise production in the integument could be developed concomitantly with these other protective features, or even subsequent to them, a more effective protective function might result. Certainly a wider range of protection might be afforded the pupae, since presumably, additional sensory systems of predators (sound and tactile vibration) would be negatively stimulated.

The intimate association of sound with other protective functions, and even a possible time sequence, was derived by Hinton (1970a: 91). He noted that adults of certain mutillid wasps possess a structure which was "almost certainly first evolved as part of a stridulatory file. "This latter became modified, while still capable of sound production, to diffract light. The latter enhanced warning colors caused by pigments on other parts of the wasp body.

While Hinton did not indicate his feelings on the original function of sound in these wasps, it is our contention that sound in this case has, at the very least, some protective function, as has the diffraction grating producing the color. We call attention to the reinforcement or "back-up" nature of this second system of protection.

Rather than arguing for any inefficiency of the first system, one might speculate that, in the above case, it might require less evolutionary energy for the wasp to develop two methods of solving a problem (with one set of morphological features) than by increasing the efficiency of one system. Again, a difficulty involved in the latter is that in over-specialization for protection there could be interference with other requirements of the pupae.

We submit that the above situation in wasps is analogous to the situation in lycaenid pupae, where sound production, originally evolved or functioning in a protective way, may still be useful in this capacity. Additionally, however, it has taken on a communicative function with ants, which likewise serve to protect the pupae from predators. Thus considered, a rather complex series of structures and relationships can be reduced to a simple model, and we can conceive of their evolution with a rather modest evolutionary (genetic) investment.

We are left with many questions and suppositions which vigorous experimentation should eventually clarify. For example, the predators and parasites of pupal Lycaenidae are little known. Only slightly more than one percent (140 species) of this family have identified parasites reported from any life cycle stage. These parasites include over 100 species of Hymenoptera and 41 species of Diptera. We know significantly less about the behavior of the parasites and predators, and about the interactions between host and parasite, than about their identity. Thus, does sound really protect the pupae? How much "protection" is really needed in this stage of development? Indeed, many of the internal parasites which emerge from the pupae, result from eggs laid on the larvae, so it is the latter stage which may require relatively greater adaptive "protection."

It has been argued that a high rate of parasitism in some populations, even those protected by ants, is evidence for the lack of benefit from the latter, or that it infers little protective adaptation of any sort. We submit on the other hand, that little can be determined by the incidence or rate of parasitism between or among populations until all facts are known. Downey (1962) has found egg parasitism in different *Plebejus icarioides* populations varying from 0 to as high as 88%. Yet even the latter population, losing 88% of its eggs and additionally suffering predation and parasitism on larvae, pupae and adults, seemed to be flourishing. The point to be made here is that a very minute statistical advantage may be very significant in an evolutionary sense. It is possible that every pupa encountered by females of a particular wasp species may be killed by it. However, sporadic and irregular bursts of sound, or the integumental vibrations accompanied by sound, could interrupt or delay the oviposition response in the parasite. These accumulated delays, or interruptions in behavioral responses of the parasite, would increase the length of time on one host, and restrict the total number of hosts a parasite could visit during its life. Thus the response is adaptive and beneficial for the species, and not necessarily for the individual who exhibits the protective feature.

Function and Ants

Sound production is not limited to the Lycaenidae in the Lepidoptera, but has been reported from a limited number of Papilionidae, Danaidae and Hesperiidae. If it functioned principally for protection, and the structures producing the sound have an origin in morphological structures common to all butterfly groups, one would generalize that more species and families would utilize such an adaptation. Since the pupae of practically all the Lycaenidae and Riodinidae produce the sound, and most of the other families do not, and since most of the Lycaenidae and Riodinidae are, or have been, associated with ants, while other families are not, we conclude there is some beneficial relationship involved. This supposition is demonstrable in some species (i. e. *Anatole rossi*), but is unknown or not reported in the vast number of lycaenids whose life histories are at least partially known. In most of the latter cases however, observations have been made in temperate regions where mymecophilous associations may be less obligate (and more casual). There are cases, such as *Feniseca tarquinius,* which cannot, at least as yet, be explained in the same terms. Such species are rather far from being a typical lycaenid in many other aspects of their life cycle, so that we may assume their sound production may likewise be rather atypical.

Perhaps it is a mistake to look for single causes and explanations, yet we feel that in presenting some thoughts and alternatives, enterprising naturalists with living material at hand may be stimulated to consider and test the variables.

Empty pupal cases of *Ogyris genoveva gela* Waterh. are destroyed by the sugar ant *Camponotus,* in whose nest the larvae have pupated (Common, 1964). Elfferich (1966) likewise reports that ants destroy the pupal pelts of *Maculinea.*[2] In Mexico, according to Ross (1966), Camponotus ants remove the old larval skin from pupal pens of *Anatole rossi* Clench, and deposited them on the ground a short distance away from the shelters. It is interesting to speculate that the larvae may have induced the ant response through odors which persisted even in the cast skin, while the pupal pelts, lacking appropriate signal odors or the mechanism to communicate by sound, were destroyed by eating, rather than removal.

Chapman (1912: 394) notes that the amyrmecophilous larvae of *V. optilete* and *C. orbitulus* have rather "eccentric" food-plants (Ericaceae, Primulaceae). Although we would have to debate the premise that these two plant families are atypical lycaenid fare, the implication that the diet may effect or control ant association is well taken. Edwards (1878: 5) earlier noted that ants likewise "preferred" larvae of a single species feeding on one plant rather than another plant species.

Conceivably then, food-plant of the larva may play a major role in symbiosis, and indirectly (since sound and symbiosis are related), contribute to or affect the sound symbiotic relations. For example, would parasites and predators neglect certain species newly adapted to a particular plant; or would they, on a host species with more catholic plant tastes, contribute to differing selective pressures in different plant niches? If this is so, how might the sound organs react in the same situations, if they are defensive in function. If they are primarily for ant associations, the same questions could be asked. Thus, of the sound producers, how different are the organs of the most eccentric feeders, and the amyrmecophiles compared with the myrmecophiles? Our present understanding of the sound organs has not enabled us to distinguish such (subtle?) differences, but we suggest that such interactions may be most revealing in the evolution of the families involved.

We must also understand much more about the ant larvae symbiosis in the family. For instance it has been noted that the larval honey glands exude a fluid. Whether the honey-like liquid is offered as an "inducement" to protective symbiosis as advanced by Thomann (1901), or to prevent aggression by ants as suggested by Lenz (1917), it would appear that there is some form of "communication" between larvae and ant and some advantage to the larvae in possessing the gland. Since some larvae lack the organ (and presumably therefore either lack the presumed protective symbiosis or are additionally subject to greater amounts of ant aggression) it would seem that they should suffer a greater loss due to predation, whichever hypothesis is true. It would seem therefore, that some experimental evidence is needed as much to prove one thesis as the other.

Malicky (1970) discovered pheromonal producing "cupolas" in association with the honey glands on the larvae, and argued that these were most important in controlling ant behavior. However, one wonders why, if the perforated cupolas were to induce palpation, and reduce aggression as he suggests, that they would lack "success" in this regard in such species as the amyrmecophilous *Feniseca tarquinius.* Indeed the long setae on the body of *Feniseca* larvae were thought by us to be ant repellant devices. The setae no doubt have additional functions in

---

[2] Sound production in the internidal pupae of various species has been reported by Elferrich (1966), Downey (1966) and the present work. It helps verify the importance of the sound in relationship to the ants, and is highly suggestive of an erroneous tribal placement of this genus with *Glaucopsyche, Philotes* and *Scolitantides,* all of whose members have tooth/cast possessing, non-stridulatory pupae.

this case, perhaps to serve as clothing hairs on which to suspend cast skins, cotton-fluff and other detritus associated with the host aphid so as to remain inconspicuous. Any advantage of being inconspicuous, however, is associated with predation, and ants are certainly one form of predator in this case.

Malicky fails to note the suggestion of Newcomer (1912) mentioned by Downey (1962), or that of Clark and Dickson (1956), that the eversible tentacles on segment eight of the larvae, may be ant deterrants. Some observers report them to be brought into play particularly when the honey-gland is "over-worked". The loosely arranged setae would certainly strike the ant if he was in the vicinity of a full erect tentacle!

We would like to speculate that these cupolas have assumed a new function in some species. If the pheromones given off by the cupolas serve as signals to the ant, we might presume this signal could be copied by other organisms in the specific niche occupied by the ants (for example, the host aphids of *Feniseca*). The secretion, for instance, could "pacify" the host aphid, who might have responded to the same chemical signal and be consumed by this odor-mimetic carnivore, rather than tended by a similar smelling, but friendly, protective ant.

Malicky has made a fine contribution to understanding these relationships. Particularly significant is the description of the cupolas and the reaction of the ants to the caterpillars. We are excited by the similarity in behavior of all of the European species he observed; their similarity is suggestive of the fact that some reactions and solutions are similar, and within easy reach.

However, while one cannot doubt the observations given by Malicky regarding the lack of known symbioses, there are a few empirically derived data regarding protection. We are impressed by the observations of Gary Ross (1966) in Mexico where 100% of the larvae not attended by friendly ants were killed by rapacious ant predators (whose mandibles interestingly enough will not permit them to imbibe inducements of honey). The "cow palaces" (brood chambers) constructed by attendant ants at the base of the food plant are not necessarily foresight, but certainly suggest a complex situtation (reaction) when attendant ants are "not able" to render full protection. It would seem plausible that the nocturnal habit is an additional means of escaping predation and leaving the plant may have been due to original habits of the larvae, rather than insufficient protection by the ant. Certainly the ants are not always successful in protection, but as the mathematical computations of Fisher have proved--it takes only a very minute advantage to exert selective pressures in a given direction. Were the symbiotic protections only "very slightly" functional they might still be protective, and of advantage to the larvae. The lack of evidence, while unfortunate, bespeaks the difficulty of proof, rather than the lack of fact.

There are many ways that an association between two species can be conceived and evolved. Not all of these require complex changes in the genetic systems of both organisms. For example, certain histerid and staphylinid beetles live in colonies of highly carnivorous army ants apparently due to the behavioral adaptation of rubbing the legs of the ant workers (Akre and Rettenmeyer, 1966). They are supposed thus to acquire and maintain the colony odor. The response to the colony odor was already evolved in *Eciton,* but the acquisition of the colony odor by the beetle conceivably lessens the chance that they will be attacked by the ant.

Other myrmecophiles may have had to develop *trichomes,* secretions highly attractive to the ants, before they developed other behavior patterns of ant association.

If we accept that sound may have more than one function, or indeed, have two effects, it will be difficult to ascertain its original function. However, the occurrence of pupal noise in other lepidoptera, particularly moths, which are in no way related to ants, suggests that a defense function came first.

SUMMARY

1. Data on pupal sound production in 60 taxa of Lycaenida and Riodinidae

are presented for the first time and additional information presented on 15 species previously known as sound producers.

2. Comparative ultrastructure of the sound organs, scars of larval structures, setal types and other abdominal structures is presented. These include scan electron observations and photographs of the sound producing and other abdominal organs of 38 species.

3. An intersegmental tooth-cast system is described. While there is both an actual and a potential relationship of these structures to stridulating devices, it was concluded that the tooth-cast system is not involved in sound production. Eight species of lycaenid are noted (*acuta, alexis, lygdamus, mohavi, rita, speciosa, thero* and *orion*) which have only the tooth-cast system, and presumably, are incapable of sound production. Additional inferences to support this contention include the occurrence of the tooth-cast system on relatively immotile segments, and the absence of noise from living pupae of three of the species.

4. Pupal movement is discussed including the relationship of abdominal flexion to pupation, adult eclosion and sound production. It is suggested that pupae in exposed environmental situations are capable of more movement, and perhaps make greater use of sound production, then pupae in more sheltered situations.

5. It is suggested that sound is primarily protective in function. It arose as a secondary system of defense (considering the pupal integument itself as the primary system) as an aftermath of required pupal movements. The use of sound as a communication system with ants served in a protective capacity also.

6. It is further suggested that pupal sound production is both universal and ancestral in the families Lycaenidae and Riodinidae. Species which have modified or lost the structures or the presumed basic functions involved, have acquired these characters secondarily in the course of their specific evolution.

7. Instances of pupal convergence are noted as are inferences on genetic and taxonomic relationships of species based on the sound organs. The following abdominal structures on the pupae have potential taxonomic and evolutionary significance (arranged in order of presumed usefulness, greater to lesser): stridulatory organs (plate, file), setae, other sensillae, spiracles, cremastral area, general sculpturing, scars and cremastral hooks.

ACKNOWLEDGMENTS

We are grateful to the following researchers and institutions for contributing pupae used in this study: Rev. A. E. Bean; Dr. I. F. B. Common, C. S. I. R. O., Camberra, Australia; H. K. Clench, Carnegie Museum; N. W. Elfferich; Dr. T. C. Emmel, University of Florida; Dr. E. T. Giles, University of New England, Armidale, Australia; G. A. Gorelick; O. Hoegh-Guldberg, Naturhistorisk Museum, Aarhus, Denmark; R. O. Kendall; Dr. C. D. MacNeil; Dr. Hans Malicky, Vienna University, Austria; L. M. Martin; D. V. McCorkle; Noel McFarland; Dr. F. D. Morgan, Waite Institute, Adelaide, Australia; E. J. Newcomer; Dr. J. A. Powell, California Insect Survey, University of California, Berkley; Dr. G. N. Ross; Oakley Shields, University of California, Davis, California; D. B. Stallings; Dr. O. R. Taylor, University of Kansas, Lawrence, Kansas; and Fred Thorne. Our thanks also to Dr. Lee D. Miller, Dr. C. G. McCollum and Mr. A. C. Haman for review and criticism of the manuscript.

LITERATURE CITED

Akre, R. D. and C. W. Rettenmeyer, 1966. Behavior of Staphylinidae associated with army ants (Formicidae: Ecitonini). Jour. Kansas Ent. Soc., 39(4): 745-782.

Chapman, T. A., 1896. On the phylogeny and evolution of the Lepidoptera from a pupal and oval standpoint. Trans. Ent. Soc. Lond., 1896:567-587

———————., 1900. Chapter V. The phylogeny of the Lepidopterous pupa, pp. 88-100. *In,* Tutt, J. W. A natural history of the British Lepidoptera. Vol. II. Pp. viii + 584. Sonnenschein & Co., London.

———————., 1905. Sculpturing of the pupa of *Thester ballus*. Entom. Rec. & Jour. Var., 17:145, pl. V.

———————., 1905a. Pupal skin of *Chrysophanus phlaeas*. Entom. Rec. & Jour. Var., 17:172-173, pl. VI.

———————., 1905b. Trumpet-hairs on the pupa of *Chrysophanus dispar*. Entom. Rec. & Jour. Var., 17:322-324, pl. XII.

———————., 1906. The pupa of *Chrysophanus dispar*. Entom. Rec. & Jour. Var., 18:15-17, pl. I.

———————., 1906a. Trumpet-hairs on the pupa of *Chrysophanus vigaureae* var. *miegii*. Entom. Rec. & Jour. Var., 18:89, pl. IV.

———————., 1906b. Pupal skin and pupal hairs of *Zephyrus quercus*. Entom. Rec. & Jour. Var., 18:237, pl. X.

———————.,1906c. Pupal skin and hairs of *Thecla w-album*. Entom. Rec. & Jour. Var., 18:263. pl. XI.

———————.,1907. The pupal skin and hairs of *Loweia (Chrysophanus) amphidamas*. Entom. Rec. & Jour. Var., 19:55, pl. III.

———————., 1907a. Notes on the cremaster of certain ruralid pupae. Entom. Rec. and Jour. Var., 19:221-222, pls. VII, VIII.

———————., 1912. On the early stages of *Albulina pheretes,* a myrmecophilous plebeiid butterfly. Trans Ent. Soc. Lond., 1912: 393-406, pls. XVIII to XXXVI.

———————., 1913. Description of the pupa case of *Heodes dorilis*. Entom. Rec. and Jour. Var., 25:133-134, pls. IX, X.

Clark, G. C. and C. G. C. Dickson, 1956. The honey gland and tubercles of larvae of the Lycaenidae. Lepid. News, 10:37-44, 3 pls.

——————————, 1971. Life histories of the South African Lycaenid butterflies. Pp. xvi + 272. Purnell, Cape Town.

Common, I. F. B., 1964. Australian butterflies. Pp. 131. Jacaranda Press, Brisbane.

——————— and D. F. Waterhouse, 1972. Butterflies of Australia. Pp. 498. Angus and Robertson, Sydney.

Dethier, V., 1963. The physiology in insect senses. Pp. ix + 266. Methuen and Co., London.

Downey, J. C., 1962. Variation in *Plebejus icarioides* (Lepid.: Lycaenidae) II. Parasites of the immature stages. Ann. Ent. Soc. Amer., 55:367-373.

———————., 1966. Sound production in pupae of Lycaeidae. Jour. Lepid Soc., 20(3):129-155.

———————., 1967. Sound production in Netherland Lycaenidae. Ent. Berichten, 27:153-157.

Edwards, W. H., 1878. Notes on *Lycaena pseudargiolus* and its larval history. Can. Ent., 10(1):1-14.

Elfferich, N. W., 1966. De Nederlandse Lycaenidae met gegevens over de biologie en de vliegplaatsen Wetenschappelijke Mededelingen, No. 66, 44 pp.

Hinton, H. E., 1946. Concealed phases in the metamorphosis of insects. Nature, 157:552-553.

———————., 1948. Sound production in lepidopterous pupae. Entomologist, 81:254-269.

———————., 1948a. On the origin and function of the pupal stage. Trans. Roy. Ent. Soc. London, 99:395-409.

————————., 1955. Protective devices of endopterygote pupae. Trans. Soc. Brit. Ent., 12(2):49-93.

————————., 1955a. Sound producing organs in the Lepidoptera. Proc. Roy. Ent. Soc. Lond. (c), 10:5-6.

————————., 1970. Some little known surface structures. Pp. 41-58. *In* Neville, A. C. Insect Ultrastructure. 185 pp. Blackwell Sci. Publ., Oxford.

————————., 1970a. Some structures of insects as seen with the scanning electron microscope. Micron, 1:84-108.

Hoegh-Guldberg, Ove., 1972. Pupal sound production of some Lycaenidae. Jour. Res. Lepid., 10(2):127-147 (1971).

Lawrence, D. A. and J. C. Downey, 1967. Morphology of the immature stages of *Everes comyntas* Godart (Lycaenidae). Jour. Res. Lepid., 5(2):61-96 (1966).

Lenz, F. , 1917. Der Erhaltungsgrund der Myrmekophilic. Z. indukt. Abst. Vererbungsl., 18:44-46.

Malicky, H., 1970. New aspects on the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymenoptera). Jour. Lepid. Soc., 24(3):190-202.

Marshal, G. F. L. and L. de Nicéville. 1882. The butterflies of India. Burmah and Ceylon. Vol. 1. Pp. vii + 327.

Newcomer, E. J., 1912. Some observations on the relations of ants and lycaenid caterpillars, and a description of the relational organs of the latter. Jour. Ent. Soc. N. Y., 20:31-36, pls. II, III.

Poulton, E. B., 1890. The external morphology of the lepidopterous pupae: its relation to that of the other stages and to the origin and history of metamorphosis. Parts I, III. Trans. Linn. Soc. Lond., 2:187-212, 245-263. Pls. xx, xxi, xxvi, xxvii.

Prell, Henrich, 1913. Uber zirpende Schmetterlingspuppen. Biol. Centralb., 33:496-501.

Ross, G. N., 1966. Life history studies on Mexican butterflies. IV. The ecology and ethology of *Anatoli rossi,* a myrmecophilous metalmark (Lepidoptera: Riodinidae). Ann. Ent. Soc., Amer., 59(5):985-1004.

Scudder, S. H., 1889. The butterflies of the eastern United States and Canada with special reference to New England. Vol. II. Pp. xi + 767-1774. Publ. by the auth., Cambridge.

Slifer, E. H., J. J. Prestage and H. W. Beams, 1959. The chemoreceptors and other sence organs on the antennal flagellum of the grasshopper. Jour. Morph., 105:145-167.

Strawn, Martha Ann, 1964. Sound production in pupae of the butterfly family Lycaenidae. Unpublished master's thesis, Southern Illinois University.

Thomann, H., 1901. Schmetterlinge und Ameisen. Beobachtungen uber eine Symbiose zwischen *Lycaena argus* L. und *Formica cinerea* Mayr. Jahresber. Nat. Gesell. Graubunden H. F., 44:1-40, 1 pl.

Tutt, J. W., 1905-1906. A natural history of British Butterflies. Vol. 1, Pp. iv + 479. Elliott Stock, London.

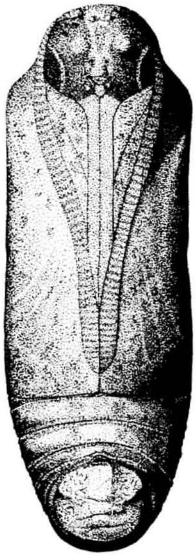
FIGURES

MAGNIFICATIONS OF SEM PHOTOS

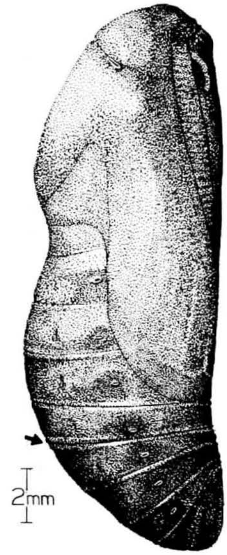
FIG.	MAG.	FIG.	MAG.	FIG.	MAG.
6	600X	30	300X	54	180X
7	300X	31	300X	55	1800X
8	480X	32	600X	56	3000X
9	300X	33	600X	57	300X
10	600X	34	300X	58	60X
11	1200X	35	350X	59	180X
12	180X	36	360X	60	650X
13	330X	37	270X	61	520X
14	600X	38	300X	62	435X
15	180X	39	120X	63	260X
16	600X	40	600X	64	870X
17	600X	41	300X	65	435X
18	1800X	42	120X	66	700X
19	1800X	43	180X	67	435X
20	1800X	44	80X	68	870X
21	1800X	45	600X	69	1300X
22	1800X	46	60X	70	960X
23	1800X	47	70X	71	1300X
24	180X	48	1200X	72	435X
25	180X	49	360X	73	435X
26	360X	50	600X	74	435X
27	240X	51	360X	75	435X
28	360X	52	180X	76	435X
29	270X	53	600X	77	435X

Note: For purposes of orientation, where possible the figures have been arranged with either the anterior or the dorsal part of the specimen toward either the top or the left of the photograph: posterior or ventral surfaces are toward the bottom, or the right of the photograph (or the intersegmental cleft of the specimens).

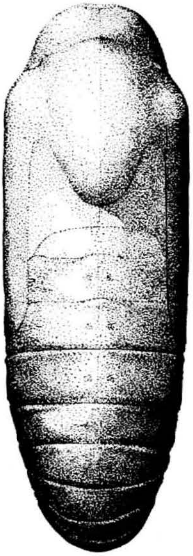
Fig. 1-5. Variation in shape of obtect pupae of Lycaenidae with stridulating organs. 1, 2, 3, ventral, lateral and dorsal view of the Australian *Ogyris genoveva gela* Waterh., from nests of a *Camponotus* ant. 4, Lateral view of Nearctic *Feniseca tarquinius* Fabr., whose larvae are amyrmecophilous and carnivorous on aphids. 5, Lateral view of Nearctic *Atlides halesus* (Cram.). The stridulating organs occur between abdominal segments 5 and 6 (marked by arrows) regardless of general shape, degree of flexibility or the position of the pupa on the substrate.



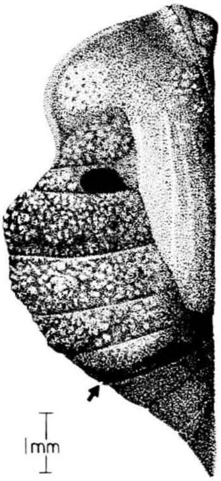
1



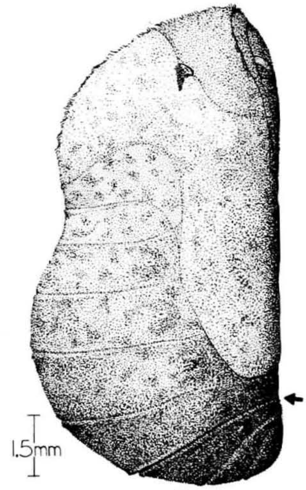
2



3

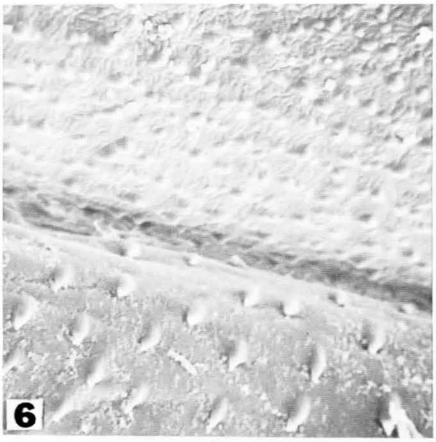


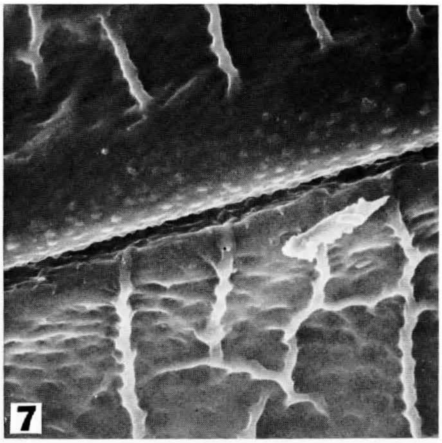
4

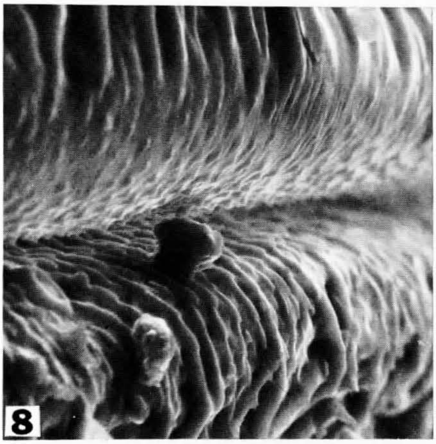


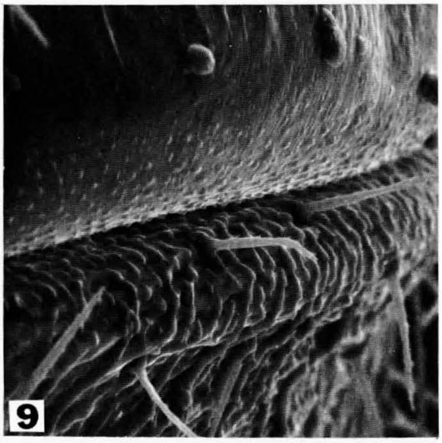
5

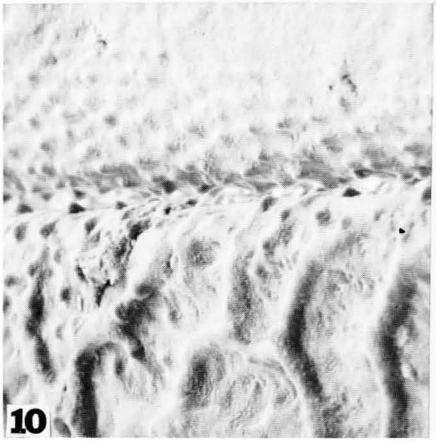
Fig. 6-11: Surface view of pupal integument showing sound-producing organs on intersegmental region, abdomen, segments 5/6. The tuberculate (grainy) nature of the stridulating plate on posterior five (above cleft) is apparent. 6, *Lycaena thoe* Guer. 7, *Polyommatus icarus* Rott. 8, *Jamides celeno* (Cram.). 9, *J. bochus* (Stoll.). 10, *Zizina otis labradus* (Godt.). 11, *Chilades laius* (Cram.).


6


7


8


9


10

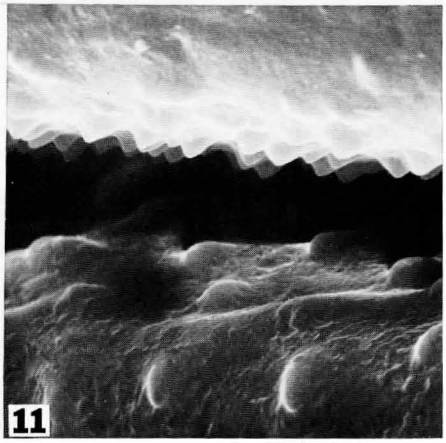
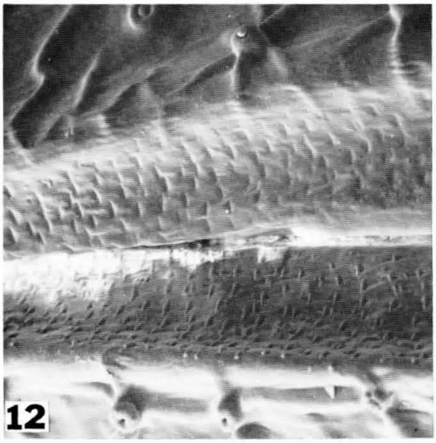
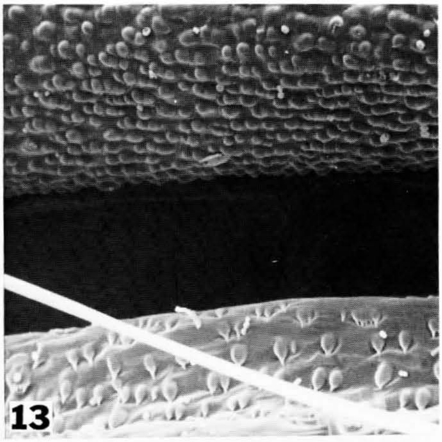
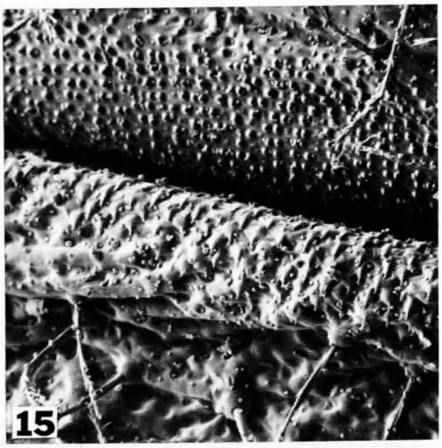

11

Fig. 12-17: Stridulatory structures of various lycaenids and riodinids. 12-15, intersegmental region 5/6. 12, *Callophrys henrici* (Gr. & Rob.). 13, *Aricia artaxerxes vandalica* Kaaber. 14, *Glaucopsyche lygdamus* (Dbldy.). 15, *Apodemia mormo* (Feld. & Feld.). 16-17, *Habrodais grunus* (Bdv.), 16, 6/7 region. 17, 4/5 region.


12


13


14


15


16

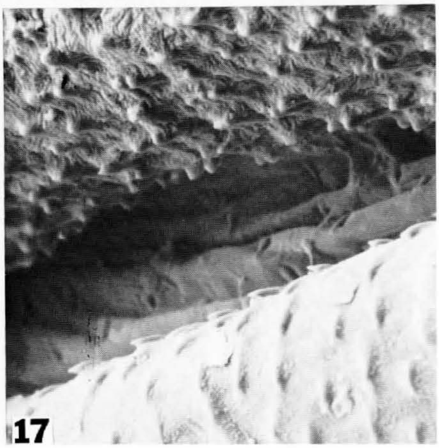
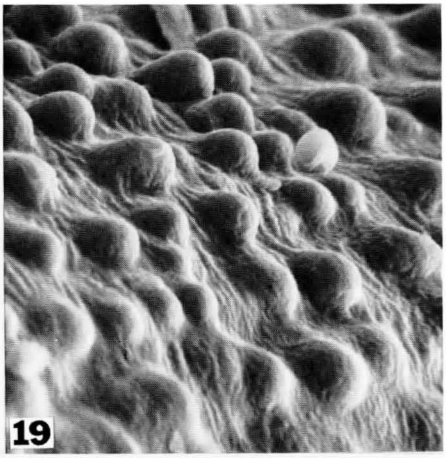
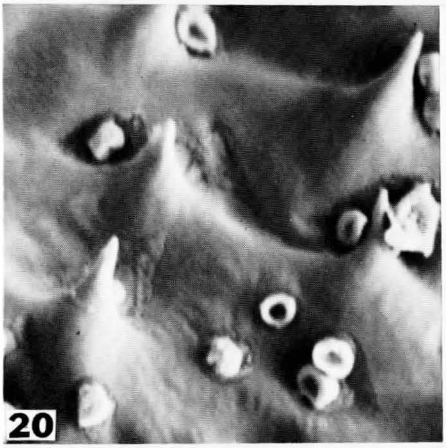
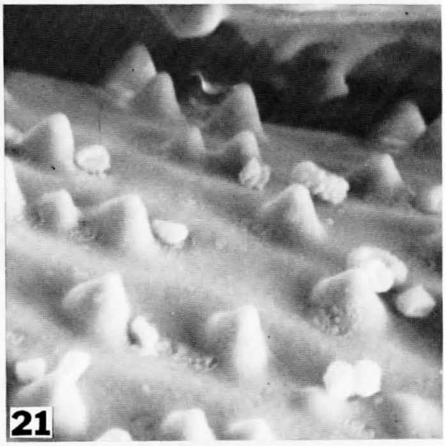

17

Fig. 18-23: 18, 20, 22, examples of teeth on files. 19, 21, 23, examples of tubercles on stridulating plates. 18, 19, *Aricia artaxerxes* F., area 5/6, dorsal. 20, 21, *Apodemia mormo* (F. & F.), area 4/5 dorsal. 22, 23, *Caria ino melicerta* Schaus, area 5/6, ventral.


18


19

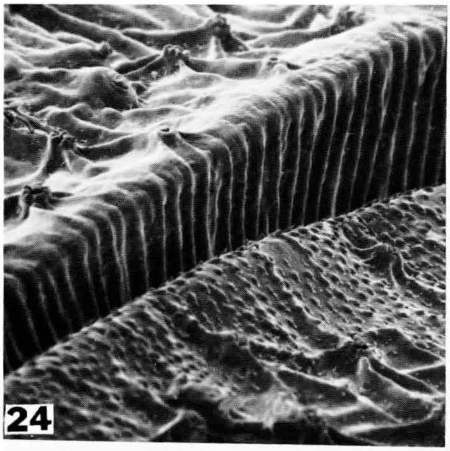

20

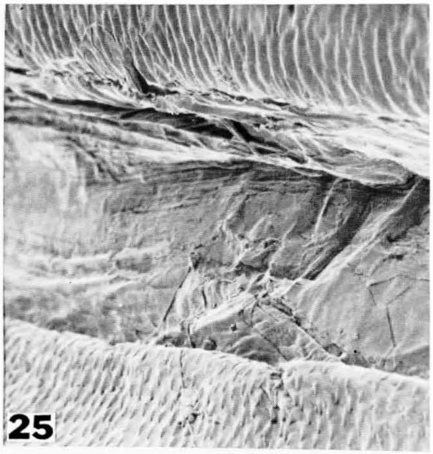

21

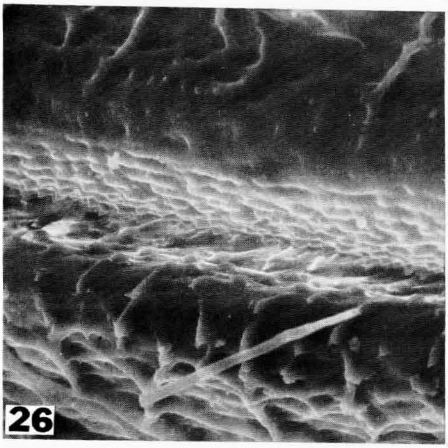

22

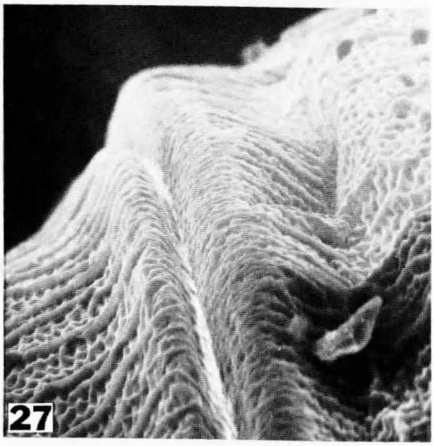

23

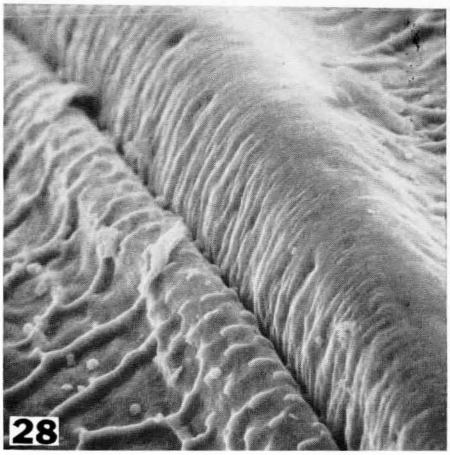
Fig. 24-29: Stridulating area between segments 5/6. 24, *Atlides halesus* (Cram.). 25, *Feniseca tarquinius* (Fabr.). 26, *Plebicula dorylas* Schiff. 27, *Nacaduba beroe gythion* Fruh. 28, *Prosotas nora suletes* (W. & L.). 29, *Nemeobius lucina* L., segments 4/5.


24


25


26


27


28

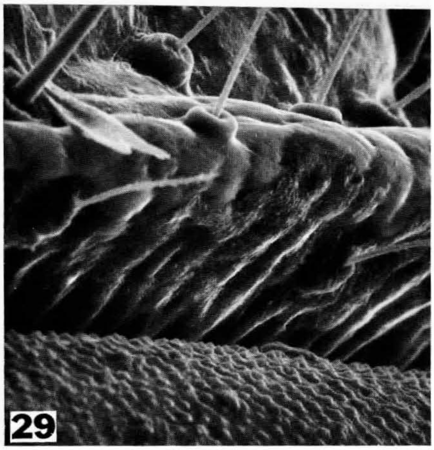
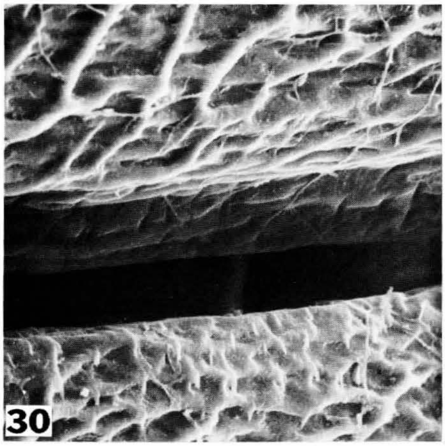
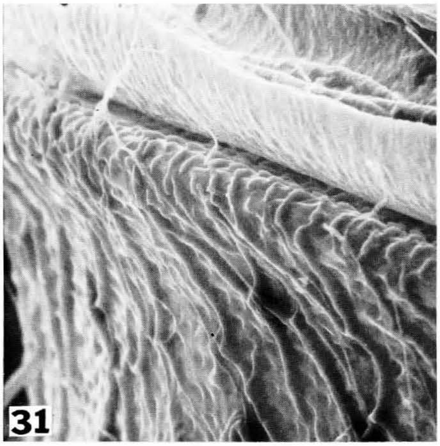
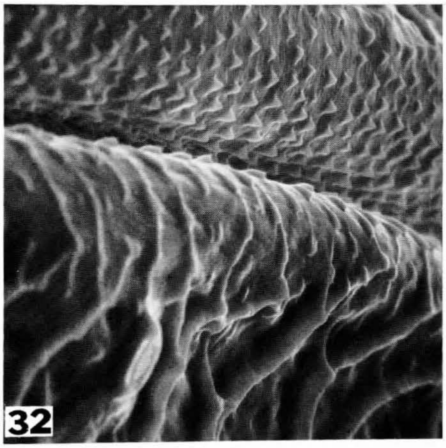
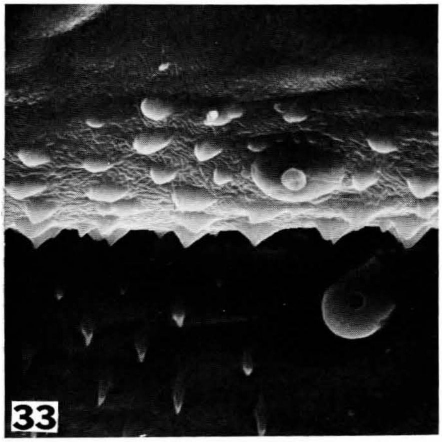

29

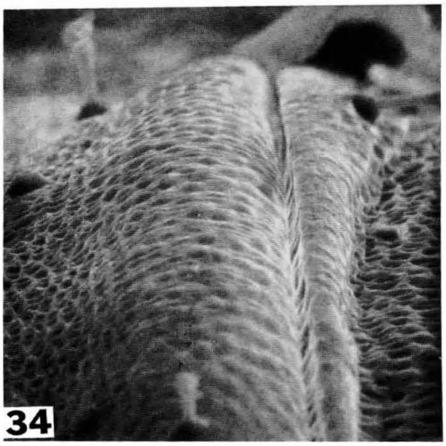
Fig. 30-35. Details of pupal morphology. 30-33, 5/6 segments. 30, *Phasis thero* (L). 31, *Prosotas dubiosa indica* (Ev.). 32, *Leptotes cassius theonus* (Lucas). 33, *Lycaena dispar* Haworth. 34, *Spindasis vulcanus* (Fabr.), 4/5 segments. 35, *Iolaus glaucus jordanus* Stgr., 7/8 segments.

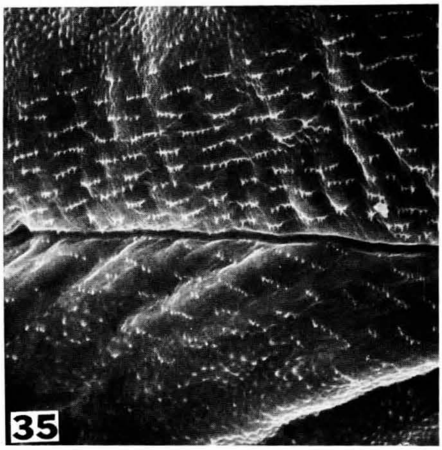

30


31

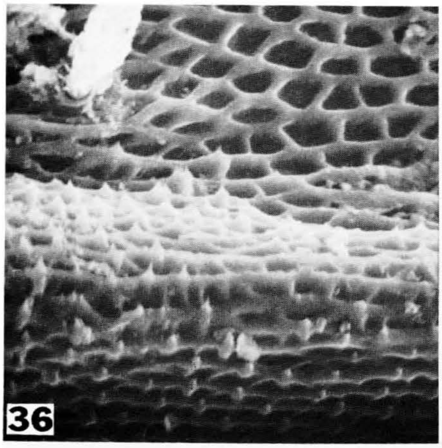

32

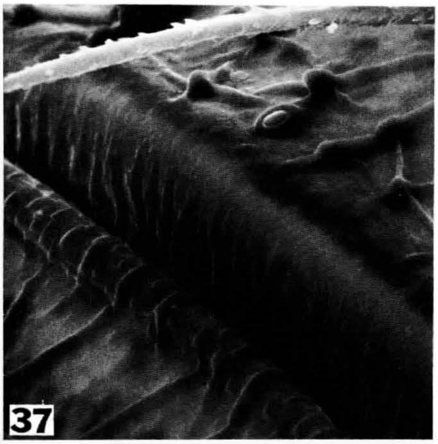

33

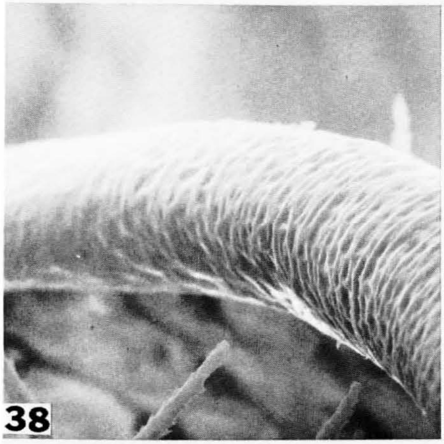

34

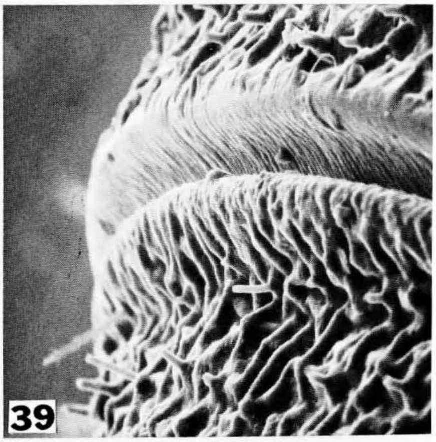

35

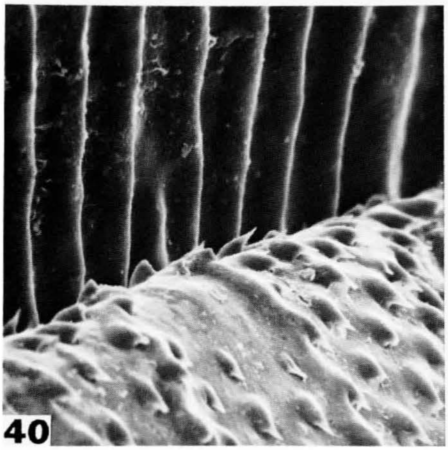
Figs. 36-41: Details of areas of stridulation. 36, *Anatole rossi* Clench, anterior segment 6. 37, *Strymon columella istapa* (Reak.), 5/6 segments. 38, *Strymon dryope* (Edw.) posterior segment 5. 39, *Callophrys dumetorum* (Bdv.), 5/6 segments. 40, *Atlides halesus* (Cram.). 5/6 segments, note smoothness of ridges on the stridulatory plate. 41, *Caria ino melicerta* Schaus, posterior segment 4, tubercles on stridulatory plate disappear toward membranous area of intersegmental region.


36


37


38


39


40

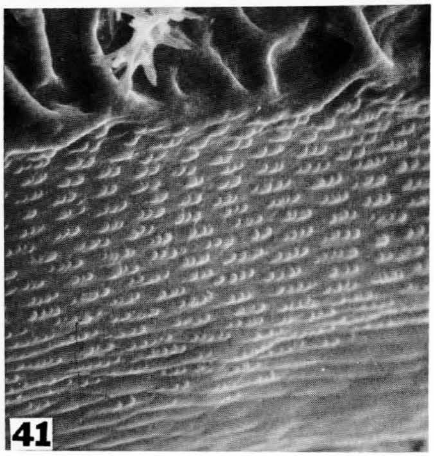
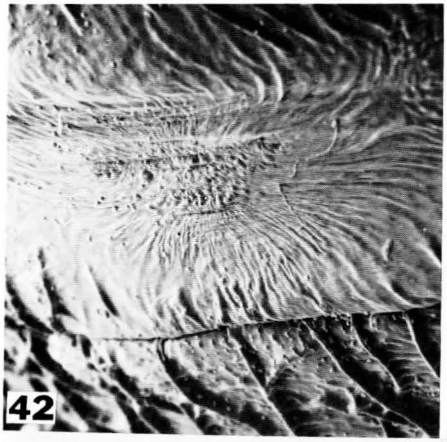
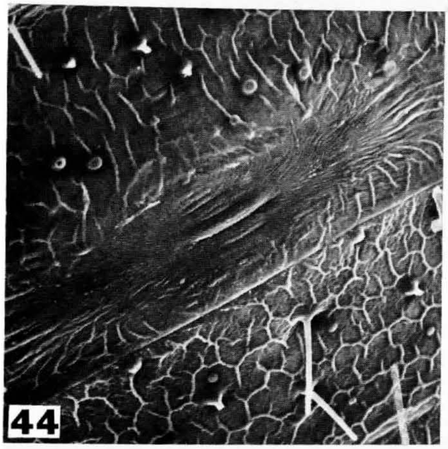
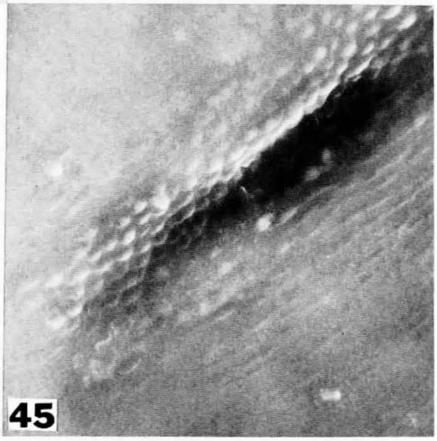

41

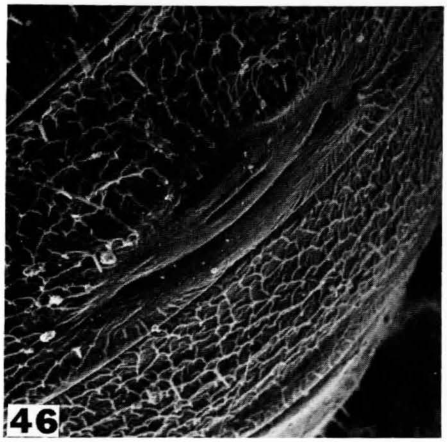
Fig. 42-47: Dorsal view, segment 7, showing scars of larval honey-gland. 42, *Aricia artaxerces* F. 43, 45, *Nacaduba biocellata* (Feld.). 44, *Polyommatus icarus* Rott. 46, *Jamides celano* (Cram.). 47, *Jamides bochus* (Stoll.).


42


43


44


45


46

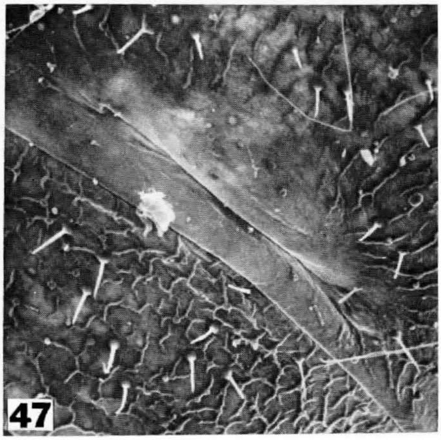
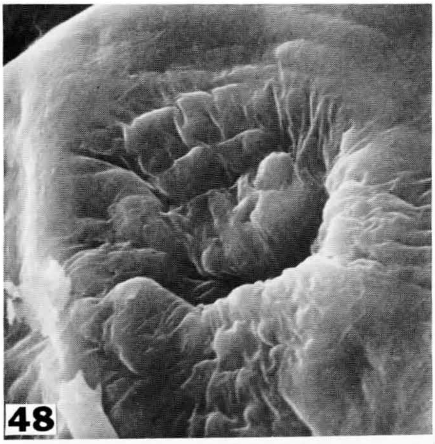
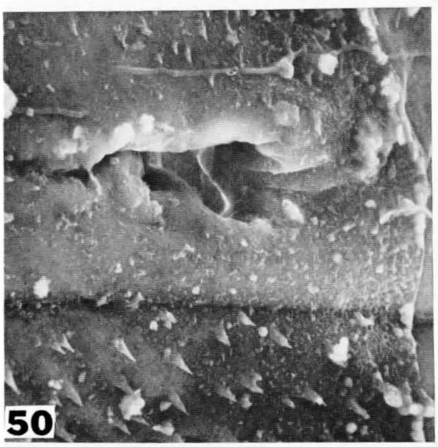
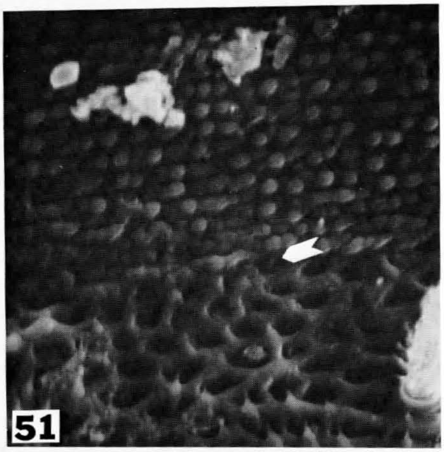

47

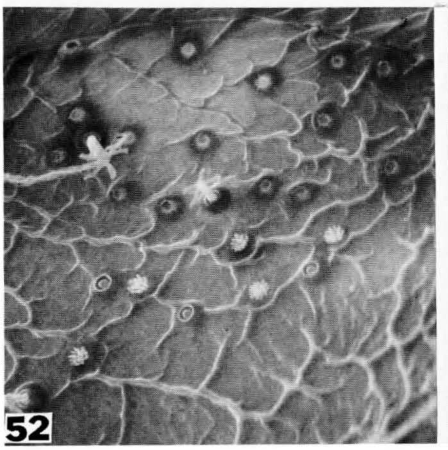
Fig. 48-50, lateral area segment 8 to show tentacle scars. 48, *Prosotas nora auletes* (W. & L.). 49, *Prosotas dubiosa indica* (Ev.). 50, *Chilades laius* (Cram.). 51, *Anatole rossi* Clench, posterior segment 5, note granular plate overlying ribbed sculpturing of segment. 52, *Calephelis nemesis* (Edw.), details of metathoracic "glandular" region. 53, *Lycaena helloides* (Bdv.) posterior segment 7 showing obsolescence of ribbing near intersegmental cleft.


48


49

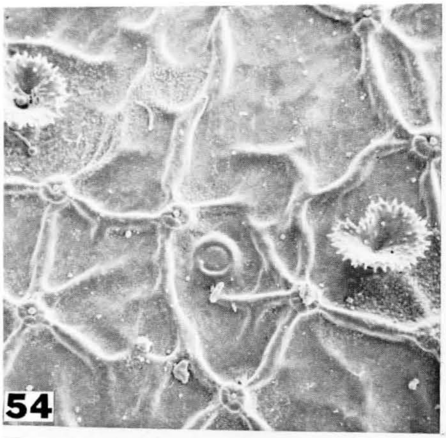

50

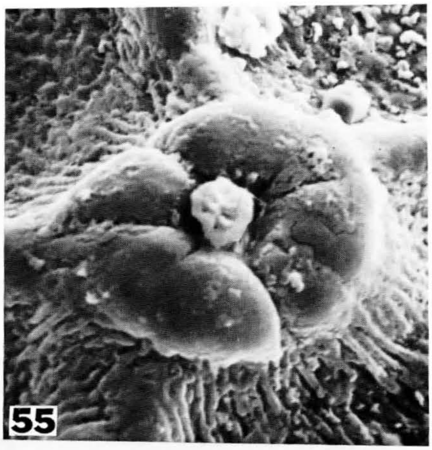

51

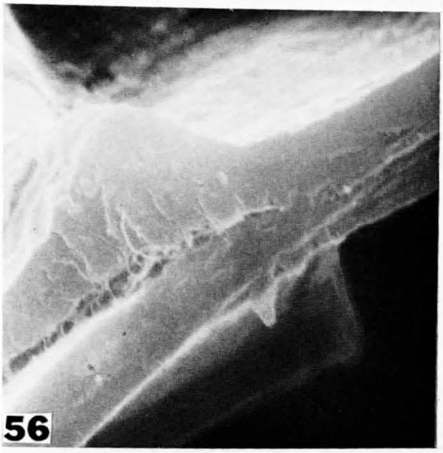

52

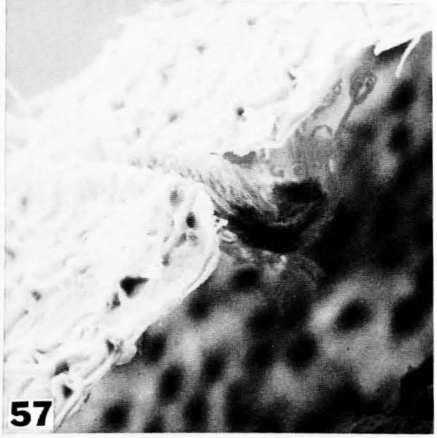

53

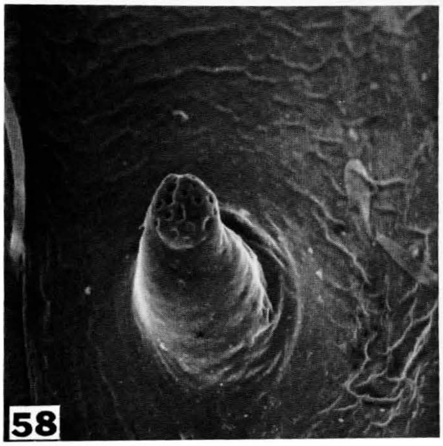
Fig. 54-59: Details of pupal morphology. 54, 55, *Lycaena thoe* Guer.; 54, elevated ribbing characteristic of pupal integument; 55, details of organ at rib junction, of typical sensilla basiconica shape. 56, 57 *Strymon dryope* (Edw.); 56, X-sec. through integument showing rib and layered nature; 57, longitudinal section showing intersegmental enfolding of segments 3/4. 58-59, *Lymnas pixe* (Bdv.); 58, dorso-lateral verruca on segment 3, note mushroom setae (see fig. 72); 59, verruca, subspiracular region, segment 4.

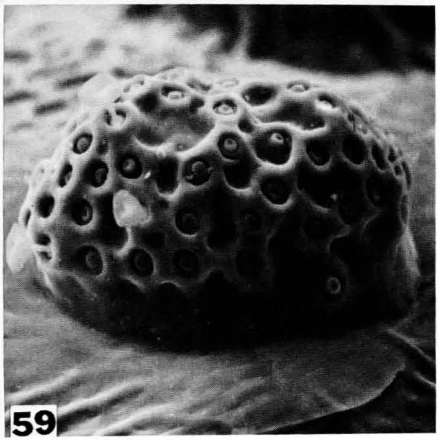

54


55


56


57


58


59

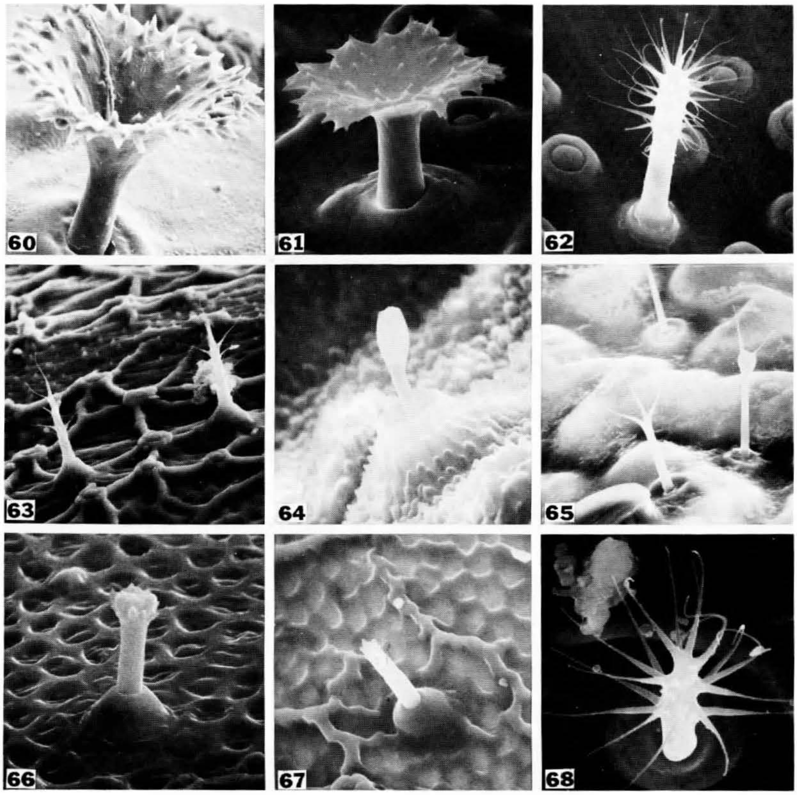


Fig. 60-68: Types of pupal setae in lycaenids. Photos taken in vicinity of spiracle on segment 6 except where indicated by segment number. 60, *Lycaena thoe* Guer. 61, *L. dispar* Haworth. 62, *Strymon columella istapa* (Reak.), note sensilla campaniformia in background, on surface of integument. 63, *Jamides celano* (Cram.). 64, *Iolaus glaucus jordanus* Stgr. 65, *Philotes speciosa* (H. Edw.). 66, *Spindasis vulcanus* (Fabr.). 67, *Nacaduba pactolus cela* W. & L., segment 5. 68, *Prosotas nora auletes* (W. & L.).

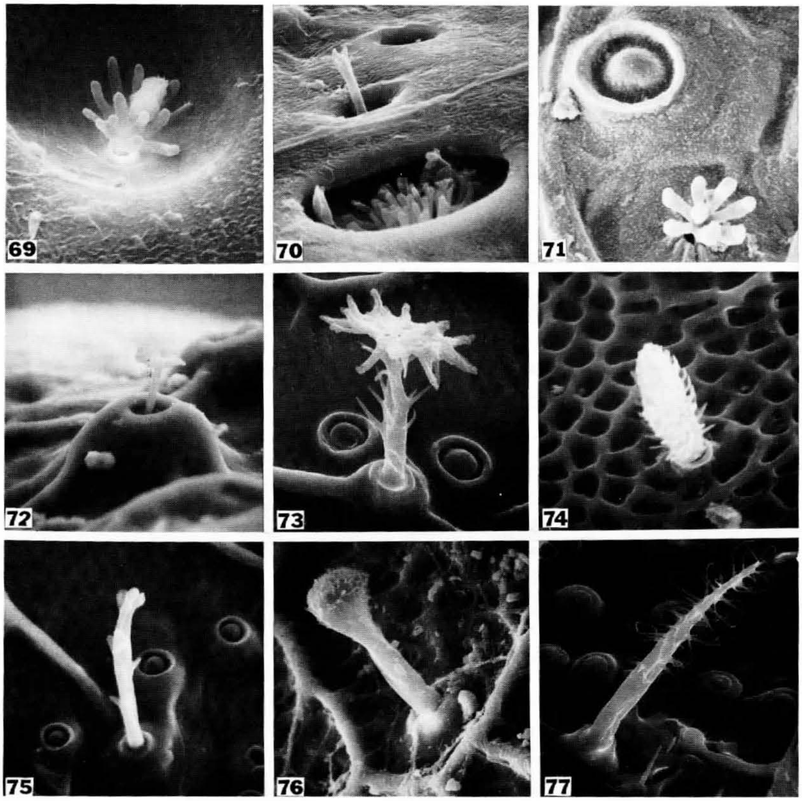


Fig. 69-77: Types of pupal setae in lycaenids and riodinids. The number indicates the abdominal segment on which the setae for the photo was taken. 69-70, *Curetis acuta paracuta* de Nice. 69, seg. 3. 70, a coeloconic sense organ from seg. 7. 71, *Lephelisca wrighti* (Holl.), 6. 72, *Lymnas pixe* (Bdv.) 4. 73, *Caria ino melicerta* Schaus, 4. 74, *Anatole rossi* Clench, 7. 75, *Calephelis nemesis* (Edw.), 3. 76, *Phasis thero* (L.) 6. 77, *Strymon bazochii* (Godt.).

ERRATA: NO. 13

p. 3, line 18: “Vilhena”, not “Velhena”.
p. 4, line 36: no semicolon at end of line.
p. 4, line 38: “Marechal”, not “Merchal”.
p. 5, line 26: “11-VII-72”, not “11pVII-72”.
p. 6, line 20: “Erichson”, not “Erickson”.
p. 7, line 3: “. . .1500 Km. of *H. e. eucoma. . .*”, not “1500 “15. .1500 Km. from *H. e. eucoma. . .*”.
p. 9, line 5 from bottom: “FW vein M_1”, not “FW vein M”.
p. 9, line 34: “(Figure 17)”, not “(Figure 18)”.
Plate III, second line from bottom: “population”, not “pupulation”.
Plate IV, lines 4 & 5: “*elevatus*”, not “*elivatus*”.