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THE UNIVERSITY OF ALBERTA

Host Selection and Gustatory Chemoreception in Three *Leptinotarsa* species

by

Janet Louise Haley

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Host Selection and Gustatory Chemoreception in Three *Leptinotarsa* species submitted by Janet Louise Haley in partial fulfilment of the requirements for the degree of Master of Science

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Date 26 September 1988

Abstract

Leptinotarsa beetles and their solanaceous host plants provide an informative system for investigating the sensory basis of host plant insect interactions. The Colorado potato beetle, *Leptinotarsa decemlineata* and two related species, *L. haldemani* and *L. texana*, were used to study interspecific differences in feeding specialization, behavioural discrimination of host plants, anatomy of galeae, responses of galeal gustatory sensilla and to assess evolutionary and genetic relationships of the beetles.

Each of the three *Leptinotarsa* species was characterized by distinctive behavioural patterns of host plant discrimination. Behavioural bioassays performed in the lab corresponded to field observations. Number of bites in 60 seconds provided the most useful indicator of host plant preferences.

Galeal sensilla were investigated as a major site of contact chemoreception. No major differences in numbers or distribution of sensilla were noted among the three species. Electrophysiological responses of galeal gustatory sensilla to four solanceous plant saps showed differences among beetle species as well as among individuals within a species. For *L. decemlineata* and *L. texana*, similarities were noted in responses to saps from three *Solanum* species while responses to *Lycopersicon* were more complex. Responses of galeal gustatory sensilla to plant saps do not clearly correspond to behavioural host plant discrimination of the same solanceous plants.

Polyacrylamide gel electrophoresis of allozymes was used to establish genetic relationships for the above three *Leptinotarsa* species as well as two others: *L. lineolata* and *L. rubiginosa*. Significant deviations from gene pool

homogeneity in several populations may indicate incipient biotypes. Relative degree of heterozygosity did not correspond to degree of feeding specialization of populations. Differences for populations within species were always substantially less than differences among species. *L. lineolata* was clearly the most divergent of the 5 species sampled. UPGMA and Wagner trees of several genetic distance measures indicate that *L. decemlineata* and *L. haldemani* are more closely related to each other than either is to *L. texana*.

The evolution of degree of feeding specialization appears to be from monophagy to oligophagy. The *Leptinotarsa-Solanum* relationship is useful for investigating a proximate behaviour within an evolutionary framework and contributes to better understanding of an economic problem: the Colorado potato beetle.

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I would like to express my sincere thanks to the members of my examining committee, B.K. Mitchell, R.H. Gooding and P.J. Albert. Their constructive criticisms permitted judicious improvements.

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D.W. Langor introduced me to starch gel electrophoresis and was a source of intellectual support during the darkest moments of electrophoresis. C. Reyes was a sympathetic office mate. J. Rollens was a patient tutor for statistics. I am particularly grateful to A. Sen for his help with SEM and for his sustained interest in the entire project. K. Smy provided a summer's worth of beetle behaviour experiments and help around the lab. C. Hilchie was especially helpful for her endless hours of beetle rearing.

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I. Introduction

Herbivorous insects are associated with specific ranges of host plants. Whether these plants belong to a single plant species or to many plant species, the evolution of insect-host plant interactions and the ability of insects to find preferred plants is intriguing.

The sensory basis of such host plant-insect interactions has often been studied and debated. Sensing and encoding of responses to compounds present in plant tissues may be based on diagnostic stimuli, ratios of deterrent and stimulant compounds or patterns of multineuronal responses. This thesis explores sensory physiology within ecological and phylogenetic constraints. Host plant choice is discussed as a complex process mediated by mixtures of chemicals perceived, in part, by contact chemoreceptors on the galeae of three chrysomelid beetles.

Phytophagous insects have developed different degrees of feeding specialization, a continuum which may be partitioned into monophagy, oligophagy and polyphagy. Monophagy and oligophagy are each sub-divided into three degrees. First degree monophagy, or specific monophagy, is used to describe feeding on a single species of plant. Second degree monophagy describes feeding on several plants within a similar section of a plant genus. Third degree monophagy, or generic monophagy describes feeding on many or all species within a given plant genus. Oligophagy describes broader feeding preferences than monophagy and extends from feeding on related plants belonging to several genera of the same family to feeding on a variety of genera belonging to different plant orders. Polyphagous insects feed on a great number of plants belonging to genera in distantly related orders (Jolivet, 1986).

Evolution of degree of feeding specialization is a result of composite selection pressures. Since host plants for phytophagous insects provide protection from biotic and abiotic factors as well as providing a source of food, costs associated with broad acceptance of host plants must be balanced with benefits of flexibility in feeding habits. Selective advantages of restricted acceptance of food plants include: less competition with other herbivores, specialized adaptations to microhabitats and host phenology, reduced metabolic costs and protection by sequestering of plant secondary compounds (Miller and Strickler, 1984). In order to take advantage of the beneficial characteristics of a plant and to avoid plants with detrimental features, the insect must be able to recognize suitable host plants. Recognition of host plants is thus an important consideration when discussing host plant-insect interactions.

The genus *Leptinotarsa* Stål is well suited to comparative studies of host plant affinities since there are relatively few species and many of the species of this genus may be considered oligophagous for some members of the plant family Solanaceae (Jacques, 1972, 1988).

From 32 *Leptinotarsa* species, I have selected three species for this study. These species were chosen on the basis of host preferences with each species having its own pattern of feeding preferences as described by Jacques (1972, 1988) and Hsiao (1974, 1976). The three species examined in this thesis are: *Leptinotarsa decemlineata* (Say), *L. haldemani* Rogers and *L. texana* (Schaeffer). Each occurs in North America and feeds on members of the plant family Solanaceae. Dependence of *Solanum* species on insects is limited to syrphid flies and Hymenoptera which function as pollinators (Symon, 1975).

Jac[illegible]88) catalogued host plants for eleven *Leptinotarsa* species occurring in the United States. *Leptinotarsa texana* is recorded only from *Solanum elaeagnifolium*. *L. decemlineata* is recorded from ten *Solanum* species, one *Physalis* species, and *Lycopersicon esculentum*. *L. haldemani* is recorded from two *Solanum* species, two *Physalis* species and *Lycopersicon esculentum*. *L. haldemani* has also been described on *Lycium* species, another genus of the family Solanaceae, and in the Benson, Arizona area, *Lycium* species may be a major host plant (*pers. obs.* and Bernon, *pers comm*). Based on these host plant records, *L. decemlineata* and *L. haldemani* are first degree oligophages and *L. texana* is a specific monophage using the criteria of Jolivet (1986).

Hsiao (1974) documented larval feeding habits of eight *Leptinotarsa* species. All eight species had similar life histories and developmental requirements. According to nutritional criteria, as measured by percent mortality, rate of development and pupal weight, *L. texana* is highly host specific, and *L. haldemani* and *L. decemlineata* are less host specific. *L.* decemlineata was considered to be the more polyphagous of the two oligophagous species despite an equal number of plant species consumed by both (Hsiao, 1974).

Although *L. decemlineata* is considered to be a first degree oligophage, biotypes of *L. decemlineata* are well known. For example, *L. decemlineata* in Benson, Arizona is adapted to *S. elaeagnifolium* (Hsaio, 1978) and in North Carolina to *S. carolinense* (Hare and Kennedy, 1986). A biotype in Colorado is also adapted to hairy nightshade, *Solanum sarrachoides*, (Horton and Capinera, 1987; Horton *et al*, 1988). The existence of biotypes has led many people to

speculate that species polyphagous over their entire range may be locally specialized (Fox and Morrow, 1981). Host location and selection may be similar for particular biotypes of *L. decemlineata* and *L. texana* even though *L. decemlineata* is considered more polyphagous as a species than *L. texana*. For the purposes of this thesis, a single population of each of the species was studied for morphological, behavioural and electrophysiological differences. *L. decemlineata* were from the Edmonton area where they feed on *S. tuberosum*. *L. haldemani* were from the Pena Blanca, Arizona region where they feed on at least two *Physalis* species and *S. douglasii*. *L. texana* were from Hidalgo county, Texas where they feed exclusively on *S. elaeagnifolium*. Electrophoretic data were gathered on several populations to assess the extent of interpopulation differences.

Phylogenetic relationships among plant species may be implicated in feeding specialization. Examples of taxonomic congruence among host plants and insects include aphids (Eastop, 1973), *Yponomeuta* moths (van Drongelen, 1979; van Drongelen and Povel, 1980) and papilionid butterflies (Miller, 1986). Phylogenetic relationships are considered to be reflected in their taxonomic arrangements.

The centre of diversity of Solanaceae at the generic level is in western and southern America (D'Arcy, 1975) which roughly corresponds to the centre of diversity of the genus *Leptinotarsa* which is in Mexico (Jacques, 1972, 1988). Taxonomic congruence between *Leptinotarsa* beetles and their solanceous host plants suggest a co-evolutionary relationship between *L. decemlineata* and its close relatives with the sub-genus *Leptostemonum* of the genus *Solanum* (Hsiao, 1981). The nature of this relationship was not investigated and the distinction between sequential

evolution (insects follow plants) and true co-evolution (each species greatly influences the other) was not made by Hsiao (1981). Unless a true co-evolutionary relationship can be described, I assume that sequential evolution is the basis of Hsiao's statement.

The plant family Solanaceae contains host plants for seven of eleven *Leptinotarsa* species found in the United States while the plant families Compositae and Zygophyllaceae contain host plants for the four other species (Jacques, 1988). The *Leptinotarsa* species considered in this thesis are restricted to plants of the family Solanaceae.

The family Solanaceae consists of 84 genera and almost 3000 species. D'Arcy (1975) placed the 84 genera into three sub-families: Solanoideae, Cestroideae and Nolanoideae. All of the described host plants of the *Leptinotarsa* species discussed in this thesis are within the sub-family Solanoideae. The sub-family Solanoideae has seven tribes. The tribe Lycieae contains the genus *Lycium* and the tribe Solaneae contains *Lycopersicon, Physalis* and *Solanum* (Hunziker, 1975).

For this study, I chose to detail responses to *Lycopersicon esculentum* Mill., *Solanum dulcamara* L., *S. elaeagnifolium* Cav. and *S. tuberosum* L. These plants cover a spectrum of larval feeding responses from unacceptable as host plant (*Leptinotarsa haldemani* on *S. elaeagnifolium*) to moderately acceptable (*L. decemlineata* on *Lycopersicon esculentum*) through to solely acceptable as host plant (*L. texana* on *S. elaeagnifolium*) (Hsiao, 1974). Within the genus *Solanum*, there are seven sub-genera (D'Arcy, 1972). *Solanum elaeagnifolium* is in the sub-genus *Leptostemonum*, section Leprophora; *S. tuberosum* and *S. dulcamara* are in the sub-genus *Potatoe*. Within the sub-genus *Potatoe*, *S. dulcamara* is in the section Dulcamara

and *S. tuberosum* is in the section Petota (D'Arcy, 1972). Although relationships among Solanaceae are uncertain, the groupings of D'Arcy (1972) imply that *Solanum dulcamara* and *S. tuberosum* are more closely related to each other than either is to *S. elaeagnifolium*. Furthermore, the implication is that species of the genus *Solanum* are more closely related to each other than to species of the genus *Lycopersicon*.

Feeding specialization theories based on taxonomic affinities may be tested by *Solanum dulcamara*, a native of Europe (House, 1934). *Leptinotarsa* species have undergone most of their evolution on the North American continent since it was not until the 20th century that they were introduced to Europe (Jacques, 1988). *Solanum dulcamara* was introduced to the North American continent from Europe by settlers, that is within the last 400 years. Until recently, *S. dulcamara* did not belong to the normal range of host plants because of its distribution. With a change in distribution of insects and plants, new host plant affinities have arisen. The presumed phylogenetic relationship between *S. dulcamara* and *S. tuberosum* (both in the sub-genus *Potatoe*) permitted recognition and use of these plants by *Leptinotarsa* species.

Factors other than geographic distribution of host plants which prevent insects from feeding on otherwise suitable plants include temperature, humidity, light or soil conditions which render the plant unacceptable or exclude suitable pupation sites. For example, *Leptinotarsa decemlineata* in the Benson, Arizona area is usually found on *Solanum elaeagnifolium*. However, *L. decemlineata* is found on *S. elaeagnifolium* only in areas such as in gullies and near free-water where moisture conditions allow pupation. Tower (1918) described a similar distribution in

Mexico where host plants are found on open plains and near stream beds while *L. decemlineata* is found only on plants near stream beds. Soil fertility may also influence beetle distribution. Among potato cultivars, foliar nitrogen is a better predictor of beetle abundance than foliar biomass (Jansson and Smilowitz, 1986). Conditions that favour high foliar nitrogen would favour high beetle populations. Host plants may also be suitable but not coincident in time. *Leptinotarsa haldemani* can grow and develop optimally on *Solanum tuberosum* in culture, however *L. haldemani* has not been described on cultivated *S. tuberosum* in field conditions. It is possible that *S. tuberosum*, a winter crop in the area where *L. haldemani* occurs naturally, is not grown while *L. haldemani* is active above ground. If *S. tuberosum* were grown earlier in the season or *L. haldemani* were present during *S. tuberosum* growing season, *L. haldemani* could potentially be found on this *Solanum* species.

Description of the evolution of feeding behaviour is not possible without consideration of oviposition behaviour. Oviposition behaviour is probably a major focus of selection and is related to feeding preferences for these beetles. May and Ahmad (1983) are of the opinion that oviposition by adult females is the point at which the most important host selection behaviour takes place. Oviposition preferences have been studied by Bongers (1970) and Hsiao and Fraenkel (1968b) for *Leptinotarsa decemlineata*.

Oviposition preferences are not necessarily influenced by the same stimuli as feeding preferences. For the genus *Leptinotarsa*, adults and larvae feed on members of the same species of host plants or even on the same host plant. Within this genus, however, despite similar nutritional requirements for larvae and adults, oviposition

preferences are not necessarily a consequence of feeding patterns. *Solanum luteum* was prefered to *S. tuberosum* in a choice test for oviposition by *L. decemlineata* even though *S. luteum* reduced fecundity in adult females. Reduction in fecundity was thought to be a result of reduced food intake even though sufficient leaf area for adequate nutrition was available (Bongers, 1970). Hsiao and Fraenkel (1968b) have also described an ovipositional preference by *L. decemlineata* for *S. nigrum* despite reduced feeding by adults and larvae. Field observations (Jacques,1972) report larvae of *L. decemlineata* on *Polygonum convolvulus, Chenopodium album* and *Amaranthus retroflexus*, none of which support continued growth of the larvae (Hsiao and Fraenkel, 1968a).

Differences between feeding and oviposition preferences appear to be of minimal importance for *Leptinotarsa decemlineata*. Newly emerged females require a pre-oviposition feeding period. Ovaries develop immediately after pupal- adult ecdysis and eggs are formed exclusively from nutrients ingested during adult life (de Wilde and de Loof, 1973). Oviposition normally takes place on the plant upon which the female has completed her maturation feeding since females feed nearly every day and gravid females are too heavy to disperse easily (Bongers, 1970). This pre-oviposition feeding period would tend to ensure that correct host plants are chosen for larvae which are even less mobile than gravid females and which would probably die if forced to take their first few meals on an unsuitable host plant. Field observations of *L. decemlineata* indicate that oviposition sites and suitable sites for larval development are linked (Moreau,1976).

The major objective of this study is to compare host selection and gustatory chemoreception of three

Leptinotarsa species. This comparative approach to sensory physiology is augmented by the inclusion of an abbreviated phylogeny for several *Leptinotarsa* species.

The first chapter of this thesis is an introduction to the beetle genus *Leptinotarsa* and its associated host plants.

Chapter two is an analysis of behavioural responses of *Leptinotarsa decemlineata*, *L. haldemani* and *L. texana* to intact leaves of four solanaceous plants. These behavioural assays characterize short range sensory discrimination of plants by beetles. Plant acceptability is ranked for the beetle species. This behavioural context is necessary for interpretation of electrophysiological data.

Chapter three describes comparative anatomy of the tip of the galea of the three *Leptinotarsa* species. Only features visible with scanning electron microscopy are described. Numbers, distribution and gross anatomy of sensilla are compared among species. This chapter serves to allow electrophysiological studies to be compared on the basis of sensitivity to plant saps alone since scanning electon micrographs for all three species are similar.

Chapter four is a discussion of differing responses of galeal contact chemoreceptors to crude extracts of plant saps. Electrophysiological responses to plant saps are compared within and among different species of beetles. Patterns of single sensillum responses are related to behavioural acceptabilty of the plant.

An abbreviated phylogeny and evolution of selected characters is briefly explored in Chapter five. This phylogeny is based on an electrophoretic survey of five *Leptinotarsa* species: *L. decemlineata*, *L. haldemani* and *L. lineolata*, *L. rubiginosa* and *L. texana*. Measures of

heterozygosity of these species are also related to degree of polyphagy.

Chapter six is a concluding discussion describing possible evolution of host plant specificity of *Leptinotarsa* species. Data from the preceeding chapters are interpreted within an evolutionary framework. Speculation on the potential usefulness of this work for pest management strategies is included.

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II. Behavioural discrimination of solanceous plants by *Leptinotarsa* beetles.

Short range sensory discrimination is involved in host plant choice. Behavioural manifestations of preference are used to establish a relative ranking for 4 solanaceous plants. The behavioural assays described in this chapter compare solanaceous plant acceptability for each of three *Leptinotarsa* species. Differences among beetle species are also compared in an effort to characterise host plant preferences for *L. decemlineata*, *L. haldemani* and *L. texana*.

Tests using beetles with no previous feeding experience are often referred to as bioassays of host plant recognition. Although host plant cognition is not host plant recognition, for the purposes of this thesis, I contend that we are dealing with host plant recognition even though the beetles tested had no feeding experience as adults. Recognition implies that a neural pattern for plant identification exists in newly emerged beetles. Recognition need not be a simple acceptance or rejection of a host plant but may be a gradation of responses. Dethier (1982) defines recognition as a set of stimuli matching a model in the neural world. The existence of a template against which stimuli can be matched is the basis of this characterisation of host plant recognition. Each beetle species could have a different host plant template against which sensory input is compared. Differences in the templates are expressed through short term behavioural assays.

Host plant preference is described as a subset of host plant recognition by Dethier (1982). Therefore, the condition of recognition is needed for a preference to manifest itself; preference implies previous cognition. The actions of an

individual are a manifestation of host plant preference. Component behavioural actions of an individual are binary as opposed to graded; a behaviour is manifest or it is not manifest. Information from behavioural assays is thus a reflection of graded sensory input in a reduced form. Preference is not an absolute measure and is influenced by pre- and post-ingestive factors.

Behavioural manifestations of preference may be grouped into two main categories: correlative studies and bioassays. Descriptions of host preference have traditionally relied on host plant records using field observations of insect-plant associations. Oviposition studies describing distribution of eggs are another index of host plant preference. Feeding behaviour is also an index of host plant preference. Long term assessments of feeding preference may be measured indirectly by larval weight gain and larval development rates, by amount of foliage consumed by larvae and adults over several hours and by fecal production. Behavioural assays of insect feeding preferences may include whole plants, intact leaves or leaf disks.

Leptinotarsa decemlineata, has been extensively studied using long and short term biaossays. Host plant records are available (Jacques, 1972,1988) and oviposition preferences have been documented (Bongers, 1970; Dimock and Tingey, 1985; Hsiao and Fraenkel, 1968). Feeding differences by larvae are manifest in total duration of feeding bout and extent of food consumption (Chin, 1950). Behaviour of adult *L. decemlineata* in the presence of presumptive feeding deterrents has been reviewed by Szentesi and Jermy (1985). They found that antifeedant effects on behaviour are exhibited by increased agitation, disproportionate egg distribution, reduced egg production and increased adult dispersal.

Host plant records are available for *L. haldemani* and *L. texana* (Jacques, 1972,1988; Neck, 1983). Neither of these species has been as extensively studied as *L. decemlineata*. Hsiao (1974) studied larval feeding of eight *Leptinotarsa* species by comparing percent mortality, rate of development and pupal weight. Hsiao ranked *L. decemlineata* as most polyphagous of the eight species, *L. haldemani* as moderately host specific and *L. texana* as highly host specific.

Host selection behaviour has two major components: searching for host plants (long range discrimination among plants) and short range recognition of appropriate host plants. Visser (1986) has demonstrated that adult *Leptinotarsa decemlineata* perceive olfactory and visual plant characteristics from a distance. These characteristics provide cues used in searching for host plants. Long (>6m) and medium (0.5-6m) range olfaction of *L. decemlineata* suggest that antennal olfactory sensilla are important in indicating the presence of a potential host plant upwind. *L. decemlineata* demonstrates positive anemotaxis which is increased in the presence of potato leaf volatiles (Visser and Thiery, 1985). Visser (1979) concludes that the initial olfactory orientation of *L. decemlineata* is directed towards solanaceous plant species. The process of host plant selection begins with distinction among potential host plants restricted by long range olfactory chemical profiles. Individuals move toward a population of plants containing a particular odour blend. No single plant attractant has been identified, instead attraction seems to be due to a mixture of plant volatiles. Relative ratios of odour components are critical for eliciting olfactory responses from the beetles (Visser, 1979). Although form of host plants has not been investigated for visual attactiveness, studies of colour attraction indicate that yellow traps with peak reflectance

at 550- 850 nm were most attractive. This range is similar to reflectance of potato leaf material (Zehnder and Speese, 1987).

Further host plant discrimination occurs when the insect is in the immediate vicinity of a potential host plant. Short range(<0.5 m), behavioural indications of preference for a plant include a tendency to spend more time in the vicinity of a particular plant, decreasing speed of locomotion, stopping and swaying antennae more frequently when presented with a particular plant (Bongers, 1970). Harrison (1985) used video recordings to describe feeding behaviour of *L. decemlineata*. Behavioural sequences of adult *L. decemlineata* when intact leaves were presented, had 4 stereotyped components: sampling, feeding, grooming and rest. Elements in the transition from sampling to feeding are described as exploration, gustatory sampling, small biting and sweep feeding. Variations in duration of each element and interruption in the pattern of component feeding behaviours are related to host plant discrimination. On plants less preferred for feeding, time spent exploring the leaf prior to gustatory sampling and time spent sampling leaf fluids increased. Re-initiation of the behavioural sequence was usual on less preferred host plants. Comparisons among these three *Leptinotarsa* species provide a framework for generalizing the clear behavioural distinctions of *L. decemlineata* to other *Leptinotarsa* species.

Quantity of food ingested as a measure of behavioural response involves both pre- and post-ingestive factors. Bioassays over a period of several hours may give strikingly different results from bioassays over several minutes. Longer term bioassays may result in severely deprived insects sampling otherwise unacceptable plants. Toxic effects of various compounds in leaves and conditioning of

the insects' response may occur in longer term bioassays. Short term bioassays presumably reduce confounding effects of satiated and famished insects and malaise due to toxic compounds contained in test materials. A bioassay over several minutes provides a more accurate measure of the sensory basis of host plant insect interactions than does a bioassay over several hours. The use of beetles with no previous feeding experience in a short term bioassay provides an approximation of differences in the innate neural pattern which is the basis of the template of host plant recognition.

METHODS AND MATERIALS

Adult beetles of *Leptinotarsa decemlineata*, *L. haldemani* and *L. texana* were used in the behavioural assay 4 - 24 hours after emergence. Consequently, age and physiological conditions of the beetles were comparable. Each beetle was tested during the third to sixth hour of the photophase to minimize effects of diel periodicity. Overhead lights were turned on to provide uniform illumination of the behavioural locale while a fibre-optic light source was directed at the leaf to enhance lighting of the insect's mouthparts. A small fan directed air across the leaf towards the beetle to standardize air flow. Temperature was maintained at 25 +/- 0.5 °C with 40-60 % relative humidity.

Individual beetles did not interact during the behavioural assay. Beetles were placed on a teflon rod of 1 cm diameter at approximately 25 cm from a slit at one end of the rod. The extreme opposite end of the rod held the edge of the leaf in a slot. Each beetle was tested for all leaves. Individuals were offered a freshly cut leaf or leaflet of one of four plant species in a haphazard order which was recorded. Time taken for a first meal by adult *L.*

decemlineata on *S. tuberosum* was 251.35 +/- 52.46 seconds (Sen, 1987). Beetles were only allowed to feed for a maximum of 60 seconds, so that satiation of the beetles was unlikely. Order of presentation of leaves did not significantly affect the approach time, exploration time or number of bites in 60 seconds indicating that satiation and short term memory were not significant factors in these bioassays.

Leaves or leaflets were selected from healthy plants. The petiole or petiolule was cut with a razor blade and placed in a vial containing tap water to maintain turgidity of the leaf during the experiment. Each leaf or leaflet was used only once to reduce variability of volatile release from damaged leaves. Similarly sized leaves and leaflets were chosen whenever possible. No effort was made to cut leaves to present an equal area of leaf material since damaged plants may release different volatiles than undamaged leaves. Samples of 10 randomly chosen sample leaves or leaflets were selected and measured for leaf area. Leaves from *Solanum dulcamara* averaged 743 mm^2, leaves from *S. elaeagnifloium* averaged 805 mm^2, leaflets from *S. tuberosum* averaged 960 mm^2 and leaflets from *Lycopersicon esculentum* averaged 1223 mm^2. Leaf area might influence rate of locomotion of the insect towards the leaf since leaves with larger leaf area would presumabley release more volatiles than leaves of a smaller leaf area. Greater release of volatiles might be more attractive to the beetles in the case of preferred plants and less attractive in the case of less preferred host plants. Since rate of locomotion towards leaves varied among beetle species and not among leaves presented to a particular beetle species, the range in leaf area is considered insignificant even though release of volatiles may be very different.

Observations were made through one way mirror to reduce the startle effect of the experimenter as she drew closer to

better observe the animals. A hand lens was used when necessary during observations. When any part of the beetle passed a red mark painted on the teflon rod at 10.8 cm from the leaf edge, a timer was started. Time to the nearest second was recorded from the time the red mark was passed to when the leaf was first touched by any part of the beetle, usually an antenna or front tarsus. This time was designated approach time. A bite was defined as squeezing the leaf with the mandibles. A second time called 'exploration time' is the time from first contact with the leaf to the time the beetle first squeezed the leaf with its mandibles to release plant sap from the interior of the intact leaf. If individuals did not feed within three minutes, they were assigned an exploration time of 180 seconds which represents a maximum value. The three minute interval was based on the minimum inter-meal interval as determined by Harrison (1985). The third factor measured was the number of bites in 60 seconds immediately following the first bite. If no bites were recorded within 180 seconds of first touching the leaf, the trial was concluded. Beetles which did not bite before 180 seconds elapsed were assigned a bite time of 180 seconds and zero bites in 60 seconds. All times were measured by Mountain clock timer installed in an Apple IIe microcomputer and appropriate software written for this experiment.

The sex of each beetle was determined at the end of each day by presence of a dimple in the last sternal sclerite if the beetle was male or by a rounded sternal sclerite for females. In cases where sex was not readily obvious, beetles were frozen and dissected to ascertain sex. Dissection to determine sex was most often necessary for *L. haldemani*.

Statistical analyses were accomplished using SPSS.X statistical package (Nie *et al.*, 1975) and UANOVA as a user-

defined procedure. UANOVA is a multivariate analysis of covariance developed at the University of Alberta by Terry Taerum. Statistical analyses used a repeated measures ANOVA with individuals nested within beetle species crossed with plant species. The beetle species had unequal sample sizes. Data were not normalized because of large sample size and reliance on ANOVA which is based on mean of distribution as a method of comparison (Denenberg, 1976). TukeyB (Tukey's alternate procedure) was used as an *a posteriori* contrast test at alpha equal to 0.05. Histograms of behavioural assays are included to demonstrate form of distribution which is not distinguished by ANOVA. Correlation of histograms is used as a measure of similarity of distribution.

RESULTS

Some individuals of each of the species displayed the four component behaviours in plant assessment and feeding as described by Harrison (1985). Percentage of individuals of the three beetle species, on four plant species, that proceeded to the stage of gustatory sampling is given in Table II.1.

Order of presentation of leaves did not significantly affect the approach time ($P<0.450$, d.f.173,545), exploration time ($P<0.733$, d.f.173,545) or number of bites in 60 seconds ($P<0.967$, d.f.173,545). Sex did not affect approach time ($P<0.601$, d.f.162,485) or exploration time ($P<0.999$, d.f.162,484) or number of bites in 60 seconds ($P<0.325$, d.f.162,483).

Table II.2 shows means and standard errors for approach times. Approach time varied among species (P< d.f.182,545). Multiple comparison using Tukey's indicat

that no significant differences were observed for *L. decemlineata* and *L. haldemani* among leaves and that *L. decemlineata* and *L. haldemani* were not significantly different from each other but that both were significantly different from *L. texana*. There were no significant differences among leaves for *L. texana*.

Exploration time (Table II.3) varied among species (P<0.000, d.f.182,544). Multiple comparisons using TukeyB indicate that exploration times for *L. decemlineata* and *L. haldemani* did not differ from each other or among plants. Exploration time for *L. texana* on *S. elaeagnifolium* did not differ from *L. decemlineata* or from *L. haldemani* however exploration time for *L. texana* on *S. dulcamara, S. tuberosum* and *L. esculentum* was significantly longer. There were no significant differences among *S. dulcamara, S. tuberosum* and *L. esculentum* for L. texana.

Bites in 60 seconds (Table II.4) varied among species (P<0.000, d.f.182,543). Multiple comparison using TukeyB were tested for host plants within species. For *L. decemlineata, S. tuberosum* and *S. dulcamara* were not significantly different from each other; *S. elaeagnifolium* and *L. esculentum* were not significantly different from each other while both *S. tuberosum* and *S. dulcamara* were different from either *S. elaeagnifolium* or *L. esculentum*. For *L. haldemani, S. tuberosum, S. dulcamara* and *L. esculentum* were not significantly different from one another while all three were significantly different from *S. elaeagnifolium*. Comparisons among plant species for *L. texana* show no significant differences despite significant differences seen when comparing *L. texana* against the other two species.

Table II.1 contains values for percentage of individuals that proceeded to the stage of gustatory sampling while Table

II.5 contains values of average number of bites for individuals which took at least one bite.

Frequency histograms of number of bites in 60 seconds demonstrate the form of distribution. None of the distributions is normal; all are heavily skewed towards values of zero bites. *L. decemlineata* on four plant species is shown by Figure II.1. Figure II.2 shows *L. haldemani* on four plant species. Figure II.3 shows *L. texana* on four plant species. Correlation values for frequency histograms among plant species for a single beetle species are shown in Table II.6.

DISCUSSION

All three species expressed the four component behaviours in plant assessment and feeding as described by Harrison (1985). Patterns of reduced exploration time and increased bites in 60 seconds were similar within beetle species although plant species were ranked differently for each beetle species.

Although approach time distinguishes *L. texana* from the other two species, approach time is not a useful distinction for host plant preferences within any of the species (Table II.2). *L. texana* approached all plant species at a slower rate than *L. decemlineata* and *L. haldemani*. The slower approach time for *L. texana* may be due to inherent differences among species or to the slightly smaller size of *L. texana* (Table II.7). Since approach time for all three species did not vary among plants within the family Solanaceae, adult beetles at 10.8 cm from an intact leaf proceed to the leaf and contacted it regardless of the subsequent acceptablity of the leaf as a food plant.

The lack of significant difference in approach time, exploration time or number of bites in 60 seconds for both sexes of *L. decemlineata* contrasts with several reports of differential mobility for male and female *L. decemlineata*. Szentesi (1985) studied distribution of adult *L. decemlineata* in experimental plots and concluded that males are more mobile than females. This difference in mobility was not manifest in this behavioural assay. Increased mobility may be evident in a field situation where reproductive as well as feeding behaviour have active roles in determining mobility.

Morphological differences which might account for differential mobility between males and females are size and tarsal sensilla. Sexual dimorphism, females being slightly larger than males, is described by Jacques (1972). Tarsal hairs which adhere to smooth surfaces and impede locomotion of *L. decemlineata* were described on tarsi of males by Pelletier and Smilowitz (1987). In both of these instances, females would presumably be the more mobile sex. Since no significant differences were evident between the sexes, potential differences in locomotion due to larger size of females were insignificant for these bioassays. The roughened surface of the teflon bar may have reduced differences in adherence by males and females. The horizontal aspect of the bar could also have reduced differences in locomotion since adherence to smooth surfaces was most obvious when male beetles were upside-down (Pelletier and Smilowitz, 1987). Significant differences in mobility, could have been confounded by interactions between inherently greater mobility of males (Szentesi, 1985) and restrictions in mobility imposed by morphological differences between males and females (Pelletier and Smilowitz, 1987). Lack of significant differences for all parameters measured by these bioassays is similar to results of Harrison (1985), Sen (1987) and Visser and

Thiery (1985), all of whom found no effects of sex on feeding behaviour.

When approach times are averaged and divided by the distance to the leaf edge, *L. decemlineata* approached at approximately 7.05 mm per second. This is much slower than the rate described by Visser and Thiery (1985), using a locomotion compensator. On average, the Dutch beetles walked at a rate of 13.6 mm/sec with air flow of 800 mm/sec and at 17.4 mm/sec in wind plus potato volatiles emanating from 6 fully grown potato plants. Differences between rates of locomotion as measured by Visser and Thiery (1985) and rates recorded in this study are most probably due to use of different measuring systems, for example, Dutch beetles may have been tested at a higher ambient temperature and on a different substrate. Differences in rate of locomotion between populations is also possible.

Harrison (1985) described the behavioural category of 'explore' as including walking, palpating and antennal waving. All of these active movements were seen for the 3 beetle species. Exploration times, for the present study, when averaged for all individuals regardless of eventual consumption of leaf material, were similar among plants for *L. decemlineata* and *L. haldemani*. Only for *L. texana*, was average exploration time indicative of eventual host plant acceptance. Exploration time for *L. texana* on *S. elaeagnifolium*, its only described host plant, did not differ from exploration times of *L. decemlineata* or *L. haldemani*. However, exploration times for *L. texana* on *S. dulcamara*, *S. tuberosum* and *L. esculentum* were significantly longer than exploration time on *S. elaeagnifolium*. Differences among exploration times for *S. dulcamara*, *S. tuberosum* and *L. esculentum* were not significant for *L. texana*. *L. texana* was more discriminating earlier in the behavioural

[redacted] at approximately the same time interval as *L. decemlineata* and *L. haldemani*.

Values for exploration time for Alberta *L. decemlineata* in these bioassays are much longer than values given by Harrison (1987) and Sen (1987). Harrison's values are only for individuals who proceeded to consume the leaves. When exploration times for individuals who proceeded to bite the leaf are counted, exploration times are comparable to values given by Harrison. In Sen's bioassay, leaves were cut and therefore a greater concentration of volatiles would have been present for the beetles to sample. The cut edges may also have released liquid that could have been sampled during palpation without having to break the integrity of the leaf surface.

Schneider (1987) states that gustatory sampling is an important step in host plant discrimination. In support of this statement, number of bites in 60 seconds, an indication of gustatory sampling, allows ranking of plant species for all beetle species. Ranking of plant species based on gustatory sampling is given in Figure II.4. For *L. decemlineata*, *S. tuberosum* and *S. dulcamara* are ranked higher than *S. elaeagnifolium* and *L. esculentum*. For *L. haldemani*, *S. tuberosum*, *S. dulcamara* and *L. esculentum* are not significantly different from one another while all three are significantly different from *S. elaeagnifolium*. For *L. texana*, *S. elaeagnifolium*, is ranked higher than *S. tuberosum*, *S. dulcamara* and *S. esculentum*. Adult ranking of host plants correspond exactly with larval rankings with two exceptions (Table II.8). For *L. decemlineata*, *L. esculentum* is ranked lower by larvae than by adults. For *L. texana*, the gradation

in reponse which is evident for larvae is not seen the bioassays using adults.

When number of bites in 60 seconds is calculated excluding values of zero, the same ranking of host plant acceptability is found as with number of bites in 60 seconds including values of zero with one exception (Table II.5). *S. dulcamara* moves up in ranking for *L. texana* when only individuals who proceeded to the stage of gustatory sampling are included. Possibly, for *L. texana* gustatory sampling of *S. dulcamara* provides similar stimuli to *S. elaeagnifolium*. If a beetle proceeds to the stage of gustatory sampling, *S. dulcamara* becomes nearly as acceptable as *S. elaeagnifolium*. Preliminary results with 10 *L. texana* indicate that if adults are starved for longer than 72 hours, no adult *L. texana* bit *L. esculentum* while 2 out of 10 adult *L. texana* consumed *S. tuberosum*. These results, although preliminary, suggest that Hsaio's results with larvae correspond to adult food choice.

Percent beetles proceeding to the stage of gustatory sampling is not significantly different for *L. decemlineata* on *S. tuberosum*, *S. dulcamara* or *L. esculentum*. In light of the possibility of emerging *L. decemlineata* biotypes specialised for *L. esculentum* (Kennedy *et al.*, 1985), these behavioural data suggest that until the stage of gustatory sampling, these three plants provide similar behavioural cues. *L. haldemani* proceeded to the stage of gustatory sampling at approximately the same rate regardless of the eventual ranking of the plant species. This corresponds to the more polyphagous feeding habits of *L. haldemani*. Behaviourally, *L. haldemani* may be cued to sample leaves and make its decisions based on gustatory samples. The majority of *L. texana* did not proceed to the stage of gustatory sampling in less than three minutes. This behavioural finickiness (terminology of Dethier, 1982)

demonstrated by *L. texana* corresponds to its monophagous lifestyle.

Histograms of number of bites in 60 seconds provide information that is otherwise lost in ANOVA analyses. Table II.6 lists correlation values among graphs, arranged in descending order of correlation coefficients. The arrangement of histograms indicates decreasing similarity in form of distribution. Form of distribution is another factor which links equally ranked host plants. *S. elaeagnifolium* and *L. esculentum*, two lower ranked host plants, are most similar followed by the two highest ranked host plants, *S. tuberosum* and *S. dulcamara*. Highly ranked host plants compared to less highly ranked host plants have the lowest correlation coefficients. For *L. decemlineata* which sampled the leaf in less than three minutes, the distribution approximates normal except for *L. esculentum* which tends towards bimodality. This suggests that *L. esculentum* may be an interesting plant to study for Alberta *L. decemlineata*. The Alberta population may consist of two groups of individuals, those which reject *L. esculentum* after few bites and those which do not. This distribution may favour the development of new biotypes by providing two groups which could become sympatrically isolated on two host plants in relatively few generations. Figure II.2 shows form of distribution for *L. haldemani* on four plant species. The trend of highly ranked host plants being most similar in distribution when compared to other each other and least similar when compared to the lowest ranked host plant is upheld. *S. elaeagnifolium* is the least similar for each paired comparison. Histograms for *L. texana* (Figure II.3) are based on very few individuals therefore, no firm conclusions can be drawn.

Behavioural assays described in this chapter establish relative rankings of 4 solanaceous plants for *L. decemlineata, L. haldemani* and *L. texana* (Figure II.4). Number of bites in 60 seconds is the most reliable indicator of host plant ranking, suggesting that gustatory sampling provides the best opportunity for discriminating among host plants. Ranking of preferred host plants based on adherence to stereotyped feeding sequences corresponds to ranking of these host plant using larval nutritional criteria (Table II.8). Heritable variability between individuals within a population provides an excellent substrate for natural selection to act upon.

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Table II.1. Percent beetles sampling leaf in less than 180 seconds for 3 beetle species on 4 solanaceous plant leaves.

	beetle species		
	L. decemlineata	*L. haldemani*	*L. texana*
plant species	N = 82	N = 65	N = 38
S. tuberosum	61%	51%	13%
S. dulcamara	61%	40%	16%
S. elaeagnifolium	45%	42%	42%
L. esculentum	55%	48%	16%

Table 11.2. Time in seconds for 3 beetle species to approach 4 solanaceous plant leaves. Values given are mean ± standard error. Mean values followed by the same letter are not significantly different using TukeyB multiple range comparison.

	beetle species		
	L. decemlineata	*L. haldemani*	*L. texana*
plant species	N = 82	N = 65	N = 38
S. tuberosum	15 ± 1 a	11 ± 1 a	20 ± 3 b
S. dulcamara	16 ± 2 a	10 ± 1 a	26 ± 6 b
S. elaeagnifolium	16 ± 2 a	12 ± 1 a	23 ± 5 b
L. esculentum	14 ± 1 a	11 ± 2 a	23 ± 3 b

Table 11.3. Time in seconds for 3 beetle species to explore 4 solanaceous plant leaves. Values given are mean ± standard error. Mean values followed by the same letter are not significantly different using TukeyB multiple range comparison.

	beetle species		
	L. decemlineata	*L. haldemani*	*L. texana*
plant species	N = 82	N = 65	N = 38
S. tuberosum	95 ± 8 a	108 ± 10 a	168 ± 6 b
S. dulcamara	100 ± 8 a	127 ± 9 a	163 ± 7 b
S. elaeagnifolium	125 ± 8 a	118 ± 10 a	118 ± 12 a
L. esculentum	110 ± 8 a	111 ± 9 a	160 ± 8 b

Table 11.4. Average number of bites in 60 seconds for 3 beetle species on 4 solanaceous plant leaves. Values given are mean ± standard error. Mean values followed by the same letter are not significantly different using TukeyB multiple range comparison.

	beetle species		
	L. decemlineata	*L. heidemani*	*L. texana*
plant species	N = 82	N = 65	N = 38
S. tuberosum	36 ± 4 a	26 ± 4 b	1 ± 1 c
S. dulcamara	40 ± 4 a	22 ± 4 b	3 ± 1 c
S. elaeagnifolium	15 ± 2 b	5 ± 1 c	11 ± 3 b,c*
L. esculentum	21 ± 3 b	18 ± 3 b	1 ± 0 c

*Member of groups b and c. Included with group b for comparisons among species. Not significantly different within L. texana.

Table 11.5. Average number of bites in 60 seconds for beetles biting 4 solanaceous plant leaves. Only values greater than zero are included. Values given are mean ± standard error. Mean values followed by the same letter are not significantly different using TukeyB multiple range comparison.

	beetle species								
	L. decemlineata			*L. haldemani*			*L. texana*		
plant species									
S. tuberosum	51 ± 3	N=50	a	50 ± 4	N=33	a	8 ± 3	N=5	d
S. dulcamara	65 ± 3	N=50	a	55 ± 5	N=26	a	18 ± 6	N=6	c
S. elaeagnifolium	34 ± 3	N=37	b	11 ± 1	N=27	d	27 ± 5	N=16	c
L. esculentum	39 ± 4	N=45	b	41 ± 5	N=31	b	5 ± 1	N=6	d

Table II.6. Correlation values among frequency histograms of bites in 60 seconds.

L. decemlineata	
elaeagnifolium/esculentum	0.95
tuberosum/dulcamara	0.92
tuberosum/esculentum	0.91
tuberosum/elaeagnifolium	0.89
dulcamara/esculentum	0.83
dulcamara/elaeagnifolium	0.80

L. haldemani	
tuberosum/dulcamara	0.97
dulcamara/esculentum	0.96
tuberosum/esculentum	0.95
elaeagnifolium/esculentum	0.93
dulcamará/elaeagnifolium	0.88
tuberosum/elaeagnifolium	0.89

L. texana	
tuberosum/dulcamara	1.00
tuberosum/esculentum	1.00
dulcamara/esculentum	0.99
tuberosum/elaeagnifolium	0.98
dulcamara/elaeagnifolium	0.98
elaeagnifolium/esculentum	0.97

Table II.7. Comparison of size of beetles. (adapted from Jacques, 1972). Horizontal bars indicate no significant difference.

	L.decemlineata	*L. haldemani*	*L. texana*
number of individuals	349	149	96
total length			
mean ± se	10.00 mm ± 0.62	9.5 mm ± 0.56	8.5 mm ± 0.75
(range)	(9.0-11.5 mm)	(8.8-11.0 mm)	(7.1-10.0 mm)
greatest width			
mean ± se	6.8 mm ± 0.42	6.9 mm ± 0.47	6.2 mm ± 0.50
(range)	(6.1-7.6 mm)	(6.2-7.8 mm)	(5.3-7.00 mm)

Table 11.8. Food spectra of Leptinotarsa species among solanaceous plants based on rate of development, % mortality and pupal weight (adapted from Hsiao, 1974).

plant species	beetle species		
	L. decemlineata	*L. haldemani*	*L. texana*
S. tuberosum	+++	+++	+
S. dulcamara	+++	+++	++
S. elaeagnifolium	++	–	+++
L. esculentum	+	+++	–

+++ optimal feeding and growth
++ moderate feeding and growth
+ some feeding and growth
– not acceptable and no growth

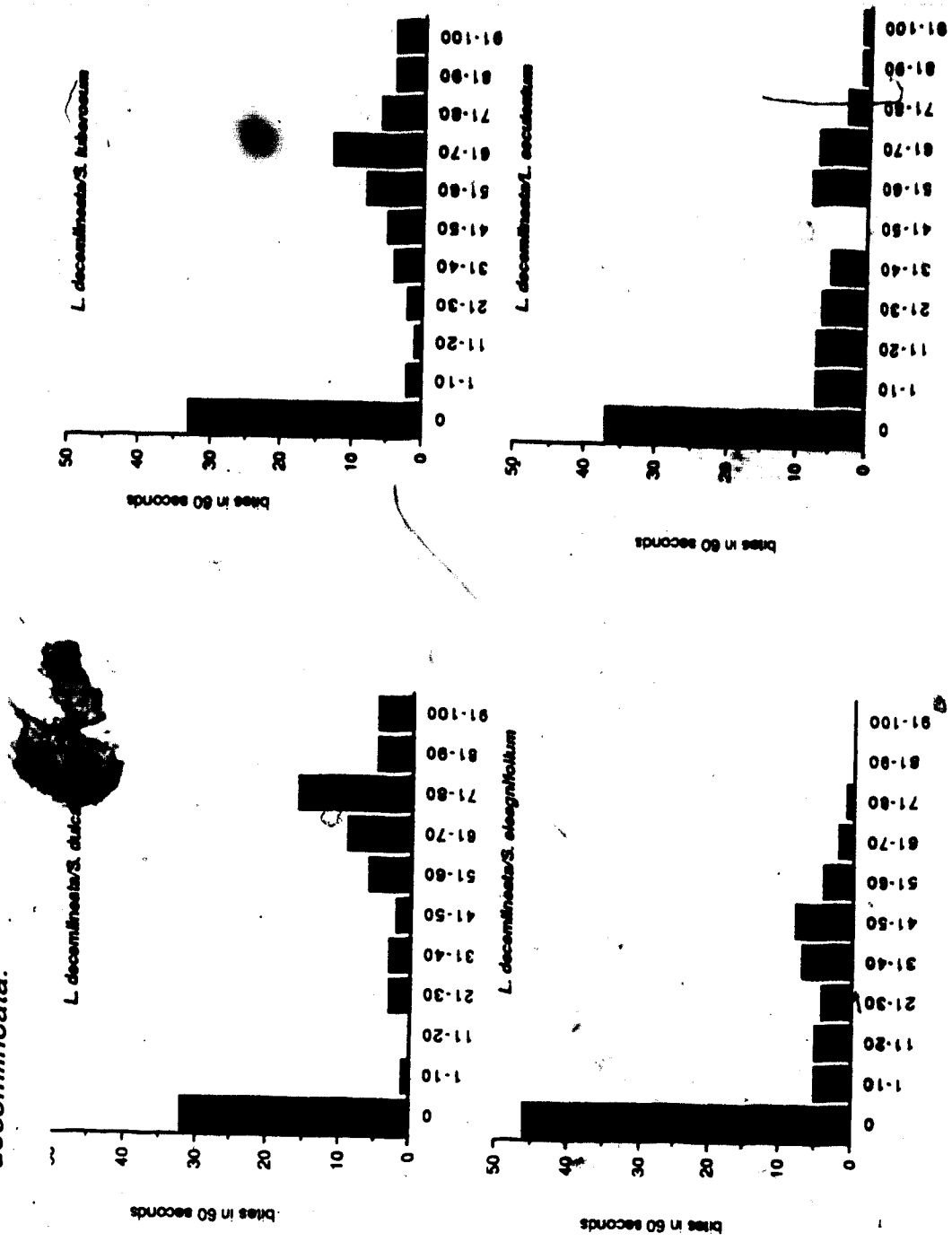
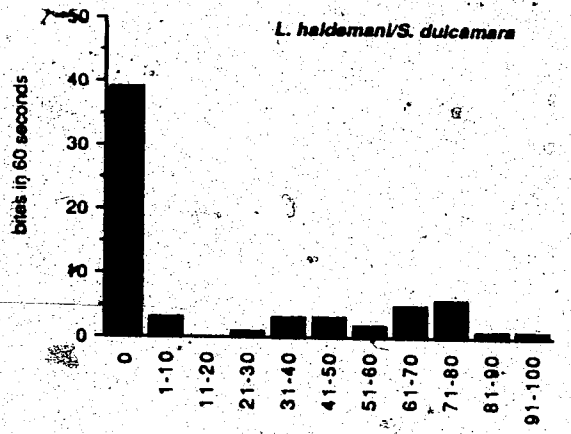
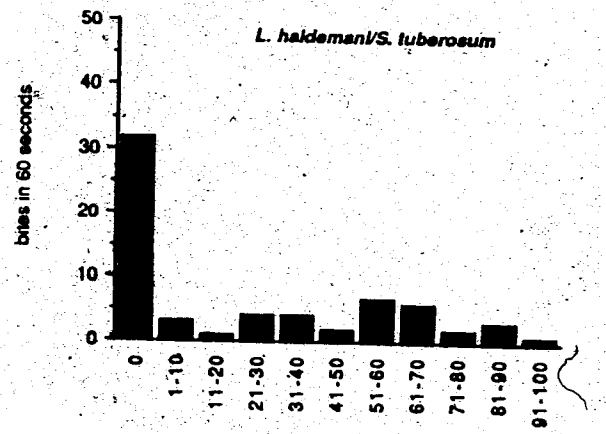
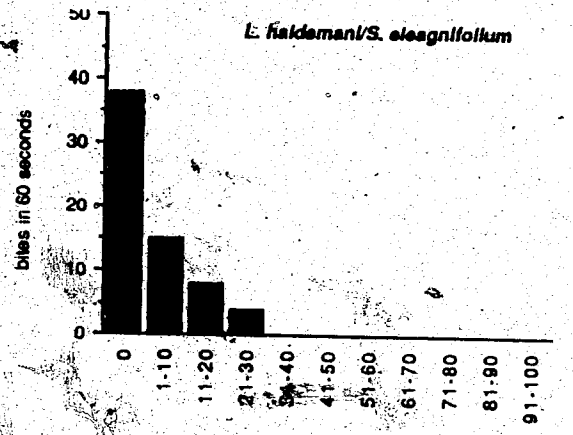


Figure II.1. Frequency histograms of number of bites in 60 seconds for *L. decemlineata*.

Figure II.2. Frequency histograms of number of bites in 60 seconds for *L. haldemani*.







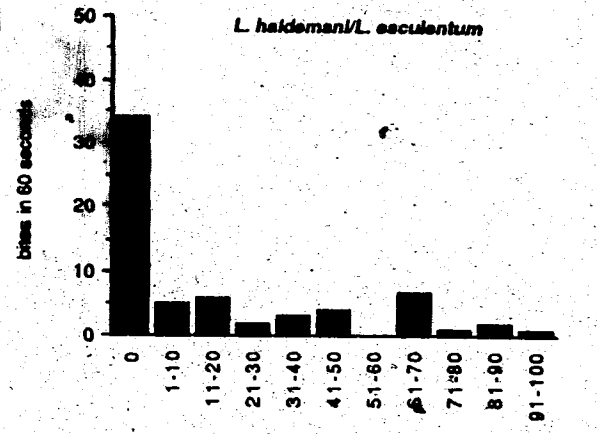


Figure II.3. Frequency histogram of number of bites in 60 seconds for *L. texana*.

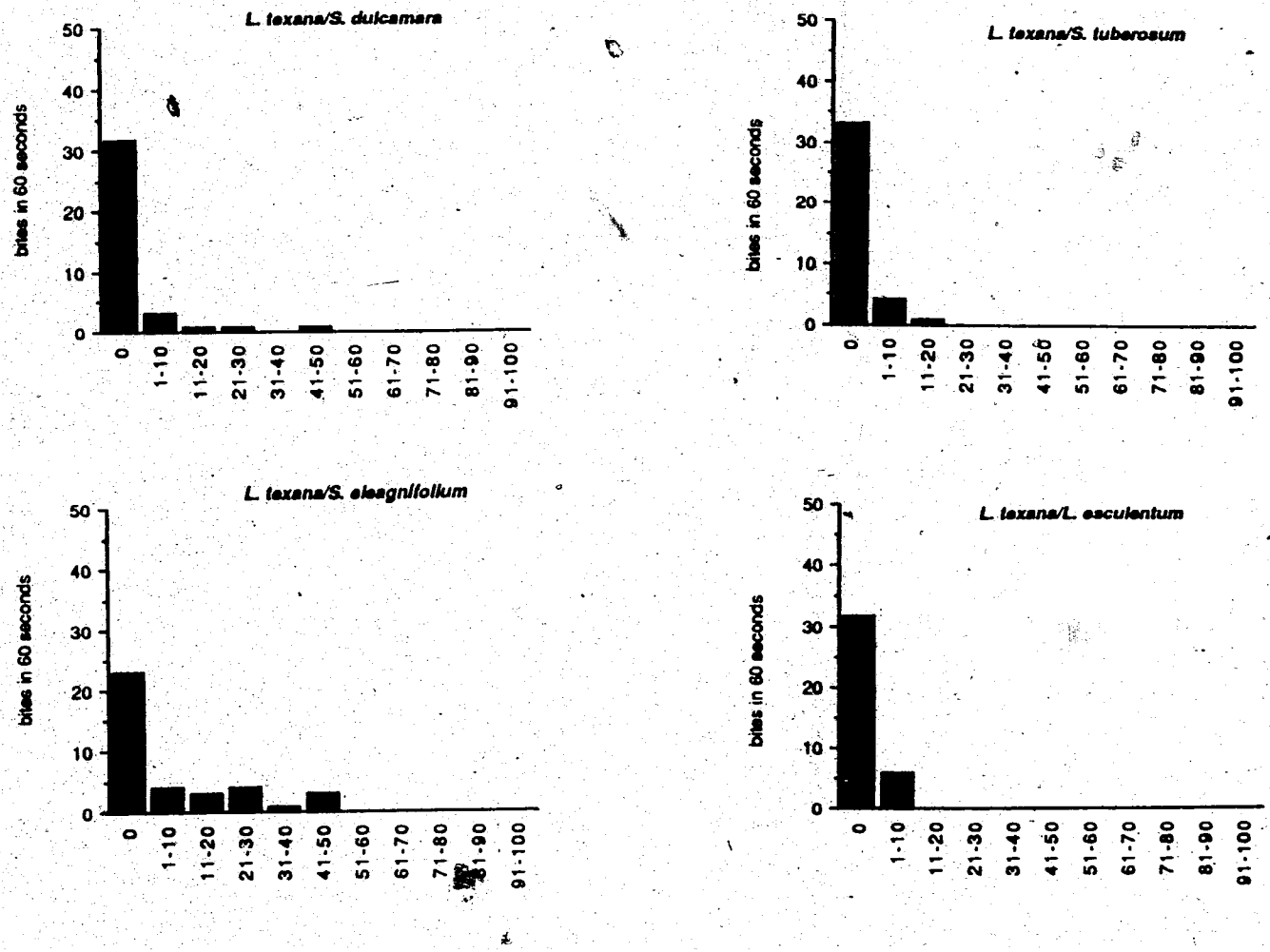


Figure II.4. Ranking of plant species by beetle species based on number of bites in 60 seconds. Plant species on the same line do not differ significantly Plant species on different lines differ in ranking within each beetle species.

L. decemlineata

S. tuberosum, S. dulcamara
S. elaeagnifolium, L. esculentum

L. haldemani

S. tuberosum, S. dulcamara, L. esculentum
S. elaeagnifolium

L. texana

S. elaeagnifolium
S. dulcamara, S. tuberosum, L. esculentum

III. Comparative external morphology of the tip of galeae

The gustatory sensory complex of *Leptinotarsa* beetles includes chemoreceptors on tarsi (Mitchell and Harrison, 1985), antennae (Sen, *pers comm*), and mouthparts (Sen, 1987). The galeae contact leaf saps intermittently during maceration of leaf material (Mitchell, 1988) providing opportunity for partial sensory information to the beetle. The sensory totality perceived by the insect has not been measured and therefore the exact contribution of galeal chemoreceptors to information on leaf saps is unknown.

Observations of living chrysomelid beetles demonstrate the mechanical importance of the galeae in feeding behaviour. For *Entomoscelis americana*, movements of the galeae and laciniae are essential for efficient transfer of food to the food canal (Sutcliffe and Mitchell, 1980). Observations of living *Leptinotarsa* beetles suggest a similar role of galeae for members of this genus. From among the mouthparts of adult *Leptinotarsa* beetles, the galeae were chosen for study since they are in a position to monitor food during feeding and are easily accessible for electrophysiological recordings using the technique described by Sutcliffe and Mitchell (1982).

The galeae of *Leptinotarsa decemlineata* have been examined structurally and electrophysiologically. They possess numerous mechanosensilla as well as approximately 15 chemosensilla (Mitchell and Harrison, 1985; Sen and Mitchell, 1987). Mechanosensitive hairs are more densely distributed on the dorsal side of the galea than on the ventral side (Sen, 1987). Chemosensitive sensilla of the galeae of *L. decemlineata* are divided into two groups, apical pegs and apical hairs, based on size, structure of the pore and ultrastructure. There are 11 to 15 uniporous,

cylindrical apical pegs which are arranged on the tip of the galeae in an irregular fashion. Most apical pegs are innervated by 5 neurons: 1 mechanosensitive dendrite and 4 chemosensitive dendrites. Among the apical pegs is a sensillum, indistinguishable with the scanning electron microscope, but which differs in ultrastructure from the other pegs. This sensillum has 1 mechanosensitive dendrite and 3 rather than 4 chemosensitive dendrites. There are 2 apical hairs in addition to the apical pegs. Apical hairs differ from apical pegs in that they are shorter and have finger-like projections at the tip. They have two lumina and no apparent dendritic sheath. These apical hairs may be mechanosensitive, chemosensitive or both. No firm conclusions have been drawn regarding their function (Sen and Mitchell, 1987).

The apical pegs described by Sen and Mitchell (1987) may be divided into two physiologically different classes. Most are sensitive to sucrose and only a few respond to gamma amino butyric acid (GABA) and L-alanine (Mitchell and Harrison, 1984, 1985; Mitchell, 1987). One apical peg, the alpha-sensillum, responds especially well to GABA and L-alanine and has a higher conductance than the other apical pegs (Mitchell and Harrison, 1984).

This chapter is a survey of chemosensilla which are distinguishable on the basis of scanning electron micrographs. Numbers, distribution and gross anatomy of sensilla are compared among species. Descriptions for *L. decemlineata* are based on the work of Sen (1987). Only the galeae of *L. haldemani* and *L. texana* are included in this study. Comparisons of the galeae of *L. decemlineata*, *L. haldemani* and *L. texana* were undertaken to determine if gross anatomical differences exist among beetle species. Characterisation of the tip of the galeae using electron

micrographs permits choice of presumably homologous sensilla for electrophysiological studies.

Methods and Materials

All specimens were studied using a Cambridge Stereoscan 250 electron microscope. Heads of 6 newly emerged male and 6 newly emerged female *L. haldemani* and *L. texana* were examined. Heads were removed and placed in warm soapy water. The left maxillae were removed from the head and sonicated for 3 minutes in warm soapy water, washed in tap water then sonicated for 1 minute in distilled water. The maxillae were then placed in carbon tetrachloride and soaked for 5 minutes, sonicated in carbon tetrachloride for 2 minutes, and following a change of carbon tetrachloride, sonicated for a further 2 minutes. Specimens were air dried and mounted on stubs by inserting the base of maxillae into a drop of silver conducting paint. All specimens were sputter coated with gold in a Nanotech Semprep 2.

Distribution of sensilla were observed at high magnification. Short, robust sensilla with a uniporous tip were classed as contact chemoreceptors. Long slender sensilla with no visible pores were classed as mechanosensilla (classification of Zackaruk, 1980).

RESULTS

The maxillae of *Leptinotarsa haldemani* and *L. texana* have a lateral, 4 segmented palpus, medial galea and cutlass shaped lacinia (Figure III.1). The galea is approximately 300 um long. Numbers and distribution of sensilla on the galea did not differ between sexes. The cuticle is relatively smooth and has numerous glandular openings (Figure III.2).

The galeae of both *L. haldemani* and *L. texana* have numerous mechanosensitive hairs on the dorsal side and fewer on the ventral side (Figures III.3 and III.4).

There are 10 to 15 chemosensilla arranged at irregular intervals on the tip of the galeae of *L. haldemani* and *L. texana* (Figures III.5 and III.6). Each chemosensillum arises from a simple socket (Figure III.2).

The pore of chemosensilla examined is in the side of the tip of the sensillum (Figure III.7).

DISCUSSION

The maxillae of *L. decemlineata*, *L. haldemani* and *L. texana* appear similar using SEM technique. The galeae possess mechanosensitive sensilla and apical pegs. Distributions of galeal sensilla did not vary with sex for any of the three species. Since male and female beetles do not differ in short term feeding behaviour (see chapter 2; Harrison, 1985; Sen, 1987) similar distribution of sensilla on the galeae is expected. There are no data to support the use of galeae in functions other than feeding behaviour.

Pores distributed over the surface of the cuticle are similar to those found in *Entomoscelis americana* (Sutcliffe and Mitchell, 1980). For *E. americana*, ultrastructural evidence indicates that the pores are associated with secretory glands. The function of the secretions is not known.

The role of mechanosensilla in discrimination of host plants is unknown. Mechanosensilla could function in discrimination of host plants before the stage of gustatory

sampling by providing information on texture of leaves. Mechanosensilla may be useful in reducing abrasion on the chemosensilla since specimens older than 20 days had worn mechanosensilla (unpublished SEM observations). Mechanosensilla may also monitor the passage of food as suggested by Sutcliffe and Mitchell (1980).

L. *haldemani* and *L. texana* have 10 to 15 chemosensilla at irregular intervals on the tip of the galea, similar to *L. decemlineata* which is described as having 11 to 15 chemosensilla. Gross anatomy of chemosensilla of all three species is similar.

L. haldemani and *L. texana* may possess alpha-sensilla and apical hairs as described for *L. decemlineata*. However, confirmation these sensilla awaits electrophysiological and ultrastructural evidence.

Indirect evidence suggests that the ultrastructure of galeal sensilla is also comparable among these three species. Conclusions on ultrastructure of the sensilla await TEM studies, however SEM micrographs give partial evidence of similar ultrastructure. Several specimens examined with SEM had sensilla broken off at the base, revealing 5 apertures within the larger ring of the socket. One aperture was larger than the four others. Comparing these observations to ultrastructural details of *L. decemlineata* described by Sen and Mitchell (1987), the larger aperture probably contained the remains of a mechanosensitive dendrite while the 4 smaller apertures probably contained the remains of chemosensitive dendrites. TEM studies of the galeae of *L. decemlineata* show that most apical pegs are innervated by 5 neurons, 4 of which extend their sensory processes up to the tip of the peg while the alpha-sensillum has only 3 chemosensitive dendrites (Sen and Mitchell, 1987). Four chemosensitive dendrites have been

described by Mitchell *et al.* (1979) and Sutcliffe and Mitchell (1980) for larvae and adult galeae of *E. americana*. Therefore, the working hypothesis derived from these SEM observations is that *L. haldemani* and *L. texana* possess 10 to 15 apical pegs with 4 chemosensitive dendrites and 1 mechanosensitive dendrite. Since gross anatomy of the tips of the galeae of *L. decemlineata*, *L. haldemani* and *L. texana* is similar among species, electrophysiological studies (Chapter four) are assumed to have been performed on homologous sensilla.

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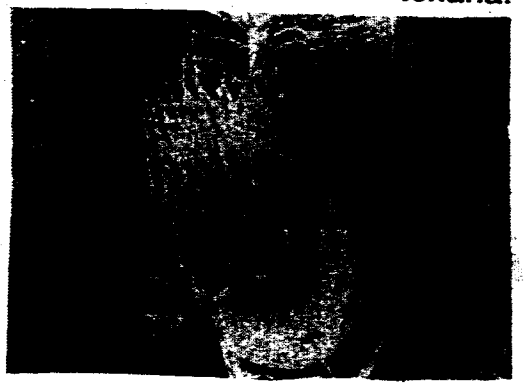
Figure III.1 Maxilla of *L. texana*.
M= mandible, L= lacinea,
G= galea, P= palpus

100 µm

Figure III.2 Glandular openings on galea of *L. texana*.

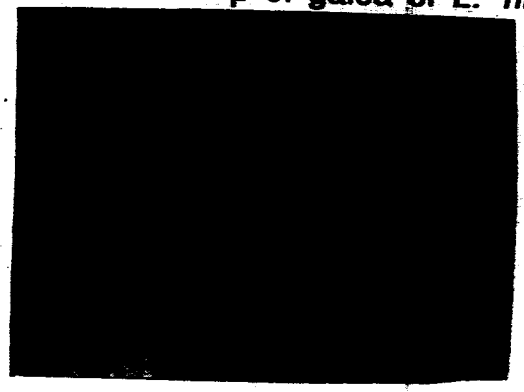
5 µm

Figure III.3 Galea of *L. texana*.



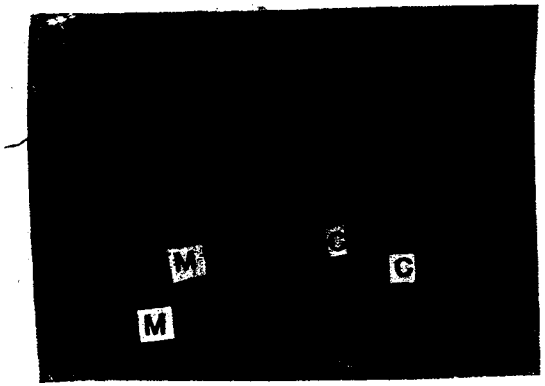
100 µm

Figure III.4 Tip of galea of *L. haldemani*.



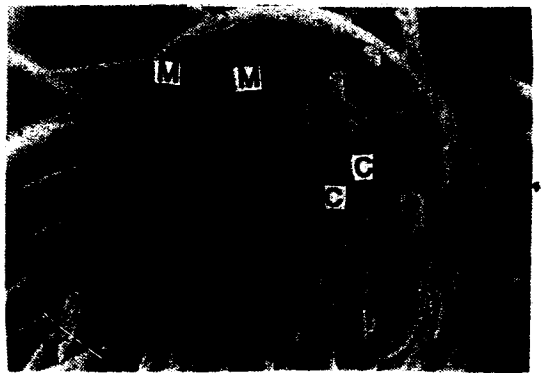
50 µm

Figure III.5 Tip of galea of *L. haldemani*.
C= uniporous chemosensilla
M= mechanosensilla



25 µm

Figure III.6 Tip of galea of *L. texana*.
C= uniporous chemosensilla
M= mechanosensilla



50 µm

Figure III.7 Pore of chemosensillum of *L. haldemani*.

2 µm

IV. Responses of galeal gustatory sensilla to plant saps.

The galeal sensilla of adult *Leptinotarsa* beetles are well placed to contact plant saps during maceration of leaves and are assumed to be important in responses to feeding stimuli. Galeal sensilla are easily accessible for electrophysiological recordings using the method described in Sutcliffe and Mitchell (1982). The abundance and distribution of chemosensilla on the galeae do not vary substantially for *Leptinotarsa decemlineata, L. haldemani* and *L. texana* (Chapter Three) although each species differs in feeding preferences (Chapter Two). Choice of homologous sensilla is relatively simple so that variable electrophysiological responses of chemoreceptors may be related to different host plant preferences.

Insect feeding behaviour is regulated by specific messages from chemosensory cells associated with the antennae and mouthparts (Frazier, 1986). Three main theories have been proposed for peripheral nervous system (PNS) input to the central nervous system (CNS). The labelled line theory states that PNS response to a single compound may elicit a behavioural response. The direct relation of a single compound with a behavioural response has been noted for sucrose and proboscis extension as well as sodium chloride and retraction or inhibition of extension for the blowfly (Dethier, 1976a). An absolute labelled line may also exist for sinigrin provoking ovipostion in *Pieris brassicae* (Schoonhoven, 1967). Accumulated evidence does not allow such a simple model to explain the complex and dynamic interactions of most insect species with their environment. Across-fibre patterning has been proposed by Dethier (1973) as a means of providing information on qualitative differences in acceptability of host plants. Different sensory

patterns are produced across dendrites in a single sensillum. Various patterns elict behavioural responses according to this theory which has proven useful in explaining responses of *Danaus plexippus* larvae, *Manduca sexta* larvae and *Phormia regina* adults to leaf saps (Dethier, 1980). The third theory is an amalgamation of the first two theories. Sign stimuli perceived by labelled lines may be a part of the pattern of sensory input necessary for feeding behaviour. The role of these labelled lines in discriminating among plants may be relatively more or less important depending on the population of insects investigated or on the physiological states of a particular individual.

Flea beetles of the genus *Phyllotreta* are stimulated to feed on non-host leaves treated with glucosinolates, however Nielsen *et al.* (1979a) could find no correlation between plant acceptability and stimulatory activity due to isolated glucosinolate mixtures. In a subsequent study, Nielsen *et al.* (1979b) determined that flavonol glycosides in combination with sinigrin are more effective than sinigrin alone. They suggest that a combination of glucosinolate and specific flavonol glycosides could be a major cue in recognition of plant species for the *Phyllotreta* species studied. Labelled lines for sinigrin may exist in this instance, however sinigrin alone is not sufficient to explain behavioural specificity. Modification of the response to sinigrin by flavonol glycosides could be explained by interactions on a single dendrite or by across-fibre patterning more nearly matching a template for acceptability.

The sensory basis of host plant discrimination for *Leptinotarsa* beetles has been extensively debated. The role of attractants, feeding stimulants, feeding deterrents and integration of complex stimulus patterns as means of plant perception by the beetles have been reviewed by Mitchell (1988).

For *Leptinotarsa* species, four types of plant chemicals are held responsible for initiation and regulation of feeding responses. These are sign stimulants (host specific chemicals), feeding stimulants (sugars, amino acids, phospholipids), feeding cofactors (potassium and other inorganic salts) and deterrents (alkaloids) (Hsiao, 1974). Contact chemoreceptors on the galea of *L. decemlineata* respond to, or are affected by, amino acids, sugars and glycoalkaloids (Mitchell and Harrison, 1984, 1985). Galeal contact chemoreceptors of *L. haldemani* and *L. texana* also respond to amino acids and sugars (Haley, unpublished data).

Leptinotarsa species are considered sensitive to repellent and deterrent chemicals which are often assumed to be secondary plant chemicals. Within the genus *Solanum*, steroidal glycoalkaloids are claimed to have a decisive role in regulating feeding behaviour and host selection. Since the mid-1940's, glycoalkaloids have been implicated as feeding deterrents for *L. decemlineata* (see Bongers (1970) for an extensive list of references to early papers). Glycoalkaloids are thought of as token stimuli whose presence or absence influences the susceptibility or immunity of a plant to attack by *Leptinotarsa* species.

Harrison (1985) failed to demonstrate different patterns of initial host acceptance for *L. decemlineata* when steroidal glycoalkaloids were tested in behavioural assays using whole leaves. Likewise, tropane alkaloids are unlikely to restrict host range of *L. decemlineata* since plants containing tropane alkaloids may be recognised on the basis of olfaction before plant fluids containing tropane alkaloids are sampled (Harrison, 1985). Harrison concludes that alkaloids are not responsible for determining different patterns of initial host acceptance. He states that the primary constraint on host

acceptability is determined by the beetles' sensory system which functions independently of secondary plant compounds.

Mitchell and Harrison (1985) reviewed the relationship of alkaloids and their effect on the chemosensory system of *Leptinotarsa decemlineata*. Their reinvestigation of the hypothesis that *Solanum* glycoalkaloids are feeding deterrents and provide differential acceptability of host plants did not uphold the hypothesis of alkaloids as sign stimuli. They conclude that there are no specific receptors for these compounds in larval galeae, adult galeae or adult tarsal chemoreceptors and that the action of glycoalkaloids is non-specific. Responses of sensory cells support the idea that total glycoalkaloid levels are partly responsible for differences in host plant acceptance. Galeal sensilla do not appear capable of differentiating among the three alkaloids tested by Mitchell and Harrison (1985).

Compounds may interact with one another to produce several types of summed responses. Theoretically, responses of single cells may be affected through synergisms and suppressions. Inhibition of a cell responding to sucrose has been demonstrated for three alkaloids (Mitchell and Sutcliffe, 1984). Furthermore, responses of cells are not necessarily independent. Linkage of dendrites within a single sensillum is possible through tight junctions (Zackaruk, 1980).

The complex environment in which sensilla evolved must be considered when discussing host plant preferences among insect species. Leaf saps provide stimuli for the *Leptinotarsa* species in this study. Differences in sensory patterns could reflect differences among species as well as between *Solanum* and non-*Solanum* plants at the level of the PNS.

Methods and Materials

Leptinotarsa beetles used in this study were field collected and maintained in culture for no longer than one year (approximately 8 generations). The *L. decemlineata* culture was established using individuals from the Edmonton area. The *L. haldemani* culture originated from the Pena Blanca area of Arizona. *L. texana* originated from Hidalgo county, Texas. All cultures were maintained at a photoperiod of 16L:8D at 25° C +/- 1°C under full spectrum fluorescent lights. Each culture was provided every second day with flasks containing freshly cut leaves of suitable host plants. *L. decemlineata* culture was allowed free access to leaves of *Solanum tuberosum* var. Norland. *L. haldemani* was presented with leaves of *Lycopersicon esculentum* var. Earliana, *S. dulcamara* and *S. tuberosum* var. Norland. *L. texana* was allowed access to *S. elaeagnifolium*.

Newly emerged adults were collected twice daily and set aside in plastic petri dishes. Beetles were individually identified for the behavioural assays described in Chapter Two. After the behavioural assays, beetles were placed in petri dishes containing damp Kimwipes. Each petri dish contained one beetle and its assigned identification number. Beetles remained in the culture room for a further 24 hours with no access to plant leaves. A random subsample of individuals for which behavioural data were available was chosen each morning for electrophysiological testing.

Preparation of beetles for electrophysiological recording is described in Sutcliffe and Mitchell (1982) and Mitchell and Harrison (1984). The recording method is similar to the method described by Sutcliffe and Mitchell (1982) and Mitchell and Harrison (1984) with the following changes. The signal from the stimulating electrode was amplified

with a George Johnston clamping preamplier and displayed on a dual beam Tektronix 5112 oscilloscope with 5A22N differential amplifiers. Filters for less than 0.1 kHz and greater than 1 kHz were in the electrical circuit before the signals were recorded. The potentials were recorded on magnetic tape after encoding in FM with a Vetter 2D FM recording adapter. A TEAC 22-4 multitrack recorder was used.

Stimuli tested were saps from *Solanum dulcamara*, *S. elaeagnifolium*, *S. tuberosum* var. Norland and *Lycopersicon esculentum* var. Earliana, as well as 100 mM KCl. Leaf saps were prepared by grinding freshly cut leaves in liquid nitrogen with a mortar and pestel. 20.0 g frozen leaf powder was mixed with 40 ml cold 100 mM KCl. The resultant slurry was centrifuged at 2000 g's for 5 minutes. Liquid leaf sap was placed in 1.5 ml Eppendorf tubes which were immediately frozen in liquid nitrogen and stored on dry ice. Chlorophyll content of leaf homogenate was determined using the method of Bruinsma (1963). Spectrophotometric measures were taken at three absorbances: 645, 652 and 663, using an HP 8451A spectrophotometer. Milligrams per litre chlorophyll a and chlorophyll b were determined as well as chlorophyll a+b. If differences exceeding 5% were found between the two determinations, saps were rejected as containing excessive breakdown products. Saps were made at two week intervals so that no saps used had been stored for longer than two weeks.

100 mM KCl was used to dilute plant saps to reduce plugging of the tip of the micropipet. *L. decemlineata* rarely responds to this concentration of KCl. *L. haldemani* and *L. texana* are slightly more responsive at this concentration so that all individuals were tested for response to KCl. Individuals which responded vigorously to KCl were rejected.

Electrophysiological recording took place between the third and seventh hours of the photophase. This corresponds to the hours for which behavioural results are available. Response to saps was recorded for a total of 15 seconds. Sap from an acceptable host plant was used to select a sensillum with a good signal to noise ratio. *S. tuberosum* was used for *L. decemlineata*, *S. dulcamara* for *L. haldemani* and *S. elaeagnifolium* for *L. texana*. The next stimulus was 100 mM KCl. If an excessive response was found to KCl, the sensillum was rejected. The next two plant saps were selected randomly with the exception of *L. esculentum*. *L. esculentum* was always the second to last stimulus. Bursting responses of cells to *L. esculentum* sap were often noted several seconds after the stimulus onset. Since this bursting pattern has been described as indicative of cell damage, the first sap of the series was re-tested. A disadaptation period of 3 minutes was allowed between *Solanum* saps. After *L. esculentum*, a 5 minute disadaptation period was allowed.

Action potentials were separated using height as a parameter. Data were digitised using the method of Mitchell and McIntyre (1986), modified for use on a Zenith 286 computer equipped with a Metrabyte DASH-16 A/D card. Digital records of responses were visually inspected and action potentials manually selected. Spike heights and time of occurrence were criteria for rejecting spikes as probable superpositions. Spikes representing single action potentials were measured for height from peak to trough and the resulting heights were plotted as frequency histograms with 25 possible classes, referred to as bins. Each bin contains the mean number of spikes for each height category for all individuals. Standard error bars are shown for each bin.

The middle 500 millliseconds of the first second of response were analysed for 18 *L. decemlineata*, 18 *L. haldemani* and

23 *L. texana* for each of 4 plant saps. Also, the entire second between the third and fifth seconds was analysed for 9 *L. decemlineata*, 12 *L. haldemani* and 11 *L. texana*

The time of occurrence of bursting patterns is also described for all plant saps. Bursting patterns are identified by repeated, vigorous firing of one or more cells.

The acceptability ranking for each sap is based on the rankings assigned in Chapter Two, Figure II.4.

Results

Figure IV.1 shows electrophysiological records for randomly selected individuals of the three beetle species in response to each of four plant saps. The sensillum representing the beetle species is the same for all four plant saps. Responses to plant saps are different for the three beetle species. Of the three beetle species, the response of *L. decemlineata* is the least complex for all *Solanum* species; a single cell producing a large spike dominates the response. No single pattern emerges for acceptable or less acceptable host plants for all three beetle species.

Figures IV.2 through IV.4 describe responses of four randomly chosen individuals to each of the four plant saps. The order of presentation of the traces indicates the same individual beetle: the first trace for each sap is from the first beetle; the second trace for each sap is from the second beetle and *et seq.* *L. decemlineata* (Figure IV.2) is predictable in its responses to *Solanum* saps, however, responses of the individuals vary greatly for *L. esculentum*. A cell producing large spikes is easily identifiable on the basis of spike height and regularity of firing for all *Solanum* saps. Figure IV.3 indicates a similar trend for *L. texana*

although the complexity of response cannot be predicted as precisely as with *L. decemlineata*. The cell producing large spikes can be identified for most traces. Responses of *L. haldemani* (Figure IV.4) are extremely complex. Identification of the cell producing large spikes is not always possible for all saps.

Figure IV.5 shows four histograms describing responses of 18 *L. decemlineata* to the four plant saps. The responses to the three *Solanum* saps are similar while the response to *L. esculentum* is more complex. A cell producing a large spike is predominant in the response to *S. tuberosum*, although a cell producing a smaller spike contributes to the response. Responses to *S. dulcamara* show an increased contribution of this second cell. The histogram for *S. elaeagnifolium* is simliar to the histogram representing responses to *S. dulcamara*: two cells firing, with the cell producing a large spike firing more often than the cell producing a smaller spike, Responses to *L. esculentum* do not permit determination of the number of cells firing.

Histograms in Figure IV.6 summarize responses of *L. texana* to four plant saps. Responses to *Solanum* saps are qualitatively similar for *L. texana* while responses to *L. esculentum* are more complex. Two or possibly three cells are involved in the response to *Solanum* saps. The cell producing the largest spike fires more frequently than the other cells. One or more cells producing smaller spikes contribute to the response to *Solanum* saps. Response to *L. esculentum* does not allow determination of the number of cells firing. Histograms for *L. texana* are remarkably similar to histograms generated by averaging responses of *L. decemlineata*.

Responses of *L. haldemani* to plant saps are shown in Figure IV.7. All four histograms are complex when compared with those for the other *Leptinotarsa* species. A minimum of three cells is involved in the responses to all four plant saps. Responses to *L. esculentum* are similar to responses to *S. elaeagnifolium* and responses to *S. tuberosum* and *S. dulcamara* are similar to each other based on these histograms.

Relating histograms describing responses of *L. decemlineata* to behavioural ranking of host plants indicates a regular arrangement of acceptability of host plant and least complexity of response. This arrangement cannot be generalised to the other two *Leptinotarsa* species. For *L. texana*, the response to its preferred host plant, *S. elaeagnifolium*, appears more complex than the response to *S. dulcamara* or *S. tuberosum*. Responses of *L. texana* to *S. elaeagnifolium* are not the same as responses of *L. decemlineata* to *S. elaeagnifolium* nor *S. tuberosum*, the preferred host plant for *L. decemlineata*. Although *Solanum* saps may be distinguished from responses to *L. esculentum* for both of these beetle species, no single response of these two *Leptinotarsa* species indicates a behaviourally acceptable or unacceptable host plant. Electrophysiological responses of *L. haldemani* do not correspond to responses by either *L. decemlineata* or *L. texana*. Responses of *L. haldemani* cannot be related to acceptablity of plants for feeding.

Figure IV.8 shows selected responses to the four saps for an individual of each beetle species during the fourth second of response. The entire second of response is shown. Fewer superpositions of spikes are seen during the fourth second and identification of individual cells based on time interval between firing is less difficult than during the first second

response when cells are firing more rapidly. Histograms of fourth second data are shown in Figures IV.9 through IV.11. The patterns that emerged during the first second are still present during the fourth second. Histograms describing responses to *L. esculentum* are equally complex during the first and fourth seconds with the fourth second response showing a much reduced total height for *L. esculentum* when compared to the *Solanum* saps. The response to *L. esculentum* appears to diminish more quickly during the fourth second than the responses to the *Solanum* saps.

Responses of each beetle species to *L. esculentum* are distinguished from responses of these beetle species to the three *Solanum* saps by bursting patterns. Bursting patterns were only seen for *L. esculentum* for all beetle species with the exception of one individual of *L. texana* which showed bursting patterns for *S. dulcamara* as well as *L. esculentum*. Number of individuals showing bursting patterns for *L. esculentum* and time of occurrence of bursting patterns are given in Table IV.1. *L. texana* had significantly more individuals showing the bursting patterns than either of the other two species. *L. decemlineata* and *L. haldemani* had approximately equal numbers of individuals demonstrating bursting patterns. Although no significant differences were noted, a general trend exists showing *L. haldemani* has the greatest time interval before bursting patterns were seen.

Discussion

Specific messages based on number of cells firing in a single galeal gustatory sensillum are sufficient to distinguish *Solanum* saps from *L. esculentum* sap for *L. decemlineata* and *L. texana*. Distinct patterns distinguishing preferred from less preferred *Solanum* species are not evident. For *L. texana* and *L. decemlineata*, the peripheral gustatory

system may have the major role in discriminating among solanaceous and non-solanaceous plant species.

Messages using number of cells firing in a galeal gustatory sensillum are not sufficient to distinguish among these saps for *L. haldemani*. Sensilla on other mouthparts, antennae or tarsi may be crucial for discrimination of plant saps by *L. haldemani*.

Mitchell *et al.* (*in press*) demonstrated that the response of a single galeal contact-chemoreceptor to potato sap is uniform and has a single cell predominating for *L. decemlineata*. Responses have low variability among sensilla tested as well as low variability with multiple hits on a single sensillum. Mitchell and McCashin suggest that firing of the cell producing the large spike may be an important part of the code signalling an acceptable host plant. For these three *Leptinotarsa* species, the total number of cells firing as well as the variability across sensilla could provide a total sensory firing pattern which correlates with host plant preference.

Behavioural correlations for host plant acceptability and electrophysiological responses of *L. decemlineata* are possible, however, this trend cannot be generalised to the other *Leptinotarsa* species. Responses of *L. texana* provide an interesting comparison since, electrophysiologically, distinctions between *Solanum* and *Lycopersicon* species are clear. *L. texana* provides an especially clear behavioural plant-insect relationship and yet electrophysiological records cannot be used to predict the behavioural patterns. In addition to clear behavioural differentiation of plants, *L. texana* is also distinguished by its high signal to noise ratio compared to *L. decemlineata* and *L. haldemani*. *L. texana*

would be a good species to chose for further work on neural coding.

A comparison of the responses of beetles during the first and fourth seconds indicates that the pattern established during the first second is still seen during the fourth second. It is uncertain if beetles are using information obtained early in the response, later in the response or perhaps a contrast between early patterns and later patterns. The fourth second response shows a slower firing rate of the cells when compared to the first second. Adaptation rates have not been established for any of the cells; each cell could have the same or a different exponential decay rate. Until decay rates have been established, the fourth and first second data cannot be adequately compared. Adaptation rates of cells compared within an across-fibre pattern could contain necessary information for plant discrimination and should be established as a response parameter. The most important information necessary for this analysis is a certain identification of cells in a trace. Only the cell producing the largest spike height can be accurately identified for *L. decemlineata*.

Responses of *L. haldemani* do not allow behaviour to be correlated with electrophysiological responses as measured by spike height. The use of saps of natural host plants, for example *Solanum douglasii, Physalis* and *Lycium* species, may allow clearer relationships between electrophysiological responses and behavioural responses to emerge. The clear relationship evidenced by *L. decemlineata* is unlikely since the relationships of *L. decemlineata* and *Solanum* species exist even when the biotype of *L. decemlineata* has never been presented with leaves of *S. elaeagnifolium*. A further investigation of *L. haldemani* should include studies on circadian rhythm of feeding activity. *L. haldemani* in Texas

may be nocturnal feeders (Dickinson, *pers comm*). If *L. haldemani* is truly a nocturnal feeder, patterns of electrophysiological responses may emerge during the scotophase that differ from patterns generated during the photophase. *L. haldemani* may make its host plant choices during the scotophase and remain on the selected host plant during the photophase without acute sensory discrimination of host plants.

The significance of bursts of impulses (bursting) has been reviewed by Dethier (1976b). Several instances of bursting being considered a pathological condition are discussed as well as a report of bursting being a regular phenomen for grasshopppers (Haskell and Schoonhoven, 1969). Behavioural significance of bursting has been demonstrated by (McCutchan, 1969) who relates bursting patterns to behavioural aversion. Bursting patterns correlate with behavioural acceptance of *L. esculentum*. *L. texana* had the greatest number of individuals demonstrating bursting patterns as well as the shortest time before bursting patterns were evidenced. *L. esculentum* is an unacceptable host plant for *L. texana* based on behavioural assays and larval nutrition. No significant differences were noted between *L. decemlineata* and *L. haldemani* with regard to bursting patterns, however *L. haldemani* tended to have the longest time interval before bursting patterns. *L. esculentum* is behaviourally accepted by *L. haldemani* and is only marginally acceptable to *L. decemlineata*. Further evidence that bursting patterns are associated with a damage response is the much lengthened recovery time for the sensillum when bursting patterns were seen.

Antifeedants, substances that prevent or inhibit feeding, may disrupt normal behaviour by creating sensory input that fails to stimulate feeding or by causing a sensory input

other behaviours. Antifeedants identified for *L. decemlineata* include inorganic salts (Jermy, 1961), tannins (Pospisil, 1982, Drummond and Casagrande, 1985), fungicides (Hare *et al.*, 1983) and the organophosphorus miticide, dicofol (Walgenbach and Wyman, 1987). The presence or absence of a single receptor for each of these compounds could be investigated by description of responses based on a dose-response curve. Examination of saps from leaves treated with each of these antifeedants would provide interesting electrophysiological results. If responses of *L. decemlineata* become less complicated than responses to saps from untreated leaves, blocking of receptors might be a valuable avenue for investigation. If responses to treated leaves become more complicated than responses to untreated leaves, the patterning of responses as indicated by the across-fibre patterning theory would be upheld. The simple pattern of a single cell predominating with lesser contributions from another cell would be the pattern for an acceptable host plant. A more complicated pattern would indicate a less acceptable host plant. Investigations using treated leaves are of particular interest since the effects of the mixture of compounds would be the same except for the effect of the compound under investigation. Synergism, suppression and other means of affecting mixtures could be scrutinised.

Ma (1972) and Blom (1978) illustrated the importance of considering combined input from several sense organs when discussing behaviour. No correlation existed for sucrose and feeding behaviour however when responses from all sensilla were added together, a linear input/output relation emerged. These *Leptinotarsa* beetles provide an especially useful model system for describing reponses for summed inputs. Electrophysiological recordings are accomplished on live beetles so that a single preparation easily lasts for an entire day. Sufficient time for recording of

numerous contact sensilla on mouthparts and tarsi is available. Input from antennal sensilla would also be of interest since behavioural observations indicate that the beetles touch leaves with their antennae before sampling and during gustatory sampling maintain their antennae over the broken leaf surface.

de Boer and Hanson (1987), in a study of host and non-host plants for *Manduca sexta*, concluded that sensory organs necessary for host plant discrimination vary with the plant species tested. Both olfactory and gustatory stimuli are needed for normal behavioural acceptance of *Lycopersicon esculentum*, the host plant of *M. sextd.* Either olfactory or gustatory organs are sufficient to distinguish *Brassica napus* from the normal host plant. Gustation alone is sufficient to manifest complete rejection behaviour for *Canna generalis*. Roles of chemosensory organs could differ among these *Leptinotarsa* species. Contributions of olfactory and gustatory organs could result in comprehensive input of differing ratios for the three species. For example, gustatory sensilla may have a minor role for *L. haldemani* in production of sensory codes for plant species. Alternatively, *L. decemlineata* may rely heavily on gustatory input.

Taxonomic relationships of *Yponomeuta* moths are reflected in gustatory sensitivities to selected compounds (van Drongelen and Povel, 1980). Most *Yponomeuta* species can be identified on the basis of their chemosensory patterns (van Drongelen, 1979). A further study of these *Leptinotarsa* species including sensitivities to single compounds and characterization of response spectra for each cell would provide a further refinement on PNS capabilities of these species.

Variability at the level of the PNS with conservative changes in CNS integration have thus far been assumed. This

assumption is still untested. Variability in PNS responses of these insects is remarkable and may represent an enormous source for selection to act upon. Conversely, until CNS integration of signals is elucidated, it may be argued that PNS variability may be a trivial reflection of even greater variability in CNS capabilities.

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Table [illegible] bursting patterns for Lycopersicon esculentum sap for Leptinotarsa decemlineata, L. haldemani and L. texana.

	# bursting/ (% bursting)	mins to bursting mean seconds ± se
L. decemlineata	4/18 (22%)	[illegible] ± 3.1
L. haldemani	3/18 (17%)	12.5 ± 1.8
L. texana	16/23 (70%)	9.3 ± 1.1

Figure IV.1 Selected traces representing first second responses of *L. decemlineata*, *L. haldemani* and *L. texana* to *Solanum dulcamara*, *S. elaeagnifolium*, *S. tuberosum* and *Lycopersicon esculentum*.

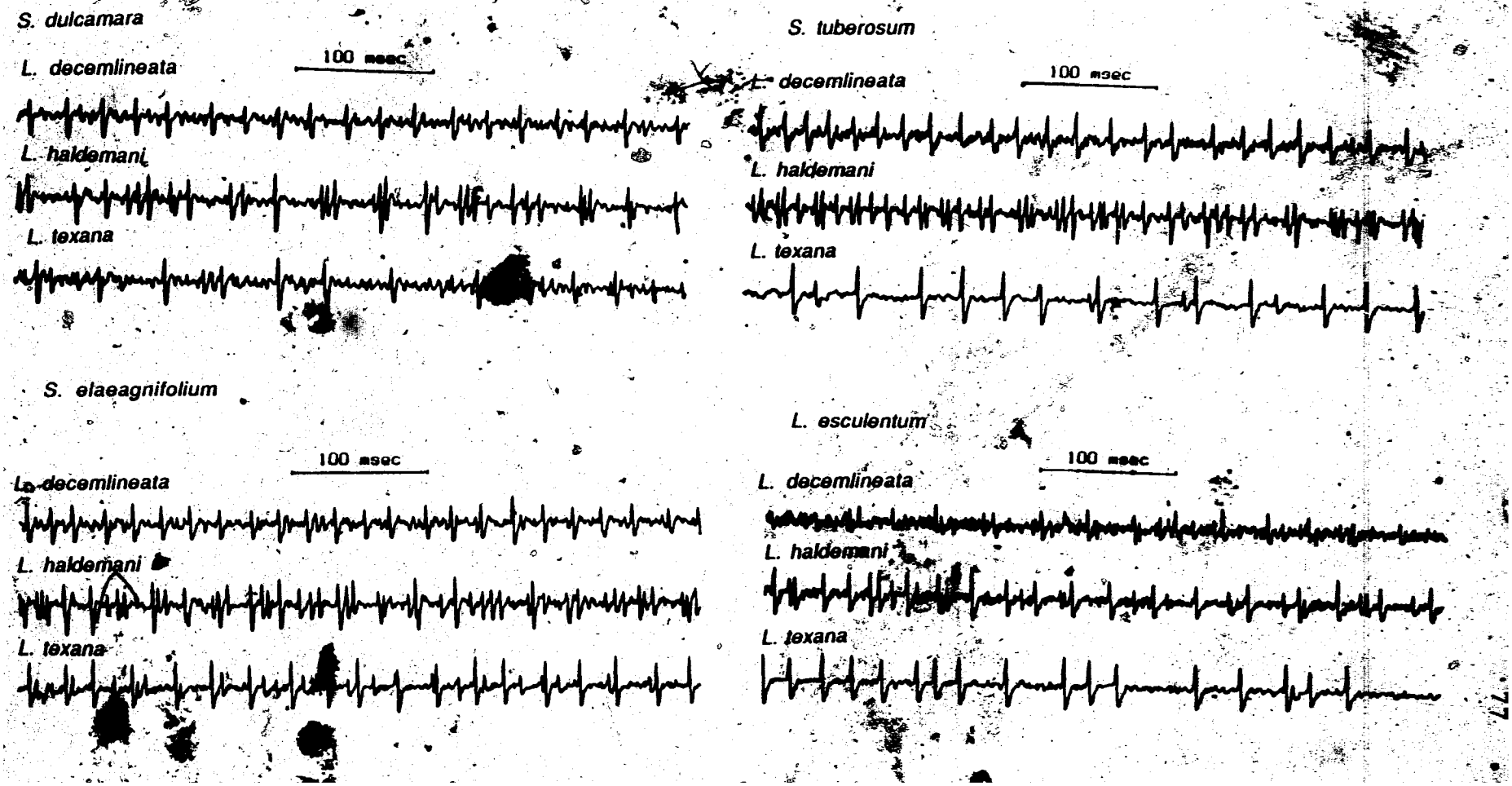


Figure IV.2 Selected traces representing responses of 4 randomly chosen individuals of *L. decemlineata* to *Solanum dulcamara, S. elaeagnifolium, S. tuberosum* and *Lycopersicon esculentum.*

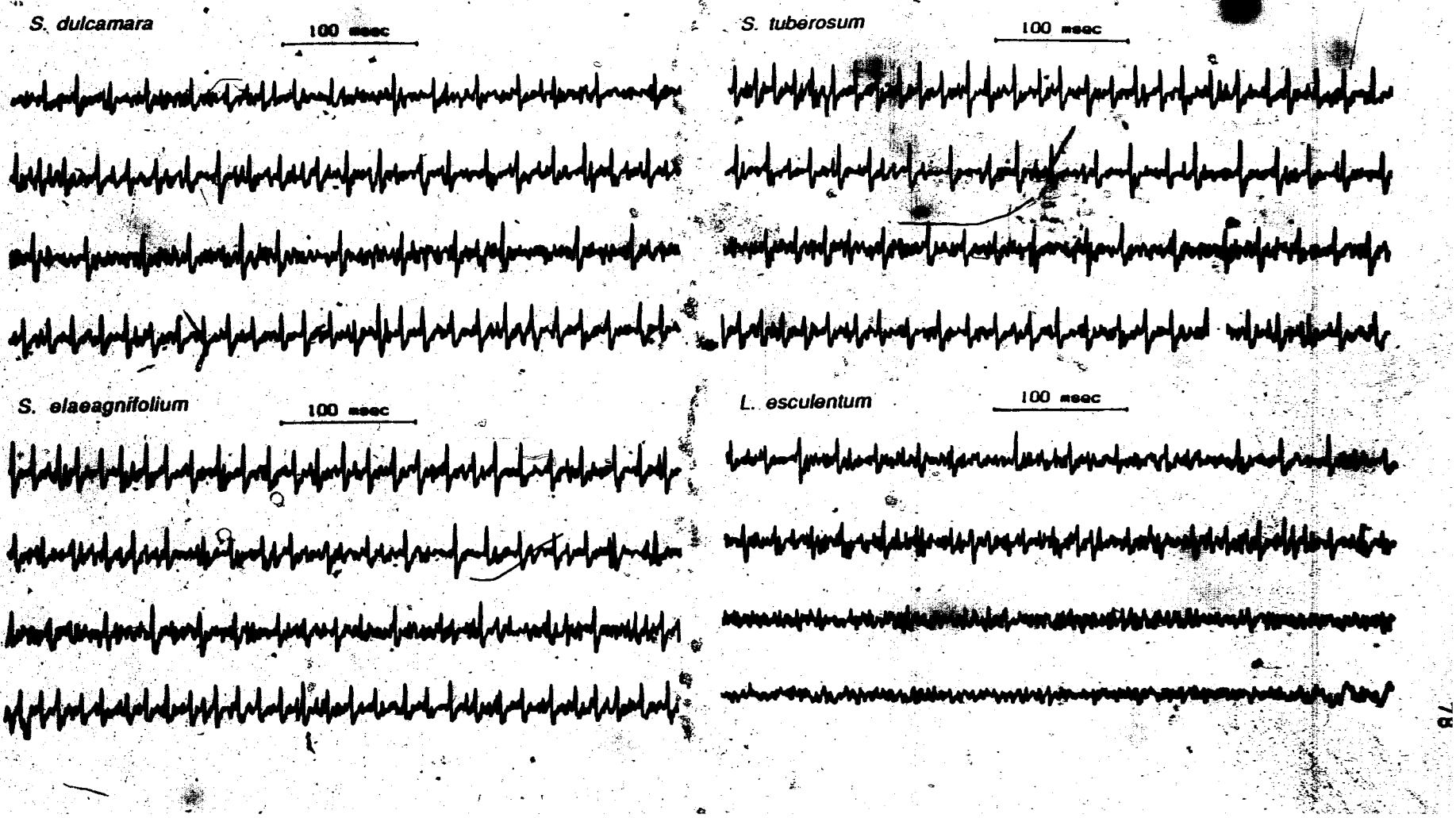


Figure IV.3 Selected traces representing responses of 4 randomly chosen individuals of *L. texana* to *Solanum dulcamara*, *S. elaeagnifolium*, *S. tuberosum* and *Lycopersicon esculentum*.

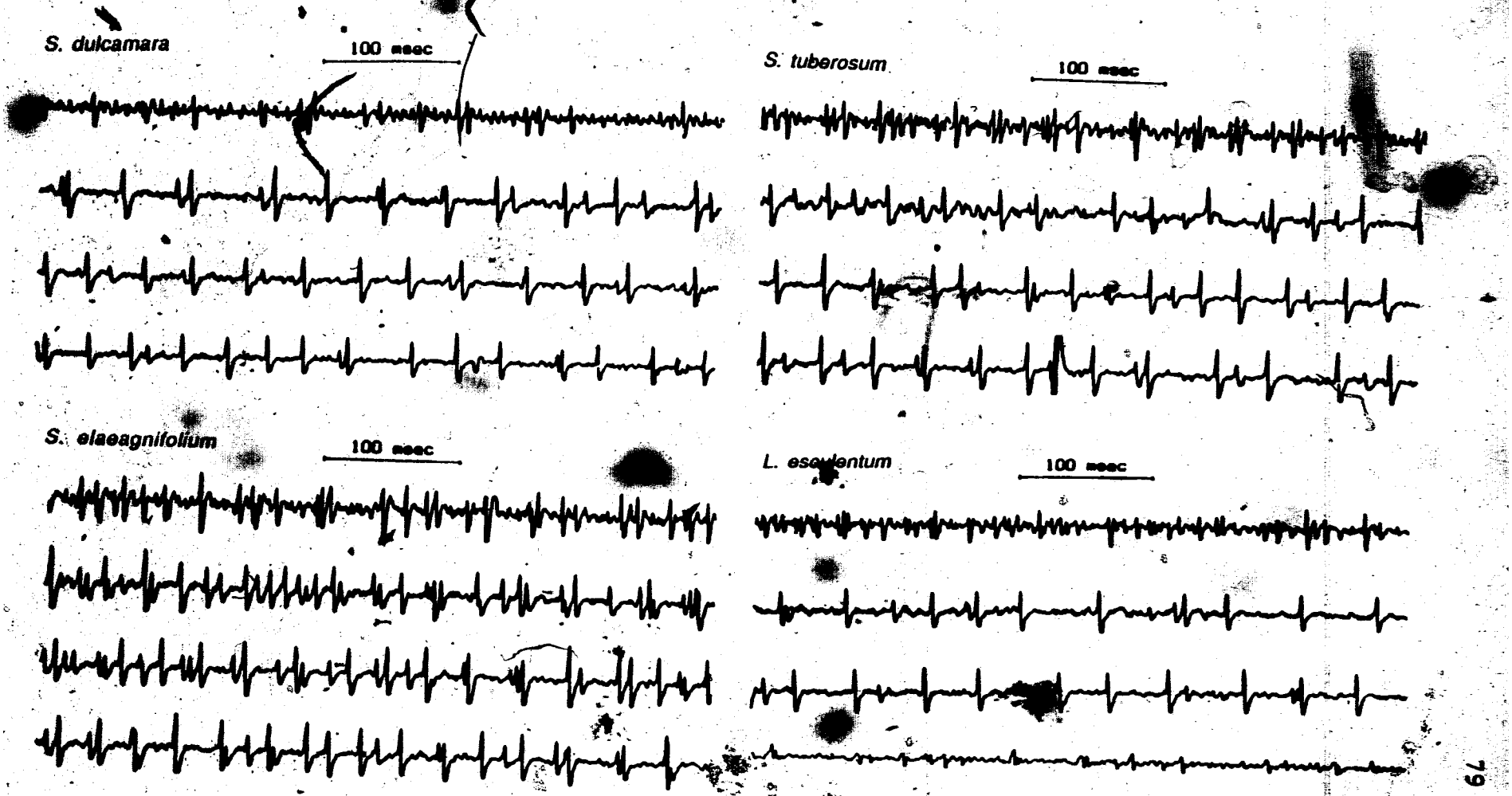


Figure IV.4 Selected traces representing responses of 4 randomly chosen individuals of *L. haldemani* to *Solanum dulcamara*, *S. elaeagnifolium*, *S. tuberosum* and *Lycopersicon esculentum*.

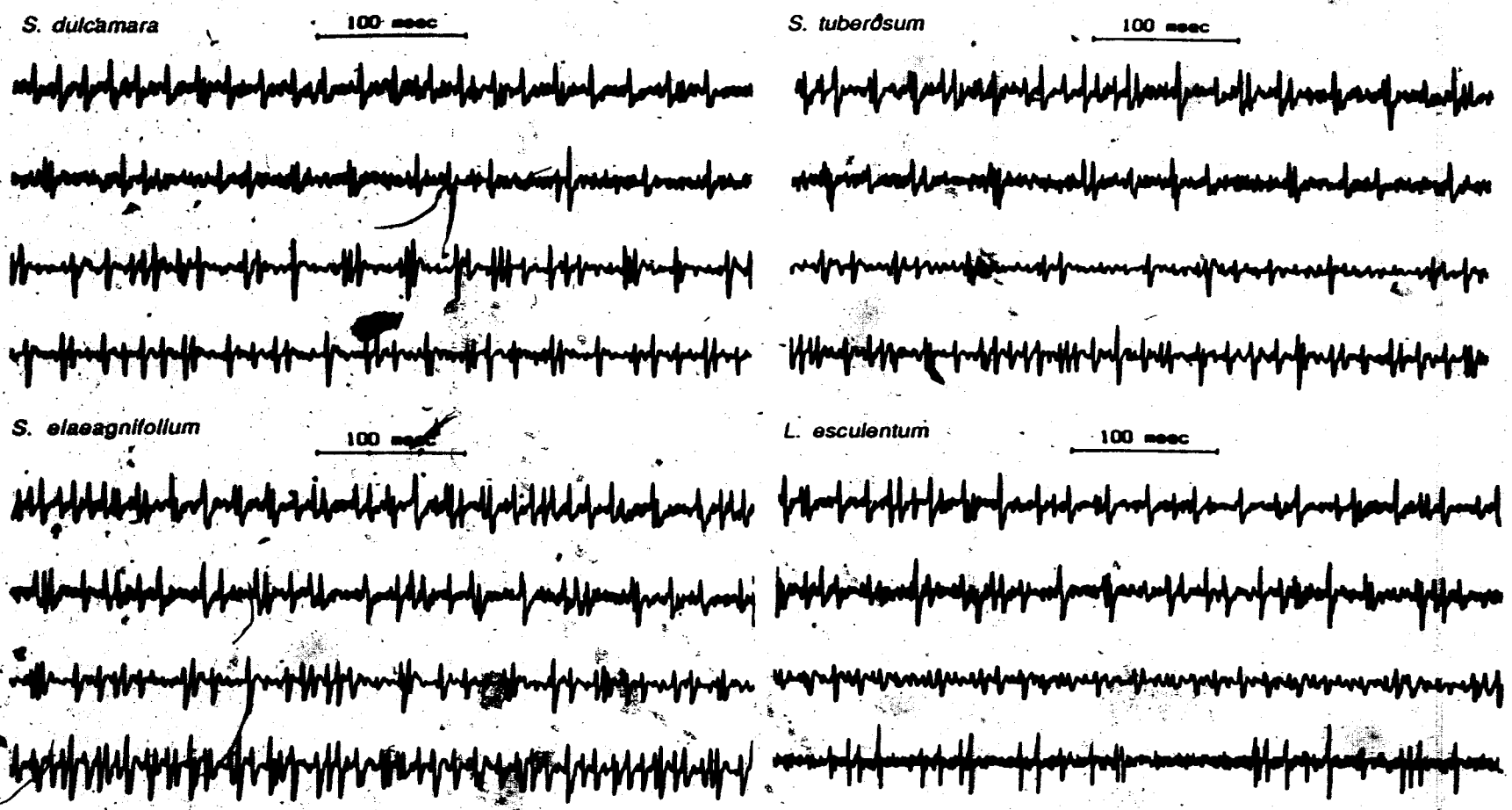
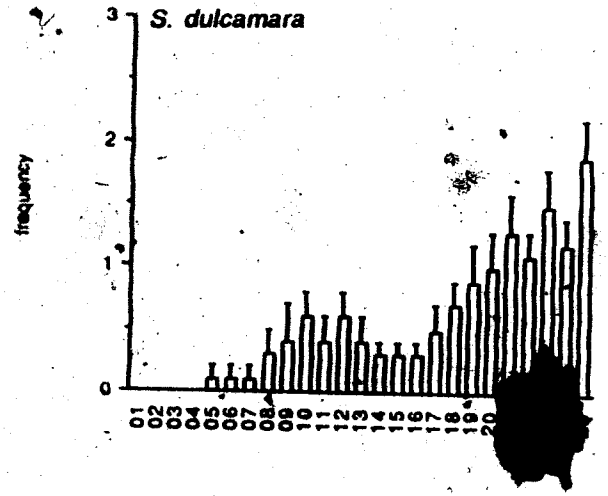
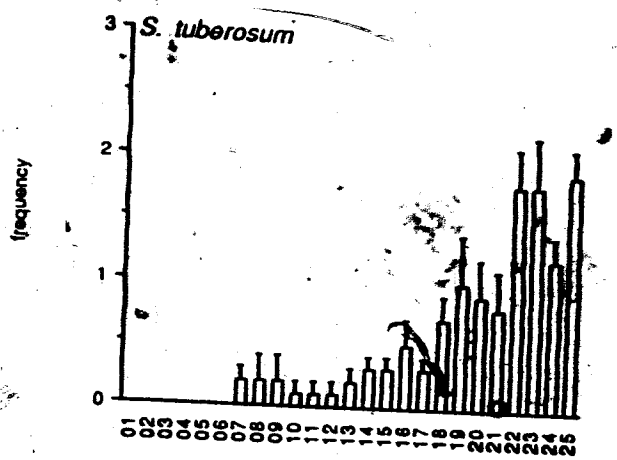
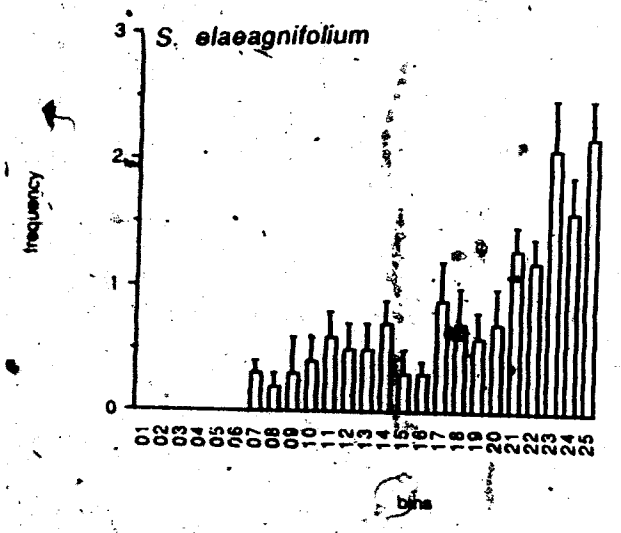


Figure IV.5 Histograms representing first second responses of 18 *L. decemlineata* to *Solanum dulcamara*, *S. elaeagnifolium*, *S. tuberosum* and *Lycopersicon esculentum*.







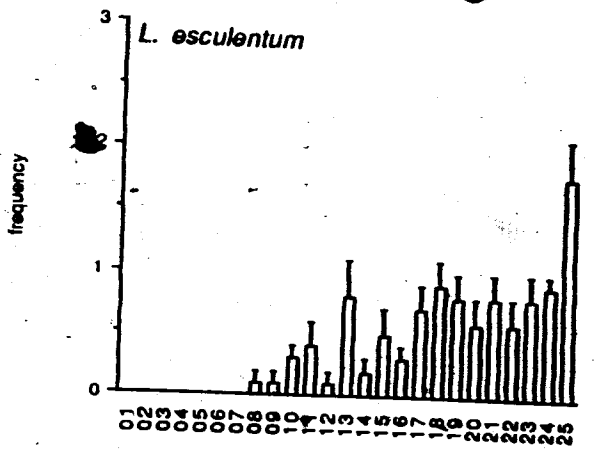


Figure IV.6 Histograms representing first second responses of 23 *L. texana* to *Solanum dulcamara, S. elaeagnifolium, S. tuberosum* and *Lycopersicon esculentum.*

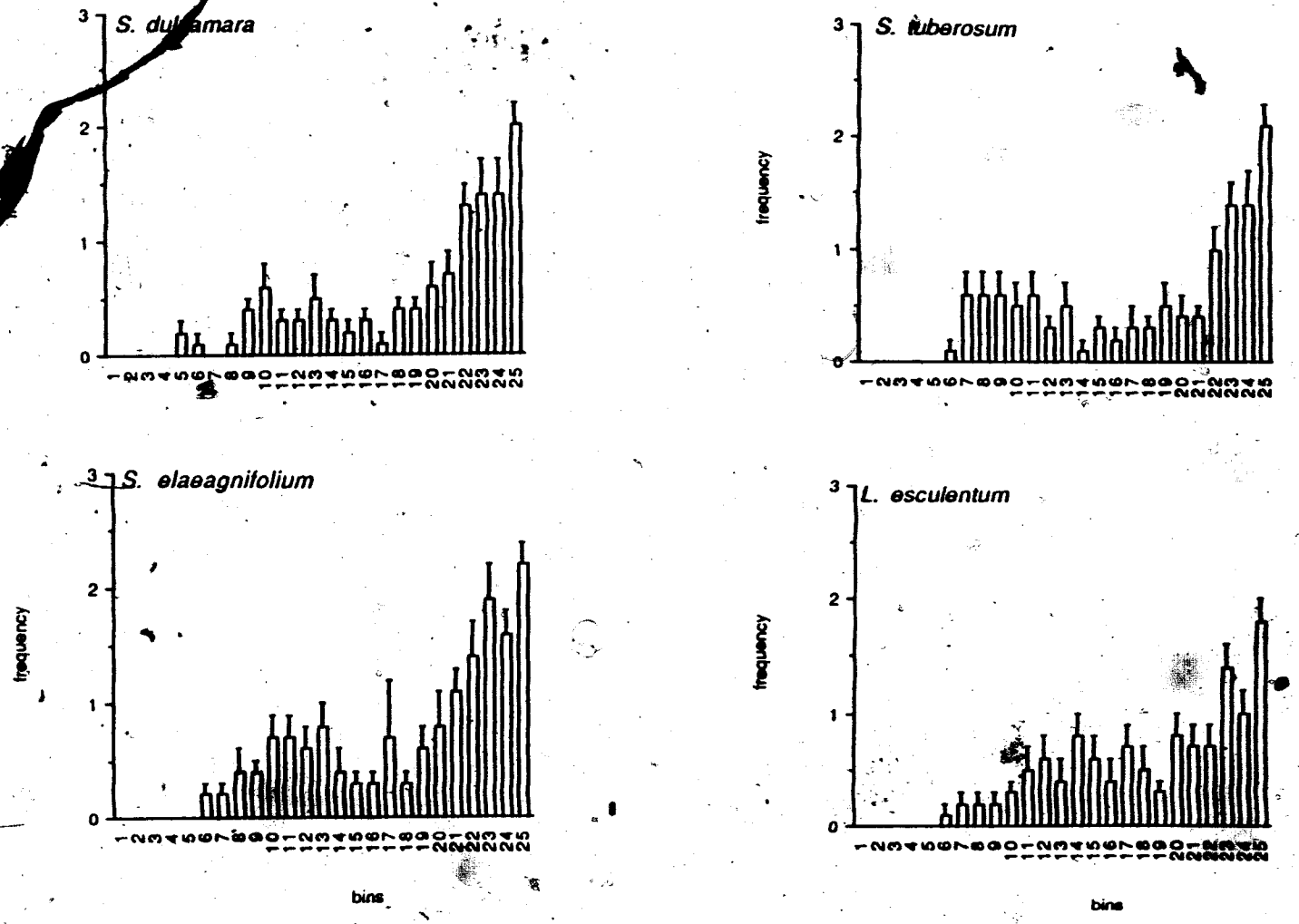


Figure IV.7 Histograms representing first second responses of 18 *L. haldemani* to *Solanum dulcamara, S. elaeagnifolium, S. tuberosum* and *Lycopersicon esculentum.*

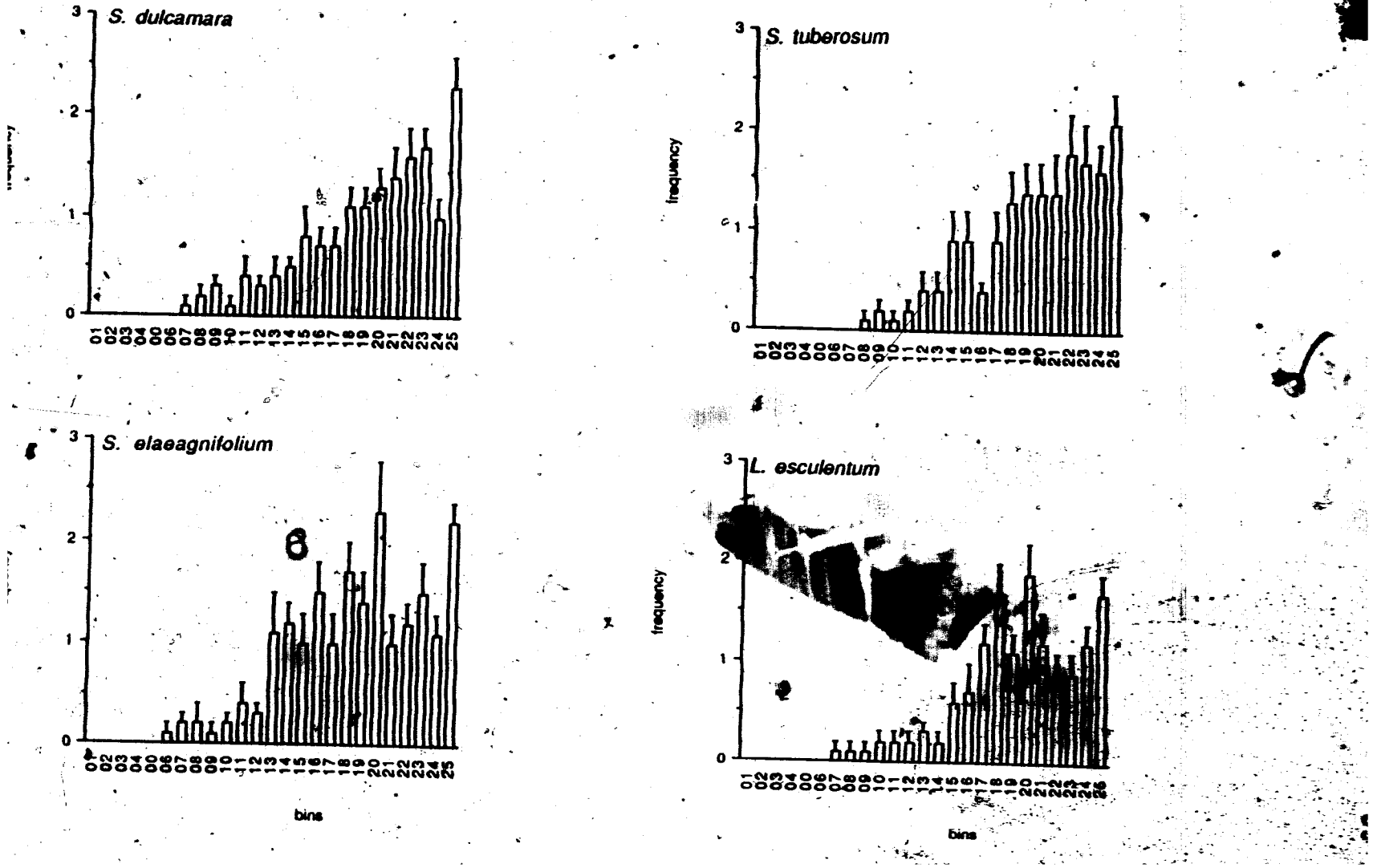


Figure IV.8 Selected traces representing fourth second responses of *L. decemlineata, L. haldemani* and *L. texana* to *Solanum dulcamara, S. elaeagnifolium, S. tuberosum* and *Lycopersicon esculentum.*

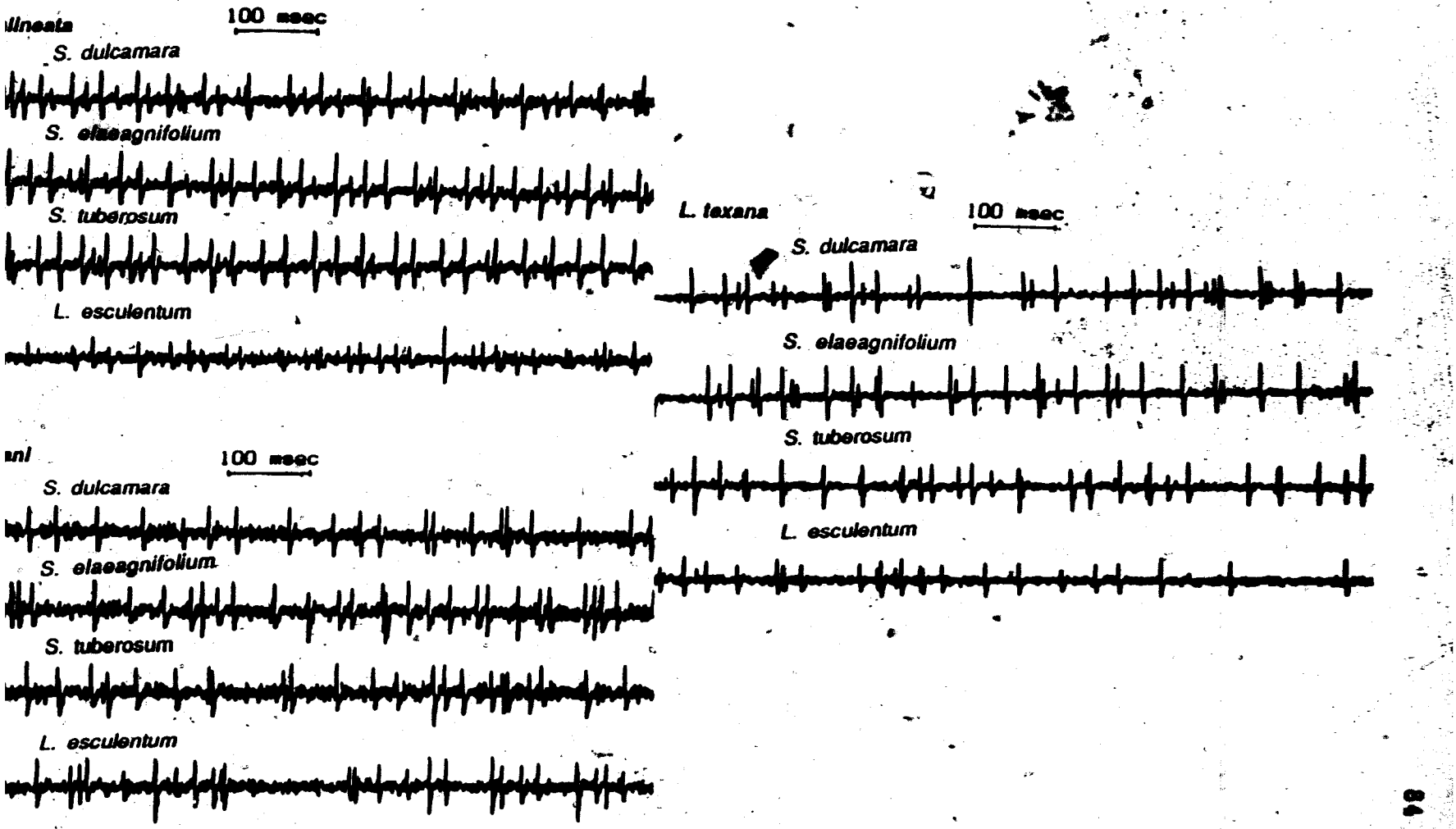
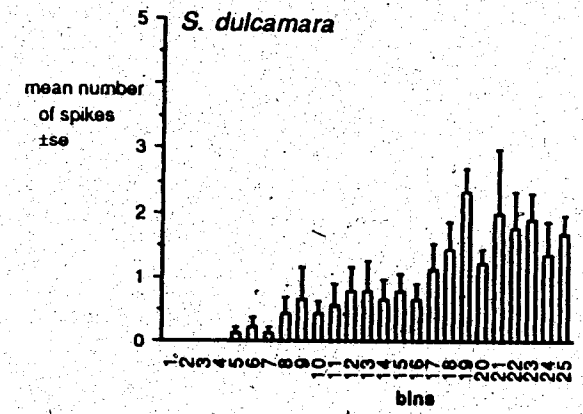
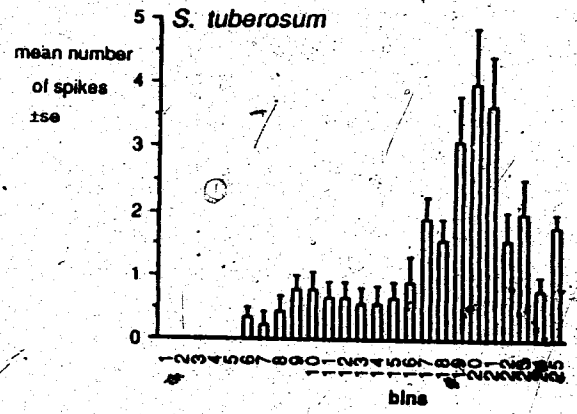
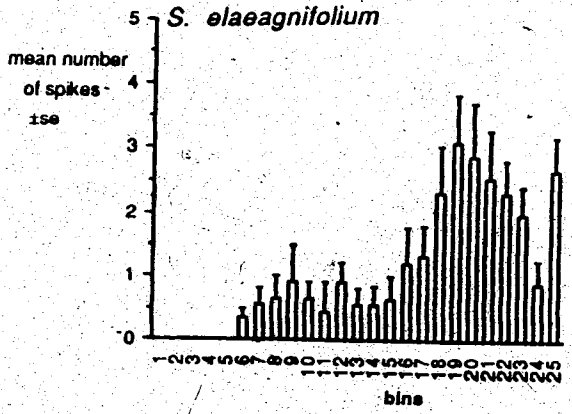


Figure IV.9 Histograms representing fourth second responses of 9 *L. decemlineata* to *Solanum dulcamara*, *S. elaeagnifolium*, *S. tuberosum* and *Lycopersicon esculentum*.







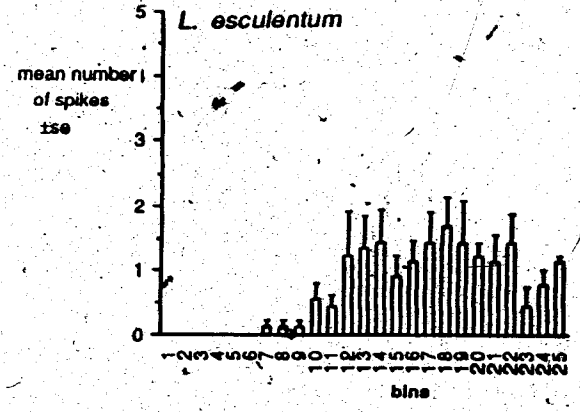
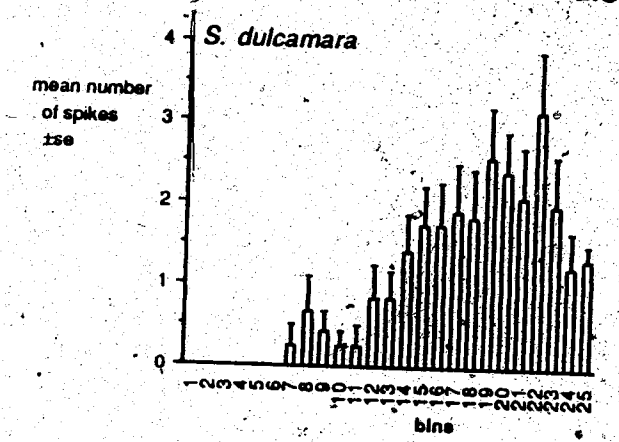
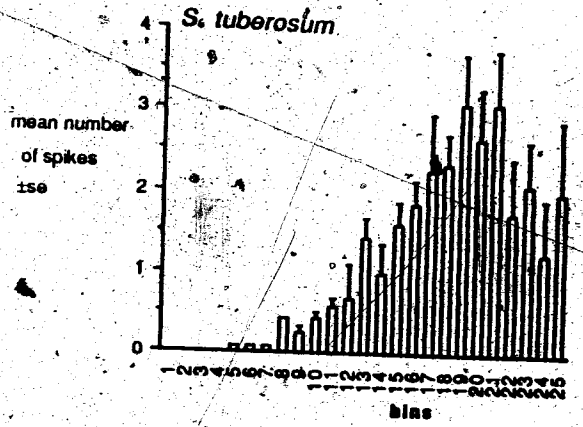
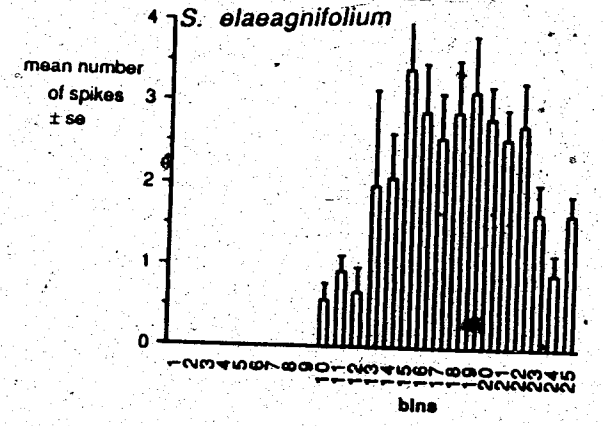


Figure IV.10 Histograms representing first second responses of 12 *L. haldemani* to *Solanum dulcamara*, *S. elaeagnifolium*, *S. tuberosum* and *Lycopersicon esculentum*.







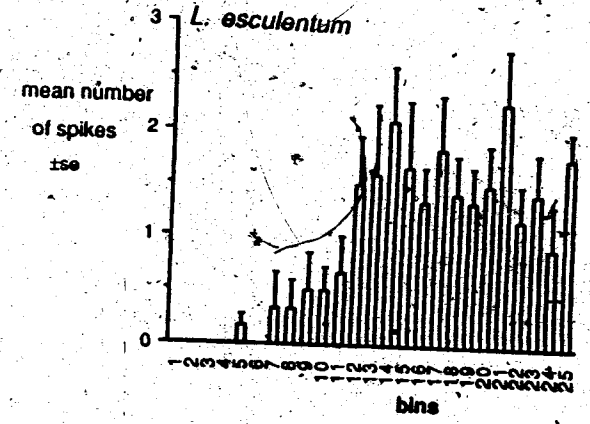
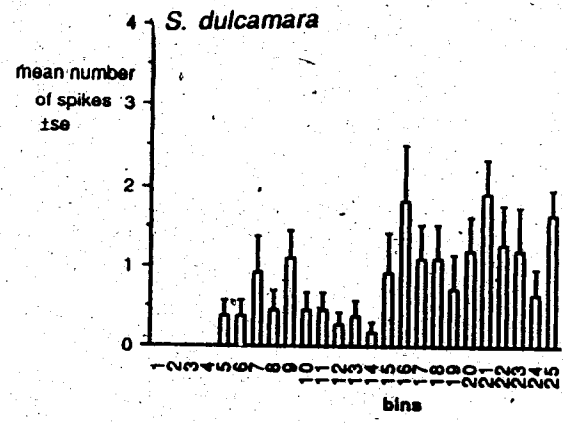
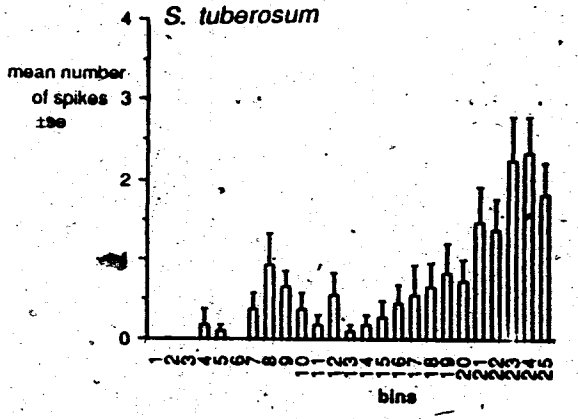
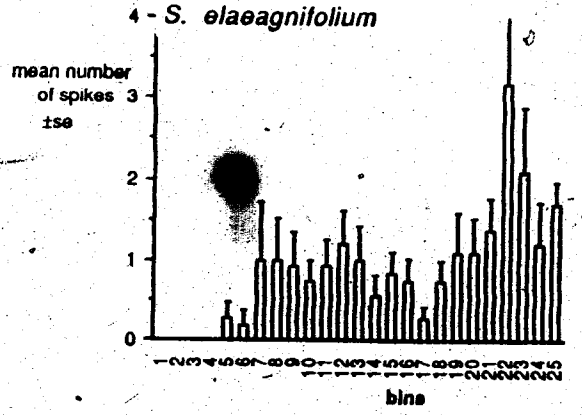
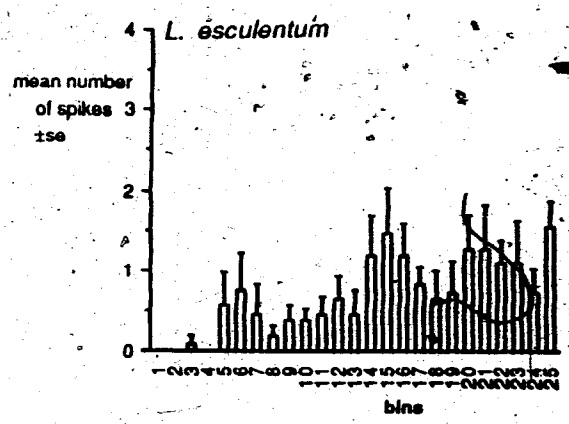


Figure IV.11 Histograms representing fourth second responses of 11 *L. texana* to *Solanum dulcamara, S. elaeagnifolium, S. tuberosum* and *Lycopersicon esculentum.*









V. Reconstruction of phylogenetic relationships among 5 *Leptinotarsa* species.

Species comparisons should include a phylogenetic context since interpretation of complex comparative results is most likely to be meaningful when the contribution of evolutionary history is taken into account. The phylogeny proposed in this chapter is intended as an aid for interpretation of present characteristics of *Leptinotarsa* species. Present properties of an organism are, in part, a product of the organism's phylogenetic past. These properties may or may not have emerged as adaptations to past conditions. However these properties are not necessarily explicable by reference to present environmental conditions.

The taxonomy of the genus *Leptinotarsa* Stål was most recently revised by Jacques (1972, 1988). Jacques described 32 species based on morphological characters, including genitalia. No phylogeny for *Leptinotarsa* species is suggested in his work. Using species descriptions provided by Jacques, only a few morphological characters allow comparisons, since character sets described are not always complete for each of the species. Furthermore, no polarization of characters into ancestral or derived states is suggested. The absence of a phylogeny for *Leptinotarsa* species and the inherent difficulties of comparing characters available in the literature provided the impetus for construction of this abbreviated phylogeny.

Ting Hsiao of Utah State University is preparing a phylogeny of many of the *Leptinotarsa* species found in Mexico and North America (*pers comm*). The phylogeny proposed in this chapter is intended as an interim hypothesis until Hsaio publishes his more complete phylogeny.

The genus *Leptinotarsa* is a member of the tribe Doryphorini of the family Chrysomelidae. The tribe Doryphorini consists of four genera: *Calligrapha*, *Chrysolina*, *Labidomera* and *Leptinotarsa* (Arnett, 1963). The genus *Leptinotarsa* is considered to be most closely related to the genus *Labidomera* (Jacques, 1972, 1988). All species of the genus *Leptinotarsa* are phytophagous as larvae and adults. Of the 32 species described by Jacques (1988), host plant records are available for 14 species. Of these fourteen species, 10 species feed on Solanaceae, 2 species on Compositae and 2 species on Zygophyllaceae.

Tower (1906) attempted to describe 'natural' groupings of the genus *Leptinotarsa*. The usefulness of these groupings is questionable since their derivation is unclear. It would appear that the groupings are based on elytral colour patterns. The seven groups described by Tower (1906) are as follows: flavopustulata group, haldemani group (including *L. haldemani*), lacerata group, rubiginosa group (including *L. rubiginosa*), lineata group (including *L. decemlineata*), dilecta group (including *L. lineolata*) and zetterstedti group. Tower (1918) concentrates on the lineata group and includes both *L. decemlineata* and *L. texana* in this group. If these 'natural' groups may be considered to reflect a common evolutionary history, *L. decemlineata* and *L. texana* are more closely related to each other than to the other species considered in this thesis. Their relationship to the other species is uncertain since no indication of relationships among groups is provided.

Tower (1906) described southern Mexico as the centre of origin for *Leptinotarsa*. This was based on greatest specific differentiation, greatest abundance of individuals, location of closely related forms and of lines of dispersal. Tower also suggested polyphagy and least dependence on a restricted

habitat are derived, not ancestral characteristics. The Mexican origin for *Leptinotarsa* species has recently been questioned (Neck, 1983). Whether *Leptinotarsa* species originated in Mexico (Tower, 1906) or the southern United States (Neck, 1983) is not of critical importance to this study. Distributions of these species do however suggest that the populations sampled are at the northern limits of species ranges.

Data on the distibution of *L. decemlineata*, *L. haldemani* and *L. texana* are found in Table V.1. Distributions of these three *Leptinotarsa* species suggest that they are genetically isolated. *L. decemlineata* and *L. texana* are sympatric in Texas where both feed on *Solanum elaeagnifolium*. *L. decemlineata* is also found on other *Solanum* species in Texas (Neck, 1983). Hybrids of *L. texana* and *L. decemlineata* are not reported (Brown, 1961; Neck, 1983; Tower, 1918). *L. haldemani* and *L. decemlineata* are sympatric in the Benson area of Arizona. In the Benson area, *L. decemlineata* is found primarily on *S. elaeagnifolium* while *L. haldemani* is found primarily on *Lycium* species (*pers obs*). Hybrids of these two species have not been recorded.

There are four possible schemes for relating these three *Leptinotarsa* species: all three species may be equally distantly related, *L. decemlineata* and *L. texana* may be more closely related, *L. decemlineata* and *L. haldemani* may be more closely related or *L. texana* and *L. haldemani* may be more closely related . Elytral colour patterns suggest that *L. decemlineata* (striped) and *L. texana* (striped) are more similar to each other than either is to *L. haldemani* (unicoloured). Based on degree of polyphagy, one might hypothesize that *L. decemlineata* (oligophagous) and *L. haldemani* (oligophagous) are more similar to one another than to *L. texana* (monophagous). However, these characters

may simply reflect ancestral states; relationships are still open to question.

Chromosome analysis of 13 *Leptinotarsa* species and 2 *Labidomera* species provides information which is potentially useful for establishing relationships among these species (Hsiao and Hsiao, 1983). Chromosome numbers, karyotypes and chiasma frequencies are compared with host plant utilisation and geographic distribution. Hsiao and Hsiao did not use these data to establish phylogenetic relationships.

A complete morphometric study of these three species is outside the scope of this thesis. An alternative strategy for determining relationships is the use of electrophoretic characters as the basis for comparison. Although genetic relationships do not necessarily imply phylogenetic relationships, phenograms based on overall similarity of electrophoretic characters may be used to estimate phylogenetic trees (Ferguson, 1980; Richardson *et al.*, 1986). A major advantage in using electrophoretic characters for otherwise morphologically distinct species is that the extent of genetic divergence may be quantified; the use of morphometric characters results in more subjective indices of extent of divergence. Coupled with its use in estimation of phylogenetic relationships, electrophoretic data also discloses information on heterozygosity. The ecological significance of heterozygosity is discussed.

Electrophoretic characters were available for 5 *Leptinotarsa* species: *L. decemlineata*, *L. haldemani*, *L. lineolata*, *L. rubiginosa* and *L. texana*. UPGMA phenograms and Wagner trees based on electrophoretic data are presented. A

cladogram is included, based on published information and independent of electrophoretic data.

Methods and Materials

Adult *Leptinotarsa* were collected in late August of 1986 and 1987. Distribution of each species, number of specimens collected from each locality, host plant upon which they were found and years collected are listed in Table V.1. Voucher specimens from [redacted] have been deposited in the Strickland Museum at [redacted] iversity of [redacted]

Adult beetles were collected live and kept in culture on host plants for a minimum of 7 days before electrophoresis. This ensured that all individuals were at least a week old before preparation for electrophoresis. Thoracic tissue was obtained by first removing elytra and wings, cutting off the abdomen and then the head. Abdomens were retained for later dissection to determine sex of the individual. Thoraces were split before homogenation for ease of handling. Homogenizing buffer contained 300 mg polyvinylpyrolidine, 10 mg dithiothreitol, 7 ml distilled water and 1 ml phosphate buffer pH 6.7. 600 ul of homogenizing buffer was used for each individual in 3 aliquots. Homogenate not used immediately was frozen and stored at -20 °C.

Homogenate was electrophoresed in 9 % or 11% polyacrylamide gels, Tris-HCl pH 8.9, under conditions described in Rolseth and Gooding (1978) and Sperling (1987). For fresh homogenate, 30 ul was used. When homogenate had been frozen and then thawed before use, 60 ul was injected into each slot in the stacking gel.

Staining of gels used standard modifications of Shaw and Prasad (1970), Brewer (1970) or Richardson *et al.* (1986). Filter paper overlays were used instead of agar. Overlays

were dipped into staining solutions and then squeezed firmly between 2 layers of paper towel to remove excess staining solution.

Individuals of each of the 5 species gave interpretable bands at 18 loci. Enzyme Commission (EC) numbers are from the Nomenclature Committee of the International Union of Biochemistry, 1984. The data used for phylogenetic analysis and estimates of heterozygosity were from the following enzymes: adenylate kinase (AK, EC 2.7.4.3), arginine phosphokinase (APK, EC 2.7.3.3), ethanol dehydrogenase (ETOH, EC 1.1.1.1), fucose dehyrogenase (FUDH, EC 1.1.1.122), fumarate hydratase (FUM, EC 4.2.1.2), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), gylcerol dehyrogenase (GLYDH, EC 1.1.1.72), hexokinase (HK, EC 2.7.1.1), hydroxybutyrate dehydrogenase (HBDH, EC 1.1.1.30), isobutanol dehydrogenase (IBDH, EC 1.1.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), isopropyl dehydrogenase (IPDH, EC 1.1.1.80), malic enzyme (ME, EC 1.1.1.40), octanol dehdrogenase (ODH, EC 1.1.1.73) using 1-octanol, phosphoglucose isomerase (PGI, EC 5.3.1.9), sorbitol dehydrogenase (SODH, EC 1.1.1.14), xanthine oxidase (XO, EC 1.2.3.2). Two loci were scored for hexokinase (HK.1, HK.2). Banding patterns suggest that 4 loci produce monomers (AK, APK, HK.1, HK.2), one locus produced a tetramer (ME), two loci produced undetermined multimers (SODH, XO) and the rest produced dimers.

Enzymes assayed which did not yield interpretable bands include aldolase, aldehyde oxidase using heptaldehyde as well as benzaldehyde, glucose oxidase, alpha-glycerophoshate dehydrogenase, glucose-6-phoshate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, octanol dehydrogenase using 3-octanol, phosphoglucomutase, succinate dehydrogenase, superoxide

diaphorase and esterases. Esterases displayed distinct bands for at least 3 loci. Homologies for esterases could not be established among species so that esterases could not be used for constructing a phylogeny. Smeared banding patterns were the most common cause of difficulty in interpreting loci consistantly.

Electrophoretic data were analyzed using BIOSYS-1 (Swofford and Selander, 1981) to produce allele frequencies, tests for Hardy-Weinberg equilibrium, genetic distance measures, UPGMA phenograms and Wagner trees. T-tests and simple regression for heterozygosity measures were calculated using Statworks TM version 1.2 (© Cricket Software).

Chromosome comparisons for *L. decemlineata*, *L. haldemani*, *L. lineolata*, *L. rubiginosa*, *L. texana* and 2 *Labidomera* species were determined by examination of Figure 37 of Hsiao and Hsiao (1983). Homologous chromosome sets could not be determined for all chromosomes described. Chromosomes with secondary constrictions were assumed to be homologous. Total length in millimetres for chromosomes with secondary constrictions was used as a character. The presence of two chromosomes with secondary constrictions distinguished *L. decemlineata*. The secondarily constricted chromosome closest in length to the secondarily constricted chromosomes of the other species was chosen for the character state of *L. decemlineata*. X chromosomes were also assumed homologous. The total length of the X chromosome was used as another character.

Morphological data was based on descriptions found in Jacques (1972) for *Leptinotarsa* species. *Labidomera* species descriptions were taken from Blatchley (1910), Brown (1961), and Headstrom (1977). Table V.2 contains

descriptions of character states for each of the species. MacClade Version 2.1(© W.P. Maddison and D.R. Maddison) was used to determine tree length of cladograms based on published data. All possible combinations were tested and the most parsimonious cladogram selected.

Results

Allele frequencies were calculated for 5 *Leptinotarsa* species (Table V.3). Complete allelic substitutions among species were not found. The conditions of Hardy Weinberg equilibrium were not met in 35 loci of 180 tests performed using exact probability measures (alpha=0.05). Genetic variability measured by mean number of alleles per locus, percent polymorphic loci and mean heterozygosity (proportion of individuals sampled which are heterozygous (direct count) and Hardy-Weinberg expected) are contained in Table V.4.

Biased estimate of mean heterozygosity per locus (1-sum of chi^2 averaged over 18 loci) and number of food plants associated with each species or biotype are contained in Table V.5. Mean heterozygosity for populations with one host plant ($H\bar{x}$=0.5) and greater than one host plant ($H\bar{x}$=0.5) did not differ significantly using a T-test at alpha= 0.05. Simple regression of heterozygosity for populations with one host plant and greater than one host plant had a coefficient of determination of R^2=0.3 (n=10 populations).

Genetic similarity and distance measures were calculated for 13 measures of genetic similarity and distance. Nei's (1972) identity (I) and Nei's (1972) distance (D) are given in Table V.6. For Nei's I, values ranged from 0.878 (intraspecific comparisons) to 0.294 (interspecific comparisons).

UPGMA phenograms were calculated for 13 measures of genetic similarity and distance available in Biosys-1. Table V.7 groups the phenograms according to similarity in topology. UPGMA of Nei's (1972) genetic distance is shown in Figure V.1. Disregarding variation among populations of *L. lineolata*, 2 major topologies emerge. Topology one is the same as Nei's (1972) genetic distance. Topology two groups *L. haldemani* with *L. decemlineata* rather than *L. rubiginosa*.

Wagner trees were computed for all possible coefficients. The optimised Wagner tree of modified Rogers distances (Wright, 1978) is shown in Figure V.2.

Characters extracted from the literature for establishing an independent cladogram are shown in Table V.2. The states shared with *Labidomera clivicollis* or *L. suturella* were designated as ancestral states. Ancestral states were necessary to polarise characters. The most parsimonious cladogram for 5 *Leptinotarsa* species and a combination of 2 *Labidomera* species is shown in Figure V.3. This cladogram suggests that *L. decemlineata* is more closely related to *L. texana* than to *L. haldemani*.

Discussion

A. Hardy-Weinberg equilibrium

Hardy-Weinberg equilibrium expectations were not met for 35 loci over all populations (Table V.3). There are a number of possible methodological problems which could contribute to an apparent deviation from Hardy-Weinberg expectations, such as the presence of null alleles, samples composed of a few large groups of siblings, or heterogeneous distributions of alleles in space or time (Wahlund effect).

Efforts were made to detect these factors, all of which could have contributed to the excess of homozygotes that was found in some population samples in this study. Null alleles were probably not present at appreciable frequency, if at all, since there was no indication that the failure of some individuals to produce bands for a particular locus was due to anything other than degraded homogenate. It is possible that a number of individuals were siblings, since collections were made from relatively restricted areas. However, beetles were usually sampled at 2 to 3 beetles per plant, with plants separated by several meters. Use of individuals collected during 2 field seasons may also have contributed to the deviations from Hardy -Weinberg equlibrium. However, results were analysed for 1986 and for the pooled results of 1986 and 1987, and both analyses showed significant deviations from Hardy-Weinberg expectations. It is most likely that deviations from Hardy-Weinberg expectations are biological in origin rather than methodological. Methodological problems would tend to result in deviations in Hardy-Weinberg expectations at loci rather than among populations. Four loci are necessary to account for over half the deviations: ME (7 deviations), SODH (5 deviations), FUDH (4 deviations) and ODH (3 deviations). Over half the deviations are accounted for by only three populations: *L. decemlineata*, Edmonton (6 deviations), *L. lineolata*, Gardener Canyon (7 deviations), and *L. texana*, Hidalgo county (5 deviations) (Table V.3).

Explanations for real deviations from Hardy-Weinberg expectations could involve any factor causing non-random association of alleles, such as direct natural or sexual selection on alleles, hitchhiking of alleles with selected characters, or assortative mating. *L. decemlineata* is known to contain biotypes (Hare and Kenndy, 1986; Horton and Capinera, 1987; Horton *et al.*, 1988; Hsiao,1981). Detection of incipient host races for the other *Leptinotarsa* species would

be intriguing. In any case, the absence of Hardy-Weinberg equilibrium suggests that panmixia may not be the most appropriate population model.

B. Heterozygosity

Absolute heterozygosity and polymorphism measures for the *Leptinotarsa* samples (Tables V.4 and V.5) were consistently high, compared both to invertebrates in general (Richardson *et al.*,1986) and to other studies on *Leptinotarsa*. Jacobson and Hsiao (1983) showed a mean heterozygosity for *L. decemlineata* of 0.206, compared to the mean value of 0.402 for *L. decemlineata* found in this study.

However, Jacobson and Hsiao (1983) did propose an inherently high heterozygosity for *L. decemlineata*. Also, all populations sampled in my study were from the geographic extremes of species ranges. High levels of heterozygosity could be expected for *Leptinotarsa* populations at geographic extremes which are associated with extreme temperatures. For *Drosophila melanogaster*, alcohol dehyrogenase has a greater number of alleles present in environments with greater extremes in temperature (Pipkin *et al.*, 1973).

Part of the difference between Jacobson and Hsiao's and my study may also have been due to my use of polyacrylamide rather than starch gels. Starch gels are known to provide poorer resolution of allelic variants (Ferguson, 1980). Furthermore, samples used in this study were field-caught as adults. Jacobson and Hsiao's samples were obtained from lab colonies, the initial size of which was not specified. However, some of the biases introduced by my methods would have caused an apparent reduction in heterozygosity. Esterases were not scored in this study due to difficulties in

homologizing loci. Since esterases are highly polymorphic (Gilespie and Kojima, 1968), inclusion of esterase loci would probably have increased levels of heterozygosity found in this study. Also, overlapping alleles and smeared loci were not scored in this study. If techniques for homologizing loci and discriminating among alleles had been further refined, even greater heterozygosity may have been described.

I consider it likely that the high heterozygosity and polymorphism estimates obtained in this study are more easily explained by methodological biases than by an unusual biological situation. Since the initial choice of loci was intended to discriminate among species, I suspect the high percentages of polymorphic loci may be due to a biased choice of loci. Since absolute values of heterozgosity may have been inflated, comparisons will be restricted to relative variation in heterozygosity among populations within this study.

Jacobson and Hsiao (1983) provided estimates of relative variation in heterozygosity between *L. decemlineata* populations. They did not find significant differences in heterozygosity between different field populations, and also between field populations and a population which had been in culture for an estimated 150 generations. In the present study, heterozygosity measures were also found to be similar for all populations (Table V.5), even for the population of *L. decemlineata* in the Edmonton area, which is known to have undergone a rapid recent expansion into the area (circa 1925, see Hsiao, 1981). The only species that was significantly different was *L. texana*, which had a lower heterozygosity than the other species.

Heterozygosity and polymorphism within species may be expected to be influenced by environmental heterogeneity. If allelic variants reflect different enzyme function, greater

heterozygosity could provide selective advantages to individuals encountering variable habitats. Examples of individual fitness correlating with allozyme heterozygosity include reports by Koehn and Gaffney (1984), Turelli and Ginzberg (1983) and Zouros *et al.*, (1980). Heterozygous snails have been demonstrated to have lower routine metabolic costs than more homozygous individuals (Garton, 1984). Sturgeon and Mitton (1982) found that average heterozygosity was lower for mountain pine beetles, *Dendroctonus ponderosae*, from more restricted habitats than beetles from more variable habitats. However, examples abound showing no correlation or even a negative correlation of heterozygosity and fitness. Mukai *et al.* (1974) were unable to find any significant correlations in *Drosophila melanogaster*. Studies in rodent populations (Gaines *et al.*, 1978) demonstrated significant negative correlation between heterozygosity at 5 allozyme loci and survival and growth rate.

In the context of environmental heterogeneity, extent of heterozygosity may be related to degree of feeding specialization. Since monophagous insects presumably have a more restricted habitat than their more polyphagous relatives, heterozygosity could be expected to be higher for the more polyphagous populations. In the present study, regression of heterozygosity measures against degree of monophagy did not demonstrate higher levels of heterozygosity in the more polyphagous populations of *Leptinotarsa* species. However, one reason why these populations did not conform to the expected trend may have been that degree of feeding specializaton was not accurately assessed. Species considered polyphagous over their geographic range may be locally specialised (Pashley, 1988; Scriber, 1986). More polyphagous populations of *Leptinotarsa* may in reality be a collection of numerous specialized populations.

Since no correlation was found between levels of heterozygosity and relative degree of host plant specialization of species or populations, there is no evidence to support heterozygote advantage for different allelic forms of the enzymes studied. Instead, interpretation of high heterozygosity measures in the context of expected genotype frequencies suggests that heterozygosity may be a result of high allelic polymorphism. Expected values under Hardy-Weinberg equilibrium were higher than observed values for the heterozygote class for each population, with the exception of the Gleeson population of *L. lineolata* (Table V.3). The functional significance of high allelic polymorphism is unclear, though it may be related to the formation of biotypes. There is however, no evidence to support the idea that polyphagous species of *Leptinotarsa* have greater numbers of biotypes than monophagous species.

C. Phylogeny

The main aim of this phylogenetic reconstruction is to establish the relative evolutionary branching pattern for *L. decemlineata, L. haldemani* and *L. texana*. These species may be composed of several biotypes, as evidenced in the previous section on Hardy-Weinberg analyses. However, phylogenetic reconstruction using electrophoretic characters does not necessarily require Hardy-Weinberg equilibrium, especially when population level differences are substantially less than species level differences.

Phylogenetic reconstruction using phenetic analysis of electrophoretic data is used to provide insights into relationships among these *Leptinotarsa* species. Cladistic methods are an alternative approach to the phenetic analysis of these data. In some cases, cladistics is preferable

to phenetics since it allows an estimation of phylogeny regardless of whether events at speciation, such as genetic drift in small populations, result in an inaccurate representation of the ancestral gene pool. However, cladistic analysis of these data was not possible because of the high levels of polymorphism evidenced. A method often suggested for dealing with high levels of polymorphism in cladistic analyses is to perform analyses only on the basis of alleles present above a certain frequency, for example 5% (Mickevitch and Mitter, 1981). This method was not used for analysis of these frequency data. The major objection to the use of this method is that neglecting to count alleles which are present negates the power of the cladistic analysis.

The assumption used in phylogenetic reconstruction based on these data is that cladistic analyses are not the only acceptable way of deriving phylogenetic information. Overall similarity of *Leptinotarsa* species is used for interpreting electrophoretic results. Overall similarity is sometimes misleading for phylogenetic reconstructions since equal rates of evolution among lineages is an underlying assumption of phenetic analyses. Use of minimum length Wagner trees is a means of including unequal evolutionary rates in the construction of the tree. A cladistic analysis of morphological and chromosome characters available in the literature is presented as a further test of the phenetic reconstruction provided by the electrophoretic data.

Nei's (1972) genetic distance values are presented in Table V.6. Genetic distances range from 0.130 to 1.223. The genetic distance coefficient for *L. decemlineata* and *L. haldemani* averaged across the four populations is 0.413. Jacobson and Hsiao (1983) included *L. haldemani* in their study of population differences in *L. decemlineata*. Nei's (1972) genetic distance for *L. decemlineata* and *L. haldemani* was 0.439 (Jacobson and Hsiao,1983).

Nei's (1972) identity has frequently been used to compare species elecrophoretically. Thorpe (1982) compared numerous values of Nei's (1972) identity and found that 85% of identity values for congeneric species studied electrophoretically exceed 0.35. Identity values for all pairs of *Leptinotarsa* populations (Table V.6) exceed 0.35, with the exception of *L. lineolata*. Based on these values, further study of this genus should include the possibility of placing *L. lineolata* in a separate genus.

Comparisons within single species show that values for Nei's genetic identity exceed 0.85 for most cases (Thorpe, 1982). *L. lineolata* populations did not always exceed 0.85, however genetic identity values among populations always exceeded genetic identity values among species. The genetic identity value between Benson and Pena Blanca populations of *L. haldemani* (I=0.851) is similar to Patagonia and Gardener Canyon populations of *L. lineolata* (I=0.847). Similarity of values among populations regardless of species implies that populations of the same species were sampled (Table V.6). The only taxonomically dubious result indicated by these data involves *L. lineolata*. Sampling of *L. lineolata* populations and comparisons with other congeneric species would be informative for revision of this genus.

The unweighted pair group method of analysis (UPGMA) of Sneath and Sokal (1973) is commonly used to construct phenograms of electrophoretic data (Berlocher, 1984). UPGMA trees were calculated for 13 measures of genetic similarity and distance (Table V.7). Exact branch lengths of UPGMA trees do not necessarily indicate relative divergence times since constant average evolutionary rates among lineages cannot be assumed. The 13 UPGMA trees produced were compared on the basis of topology. Four

topologies resulted from the UPGMA trees. Grouping of similarity and distance coefficients on the basis of resulting topology of UPGMA trees is shown in Table V.7. The topology of group one coefficients is illustrated in Figure V.1. Nei's (1972) genetic distance was chosen for illustration since this coefficient is standard in the literature. Group two topologies differ from group one topologies only in the relationships among *L. lineolata* populations. The exact relationships among *L. lineolata* populations is of minor concern for the construction of this phenogram so that groups one and two may be grouped together and described as topology one. Differences between group three and four are also in presentation of *L. lineolata* populations. These groups may be pooled to give topology two. Topology two differs from topology one in the placement of *L. haldemani*. Topology one links *L. haldemani* first with *L. rubiginosa* while topology two links *L. haldemani* first with *L. decemlineata*. Rogers (1986) compared nine genetic distance measures for their usefulness in construction of phylogentic trees. The genetic measures preferred by Rogers suggest that topology one (Figure V.1) is the most reliable representation of phenetic results.

The distance Wagner method of presenting electrophoretic data may be considered an approximation of phylogenetic relationships since it aims for a tree of minimum total length. Taxonomic units once joined are considered 'hypothetical taxonomic units'. Divergence of each branch is from the hypothetical ancestor. Farris (1981) states that the distance Wagner method is compatable with cladistic procedures, and is the best available clustering method for arriving at geneologies efficiently. Optimised Wagner trees are tested for minimum total length. Optimised trees are the average of all cycles computed and are especially good for indicating branch length (Swofford, 1981). According to Rogers (1986), coefficients especially useful for Wagner

trees are modified Cavalli-Svorza and Edwards (1967) arc and chord and modified Rogers distance (Wright, 1978). Modified Rogers (Wright, 1978) was chosen as the coefficient for computation of the Wagner tree since it was the only one of these three available in BIOSYS-1.

Interpopulation Roger's modified similarity coefficients (Wright, 1978) are shown graphically in Figure V.2 as a Wagner tree. The Wagner tree is consistant with one of the two major topologies obtained with phenetic analyses. Branch lengths indicate that evolution has not been constant for all lineages, although differences in rates of evolution have not been sufficiently divergent to confuse phenetic results. For these *Leptinotarsa* species, the UPGMA analyses and optimised Wagner trees indicate the same relationships. Hence, branching pattern of phenograms and Wagner trees substantiate a phylogeny in which *L. decemlineata* is more closely related to *L. haldemani* than to *L. texana*.

Morphological data was obtained from various published sources (Table V.2). The most parsimonious tree, calculated using MacClade to estimate tree length, is shown in Figure V.3. This cladogram is proposed as an estimation of phylogenetic history based on morphological characters. When only characters from Hsiao and Hsiao (1983) are considered, *L. decemlineata* and *L. texana* are more closely related than either is to *L. haldemani*. Considering only those characters described in Jacques (1972), the same relationships exist. The use of *Labidomera* characters for these cladograms was for outgroup polarisation, since *Labidomera* has been described as the most closely related genus to *Leptinotarsa* (Jacques, 1972). Inclusion of *Labidomera* in this cladogram supports the use of *L. lineolata* as the outgroup for electrophoretic comparisons. When considering chromosome characteristics or

morphological characteristics, *L. lineolata* consistently branches off before the other *Leptinotarsa* species in this study.

The cladogram presented in Figure V.3 is the most parsimonious for the chromosomal and morphological data available. This cladogram conflicts with the Wagner and UPGMA trees for electrophoretic data but agrees with the relationships suggested by Tower (1906, 1918). The cladogram indicates *L. decemlineata* is more closely related to *L. texana* than to *L. haldemani*.

If morphological data excluding colour patterns on elytra are eliminated from the data matrix, the resulting cladogram indicates that *L. decemlineata* is equally distant from *L. texana* and *L. haldemani*. Since colour patterns may be subject to intense selection pressures because of fundamental roles in camoflage, deception or advertisement (Hoffman, 1985), the use of colour patterns as critical characters in relating *Leptinotarsa* species is questionable. Colour patterns may be the result of convergent evolution. For *L. decemlineata* and *L. texana*, the lined pattern on the elytra may be useful in breaking up the continuity of surface and recognisable outline. The red and blue colours of *L. rubiginosa* and *L. haldemani* suggest that either may be berry mimics or possibly warning colours (Evans, 1984; Hinton, 1976). Variation within a single species is also considerable. Colour morphs of *L. decemlineata* have been described as red (Tower, 1906; Boiteau, 1987), white (Boiteau, 1980; Hsiao and Hsiao, 1982), and black (Boiteau, 1985). *L. haldemani* is described as being blue, green, blue-black or violet (Jacques, 1972).

Problems with reconciling electrophoretic and morphological data indicate that there are theoretical or procedural problems with one or both analyses or that additional data

are needed to resolve the phylogenetic relationships. Hillis (1987) reviewed advantages of morphological and molecular approaches to systematics. Advantages of morphological methods were applicablity to museum and fossil specimens, use of ontogenetic characters and cost. For this study, the use of ontogenetic characters is the only compelling advantage for morphological data. Environmental influences on phenotype are a serious consideration for morphological characters. Non-heritable variation is primarily a problem for morphological characters. In general, biomolecular data are less confounded by environmental influences than are morphological data (Hillis, 1987).

The electrophoretic data set presented here is substantially larger than the morphological data set. The morphological data set is also subject to the limitation of reliance on species descriptions available in the literature. The validity of the cladogram produced is thus questioned. A larger data set for morphological characters and identical measurements for each character would be especially valuable for resolving phylogenetic differences proposed by these two data sets.

In the absence of a more definitive cladogram for morphological characters, I consider the electrophoretic data set to provide the most reliable estimate of phylogeny. In this phylogeny, *L. haldemani* and *L. decemlineata* are more closely related to each other than either is to *L. texana*.

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Table V.1. Species of Leptinotarsa collected, distribution in North America (Jacques, 1988), locality sampled and foodplant collected from.

species	distribution in North America	localities sampled	foodplant	sample size (yr collected)
L. decemlineata	widespread in North America and Mexico	Edmonton, Alberta	*S. tuberosum*	7 (1986) + 51 (1987)
		Benson, Arizona	*S. elaeagnifolium*	16 (1986) + 27 (1987)
L. haldemani	Arizona,Oklahoma, Texas, Mexico	Pena Blanca, Arizona	*Physalis spp.*	
			S. douglasii	22 (1986) + 10 (1987)
		Benson, Arizona	*Lycium sp.*	37 (1987)
L. lineolata	Arizona, New Mexico, Texas, Mexico	Gardener Canyon, Arizona	*H. monogyra*	8 (1986) + 23 (1987)
		Gleeson, Arizona	*H. monogyra*	13 (1987)
		Patagonia, Arizona	*H. monogyra*	27 (1987)
		Pena Blanca, Arizona	*H. monogyra*	6 (1986) + 26 (1987)
L. rubiginosa	Arizona, New Mexico, Texas, Mexico	Pena Blanca, Arizona	*Physalis sp.*	11 (1987)
L. texana	Texas, Mexico	Hildago county, Texas	*S. elaeagnifolium*	10 (1986) + 32 (1987)

Table V.2. Characters used in cladistic analysis with source in brackets. (1)=Blatchley (1910); (2)= Brown (1961); (3)=Headstrom (1977); (4)=Hsiao and Hsiao (1983); (5)= Jacques (1972).

		length of X chromosome (mm)	no. chromosomes with 2e constrictions (mm)	length of 2e constricted (mm)	elytral marking	pronotum punctation	pronotum punctation
Leptinotarsa	decemlineata	15 (4)	15 (4)	15 (4)	striped (5)	fine-coarse (5)	dense (5)
L.	haldemani	13 (4)	15 (4)	15 (4)	unicoloured (5	fine (5)	sparse (5)
L.	lineolata	13 (4)	15 (4)	15 (4)	striped (5)	fine-coarse (5)	dense (5)
L.	rubiginosa	14 (4)	16 (4)	16 (4)	unicoloured(5)	fine-coarse (5)	sparse (5)
L.	texana	15 (4)	15 (4)	15 (4)	striped(5)	fine-coarse (5)	dense (5)
Labidomera	clivicollis	13 (4)	14 (4)	14 (4)	striped (2,3)	fine (1,3)	sparse (1,3)
Labidomera	suterella	13 (4)	14 (4)	14 (4)	striped (1)		

		elytral punctation	elytral punctation	aedeagus arch	aedeagus apex	aedeagus long as wide	interocular width (mm)
Leptinotarsa	decemlineata	coarse (5)	irregular rows (5)	greatly (5)	pointed (5)	3 X (5)	1.7 (5)
L.	haldemani	fine (5)	irregular rows (5)	arched (5)	rounded (5)	4 X (5)	1.8 (5)
L.	lineolata	coarse (5)	no rows (5)	slightly (5)	rounded (5)	?	1.4 (5)
L.	rubiginosa	coarse (5)	irregular rows (5)	arched (5)	rounded (5)	4 X (5)	1.8 (5)
L.	texana	coarse (5)	regular rows (5)	slightly (5)	rounded (5)	4 X (5)	1.7 (5)
Labidomera	clivicollis	?	?	?	?	?	?
Labidomera	suterella	fine (1,3)	irregular rows (1,3)	slightly (2)	pointed (2)	?	?

Table V.3. Allozyme frequencies detected at 18 polymorphic loci in 5 Leptinotarsa species. Asterisks indicate loci not in Hardy-Weinberg equilibrium (P< 0.05).

locus	alleles	L. decem (Ben)	L. decem (Edm)	L. halde (P Bl)	L. halde (Ben)	L. lineol (Gard C)	L. lineol (Glee)	L. lineol (Pata)	L. lineol (P Bl)	L. rubig (P Bl)	L. tex (Hid cnty)
AK	f(A)	-	-	0.04	-	-	-	-	-	0.36	0.80
	f(B)	-	-	0.17	0.50	-	-	-	-	0.05	0.20
	f(C)	0.27	0.17	0.79	0.44	-	-	-	-	0.41	-
	f(D)	0.59	0.66	-	0.06	0.32	-	-	0.39	0.18	-
	f(E)	0.14	0.17	-	-	0.22	0.46	0.23	0.52	-	-
	f(F)	-	-	-	-	0.26	0.54	0.73	0.08	-	-
	f(G)	-	-	-	-	0.20	-	0.04	0.01	-	-
APK	f(A)	0.02	0.12*	-	0.16	-	-	0.22	0.12	0.10	-
	f(B)	0.98	0.88*	1.00	0.84	1.00	1.00	0.78	0.88	0.90	1.00
ETOH	f(A)	-	-	-	-	-	-	-	-	-	0.59
	f(B)	-	-	0.24	0.27	-	-	-	-	-	0.41
	f(C)	0.17	0.67	0.26	0.41	-	-	0.37	0.14	-	-
	f(D)	0.40	0.33	0.50	0.31	0.31	0.67	0.61	0.50	0.64	-
	f(E)	0.43	-	-	0.01	0.63	0.29	0.02	0.36	0.36	-
	f(F)	-	-	-	-	0.06	0.04	-	-	-	-
FUDH	f(A)	0.36*	0.23*	-	-	0.43*	0.25	0.23	0.13	-	0.26*
	f(B)	0.45*	0.54*	-	0.50	0.37*	0.50	0.73	0.71	0.28	0.20*
	f(C)	0.19*	0.23*	0.29	0.47	0.20*	0.25	0.04	0.16	0.66	0.54*
	f(D)	-	-	0.53	0.03	-	-	-	-	0.06	-
	f(E)	-	-	0.18	-	-	-	-	-	-	-

Table V.3 continued.

locus	alleles	L. decem (Ben)	L. decem (Edm)	L. halde (P Bl)	L. halde (Ben)	L. lineol (Gard C)	L. lineol (Glee)	L. lineol (Pata)	L. lineol (P Bl)	L. rubig (P Bl)	L. tex (Hid cnty)
FUM	f(A)	0.50	0.52	-	0.07	-	-	-	-	-	0.03
	f(B)	0.50	0.48	0.53	0.59	-	-	-	-	-	0.97
	f(C)	-	-	0.47	0.34	-	-	-	-	0.80	-
	f(D)	-	-	-	-	0.33	0.40	0.50	0.66	0.20	-
	f(E)	-	-	-	-	0.67	0.60	0.50	0.34	-	-
GOT	f(A)	-	-	-	-	-	-	-	-	0.50	-
	f(B)	0.37	0.48	0.72	0.59	-	-	-	-	0.50	-
	f(C)	0.63	0.52	0.28	0.41	-	-	-	0.02	-	1.00
	f(D)	-	-	-	-	0.70	0.70	0.81	0.68	-	-
	f(E)	-	-	-	-	0.30	0.30	0.19	0.30	-	-
GLYDH	f(A)	-	0.14	-	-	-	-	-	-	-	0.44
	f(B)	0.33	0.31	-	-	-	-	-	-	0.27	0.56
	f(C)	0.67	0.52	0.57	0.61	0.56	0.42	0.31	0.52	0.68	-
	f(D)	-	0.03	0.43	0.39	0.44	0.58	0.69	0.48	0.05	-
HK.1	f(A)	-	-	-	-	-	-	0.24	-	-	-
	f(B)	0.07	-	-	-	0.50	0.38	0.42	0.90	-	-
	f(C)	0.68	0.38	0.48	-	0.50	0.62	0.34	0.10	-	0.37
	f(D)	0.25	0.62	0.25	0.67	-	-	-	-	0.45	0.63
	f(E)	-	-	0.27	0.33	-	-	-	-	0.55	-
HK.2	f(A)	-	-	-	-	0.50	0.54	0.29	0.50	-	0.48
	f(B)	0	0.15	0.29	-	0.50	0.46	0.71	0.50	-	0.52
	f(C)	0.60	0.50	0.50	0.40	-	-	-	-	0.28	-
	f(D)	0.40	0.35	0.21	0.60	-	-	-	-	0.67	-
	f(E)	-	-	-	-	-	-	-	-	0.05	-
HBDH	f(A)	0.50	0.56	-	-	-	-	-	-	-	0.69
	f(B)	0.50	0.44	0.48	0.60	-	-	-	-	0.14	0.31
	f(C)	-	-	0.52	0.40	0.67	0.68	0.54	0.52	0.55	-
	f(D)	-	-	-	-	0.33	0.32	0.46	0.48	0.31	-

Table V.3 continued.

locus	alleles	L. decem (Ben)	L. decem (Edm)	L. halde (P Bl)	L. halde (Ben)	L. lineol (Gard C)	L. lineol (Glee)	L. lineol (Pata)	L. lineol (P Bl)	L. rubig (P Bl)	L. tex (Hid cnty)
IBDH	f(A)	0.29	-	-	-	0.15	0.65	-	0.12	-	-
	f(B)	0.21	0.59	-	-	0.50	0.35	0.22	0.27	-	0.76
	f(C)	0.50	0.41	0.20	0.55	0.35	-	0.78	0.61	0.60	0.24
	f(D)	-	-	0.80	0.45	-	-	-	-	0.40	-
IDH	f(A)	0.03	0.10	0.50	0.36	-	0.67	0.25	0.66	0.32	0.47
	f(B)	0.35	0.77	0.50	0.35	0.46	0.33	0.31	0.34	0.23	-
	f(C)	-	-	-	0.29	0.54	-	0.44	-	0.45	0.53
	f(D)	0.62	0.13	-	-	-	-	-	-	-	-
IPDH	f(A)	-	-	-	-	-	-	-	-	-	0.70
	f(B)	-	-	-	-	0.37	0.69	0.34	0.44	-	0.30
	f(C)	-	-	-	-	0.63	0.31	0.66	0.56	-	-
	f(D)	0.73	0.33	0.16	0.03	-	-	-	-	-	-
	f(E)	0.27	0.67	0.40	0.64	-	-	-	-	-	-
	f(F)	-	-	0.44	0.33	-	-	-	-	0.82	-
	f(G)	-	-	-	-	-	-	-	-	0.18	-
ME	f(A)	-	0.21	-	-	-	-	-	-	0.39	0.33
	f(B)	0.49	0.63	0.73	0.59	-	-	-	-	0.61	0.67
	f(C)	0.51	0.16	0.27	0.41	0.02	0.69	-	0.61	-	-
		-	-	-	-	0.56	0.31	0.56	0.39	-	-
		-	-	-	-	0.42	-	0.44	-	-	-
ODH	f(A)		-	-	-	-	-	-	-	-	0.54
	f(B)		-	-	-	0.10	0.20	0.27	0.50	-	0.46
		.36	0.56	-	-	0.50	0.65	0.60	0.44	-	-
		0.54	0.31	-	-	0.40	0.15	0.13	0.04	0.45	-
		0.10	0.13	0.10	0.47	-	-	-	0.02	0.55	-
	f(F)	-	-	0.54	0.50	-	-	-	-	-	-
	f(G)	-	-	0.36	0.03	-	-	-	-	-	-

Table V.3 continued.

locus	alleles	L. decem (Ben)	L. decem (Edm)	L. halde (P Bl)	L. halde (Ben)	L. lineol (Gard C)	L. lineol (Glee)	L. lineol (Pata)	L. lineol (P Bl)	L. rubig (P Bl)	L. tex (Hid cnty)
PGI	f(A)	0.38	0.57	-	-	-	-	-	-	0.64	0.51
	f(B)	0.62	0.38	0.65	0.51	-	-	-	-	0.36	0.49
	f(C)	-	0.05	0.35	0.46	0.05	-	0.56	0.45	-	-
	f(D)	-	-	-	-	0.59	0.54	0.44	0.55	-	-
	f(E)	-	-	-	-	0.36	0.46	-	-	-	-
SODH	f(A)	-	-	-	-	0.48	0.04	0.20	0.24	-	-
	f(B)	-	0.31	-	-	0.32	0.63	0.63	0.57	0.36	-
	f(C)	0.47	0.31	0.48	0.42	0.20	0.33	0.17	0.19	0.46	0.36
	f(D)	0.38	0.35	0.52	0.58	-	-	-	-	0.18	0.32
	f(E)	0.15	0.03	-	-	-	-	-	-	-	0.32
XO	f(A)	-	-	-	-	0.24	-	0.26	0.35	-	0.65
	f(B)	-	-	-	-	0.56	0.27	0.60	0.42	-	0.35
	f(C)	0.59	0.30	0.48	0.09	0.20	0.41	0.14	0.23	-	-
	f(D)	0.40	0.49	0.52	0.55	-	0.32	-	-	0.40	-
	f(E)	0.01	0.21	-	0.36	-	-	-	-	0.30	-
	f(F)	-	-	-	-	-	-	-	-	0.30	-

Table V.4. Genetic variability at 18 loci for 5 Leptinotarsa species.

species	mean sample size per locus	mean number of alleles at each locus	% loci polymorphic*	mean heterozygosity direct count	mean heterozygosity Hardy-Weinberg expected
L. decemlineata (Edmonton)	40.0 ± 2.8	2.7 ± 0.2	100	0.372 ± 0.034	0.520 ± 0.025
L. decemlineata (Benson)	28.0 ± 2.4	2.5 ± 0.1	94.4	0.413 ± 0.044	0.506 ± 0.032
L. haldemani (Pena Blanca)	22.8 ± 1.6	2.4 ± 0.1	94.4	0.355 ± 0.034	0.491 ±0.036
L. haldemani (Benson)	23.3 ± 2.0	2.6 ± 0.2	100	0.424 ± 0.04[illegible]	0.520 ± 0.020
L. lineolata (Gardener Canyon)	22.1 ± 1.6	2.5 ± 0.2	94.4	0.447 ± 0.056	0.518 ± 0.036
L. lineolata (Gleeson)	11.5 ± 0.4	2.2 ± 0.1	94.4	0.506 ± 0.055	0.484 ± 0.033
L. lineolata (Patagonia)	18.0 ± 1.8	2.5 ± 0.1	100	0.435 ± 0.052	0.490 ± 0.026
L. lineolata (Pena Blanca)	23.4 ± 1.8	2.6 ± 0.2	100	0.407 ± 0.048	0.505 ± 0.029
L. rubiginosa (Pena Blanca)	10.4 ± 0.2	2.5 ±0.1	100	0.400 ± 0.040	0.512 ±0.031
L. texana (Hidalgo county)	30.9 ± 2.4	2.0 ± 0.1	83.3	0.361 ± 0.058	0.408 ± 0.046

*a locus is considered polymorphic if the frequency of the most common allele ≤ 0.95

Table V.5. Number of host plants associated with each species or biotype and mean heterozygosity per locus (biased measure).

species	locality	foodplants associated with species	heterozygosity
L. decemlineata	Edmonton, Alberta	*12 Solanum spp., 1 Physalis sp. Lycopersicon sp.*	0.513 ± 0.025
L. decemlineata	Benson, Arizona	*1 Solanum sp. (S. elaeagnifolium)**	0.494 ± 0.032
L. haldemani	Pena Blanca, Arizona	*2 Solanum spp., 2 Physalis spp., 1Lycopersicon sp.*	0.478 ± 0.036
L. haldemani	Benson, Arizona	*1 Lycium sp.*	0.507 ± 0.020
L. lineolata	Gardener Canyon, Arizona	*1 Hymenoclea sp.*	0.504 ± 0.035
L. lineolata	Gleeson, Arizona	*1 Hymenoclea sp.*	0.462 ± 0.031
L. lineolata	Patagonia, Arizona	*1 Hymenoclea sp.*	0.472 ± 0.025
L. lineolata	Pena Blanca, Arizona	*1 Hymenoclea sp.*	0.485 ± 0.027
L. rubiginosa	Pena Blanca, Arizona	*1 Solanum sp., 1 Physalis sp.*	0.487 ± 0.030
L. texana	Hildago county, Texas	*1 Solanum sp.*	0.400 ± 0.045

*This biotype of L. decemlineata is described as monophagous (Hsiao, 1981).

Table V.6. Matrix of Nei's (1972) genetic identity above diagonal and Nei's (1972) genetic distance below diagonal.

	L. decem (Edm)	L. decem (Ben)	L. halde (P Bl)	L. halde (Ben)	L. lineol (Gard C)	L. lineol (Glee)	L. lineol (Pata)	L. lineol (P Bl)	L. rubig (P Bl)	L. tex (Hid cnty)
L. decem (Edm)	***	0.878	0.631	0.711	0.404	0.409	0.375	0.408	0.565	0.549
L. decem (Ben)	0.130	***	0.651	0.656	0.431	0.448	0.321	0.307	0.549	0.501
L. halde (P Bl)	0.460	0.430	***	0.851	0.325	0.407	0.385	0.358	0.655	0.368
L. halde (Ben)	0.342	0.421	0.162	***	0.304	0.348	0.359	0.386	0.708	0.398
L. lineol (Gard C)	0.906	0.842	1.125	1.192	***	0.821	0.847	0.824	0.387	0.337
L. lineol (Glee)	0.894	0.802	0.899	1.057	0.197	***	0.775	0.849	0.369	0.305
L. lineol (Pata)	0.980	1.136	1.093	1.024	0.166	0.255	***	0.841	0.352	0.294
L. lineol (P Bl)	0.895	0.899	1.028	0.953	0.194	0.164	0.173	***	0.386	0.316
L. rubig (P Bl)	0.572	0.599	0.424	0.345	0.950	0.996	1.044	0.952	***	0.388
L. tex (Hid cnty)	0.599	0.692	1.000	0.921	1.087	1.186	1.223	1.087	0.948	***

Table V.7. UPGMA phenograms based on similarity and distance coefficients grouped according to topology. Disregarding variation for topology among populations of L. lineolata, two major topologies emerge. Groups one and two make up topology one. Groups three and four make up topology two.

Group one	Group two
Nei (1972) identity Nei (1972) distance Nei (1978) identity Nei (1978) distance Nei (1972) minimum distance Nei (1978) minimum distance Modified Rogers distance (Wright, 1978)	Cavalli-Sforza and Edwards(1967) chord Cavalli-Sforza and Edwards(1967) arc Edwards (1971, 1974) distance

Group three	Group four
Rogers (1972) similarity Rogers (1972) genetic distance	Prevosti distance (Wright, 1978)

Figure V.1. UPGMA phenogram of Nei (1972) genetic distances obtained with BIOSYS-1 (Swofford and Selander, 1981). *Leptinotarsa* populations are from Alberta (Edmonton), Arizona (Benson, Gardener Canyon, Gleeson, Patagonia, Pena Blanca) and Texas (Hidalgo county).

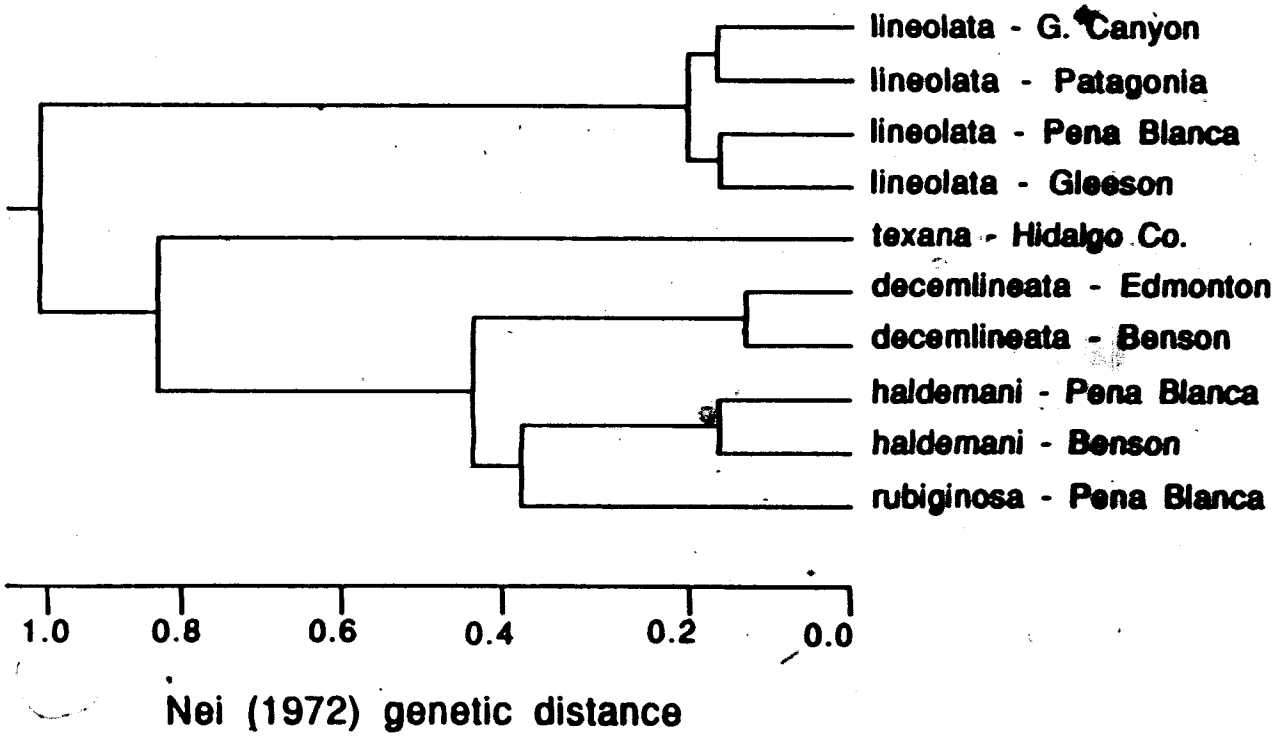


Figure V.2. Optimised Wagner tree of modified Rogers genetic similarity (Wright, 1978) obtained with BIOSYS-1 (Swofford and Selander, 1981). Data used are the same as in figure 1.

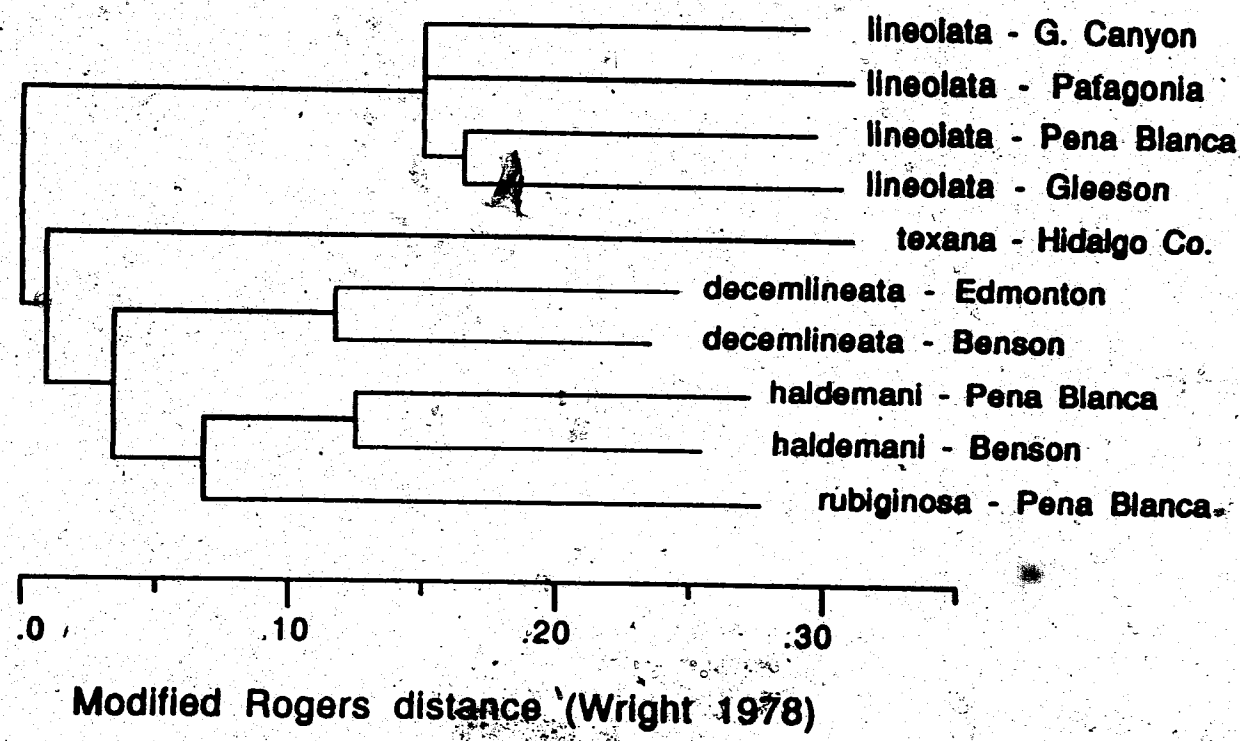
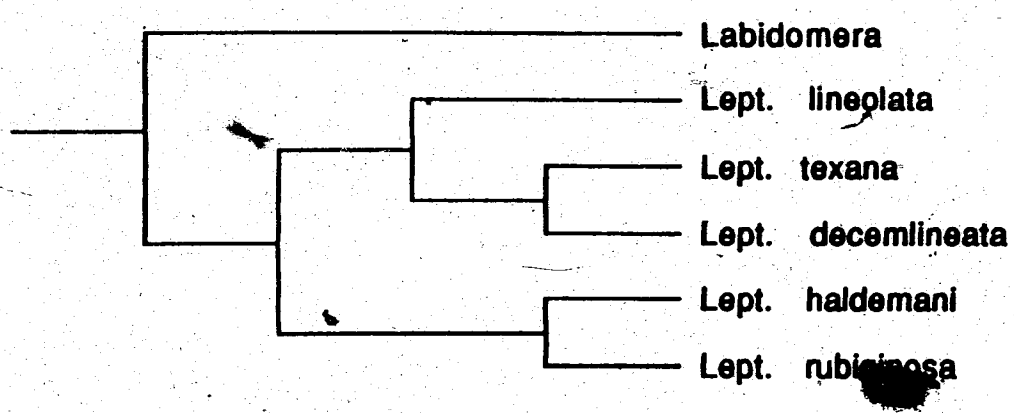


Figure V.3. Minimum length cladogram based on published characters using MacClade Version 2.1(© W.P. Maddison and D.R. Maddison). Chromosome characters are based on (Hsiao and Hsiao, 1983). Morpohological characters are based on descriptions found in Jacques (1972) for *Leptinotarsa* species. *Labidomera* species descriptions were taken from Blatchley (1910), Brown (1961), and Headstrom (1977).



VI. Evolution of host plant specificity of *Leptinotarsa* species.

Host plant specificity of *Leptinotarsa* species for solanceous plants is described using host plant records, nutritional criteria, and ovipositional choice tests (see Chapter one). Feeding behaviour (Chapter two), morphology of the galeae (Chapter three),and electrophysiological responses to plant saps (Chapter four) are examined in detail for *Leptinotarsa decemlineata*, *L. haldemani* and *L. texana*. The phylogeny presented in Chapter five allows behavioural and gustatory specificity described in chapters two and four to be discussed within an evolutionary framework.

The optimised Wagner tree shown in Chapter five, Figure V.2 is the basis of the evolutionary history presented for these beetles. The optimised Wagner tree was chosen as the best approximation of phylogenetic relationships (Farris, 1981). This phylogeny suggests that *L. haldemani* and *L. rubiginosa* have shared a more recent evolutionary past than *L. haldemani* and *L. decemlineata*. *L. texana* diverged from the lineage producing *L. decemlineata*, *L. haldemani* and *L. rubiginosa* more recently than the divergence producing *L. lineolata*. Data for the 5 *Leptinotarsa* species studied electrophoretically may be compared with host plant records and presumed degree of polyphagy.

Ecological forces are usually regarded as favouring increased specialization. Dethier (1954) describes polyphagy as a "luxury" which is only possible when competition is greatly reduced among herbivores. In discussing the co-evolution of phytophagous insects and plants, Jolivet (1986) concludes that polyphagy is ancestral to monophagy and oligophagy. His conclusions are based on the idea that exploitation of plant chemical defenses by insects is the basis for specialized associations between insects and plants.

Diet specialization theories presume that specialized insects are metabolically or ecologically more efficient at using food resources than more polyphagous species. Metabolic efficiency of specialized species over generalist species has been demonstrated for *Papilio trolius* and *P. glaucus glaucus* and yet is not adequately demonstrated for many insect species (Scriber and Slansky, 1981; Slansky and Scriber, 1981). In general, efficiency of food utilization is independent of degree of specialization and most variation is related to the nutritional quality of the plant (Slansky and Scriber, 1981). Hagen (1986), in a study of *Papilio* species, demonstrates the emergence of more polyphagous lineages from oligophagous ancestors. He concludes that organisation of insect communities and the regulation of insect populations is less dependent on interspecific competition among phytophagous species than on external constraints such as insect enemies and plant defenses. Ecological efficiency is thus proposed as an explanation of feeding specialization.

The advantages of specialization are, however, potentially counteracted by increased resource availability for more polyphagous species. Through a series of acquisitions of new host plants, a restricted relationship between aphids and plants evolved into a more polyphagous relationship (Eastop,1973). Furthermore, the balance achieved for degree of feeding specialization is not necessarily static. Insect host ranges may expand and contract over evolutionary time. A polyphagous insect may evolve from a more specialized ancestor and later give rise to specialized biotypes (Dethier,1954).

Tower (1906) suggested that monophagy is ancestral for the genus *Leptinotarsa*. The switch to oligophagy requires only one evolutionary step as indicated in Figure VI.1. The

ancestral basis for monophagy is supported by the work of Harrison (1987). The evolution of host plant choice for three geographic populations of *L. decemlineata* was through host expansions and selection for broad feeding preferences. Subsequently, *L. decemlineata*, a relatively polyphagous species, produced specialized biotypes, each limited to subsets of the ancestral host range. Further evidence for the expansion of host range by L. decemlineata is provided by Hare and Kennedy (1986) and Horton *et al.* (1988).

Neck (1983) suggested that adult feeding ranges are broader than larval feeding ranges for *L. decemlineata* and *L. texana* . This is consistant with May and Ahmad's (1983) opinion that oviposition by adult females is the point at which the most important host selection behaviour takes place. Since feeding behaviour and oviposition behaviour are linked by a pre-ovipositional feeding period and eggs are formed exclusively from nutrients ingested during adult life (de Wilde and de Loof, 1973), the role of host-choice behaviour by adults is emphasised over physiological adaptations of larvae for digestion and detoxification.

Although mechanisms of larval competition for host plant use have not been described in this study, comparisons of host plant use over the geographic distribution of these *Leptinotarsa* species provides interesting insights into possible host plant competition as a factor in sympatric speciation. *L. decemlineata* and *L. haldemani* in Benson, Arizona are found sympatrically. Although competition for host plants would appear to be a serious problem when comparing potential host plant use for all populations of each of these species, neither species is commonly found on the other's food plant. *L. haldemani*, although one of the most polyphagous of the genus, is not found on *S. elaeagnifolium*. In the Benson area where *L. decemlineata* and *L. haldemani* overlap in distribution, a biotype of *L.*

decemlineata exists which is specialized on *S. elaeagnifolium*. *S. elaeagnifolium* is an unacceptable *Solanum* species for *L. haldemani* which is found most commonly on *Lycium* species (*pers obs*).

Potential for interactions between *L. haldemani* and *L. rubiginosa* is found in the Pena Blanca region of Arizona. In this area, the two may be found on the same host plants, however, *Solanum douglasii* appears to be the most common host plant for *L. haldemani* while *L. rubiginosa* is found primarily on *Physalis* species (*pers obs*). Neck (1983) compared host plant distributions of *L. decemlineata* and *L. texana* in south-eastern North America. Whereas *L. decemlineata* is specialized on *S. elaeagnifolium* in the Benson, Arizona area where it overlaps with *L. haldemani*, *L. decemlineata* is most commonly found on *S. rostratum* in the Austin, Texas area. In the region around Austin, *L. texana* is found in much greater densities on *S. elaeagnifolium* than is *L. decemlineata* (Neck,1983). These insect-plant interactions merit further study, in particular, for metabolic and ecological efficiency of host plant use among localised populations of each species.

Evaluation of behavioural results when applied to the Figure VI.1 indicate that the behaviours in plant assessment and feeding described by Harrison (1987) are common to all three species. Number of individuals proceeding to the stage of gustatory sampling by *L. texana* was most restricted when compared to *L. decemlineata* and *L. haldemani*. *L. haldemani* proceeded to the stage of gustatory sampling regardless of eventual acceptablity of the host plant. This corresponds to the more polyphagous feeding habits of *L. haldemani*. Since behaviour may be a focus of selection for specialized feeding habits, adaptations may involve differences in time spent in various activities

rather than changes in a fundamental sensory ability. The more polyphagous *L. haldemani* does not reject a potentially suitable solanceous plant as quickly in the behavioural sequence as the more monophagous *L. texana*.

Sensory input may be similar for *L. texana* and *L. decemlineata* for *Solanum* species and yet different behaviours result. Monophagous and polyphagous adaptations may involve natural selection for changes in emphasis on fundamentally different behaviours based on different sensory input rather than sharpening of sensory ability in one mode. Monophagous insects may have no greater ability to detect compounds and yet have an extremely specialized behavioural response. Further comparison of the sensory capabilities of these species, in particular with single compounds is needed to examine the possibility of differential ability to detect compounds among *Leptinotarsa* species.

For each *Leptinotarsa* species, variability in electophysiological responses is seen among different plants as well as for a single plant sap. A trend for least variability in responses for *L. decemlineata* and greatest variability for *L. haldemani* corresponds with degree and evolution of feeding specialization (Figure VI.1). The most polyphagous species demonstrates no clear patterns of neural response. More polyphagous species may have a broader fit for the presumed neural template for behaviour than more restricted feeders. For more polyphagous species, component behaviours may result even if a series of PNS signals does not indicate a perfect match for the template for feeding behaviours.

Behavioural and electrophysiological data can also be fitted to the cladogram from chapter five, Figure V.3. The resulting cladogram, Figure VI.2 demonstrates that when number of

host plants and behavioural data are included, the most parsimonious dendrogram is the Wagner tree based on electrophoretic data (Figure VI.1). This is further evidence for the choice of the Wagner tree as the most parsimonious phylogeny for these beetles.

Feeding behaviour of these *Leptinotarsa* species cannot be directly related to electrophysiological responses. Studies investigating sensory coding across sensilla on a single galea as well as across sensilla on different body parts are especially interesting possibilities for further investigation. CNS reception of combined sensory input is also of particular interest. Comparisons among species at the level of CNS responses are relevant to debates on the conservative nature of changes in the CNS.

Oviposition preferences could be narrower than feeding preferences since larvae are restricted to feeding on the plant upon which they were laid. A study including ovipositional choice tests would explore differences between feeding and ovipositional choice. Tests involving *S. luteum* and *S. nigrum* would provide interesting comparisons, especially at the sensory level. Both *S. luteum* and *S. nigrum* are preferred for oviposition for *L. decemlineata* even though they do not support continued growth of larvae (Bongers,1970; Hsiao and Fraenkel,1968). Recordings of CNS signals from sensilla on the ovipositor as compared to the signals from sensilla on mouthparts could provide the crucial tests allowing insight into the interpretation of sensory information and the possible role of coordination of sensory information as it applies to insect behaviour.

Thompson (1988) reviewed the relationship between ovipositional choice and subsequent larval performance in the evolution of host associations among phytophagous insects. Under field conditions, the use of host plants which

do not result in optimal growth rates, often referred to as oviposition mistakes, are discussed in the light of four hypotheses. When novel host plants are recently added to the range of an insect species, associations over many generations may occur when adult host selection is refined to reduce oviposition on the novel plant species. Conversely, larvae may develop the ability to survive on the novel plant species. Oviposition on less desirable host plants may also be a result of greater availability relative to more desirable host plants in a given locality. Enemy-free space can influence ovipostional choice by changing ranking of host plants for ovipostion in the presence or absence of parasitoids and predators. Furthermore, patterns of use of plant parts and mixed species diets may vary for the instar examined. Evolution of host plant specificity by *Leptinotarsa* species for *S. luteum* and *S. nigrum* would be an interesting test of these four hypotheses under field conditions. The evolution of the sensory basis of host plant discrimination could then be discussed within the general framework provided by Thompson's paper.

The relative success of holometabolous insect species over hemimetabolous species is believed to be due mainly to specialization of life stages. Immature stages are specialized for feeding while adult stages are specialized for mating and dispersal (Evans, 1984). This idea is exemplified by Lepidoptera species such as *Pieris* species. Coleoptera species, in particular *Leptinotarsa* species which feed on the same food plants as larvae and adults, could provide a comparison within holometabolous insects, as Coleoptera are more primitive holometabolous species than are Lepidoptera (Boudreaux, 1979). *Leptinotarsa* species could be expected to have simpler host choice mechanisms than *Pieris* species since ovipositional ranges overlap with adult food choice. *Pieris* species could be expected to have tighter genetic linkage between larval and adult ranking in host plants

when compared to *Leptinotarsa* species since differences in choice for each life stage must be respected.

Linkage between ovipositional deterrents and feeding deterrents for *Pieris* species provide an excellent test system for these hypotheses. Renwick *et al.* (1988) have shown that gustatory feeding deterrents are not the same as ovipostional deterrents. Potentially, gustatory deterrents might be found in greatest concentration in leaf saps and waxes while oviposition deterrents might be found primarily in leaf waxes since *Pieris* species do not break surface integrity of leaves when testing for ovipostitional suitability. Distributions of ovipositional deterrents or stimulants for *Leptinotarsa* species would presumably be similar since the two behaviours overlap in host plant suitability with few exceptions. Comparisons of these exceptions could provide fascinating insights into relationships among the sensory bases of host plant discrimination which is vital when discussing evolution of host plant associations.

Studies of the evolution of host specificity contribute to the understanding of mechanisms of host plant shifts. Better understanding of these host shifts is significant for control of economic pests particularly since rational pest management strategies should consider the evolutionary background of a pest species. Modern factors in the evolution of *L. decemlineata* include the use of insecticides, breeding for host plant resistance and increased gene flow among previously isolated populations. The economic control of *L. decemlineata* using insecticides has been complicated by the rapid appearance of resistance to new insecticides. Resistance management techniques are of particular concern for pest species such as *L. decemlineata* whose abilities to form specialized biotypes are well known (see Chapter one).

An alternative to insecticidal control of *L. decemlineata* currently receiving much attention is breeding for host plant resistance. A major concern with this approach should be the rapid biotype evolution of *L. decemlineata*. A balance between time to develop new strains of host plant and development of biotypes of resistant *L. decemlineata* must be acheived. Groden and Casagrande (1986) have demonstrated resistance to *Solanum berthaultii* in as few as three generations. Host plant resistance should therefore be used as a component in a broadly based management program (Wright *et al.*, 1985). Consideration of increased gene flow among biotypes of *L. decemlineata* by human activity is necessary when discussing resistance management. Increased gene flow in biotypes resistant to a particular control strategy could provide susceptible genes and therefore be of potential use. In biotypes currently controlled by a given management technique, increased gene flow increases the probability of a resistant gene being introduced into the population.

The association of *Leptinotarsa* species with solanceous plants continues to provide an intriguing system to study the evolution of insect-host plant interactions and the sensory basis of such host plant-insect interactions. This study has provided information on responses of individual adult beetles within ecological and phylogenetic constraints. Host plant choice is only partially explained by PNS responses of contact chemoreceptors on the galeae to expressed plant saps. Much of the potential for further study of contact chemoreception lies in comparisons of the CNS responses of these beetles.

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Figure VI.1 Evolution of host-plant choice in 5 *Leptinotarsa* species. The phylogeny presented is the same as Figure V.2. Number of described host-plants is from Table V.4. Behavioural data is from Table II.1 using acceptability ranking of Figure II.4. Electrophysiological responses are shown in Figures IV. 2, IV.3 and IV.4

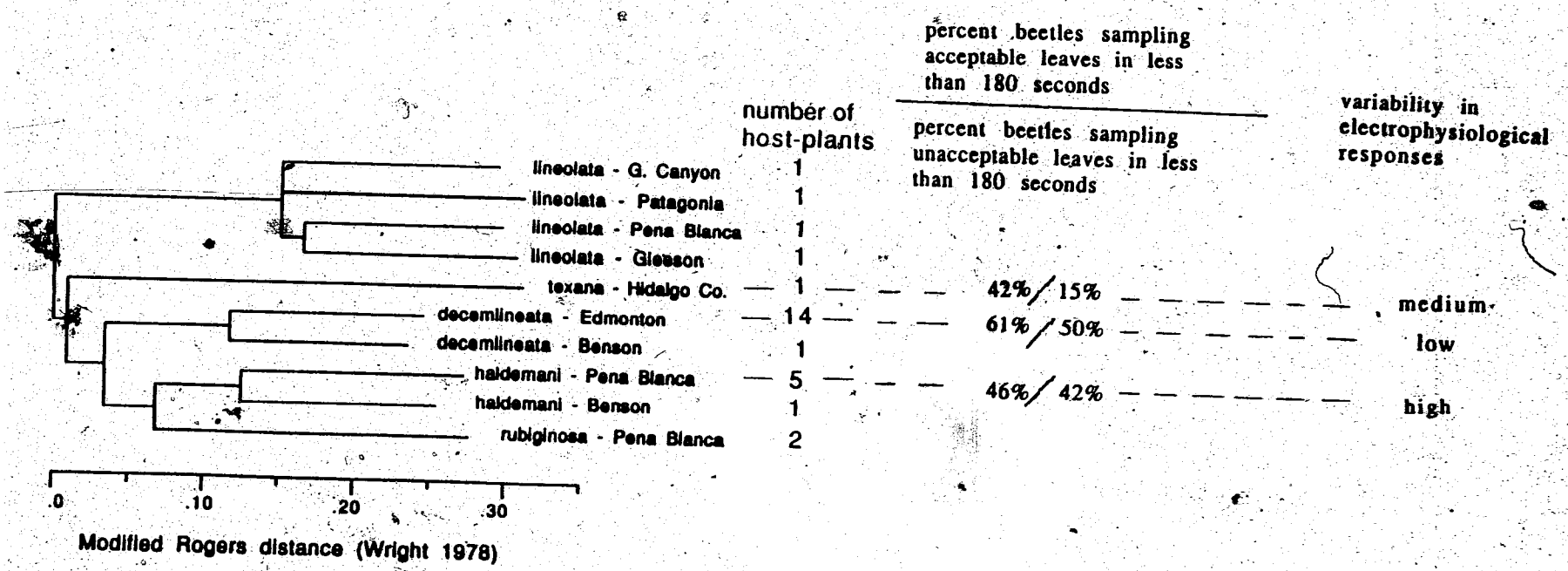

Figure VI.2 Evolution of host plant choice in 5 *Leptinotarsa* species. The phylogeny presented is the same as figure V.3. Other data is the same as for Figure VI.1