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DISTRIBUTION OF ADULT DEFENSE GLANDS IN CHRYSOMELIDS (COLEOPTERA: CHRYSOMELIDAE) AND ITS SIGNIFICANCE IN THE EVOLUTION OF DEFENSE MECHANISMS WITHIN THE FAMILY

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Abstract—Defense glands were examined in the adults of 65 species belonging to 10 different subfamilies. They were found in the pronota and elytra of members of the subfamilies Criocerinae, Chrysomelinae, Galerucinae, and Alticinae. It is suggested that these glands appeared monophyletically in the course of evolution and that the absence of glands in several species of the two most evolved subfamilies is a secondary event, explained by the presence of alternative efficient defensive behaviors: reflex bleeding in the Galerucinae and escape mechanism of jumping in the flea beetles. It is also suggested that a large distribution of the glands at the surface of the beetles is a primitive condition and that in the course of evolution only the glands most efficiently located along the edges of the pronotum and elytra were maintained. Such evolution has occurred several times. Alternative and complementary defensive mechanisms are also listed and discussed.

Key Words—Defense, glands, Coleoptera, Chrysomelidae.

INTRODUCTION

Defensive glands have, for a long time, been reported in the adults of a few species of chrysomelids, all belonging to the subfamily Chrysomelinae (Cuénot, 1896; Tower, 1906; Hollande, 1909; Patay, 1937).

When disturbed, the beetles emit a secretion from glands opening at the surface of elytra and pronotum; this behavior was considered to be responsible for their repellent quality against various vertebrate predators.

Indeed, it has been demonstrated recently that the secretion of most species of the genera *Chrysolina*, *Chrysochloa*, and *Dlochrysa* contains cardenolides which are produced by the beetles themselves and not originally present in their host plant (Pasteels and Daloze, 1977; Daloze and Pasteels, 1979; Pasteels et al., 1979).

None of these works, however, accurately describe the structure of the glands and their distribution within the insects. In a previous paper this was attempted for the Colorado beetle (Deroe and Pasteels, 1977). We would like now to look more extensively at many species of the family in order to investigate how far this defensive mechanism is distributed within the chrysomelids, and how it could have evolved.

This paper will describe the distribution of defensive glands throughout the family, as well as their precise location within the insects. Only glands present in the adult stage will be considered. Details on the structure of the glands will be given in a following paper. The results will be considered together with other mechanical, behavioral, or chemical defensive mechanisms which could occur simultaneously or independently of the exocrine secretion.

METHODS AND MATERIALS

The beetles were collected in Belgium, except for three Canadian species (*Gonioctena americana*, *Altica ambiens*, *Entomoscelis americana*) and one species from South Africa (*Diamphidia nigroornata*). We have followed the classification adopted by Derenne (1963) for the Belgian fauna and the one recently established by Jolivet (1978) for the subfamilies of the world. For the nomenclature and subdivision of the Chrysomelinae, we have followed Jolivet and Petitpierre (1976).

The presence of defensive glands was recognized by the oozing of the secretion after disturbance. In a few species, the defensive glands could not be recognized in this manner. In such cases, the glands were considered defensive if they were homologous in either their structure or location to the glands for which a defensive function was proven. They were always very easily distinguishable from the much smaller dermal glands observed in the integument of all species. For most species the identity of the glands was confirmed by histology and transmission electron microscopy. These techniques will be described in another paper dealing with the comparative structure of the glands.

The mapping of the glands was established by observing whole elytra and pronota mounted in Canada balsam after moderate digestion in 5% KOH for 4 hr at 60° C. In the figures, each gland is represented by one dot.

RESULTS

Distribution of Defensive Glands in Adults

Species from ten subfamilies were examined. In six subfamilies, none of the examined species possessed defensive glands in the adult stage. In two subfamilies defensive glands were always observed, whereas in the two others defensive glands were lacking in some species but present in others.

Subfamilies in which Defensive Glands were Never Observed. Species examined: S.F. Donaciae: *Donacia appendiculata* Ahr., *D. cinerea* Herbst; S.F. Zeugophorinae: *Zeugophora subspinosa* F.; S.F. Clytrinae: *Clytra quadripunctata* L., *Gynandrophthalma cyanea* F., *Coptocephala unifasciata* Scop.; S.F. Cryptocephalinae: *Cryptocephalus biguttatus* Scop., *C. decemmaculatus* L., *C. labiatus* L., *C. moraei* L., *C. sericeus* L., *C. trimaculatus* Ros.; S.F. Hispinae: *Hispa testacea* L., *H. astra* L.; S.F. Cassidinae: *Cassida rubiginosa* Müll., *C. sanguinosa* Suffr.

Since only a small number of species could be examined, we cannot conclude that defensive glands are absent in all the species belonging to these six subfamilies. Phylogenetic considerations discussed later make, however, this tentative hypothesis attractive.

Subfamilies in which Defensive Glands were Always Observed.

1. S.F. Criocerinae. Four species were examined: *Lema cyanella* L., *L. melanopus* L., *Lilioceris lilii* Scop., and *Crioceris asparagi* L. Figure 1

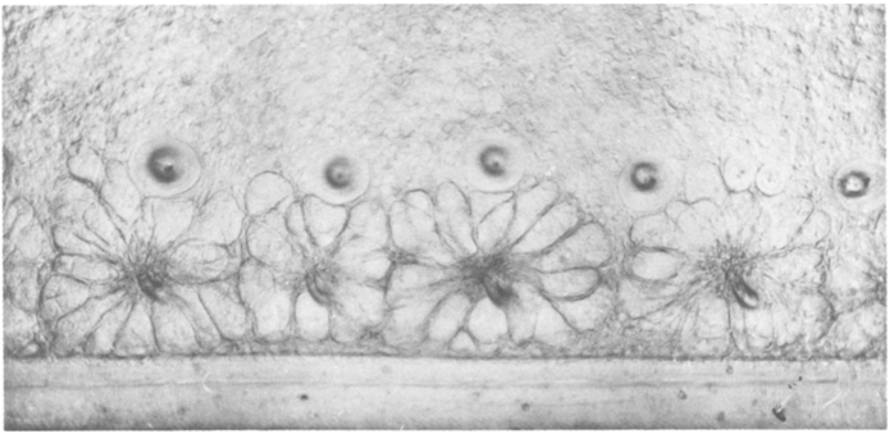

FIG. 1. Whole mount of a *Lilioceris lilii* elytron showing the lateral row of the defensive glands.

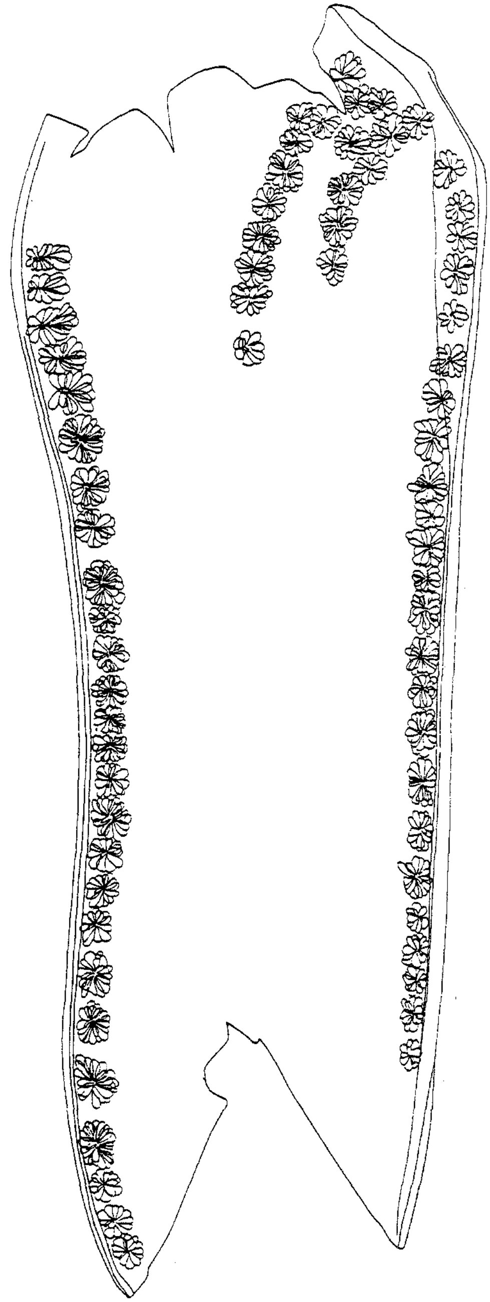

FIG. 2. Mapping of the defensive glands in an elytron of *Lilioceris lilii*. The elytron was broken at both ends during the mounting process.

illustrates how the glands can be recognized in a whole mount of the elytron of *Lilioceris lilii*, and Figure 2 gives the precise mapping of these glands. The distribution of the glands in the four species differs only by minor details. In *Lema melanopus*, the glands are largely distributed underneath both the pronotum and the elytra (Figure 3A). In the two other species, the distribution of the glands is more restricted. For example, only lateral glands were

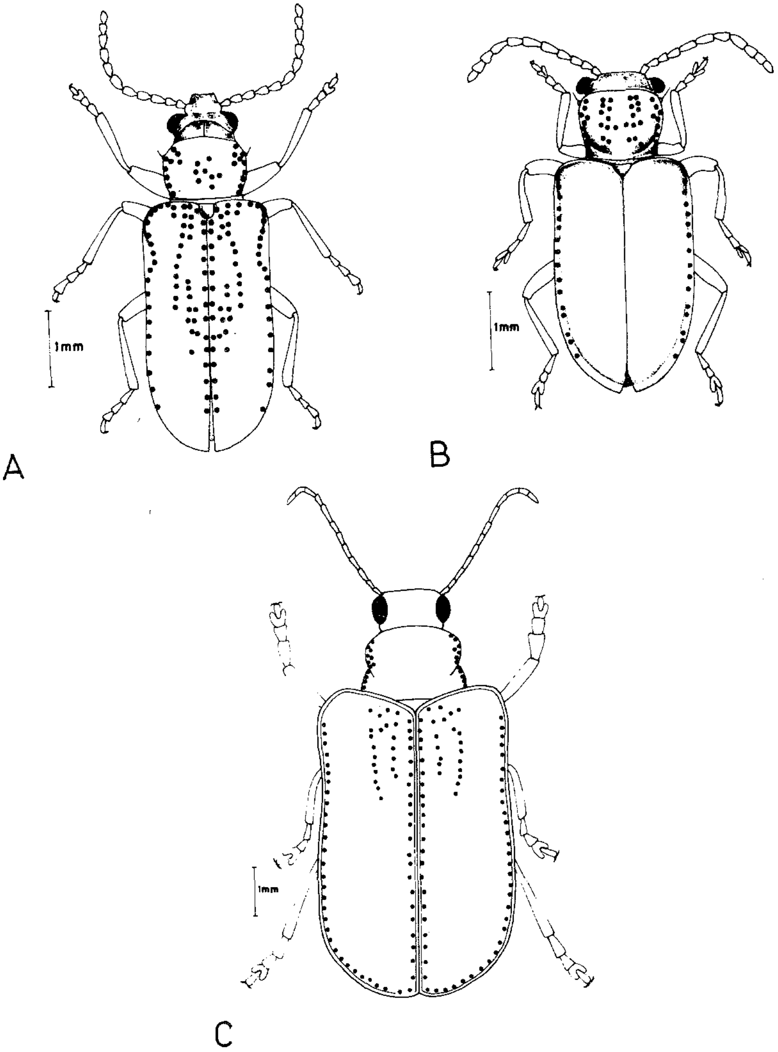

FIG. 3. Distribution of the defensive glands in selected Criocerinae: (A) *Lema melanopus*; (B) *Lema cyanella*; (C) *Lilioceris lilii*.

observed in the elytra of *Lema cyanella* and in the pronotum of both *Lilioceris lilii* (Figure 3B,C) and *Crioceris asparagi.*

2. S.F. Chrysomelinae. The following species were examined. (A) Chrysolinini: *Leptinotarsa decemlineata* Say, *Chrysolina coerulans* Scriba, *C. herbacea* Duftschm., *C. polita* L., *C. staphylea* L., *C. oricalcia* Müll., *C. varians* Schall., *C. hyperici* Förster, *C. brunsvicensis* Grav., *Dlochrysa fastuosa* Scop., *Chrysochloa cacaliae* Schrk. (B) Phaedonini: *Gastrophysa viridula* De Geer, *G. polygoni* L., *Plagiodera versicolora* Laich, *Chrysomela populi* L., *C. tremulae* F., *Hydrothassa marginella* L., *H. glabra* Herbst., *Prasocuris phellandrii* L., *Phaedon cochleariae* F., *P. veronicae* Bedel. (C) Phratorini: *Gonioctena (= Phytodecta) olivacea* Forst., *Gonioctena americana* Schaeffer, *Phratora (= Phyllodecta) vulgatissima* L., *P. laticollis* Suffr., *P. vitellinae* L. (D) Timarchini: *Timarcha goettingensis* L., *T. tenebricosa* F. (E) Entomoscelini: *Entomoscelis americana* Brown.

In the Chrysomelinae, the distribution of the glands in the insect may be more or less extensive. We were not able to recognize specific patterns which could be characteristic of a tribe. The distribution was even somewhat different between species belonging to the same genus or varied slightly from one specimen to another of the same species.

In all species, however, the glands are always present laterally in the pronotum and the elytra, where they open above the marginal groove which receives the secretion. This can be the sole location of the glands, as in the genera *Timarcha, Gastrophysa, Plagiodera, Hydrothassa, Prasocuris* (Figure 4A), *Chrysomela*, *Gonioctena americana*, some *Chrysolina* (*C. hyperici*, *C. varians*, *C. brunsvicensis*), and *Entomoscelis americana.*

In the pronotum, the lateral line of glands can be doubled by a few irregular rows of glands (*Chrysomela*). In the most extreme case (*Phaedon*), the glands are distributed on a surface reaching about one third of the pronotum on each side, (Figure 4B). Moreover, glands are often located near the anterior margin, mainly at the level of the corners of the pronotum, but they can form a nearly uninterrupted line as in *Leptinotarsa* (Deroe and Pasteels, 1977).

Furthermore, these glands are sometimes more widely distributed in the elytra too. In *Leptinotarsa decemlineata* (Deroe and Pasteels, 1977) and in *Chrysolina oricalcia* they are aligned in parallel longitudinal rows (Figure 4C). In *Chrysolina polita*, *C. coerulans*, and *C. herbacea*, one or two longitudinal rows of glands were observed along the inner margin of the elytra, and a few glands are usually close to the front margin (Figure 4D). A few incomplete longitudinal rows of glands are present also in the middle of the elytra of *Gonioctena olivacea* (Figure 5A), in this species the number of glands found in this location varies from specimen to specimen (Figure 5B).

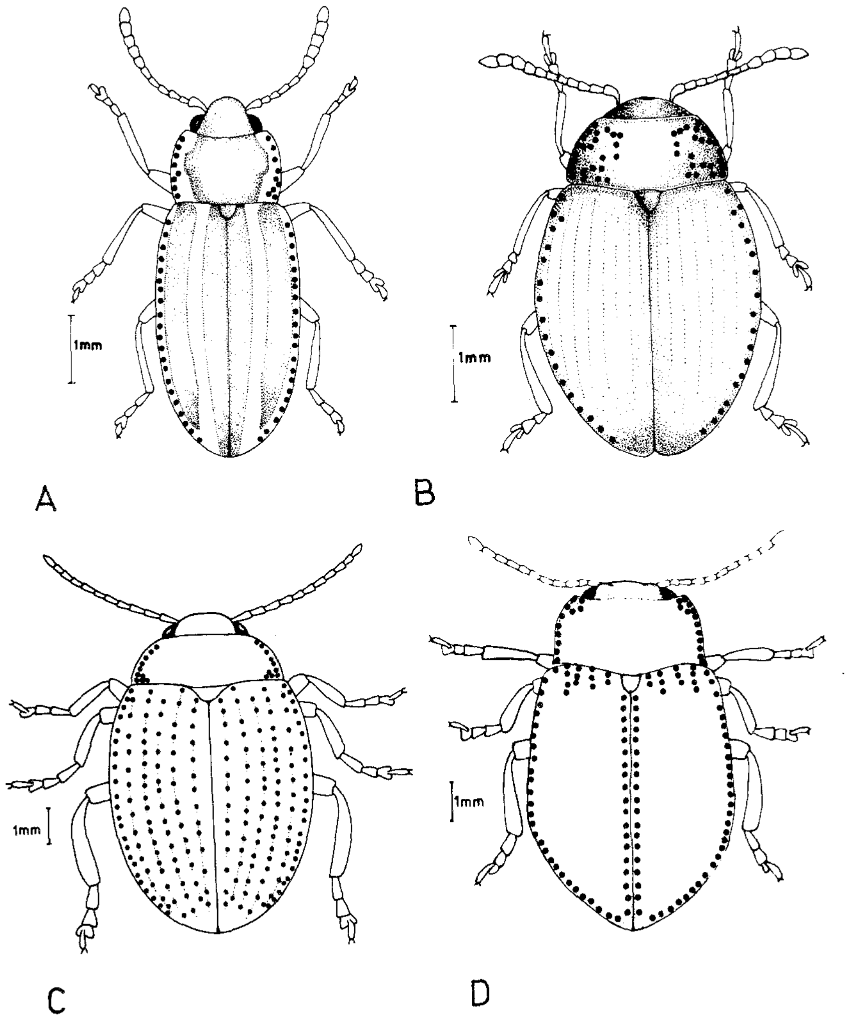

FIG. 4. Distribution of the defensive glands in Chrysomelinae: (A) *Prasocuris phellandrii;* (B) *Phaedon cochleariae;* (C) *Chrysolina oricalcia;* (D) *Chrysolina polita.*

Subfamilies in which Defensive Glands may be Present or Absent.

1. Galerucinae. The following species were examined. (A) Galerucini: *Galeruca taneceti* L., *Lochmaea suturalis* Thoms., *Galerucella calmariensis* L., *G. tenella* L. (B) Luperini: *Agelastica alni* L., *Sermylassa halensis* L., *Luperus circumfusus* Marsh, *L. longicornis* F.

In both tribes some species possess glands and others are devoid of them.

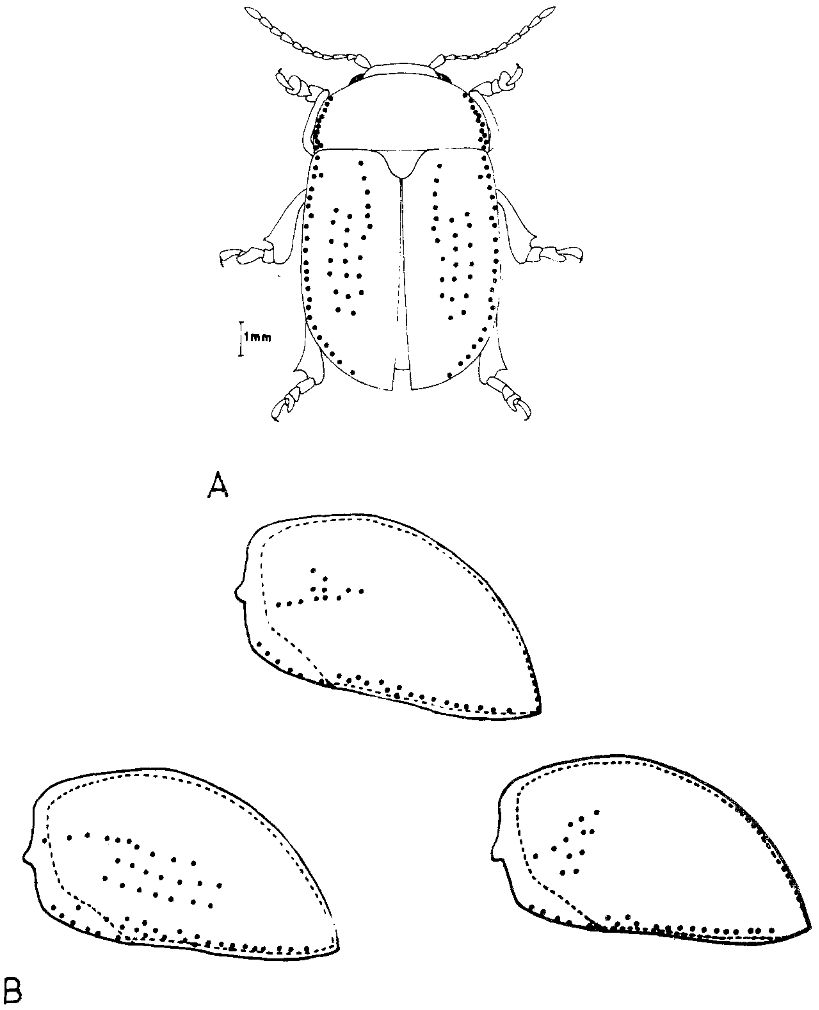

FIG. 5. (A) Distribution of the defensive glands in *Gonioctena olivacea*; (B) examples of variation in the distribution of the defensive glands within the elytra of *Gonioctena olivacea.*

Glands were found in *Lochmaea*, *Galerucella*, and *Sermylassa*, but not in other genera. In the elytra, the glands form a single lateral row in all four species. They are located anteriorly in the pronotum of *Galerucella* and *Lochmaea* (Figure 6A) but laterally in *Sermylassa* (Figure 6B).

2. Alticinae. Examined species: *Asiorestia transversa* Marsh., *Crepidodera* (= *Chalchoides*) *aurata* Marsh., *C. aurea* Geoffr., *Altica oleracea* L., *A.*

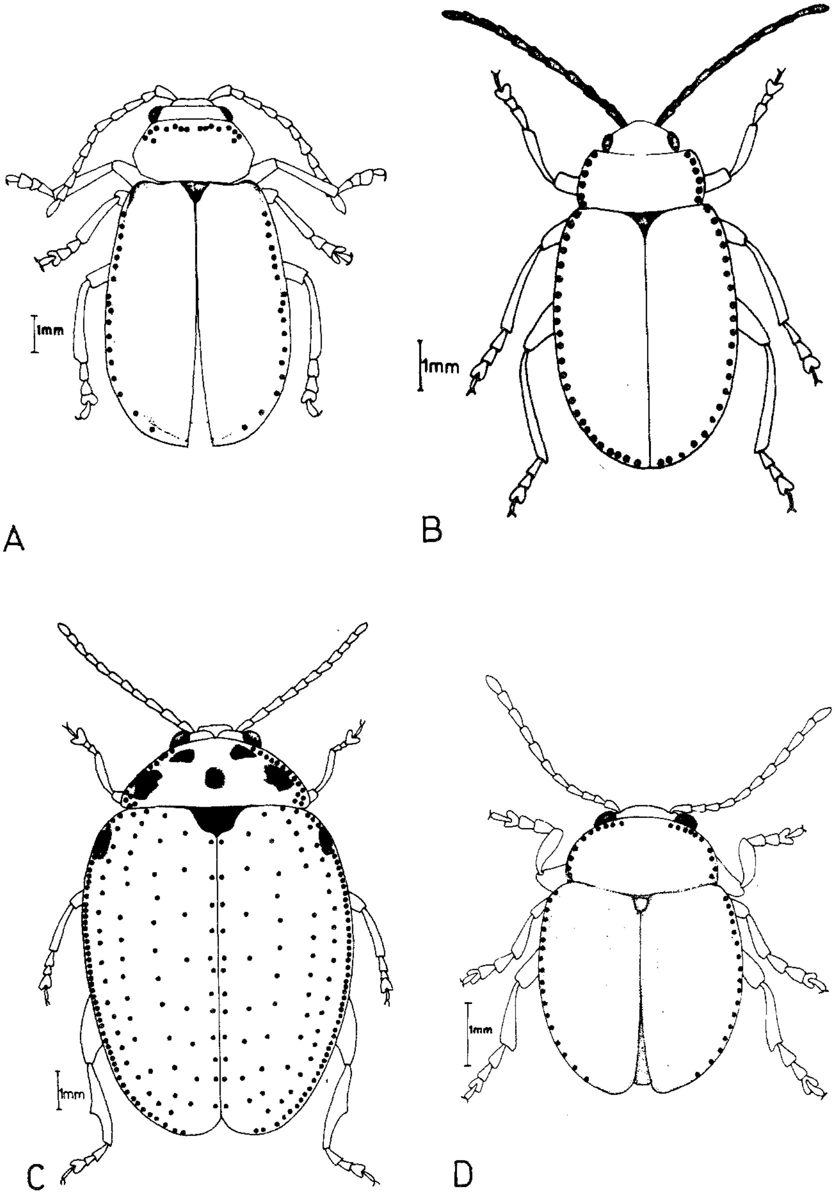

FIG. 6. Distribution of the defensive glands in selected Galerucinae (A) *Galerucella tenella* and (B) *Sermylassa halensis*, and Alticinae (C) *Diamphidia nigroornata* and (D) *Sphaeroderma testaceum*).

ambiens Le Conte, *Sphaeroderma testaceum* F., *Psylliodes affinis* Payk., *Diamphidia nigroornata* Stäl.

Glands were found in all the species except *Psylliodes affinis.* Again their distribution shows considerable variation between species. They are widely distributed in the elytra of *Diamphidia* (Figure 6C) but restricted to the lateral margin in the other species (Figure 6D). They are also located along the lateral margin of pronotum of most species but are concentrated in two patches at the posterior corner in *Altica oleracea* (Figure 7A).

Complementary and Alternative Defensive Devices

Nonglandular defensive mechanisms have not been systematically investigated. We will compile here information from the literature together with our own observations. Very little information could be found, however, with respect to tropical fauna. Again only the defensive mechanisms of adults will be considered.

Reflex Bleeding. Reflex bleeding and its efficiency against ants has been recently described for the Colorado beetle (Deroe and Pasteels, 1977). In the Chrysomelinae, buccal and sometimes tibiofemoral autohemorrhea are probably frequent in young adults when the membranes are still weak and have been reported several times [several species belonging to the genera *Chrysomela*, *Chrysolina*, *Timarcha*, and *Mesoplatys* (Cuénot, 1896; Hollande, 1909, 1911; Jolivet, 1946, 1948, personnal communication)]. Bleeding is rarely observed when the beetles are fully sclerotized and when the glandular secretion is abundant, but considerable variation exists between species. In *Timarcha*, reflex bleeding is prominent. It seems significant to us that in this species the defensive secretion can scarcely be observed and is often not emitted at all.

Reflex bleeding seems to be the main defensive mechanism in the Galerucinae, in which it is usually conspicuous. Buccal or tibiofemoral bleedings have been reported for the genera *Galeruca*, *Galerucella*, *Agelastica*, *Sermylassa*, *Luperus*, *Exosoma* (= *Malacosoma*) (Cuénot, 1896; Hollande, 1911, 1926), to which we may add *Lochmaea.*

The experiments made by Cuénot and Hollande demonstrated that several species, including Galerucinae and *Timarcha*, in which bleeding is an important defensive reflex, are chemically defended because the bleeding either repels, or is toxic to, vertebrate predators. To our knowledge, besides the Chrysomelinae and the Galerucinae, reflex bleeding has been reported only for two *Altica* (Alticinae) by Hollande (1911).

Crypsis, Aposematism, and Mimicry. In Europe, cryptic colorations seem to be the rule only in the Cassidinae, in which the flattened shape makes them even more concealed in the foliage (Figure 7B). In at least one American

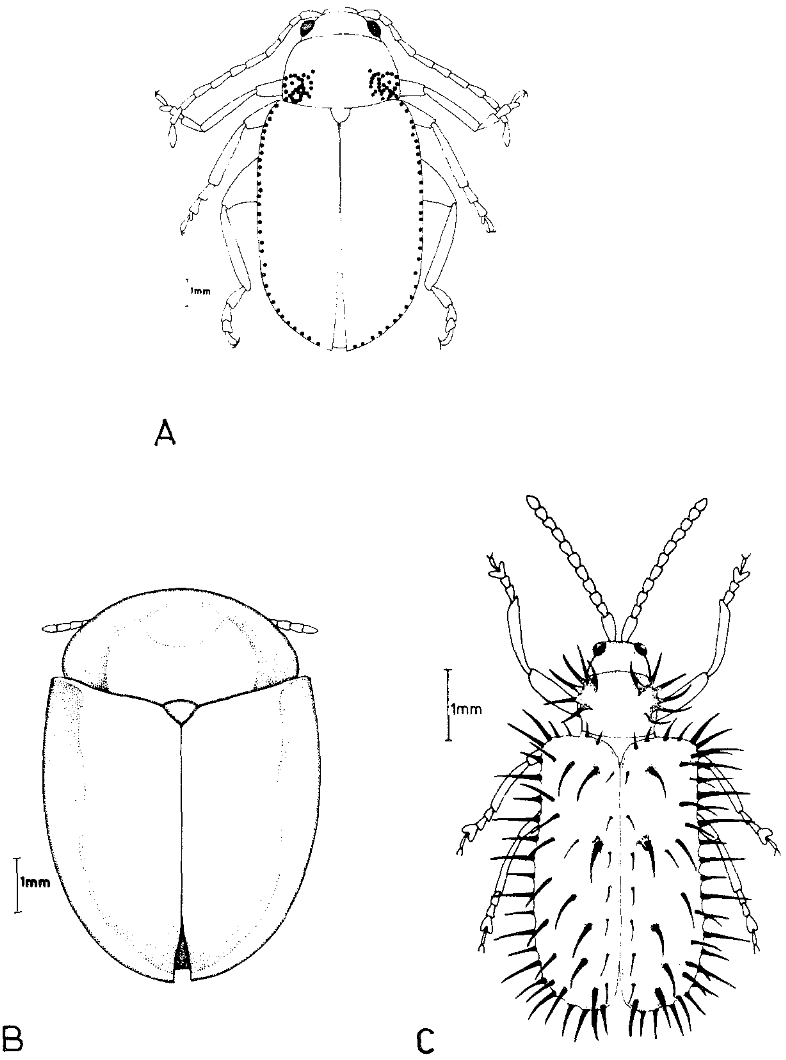

FIG. 7. (A) Distribution of the defensive glands in *Altica oleracea* (Alticinae); (B and C) alternative defensive devices in *Cassida* and *Hispa*.

species, special tarsal glands allow the beetles to have a firm grip on the substrate, which makes their capture by ants very difficult (Eisner, 1972).

As expected, aposematism is frequent in the four subfamilies in which defensive glands were observed: Criocerinae, Chrysomelinae, Galerucinae, and Alticinae. Bright coloration in these beetles, however, has not always been associated with chemical defense mechanisms. Lindroth (1971) suggested that in some flea beetles (Alticinae) aposematism is linked to their ability to disappear suddenly by jumping, and Capinera (1976) inferred from his experiments with edible models given to blue jays and chickadees that the color pattern of *Crioceris duodecimpunctata* imparts by itself some protection against avian predators.

Müllerian and Batesian mimicry must be frequent among different chrysomelids and between them and other insects. The best documented cases of mimicry concerned some *Lebia* and *Lebistina* (Carabidae) and flea beetles (Jolivet, 1967; Balsbaugh, 1967; Lindroth, 1971) as well as the chrysomelid *Mesoplatys cincta* and the carabid *Cyaneodinodes ammon* in Upper Volta (Jolivet, personnal communication).

Mimicry could also occur between the completely black *Timarcha* sp. and young *Meloë proscarabeus* observed close to each other in early spring on herbaceous plants, or between the shiny blue *Agelastica alni* and *Chrysomela aenea*, sometimes found on the same *Alnus* tree. *Chrysolina coerulans* could also play a role in this last mimicry complex, even if it lives on *Mentha* sp. It possesses the same shiny blue color and is frequently encountered close to *A. alni* and *C. aenea.*

The several metallic green species observed on different herbs could form another mimetic complex. No experiments, however, have been done to demonstrate the effectiveness of these supposed cases of mimicry and to characterize them as Batesian, Müllerian, or even Peckhamian (aggressive) as suggested by Jolivet (personal communication) for the complex *Mesoplatys–Cyaneodinodes.*

Aposematic coloration also characterizes species which do not possess defensive glands. The most obvious examples are the bright colored Cryptocephalinae and Clytrinae. We do not know yet if they are Batesian mimics or if they possess a nonglandular chemical defense mechanism.

Some aposematic chrysomelids, apparently chemically protected, may adopt a concealment behavior when a predator is at close range. According to Capinera (1976), *Crioceris asparagi* and *C. duodecimpunctata* dodge evasively to the opposite side of the asparagus stem. Both are aposematic and at least the latter is unpalatable to birds (Jones 1932). *Crioceris*, as well as many other chrysomelids, whether or not chemically protected, frequently feign death when disturbed.

Quick Escape. Most chrysomelids are slow moving and cannot fly after the dispersion flight. But there are exceptions both among species in which defense glands are present and among those devoid of them. Flight is a frequent escape mechanism in some *Crioceris,* like *C. duodecimpunctata* (Capinera 1976). Species belonging to the Donaciae and *Luperus* (Galerucinae), all devoid of defensive glands, can escape quickly by flying too.

Quick escape by jumping is, of course, well known in the flea beetles

(Alticinae). This escape seems particularly efficient for very small specimens like *Psilliodes affinis* which are devoid of defense glands.

Mechanical Protection. Mechanical protection reaches dramatic proportions in the Hispinae, *Hispa*, in which no defense glands could be found. These beetles are protected by long sharp cuticular expansions of the elytra and pronotum (Figure 7C). The efficiency of such protection has not yet been tested.

Stridulation. Stridulation has only been observed in some Criocerinae. Capinera (1976) described it for two species of *Crioceris* and we have observed it with *Lilioceris lilii.*

Regurgitation and Defecation. Regurgitation and defecation are common reactions in many insects when they are seized. The same is true for many adult chrysomelids, and notably in some Chrysomelinae, in which regurgitation can occur at the same time as buccal bleeding, both liquids are mixed together.

DISCUSSION

The defensive strategies of the chrysomelids are quite diversified and can vary even in closely related species occupying very similar ecological niches (Capinera, 1976). Moreover the beetles' protection only rarely relies on a single device as opposed to a combination of several. Therefore, it is still difficult to understand fully how the various defense mechanisms have evolved within this family. Our survey of the adults' defense glands, however, adds some elements to this study.

First of all, it seems that the development of defense glands has been a monophyletic event. Indeed, if we consider the phylogenetic tree independently proposed by Jolivet (1978), it is very significant that the defense glands were only found in the subfamilies forming one branch originating from the Aulacoscelinae, which we were unfortunately unable to study (Figure 8). No defense glands were found in some primitive subfamilies nor in other evolved ones belonging to other lines.

A second point is that the two subfamilies Alticinae and Galerucinae are the most evolved in their line. The lack of glands in some species belonging to these subfamilies must therefore be considered as a secondary event. The predominance in both subfamilies of an alternative defensive mechanism should be noted: reflex bleeding in the Galerucinae, the jumping mechanisms in the Alticinae. Even in those species, in which the glands are present, the secretion is never as abundant as in most Chrysomelinae. In this context it is interesting to recall that *Timarcha* is the genus among the observed Chrysomelinae in which glandular defense seems the less effective, judging by the amount of secretion produced. It is also in that genus that reflex bleeding is the most prominent. It therefore seems that protection is ensured by a balance of mechanisms, which could easily shift in one direction or the other in the course of evolution.

In another line of the phylogenetic tree (Figure 8), including the Hispinae and Cassidinae, alternative defensive mechanisms are obvious: chitinous expansions in the case of the Hispinae (*Hispa*) and flattened shape and cryptic colors for the Cassidinae.

As far as has been determined, no obvious defensive mechanisms is

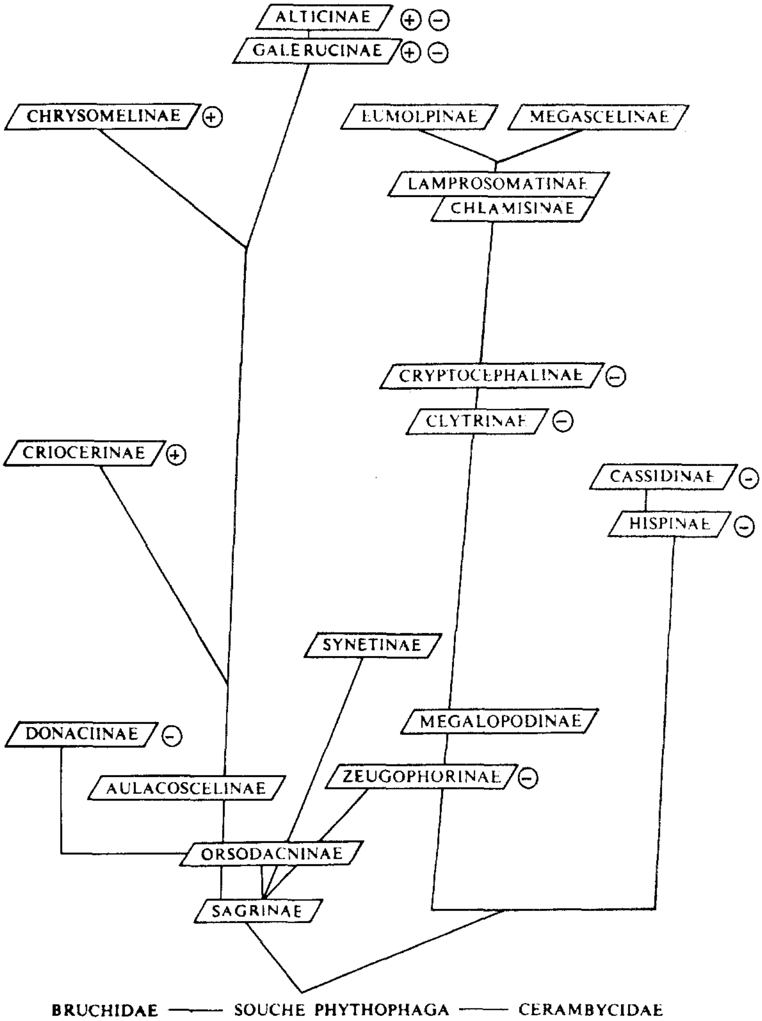

FIG. 8. Phylogenetic tree of the subfamilies of Chrysomelidae according to Jolivet (1978). The signs + or − were added where defensive glands were or were not observed.

known among adults belonging to the lines including the Zeugophorinae, Clytrinae, and Cryptocephalinae. It should be noted that the Clytrinae and to a lesser extent the Cryptocephalinae and Zeugophorinae are essentially polyphagous (Jolivet, 1978). So it could be that spectacular protection has, above all, evolved in strict phytophagous specialists which could be found much more easily by predators which can associate the beetles and their host plants.

If we consider the distribution of the glands within the beetles themselves, some interesting features also emerge. In each subfamily, the glands can be more or less widely distributed on the surface of the beetle, but they are always laterally present in both the elytra and pronotum. Even when they are more largely distributed, the lateral glands are generally more developed. It seems to us that the lateral margins of the beetles are the most efficient locations for defense glands. Small insect predators, for instance ants, will unavoidably contact the secretion which forms a chemical obstacle surrounding the beetles. Moreover, lateral secretions will remain accessible during thanatosis, but the secretion oozing from dorsal glands often will not. Finally, the lateral restriction of the glands would probably not impair the protection of the beetle against birds or other vertebrates. We tentatively conclude that the wide distribution of the glands is a primitive condition and, in the course of evolution, these glands were retained and reinforced only laterally where they are most effective, such evolution having occurred several times independently within the different subfamilies.

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