

On the Reproduction of *Prionoplus reticularis* (Coleoptera, Cerambycidae), with General Remarks on Reproduction in the Cerambycidae

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With one plate (fig. 9)

SUMMARY

Gametogenesis is completed in the pupa in both sexes and the gonads degenerate in the non-feeding adult.

Cyst wall cells, which act as trophocytes, accompany each cyst from an axial germinal region to the radiating follicles. A preliminary account is given of a fibrous structure, the lens of Demokidoff, which overlies the germinal region in the testis lobe.

The morphology of the gut and gonads of the male *Ochrocydus huttoni* are briefly described.

A distinction is drawn between the reproductive patterns of the subfamilies Prioninae and Lamiinae. In the former, where a non-feeding adult is characteristic, gametogenesis is completed in the pupa, after which the gonads degenerate, while in the Lamiinae, where the adult feeds, gamete production continues through adult life. Between these extremes, the Cerambycinae and Aseminae appear to tend toward the prionine condition, and the Lepturinae toward the lamiine.

INTRODUCTION

THE biology of the Cerambycidae has a large literature, but little is known of reproduction in the family. The limited information, nevertheless, suggests that *Prionoplus reticularis* White exemplifies a reproductive pattern typical of prionine Cerambycidae. Feeding is restricted to the larval stage; in the short-lived adult the mid-gut is a vestige and the fore-gut a dilated air-sac (Edwards, 1961). Ovulation and spermatogenesis occur early in the pupal stage and the gonads are already regressing when the adult emerges from the log-bound pupal chamber. The newly emerged gravid female is more or less sedentary, assembling males by means of an olfactory attractant; she flies only after part of her heavy load of eggs is laid. Further details of reproductive behaviour are discussed elsewhere (Edwards, 1961).

MATERIAL AND METHODS

Observations were made on live and preserved material collected in the Riverhead State Forest near Auckland, New Zealand, during the summers of 1953-4 and 1954-5. Pupae of known age were obtained from mature larvae which were collected in the field and pupated in the laboratory. Histological material was fixed in aqueous or alcoholic Bouin, sectioned at 8 or 10 μ , and [Quarterly Journal of Microscopical Science, Vol. 102, part 4, pp. 519-529, 1961.]

stained in Mayer's haemalum and eosin or Mallory's connective-tissue stain. Carnoy's fixative and acetocarmine 'squashes' were used to examine the nuclei of gonad tissue.

RESULTS

Morphology of the reproductive system

Gonadal strands develop rapidly in the prepupal stage; gonad rudiments were not located in dissections of mature larvae. The active gonad reaches maximum development in the pupa and it is mainly on this stage that the following descriptions are based.

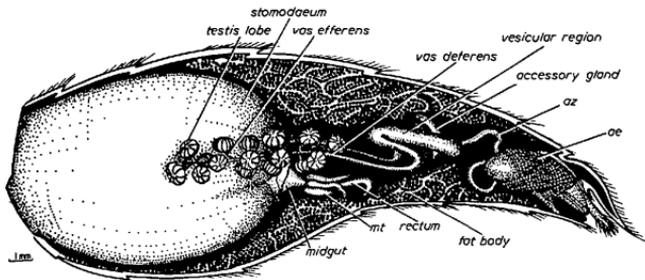


FIG. 1. Gut and reproductive system of a young adult male *Prionoplus*. *ae*, aedeagus; *az*, ductus ejaculatorius; *mt*, Malpighian tubule.

The male (fig. 1). The arborescent testis is composed of 12 to 15 lobes, connected to an axial vas deferens along its anterior third by short vasa efferentia. Each lobe, about 0.75 mm in diameter, comprises 15 follicle sectors arranged about a central cavity in the manner of a mandarin orange. The testis lobes are embedded in massive fat-body that occupies almost the entire pupal haemocoel, and are surrounded by a peritoneum which appears first as a layer 2 or 3 cells thick, distinguishable from the surrounding fat-body by the relative lack of cytoplasmic inclusions.

The peritoneum eventually condenses to form a membranous layer whose originally cellular nature is evident only where small, isolated nuclei remain. The follicular epithelium of the testis which is initially cuboidal also loses its distinctly cellular structure as development proceeds, coming finally to resemble the peritoneum. The development of the spermatic cysts and an apical structure of each testis lobe, comparable with the 'lens of Demokidoff' (Deegener, 1928), are discussed later. The vasa efferentia and terminal vasa deferentia have delicate circular muscle-layers. Posteriorly to the testis lobe region the vas deferens expands to form a storage region. Shortly before uniting with its pair the wall becomes thickly muscular and receives the duct of a crescentic, mesodermal accessory gland. The secretion of the accessory gland is an opaque, finely granular material that passes into the sperm storage region

of the vasa deferentia, where it mixes with the sperm mass. *Prionoplus* does not form a spermatophore.

The muscular ductus ejaculatorius, which reaches a length of 2.5 cm, lies tightly coiled between the apodemal wings of the aedeagus in the resting position. It is continuous with the endophallus, which in the invaginated state lies coiled with the ductus ejaculatorius. In the everted state the endophallus (fig. 2) is seen to consist of 3 regions. The basal part between the median aedeagal valves is more bulbous than the remainder; it terminates where sclerotized processes arise on each side. The lateral plates of these processes re-

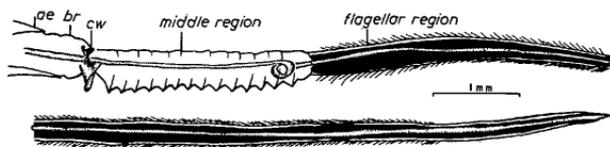


FIG. 2. Everted endophallus of *Prionoplus*. *ae*, aedeagus; *br*, basal region; *cw*, chitinous wings.

semble those figured by Duffy (1953*a*) for *Prionus coriarius*. It has been suggested that these structures act as claspers, but observation of *Prionoplus* when mating did not lend support to this view. It seems more probable that they serve to enclose the invaginated internal sac and retain it within the body by means of retractor muscles passing to the aedeagal wall, the transverse processes forming a 'double door' to the aperture of the aedeagus. The second or middle part of the endophallus is a glabrous tube, corrugated ventrally with 16 backwardly facing transverse ridges, each slightly sclerotized at the apex. This region is the first to be evaginated during pairing and serves to hold the endophallus within the ovipositor during eversion of the flagellum. Fine retrorse hairs that cover the flagellum except at the tip evidently serve to prevent it from moving back through the vagina during the movements of ejaculation. It is retracted after coitus by reinvasion. Muscle-strands from more distal points insert on the mid-ventral base of the aedeagus, and from proximal points on the anterior apodeme of the phallobase and the aedeagal wings.

Abdominal contractions that appear to be associated with the development of a hydrostatic pressure in the haemocoel for evaginating the flagellum are seen during the early stages of pairing.

The genitalia and genital musculature will be dealt with in a further paper.

The female (fig. 3). The ovaries of the adult female are more or less obliterated by the egg masses within the distended lateral oviducts. The following description therefore refers mainly to the pupal organs.

The paired ovaries, each composed of 42 to 45 polytrophic ovarioles, lie dorsolaterally in abdominal segments back to the 7th, where they attach ventrolaterally to the 7th sternite. The terminal ovarian filaments on each side are united to form paired ovarian ligaments attached to the metathoracic phragmata. During the approximately 10-day prepupal stage the external

genitalia are formed by invagination and the bifid genital duct so formed unites anteriorly on each side with the calyces, forming the cuticle-lined lateral oviducts of the mature female. The primary connexion of the mesodermal

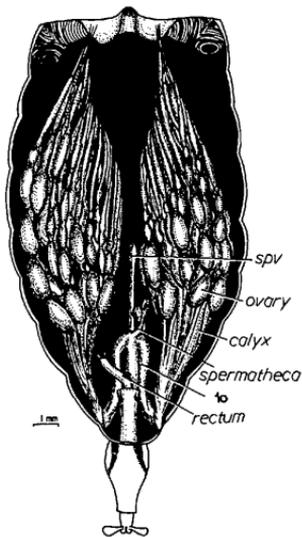


FIG. 3. Reproductive system of a female pupal *Prionoplus*. *lo*, lateral oviduct; *spv*, spiculum ventrale.

gonoduct with sternum 7 is retained throughout life as a bundle of degenerate muscle-tissue and tonofibrillae which provide a posterior anchorage to the distended calyces.

In the early pupa the calyces are stellate in cross-section, expanding as they receive eggs, and displacing fat-body until they meet in the mid-line. Finally, towards the end of the pupal period, they come to occupy the greater part of the abdominal cavity. The wall is membranous, the basement membrane and peritoneum forming the greater part of its thickness. The lateral oviducts which open to the vertical mesial walls of the calyces in segment 6 are distensible muscular ducts in which the intima is clothed with cteniform, scale-like projections (fig. 4). The spines of each scale are hollow at the base but do not appear to open at the tip. Since the membranous calyx can play no part in the extrusion of eggs to the lateral oviduct, this must be effected by the muscular oviduct walls, the spinose intima serving to grip the egg.

The median oviduct is notable for the form of the muscularis, in which a strong circular layer fuses with the intima along two diametric lines (fig. 5). Between the dorsal and ventral transverse crescentic muscle-bands and the epithelium lies a group of longitudinal muscles which have no connexions with the circular muscle and which insert to the intima at scattered points. The vagina within the ovipositor has a simple circular muscularis and resembles its male homologue, the ductus ejaculatorius. The accessory structures of the female reproductive system are associated with the anterior end of the median oviduct (fig. 6) where it fuses with the lateral oviducts in a muscular region which also receives extrinsic retractor muscles from the spiculum ventrale, sternum 8, and apodemal surfaces of segment 9 on the inner wall of the ovipositor.

The spermatheca and bursa copulatrix arise together in a muscular stalk, the bursa soon separating to form a finger-like lobe. The apex of the spermatheca is reflected and attached to the spermathecal stalk by muscle-fibres (fig. 7). In this region the outer wall is rather more heavily cuticular than the inner wall of the curve. Manipulation of fresh material suggests that contraction of these muscles, for which the curved cuticular wall acts as antagonist,

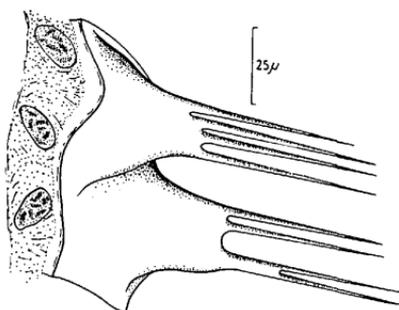


FIG. 4. Spines of lateral oviduct intima.

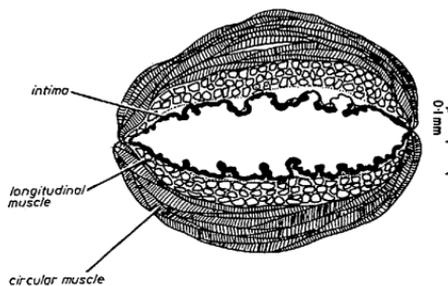


FIG. 5. Transverse section of the median oviduct in the region of divided circular muscularis.

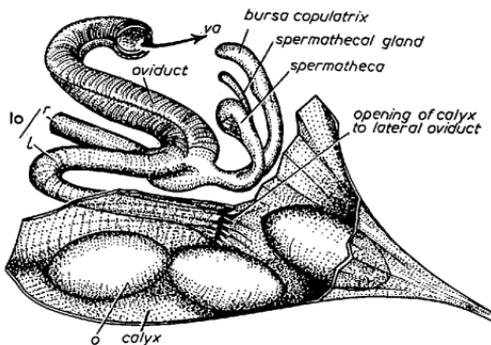


FIG. 6. Oviducts and associated structures in an adult female *Prionoplus*. *lo*, lateral oviducts (*l*, left; *r*, right); *o*, ovum; *va*, vagina.

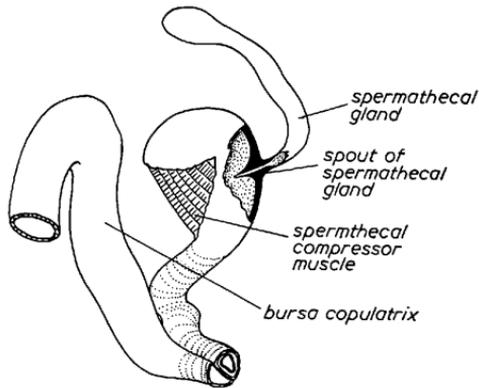


FIG. 7. Spermatheca and bursa copulatrix.

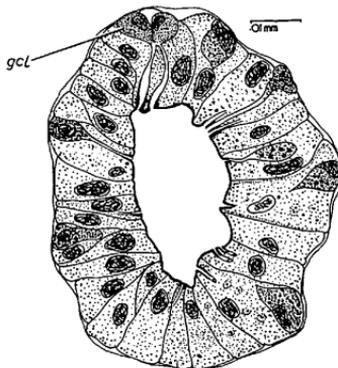


FIG. 8. Transverse section of spermathecal gland. *gcl*, gland cell.

serves to express sperm from the spermatheca. The acutely tapered spout of the spermathecal gland evidently prevents entry of sperm during the compression stroke, and possibly releases its secretion during the recovery stroke. The precise function of the compressible spermatheca in *Prionoplus* remains problematical, since the entire bursa copulatrix, spermathecal duct, and common duct are usually packed with spermatozoa in mature females.

Ductules of cellular glands are common in the wall of the spermathecal gland (fig. 8).

The long ovipositor bears a pair of subapical clavate lobes, each of which bears a terminal circling of sensory setae surrounding a group of longer sensory hairs that are employed during selection of the oviposition site.

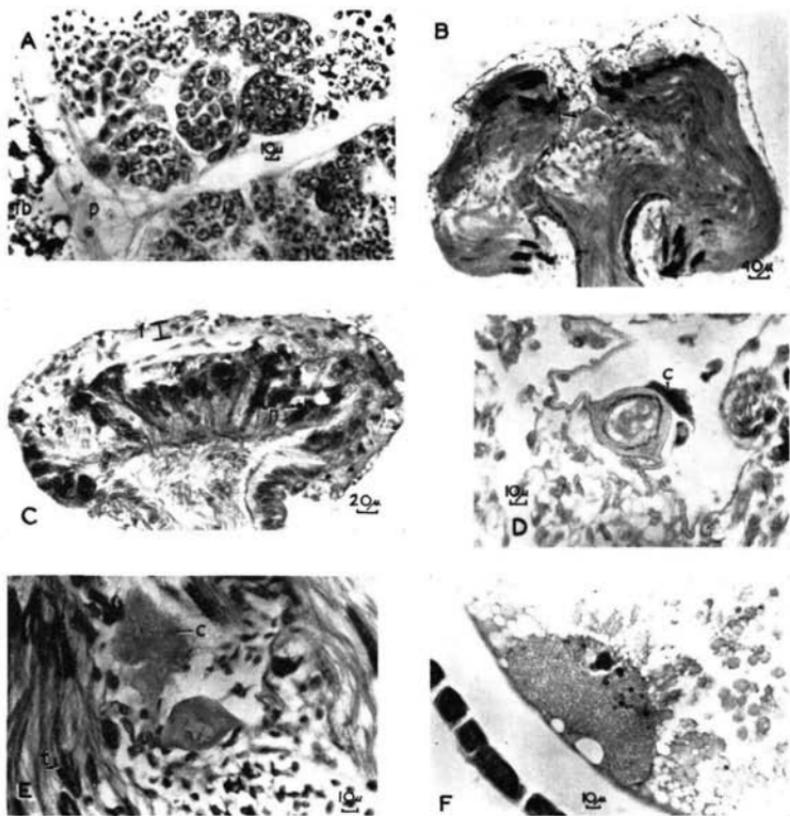


FIG. 9

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Development and senescence of the testes

The testes of *Prionoplus* are active only for a short time in the life of the individual. After rapid growth in the prepupa they reach maximum activity early in the pupal stage, and maturation of all the spermatozoa is complete before emergence of the adult.

Development of spermatozoa. The germarial region is subapical and extra-follicular, lying under the 'apical lens' to be discussed below. Cyst and germ cells move into the radiating follicles where growth and maturation takes place (fig. 9, A). Cyst formation is most rapid in the late prepupa, giving rise to a mass of cysts which are almost synchronous in development, though for some reason not yet understood the more axial and more peripheral cysts mature at a slower rate than those lying between them, and small groups of adjacent cysts may develop out of phase with those surrounding them. The majority of cysts contain primary and secondary spermatocytes by the 5th to 7th pupal day and spermatids are present by the 7th to 10th day. No dividing cells were found in acetocarmine 'squashes' of testis tissue older than 10 days. 128 spermatids are formed in each cyst. Discharge of spermatozoa to the axial cavity of the testis lobe, and so to the vas efferens, usually takes place in the late pupal or preimaginal stage (fig. 9, B). Thereafter the testis lobe decreases in diameter from a maximum of 0.75 mm to 0.4 mm or less (fig. 9, C).

By the time the hardening and darkening of the adult cuticle has occurred much of the sperm mass has reached the now dilated lower region of the vasa deferentia, and from this stage on the testis wall undergoes degenerative changes.

Up to the time of their release from the cyst the spermatozoa are linear, but as they break from the cyst and arrive in the lumen of the lobe the tail develops an open spiral form with a periodicity of about 10 μ .

Supporting tissue. The nuclei of the two cyst cells or trophocytes of each spermatid cyst are prominent and densely basiphil from the time of their arrival in the follicle. When the cyst elongates during spermatid formation, the trophocyte nucleus also lengthens and becomes flattened closely over the spermatid bundle (fig. 9, E). Unlike the cyst cells of the rutelid beetle *Popillia*

FIG. 9 (plate). Photomicrographs of material fixed in Bouin; paraffin sections, 10 μ .

A, transverse section of an early pupal testis of *Prionoplus*. Mayer's haemalum, eosin. *fb*, fat-body; *p*, peritoneum.

B, median longitudinal section of newly emerged adult *Ochrocydus huttoni*. Mayer's haemalum, eosin. *l*, lens.

C, median longitudinal section of testis of adult *Prionoplus* in advanced senescence, showing apical cap composed of lens and overlying connective tissue. Below the cap is a syncytial layer containing endomitotic nuclei. Mayer's haemalum, eosin. *f*, open connective tissue; *t*, connective tissue containing tracheae; *n*, degenerating trophocyte nucleus.

D, median longitudinal section of a late pupal testis of *Prionoplus*. Mallory's triple stain. *c*, connective tissue.

E, median longitudinal section of the lens in a late pupal testis of *Prionoplus*. Mayer's haemalum, eosin. *t*, elongated, flattened trophocyte nucleus.

F, longitudinal section of the germinal vesicle of a *Prionoplus* oocyte, showing nucleolar buds. The follicular epithelium has separated from the oocyte. Mayer's haemalum, eosin.

japonica described by Anderson (1950), which are said to accompany the spermatozoa from the testis lobes, continuing their nutritive function, those of *Prionoplus* do not leave the lobe. Their exact fate has not been determined but it is thought that the majority break down when the bundles of spermatozoa leave the cyst, though a few may remain in a group of large endomitotic nuclei in the coenocytic tissue of the senescent testis that lies under and in contact with the apical lens.

The apical lens. An apical cell of a Versonian type (Snodgrass, 1935) is not present in the testis of *Prionoplus* or *Ochrocydus*. The apical region is, however, occupied by a lenticular body similar to that briefly described by Demokidoff (1902) in the testis of *Tenebrio molitor* and termed the 'lens of Demokidoff' by Deegener (1928). The composition and function of the lens requires further examination, but a preliminary description is appropriate here.

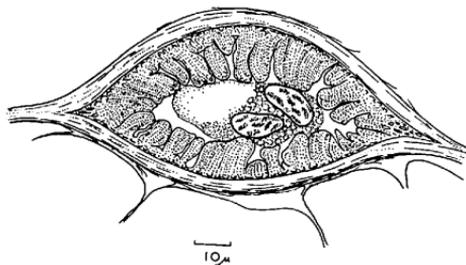


FIG. 10. The lens of a late pupal testis of *Prionoplus*.

In the active testis of the early pupa the lens overlies the germarial region. It is evidently derived from the basement membrane of the testicular epithelium, which is thickened to form a material of fibrous appearance staining weakly with eosin and giving a strong orange-red with Mallory's triple stain. Within a fibrous coat a much-folded layer, with a radiating fibrous appearance and similar staining properties to the laminate coat, surrounds a central cavity containing several nuclei. The nuclei are not markedly endomitotic. Above the lens, but not in contact with it, is a loose mass of connective tissue.

In the late pupa the lens (figs. 9, D, E; 10) is more convex, and the central cavity larger. In *Prionoplus* it is about 40 μ deep and 50 to 60 μ in greatest diameter. The lacuna, 25 μ in diameter, contains about 10 nuclei.

In the adult testis the lens material extends over the surface of the lobe as a cap up to 150 μ in diameter. It is here composed of two layers, both giving a strong orange-red colour with Mallory's triple stain. The outer region, 20 to 30 μ thick, apparently derived from previously blue-staining connective tissue originally overlying the testis, contains tracheoles and scattered nuclei. The more homogeneous inner layer, the lens proper, is seldom more than 10 μ thick. A ring of open connective-tissue, packed with convoluted tracheoles,

surrounds the lens. Below, separating it from the sperm-filled lumen of the shrunken lobe, is a layer of syncytial tissue containing large endomitotic nuclei, degenerating nuclei in which the chromatin has clumped, and numerous trapped spermatozoa. Tracheoles traverse the tissue, and there appear to be ductules passing from the endomitotic nuclei to the lumen of the lobe. The tissue appears to be undergoing autolysis; it is entirely resorbed in aged males.

The lens bears little resemblance to a typical apical cell, and its relationship to the germinal tissue in early stages of the testis and during resorption in the adult is not clear. Demokidoff's view that in *Tenebrio* it is simply a structural support (*Stützorgan*) from which connective tissue carrying tracheoles enters the testis before pupation, probably only partly accounts for its function.

Development and senescence of the ovaries

Like the testes, the ovaries are only active for a short time. The pattern of their development and regression parallels that of the testis. Delicate ovarian filaments arise in the prepupa, and by the 5th pupal day tubular germaria are present in the 2nd and 3rd abdominal segments. At 5 to 7 days several ova have enlarged in most ovarioles, producing about 400 mature eggs. More apical ova and oocytes are compressed against the metathoracic phragmata as the calyces fill and press the germarial region forward. These do not develop when oviposition begins, but are resorbed, so that there is virtually no remnant of the ovarioles in the mature adult.

In the active ovariole of the early pupa, oocytes are differentiated toward the lower end of the germarium and do not appear to be accompanied by trophocyte cells when they leave the germarium. Four to 5 oocytes in the neck, above the first separate follicle, increase from about $16\ \mu$ to $130\ \mu$ in diameter. Their germinal vesicles are central.

The follicular epithelium surrounding the enlarging ovum is composed of columnar cells with strongly basiphil cytoplasm and large endomitotic nuclei. Simultaneously with the beginning of yolk deposition the germinal vesicle moves to the wall of the primary oocyte and nucleolar budding gives rise to a number of vesicles that lie toward the inner margins of the germinal vesicle. Yolk granules have a radiating orientation about the corrugated inner surface of the germinal vesicle, suggestive of its role in yolk synthesis (Bonhag, 1958). (See fig. 9, F.)

The ovariole epithelium between follicles is columnar and extends to the centre of the ovariole but in these regions it is markedly less basiphil than the cubical epithelium surrounding the developing ovum and does not appear to have a nutritive function.

A note on Ochrocydus huttoni

The morphology of the adult gut and testis of the cerambycine *O. huttoni* closely resembles that of *Prionoplus*. The mid-gut is a functionless vestige and

the fore-gut dilated and membranous. As in *Prionoplus* the cryptonephric Malpighian tubules and rectum are little changed from the pupal condition apart from loss of muscle. The testis on each side comprises 22 to 24 lobes, arranged as in *Prionoplus*, each with 20 follicles. Maturation of spermatozoa is complete at emergence of the adult (fig. 9, B).

Cerambycid reproductive cycles

Linsley (1959), in his recent review of cerambycid biology, states that 'although some Cerambycidae (e.g. *Hylotrupes*, *Stromatium*) require little or no food in the adult stage, for most species some feeding appears to be an essential prerequisite to egg maturation and oviposition'. It is generally agreed, however (e.g. Butovitsch, 1939; Craighead, 1923; Duffy, 1953*a, b*), that the majority of Prioninae and a large number of Cerambycinae require no food as adults. The food of adult Lamiinae does suggest that they require a nitrogen source as adults for reproduction.

It is to be expected that this contrast between a long-lived feeding adult and a short-lived non-feeder should be reflected in the reproductive pattern, though this does not appear to have been recognized previously. It has been shown above that in two species, one a prionine and the other a cerambycine, gametogenesis is compressed into a comparatively short period of intense activity in the pupa and that the gonads of the adult are senescent. Silantjew (1908), found the reproductive organs of the cerambycine *Stromatium fulvum* 'fully developed' at emergence and observed that the digestive organs were weakly developed and never contained food. Ritchie (1920), on the contrary, noted that the reproductive organs of the lamiine *Saperda cackaria* (an adult feeder) are 'not quite mature' on issue of the imagines but ripen in a short time.

Ehara (1951), who examined the histology of the adult testes of 41 species of Cerambycidae from a taxonomic point of view, distinguished the Cerambycinae and Lepturinae in which he found only spermatozoa and spermatids, from the Lamiinae in which meiotic divisions were to be found throughout the adult life.

On the basis of these observations it seems possible to make a clear distinction between typical prionine and lamiine Cerambycidae. In the former, gamete formation is completed in the pupal stage in both male and female, and the adult does not feed. In the Lamiinae, on the contrary, the adult continues to feed and gamete formation continues throughout the longer adult life. Other subfamilies lie between these extremes. There is probably considerable variation within each group but the available data suggest that the Cerambycinae and Aseminae tend toward the prionine pattern and the Lepturinae toward the lamiine pattern.

I thank Dr. J. G. Pendergrast of the Department of Zoology, University of Auckland, for helpful discussion during this study.

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