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Chemical communication between lycaenid larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae).

Henning, Stephen Frank

2015-01-26
http://hdl.handle.net/10539/16772

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out rather than chewed.

For protection from ants pupation in this species takes place within the cuticle of the full grown larva.

3.2.4 Normally feeding only on secretions of ants or Homoptera or or both

Euliphyra mirifica Holland

The life history of this species was first described by Lamborn (1913). The larvae of *E. mirifica* live in the nests of the tailer ant *Oecophylla longinoda* (Latreille). From the observations made, it appears that *E. mirifica* larvae do not prey on the ant brood but are fed by the ant on regurgitated food. Pupation takes place within the ant nest.

Lachnocnema bibulus (Fabricius)

Also belonging to this group is *L. bibulus* from East Africa. Jackson (1937) and Cripps and Jackson (1940) record an entirely different life history for this species from East Africa to the one recorded by Lamborn (1914), Clark (1940), Clark and Dickson (1970), and Owen (1971) from West and Southern Africa (see p. 52).

Cripps and Jackson found that the eggs were laid on the leaves of plants *Vernonia* and *Combretum*. After the larvae hatched they were either carried into the ants nest by the host workers or deposited temporarily in holes or crevices in the bark near colonies of jassids or membracids. These larvae were constantly attended by ants (*Camponotus* sp.) and fed on the excretions of the jassids or the membracids, which they obtained by soliciting after the fashion of the ants themselves. The forelegs of the *L. bibulus* were unusually long and Cripps and Jackson believed that they were especially modified for this function. Jackson (1937) described the manner of doing this as follows: "Sitting back on its abdominal segments, the fore part of the body is raised over the anal tube of the Membracid, the forelegs are then vibrated rapidly over the insect, just the same as an ant strokes certain larvae with its antennae, when a drop of the secretion promptly appears and is readily devoured. I have never seen a Membracid actually eaten".

All the *L. bibulus* larvae were eventually taken into the ants nest by the workers. In the nest the ants fed the larvae with regurgitated food.

Pupation occured in the ants nest, and the pupae hung head downwards from the roof of a tunnel.

3.3 Discussion

In order to study the evolution of the ant/lycaenid association the feeding habits, myrmecophily and presence of glands and tubercles within the different subfamilies of the Lycaenidae were considered (see Table 4). The subfamilies were created on adult morphology and defining their limits has caused considerable controversy over the years. The arrangement of the subfamilies is that of Stempffer (1967) based on their relationships to each other.

From the table one can see that the larvae of the subfamilies Lipteninae, Epitolinae, Pentilinae and Mimacraeinae lack both honey-gland and tubercles, and they probably never occured in the ancestors of these species. The members of these subfamilies all feed on lower plants (Thallophyta) i.e. lichens and algae. The larvae while feeding on lichen growing on dark or stones, often come into contact with ants. Nevertheless several species have not been observed to be associated with ants, while even those observed with ants are ignored by them. Jackson (1937) reported that the larvae of *Deloneura ochrascens* Neave (Epitolinae) were usually found among the ants, *Crematogaster castenea* Smith, but that there was no interaction between them. Similarly both Farquharson (1922) and Jackson (1937) noted the same thing with *Iridana incredibilis* Stgr. (Lipteninae). All the larvae in these subfamilies are densely clothed with long hairs which is generally believed to provide protection, or at least act as a deterrent, against attacks by ants.

The next group of three carnivorous subfamilies, the Thestorinae, Miletinae and Spalginae, also lack both the honey-gland and tubercles. The members of these subfamilies prey mainly on Homoptera, although some feed on ants and the secretions of ants and Homoptera. The species *Thestor protumnus aridus* van Son (Thestorinae) is unusual in that it

Table 4. The feeding habits, myrmecophily, glands and duration of tne larval stages of the family Lycaenidae. Species are arranged in subfamilies according to Stempffer (1967).

Subfamilies and Species	Feeding Habits		Glands			Total duration of larval stages (days)	References
	Independent	Myrmecophilous	Perforated Cupolas	Honey-gland	Tubercles		
Subfamily Lipteninae							
Durbania amakosa natalensis van Son	Phyto-lichen		NR	-	-	NR	1
Durbania limbata Trimen	Phyto-lichen		NR	-	-	NR	1
Iridana incredibilis Stgr.		Phyto-lichen	NR	-	-	NR	2
I. perdita marina Talbot		Phyto-lichen	NR	-	-	NR	2
Subfamily Epitolinae							
Deloneura ochrascens Neave		Phyto-lichen	NR	-	-	NR	2
Hewitsonia kirbyi intermedia J. & T.	Phyto-lichen		NR	-	-	NR	?
Subfamily Pentilinae							
Pentila muhata Dew.	Phyto-lichen		NR	-	-	NR	2
Alaena amazoula Bois.	Phyto-lichen		NR	-	-	90	3
Subfamily Mimacraeinae							
Mimacraea krausei masindae B.-Bak.	Phyto-lichen		NR	-	-	NR	2
Subfamily Thestorinae							
Thestor basutus (Wall.)		Pred-Homop	NR	-	-	NR	4
Thestor dickasoni Riley		Pred-ants	NR	-	-	NR	3
Thestor protumnus aridus van Son		Pred-Homop-ants	NR	-	-	84	4
Lachnocnema bibulus (F.)		Pred-Homop	NR	-	-	21	5
Lachnocnema bibulus (F.)		Homop-excret & Ant-regurg	NR	-	-	NR	2
Subfamily Spalginae							
Fenisoca tarquinius F.	Pred-Homop		+	-	-	NR	6
Spalgis lemolea Druce	Pred-Homop		NR	-	-	NR	7
Subfamily Miletinae							
Miletus boisduvali Moore		Pred-Homop	+	-	-	NR	9
Megalopalpus zymna D.H.	Pred-Homop		NR	-	-	NR	7
Subfamily Liphyrinae							
Liphyra brassolis Westw.		Pred-ants	+	-	-	NR	8
Euliphyra mirifica Holland		Ant-regurg	NR	-	-	NR	7
Aslauga purpurascens Holl.		Pred-Homop	NR	-	+	NR	2

Table 4. -(continued)

Subfamilies and Species	Feeding Habits		Glands			Total duration of larval stages (days)	References
	Independent	Myrmecophilous	Perforated Cupolas	Honey-gland	Tubercles		
Subfamily Hamearinae							
Nemeobius lucina (L.)	Phyto-Angio		-	-	-	NR	6
Subfamily Curetinae							
Curetis thetis Drury	Phyto-Angio		NR	-	-	NR	9
Subfamily Plebejinae							
Freyeria trochilus (Freyer)		Phyto-Angio	+	+	+	35	3
Subfamily Everinae							
Azanus jesous (Guerin)		Phyto-Angio	+	+	+	19	3
Cupido minimus Fuessly		Phyto-Angio	+	+	-	NR	6
Subfamily Brephidinae							
Brephidium metophis (Wall.)	Phyto-Angio		NR	+	+	29	3
Subfamily Zizerinae							
Zizeeria knysna (Trimen)		Phyto-Angio	+	+	+	18	3
Subfamily Lycaenopsinae							
Lycaenopsis argiolus L.	Phyto-Angio		+	+	+	NR	9
Subfamily Theclinae							
Myrina silenus ficedula Tri.		Phyto-Angio	+	+	+	22	3
Iolaus trimeni Wallengren	Phyto-Angio		+	+	+	30	1
Capys disjunctus (Trimen)	Phyto-Angio		+	+	-	NR	3
Leptomyrina henningi Dickson		Phyto-Angio	+	+	-	40	1
Subfamily Aphaeinae							
Spindasis namaqua (Trimen)		Phyto-Angio	+	+	+	NR	10
Poecilmitis lycaegenes (Tri.)		Phyto-Angio	+	+	+	95	3
Aloeides aranda Wallengren		Phyto-Angio	+	+	+	197	3
Aloeides dentatis (Swierstra)		Phyto-Angio	+	-	+	200-300	11
Subfamily Lampidinae							
Euchrysops dolorosa (Trimen)		Phyto-Angio	+	+	+	19	3
Cacyreus lingeus Cramer	Phyto-Angio		+	+	-	25	3
Triclema lamias Hewitson		Pred-Homop	NR	+	-	NR	12
Lepidochrysops ignota (Tri.)		Phyto/Pred-ants	+	+	-	NR	1
Lepidochrysops trimeni (B.-Bab.)		Phyto/Pred-ants	NR	+	-	300	13
Subfamily Glaucopsychinae							
Glaucopsyche alexis Poda		Phyto-Angio	+	+	+	NR	6
Maculinea arion L.		Phyto/Pred-ants	+	+	-	NR	9
Subfamily Lycaeninae							
Lycaena dispar Haw.		Phyto-Angio	+	-	-	NR	9

Phyto = Phytophagous
Pred = Predaceous
Homop = Homoptera
NR = not recorded
+ = present
- = absent

1 = Personal observations
2 = Jackson (1937)
3 = Clark & Dickson (1971)
4 = Clark & Dickson (1960)
5 = Clark (1940)
6 = Malicky (1970)
7 = Lamborn (1914)
8 = Dodd (1902)
9 = Hinton (1951)
10 = Vissian (1976)
11 = Tite & Dickson (1973)
12 = Farquarson (1922)
13 = Claassens (1976)

feeds on coccids for its first three instars, then is either taken, or makes its own way into the nest of the host ant where it feeds on the ant brood before pupating (Clark and Dickson, 1960). Why these species are accepted by the ants is not immediately apparent. *Feniseca tarquinius* F. (Spalginae) was investigated by Malicky (1969, 1970) and found to have perforated cupolas. This is probably true for all the species in these subfamilies. Both *Thestor dicksoni* and *Lachnocnema bibulus* are carried by their host ants into the nest indicating the possible presence of some chemical substance which may induce this behaviour (see p. 83 for similar situation in *L. ignota*). Thus the perforated cupolas present in these subfamilies may produce a secretion that induces the ants, in certain instances, to carry the larvae into the nests.

A fourth carnivorous subfamily, the Liphyrinae, resembles those above in that it lacks the honey-gland, but differs from them in that it has one genus that has tubercles. They prey on ants, Homoptera and feed on ant regurgitations. The larvae of this group are all somewhat limpet-like in shape with a slightly expanded border that completely covers the legs, head and ventral portion. The cuticle of the upper surface is very tough and leathery in the *Euliphyra* and *Aslauga*, but is hard in *Liphyra*, so that ants are unable to bite through it. The larvae protect their heads and legs from attack by the ants by bringing the peripheral border of the body into contact with the substrate. In the genus *Aslauga* there are two small external tubercles on the eighth segment, but Jackson (1937) never saw the tubercles exerted and they may be non functional. This suggests that the tubercles may have been present in the ancestral form but have become non-functional or lost as they evolved the heavy 'carapace' for protection.

We then have a subfamily the Hamearinae that not only lacks both the tubercles and honey-gland, but also the perforated cupolas. *Nemeobius lucina* L. was the only species that Malicky (1970) investigated in the laboratory that failed to induce an ant association. This may be due to the lack of the perforated cupolas that are believed to produce a volatile chemical secretion that attracts ants.

The next group of subfamilies, Curetinae, Plebejinae, Everinae, Brephidinae, Zizeerinae, Lycaenopsinae, Theclinae and Lycaeninae, are all entirely phytophagous. All these subfamilies feed on Angiospermae (Embryophyta) and are usually attended by ants but are not dependent on

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them. Most species usually have both honey-gland and tubercles, although both or either may be lost. No species belonging to these subfamilies is carnivorous nor have they ever been recorded inside the nests of ants.

The species in the subfamily Aphaeinae are all phytophagous, but are dependent on their ant hosts. Several species enter ants nests for shelter and protection, but they have never been known to feed on the ant brood. The ants construct special structures on the plants to protect the larvae of certain species. *Poecilmitis brooksi* shelters in small structures resembling miniature ants nests attached to the stem of its foodplant constructed by their host ant *Crematogaster peringueyi* Emery (Dickson, 1959). *Spindasis namaqua* shelters at the base of its foodplant in "brood chambers" constructed by their host ant (see p. 45). They usually have both honey-gland and tubercles. The two species which actually shelter in an ants nest, *A. dentatis* and *A. thyra*, have both lost the honey-gland.

In the Lampidinae there are a variety of feeding habits. Some larvae are entirely phytophagous and usually attended by ants, while others are carnivorous. *Triclema lamios* Hew. feeds entirely on Homoptera (Farquharson, 1922). The most interesting genus is the *Lepidochrysops* as all species are phytophagous for two instars, then induce the ants to carry them to their nest where they feed on the ants brood. A similar situation occurs in the subfamily Glaucopsychinae which can be phytophagous or phyto-predaceous. In both these subfamilies the honey-gland is always present, but the tubercles are often lost. It appears that only two genera, the *Lepidochrysops* (Lampidinae) and the *Maculinea* (Glaucopsychinae) actually induce the ants to carry them into the nest where they consume ant brood.

From the above it appears that only three of the numerous subfamilies of the Lycaenidae are suitable for the initial study of the chemical communication between lycaenid larvae and ants. These are the Aphaeinae, Lampidinae and Glaucopsychinae. They are suitable because they are the only ones that have species that are phytophagous, enter or are carried into the ants nest, ar[illegible]us and have perforated cupolas, honey-gland and tuberc[illegible] carnivorous subfamilies are not suitable as they do not have[illegible]s or tubercles and some have developed means of protection o[illegible], chemical, for example, the hard leathery

'carapace' of the Liphyrinae. The same applies to the lichen feeders, they all lack the honey-gland and tubercles, are protected by long hair and are generally ignored by the ants. The large group of angiosperm feeders are not suitable as they are not dependent on ants, never enter their nests and are never carnivorous.

The three species to be studied in detail were selected from the Aphaeinae and Lampidinae. The Glaucopsychinae were not considered as their feeding habits are similar to that of the Lampidinae and they are also a mainly Palearctic or Nearctic subfamily not represented in Africa.

To determine the importance of chemical communication within the Aphaeinae, *Aloeides dentatis* (Swierstra) was selected as it was one of the species known to enter the nest of an ant. For the Lampidinae *Lepidochrysops ignota* (Trimen) was chosen as it belongs to the phytopredaceous genus. Also from this subfamily *Euchrysops dolorosa* (Trimen) was chosen as an example of a purely phytophagous species that has both honey-gland and tubercles but is only occasionally attended by ants on the foodplant.

4. CHEMICAL COMMUNICATION BETWEEN LYCAENID LARVAE AND ANTS

Three myrmecophilous relationships were investigated. The first was between the phytophagous *Aloeides dentatis* (Swierstra) (Aphaeinae) and its attendant ant *Acantholepis capensis* Mayr and the second was between the phyto-predaceous *Lepidochrysops ignota* (Trimen) (Lampidinae) and its host ant *Camponotus niveosetosus* Mayr. The third lycaenid species *Euchrysops dolorosa* (Trimen) (Lampidinae) was investigated as it is an example of a purely phytophagous species that has both honey-gland and tubercles but is only occasionally attended by ants on the plants. In the study area no particular ant species was observed to tend this

4.1 Materials and Methods

The colonies of ants and the lycaenid larvae were all collected at Witpoortjie, near Krugersdorp in the Transvaal. It was at this site that the field observations were undertaken.

For behavioural investigations, ant colonies were maintained in formicaria each consisting of a nest placed in a large arena (fig. 19). The nests were made following the specifications suggested by Skaife (1961) and Claassens (1974). The nest was cast as a sheet of plaster of Paris into the upper surface of which was moulded the living chamber covered by a well-fitting glass sheet divided into two parts. A trough into which water could be poured was also moulded into one end so that a suitable humidity could be maintained in the living space. When the ants were not under observation a piece of hardboard was replaced by a piece of red cellophane so that the ants and lycaenid larvae could be observed without disturbance. The nest was placed in a large arena consisting of a flat tray with hardboard base and wooden sides (fig. 19). The walls were lined with thin plastic and painted with the substance Flouon (Polytetraflouroethylene (PTFE) Dispersion) which dries into a fine powder which prevented the ants from escaping.

To study the interaction between the lycaenid larvae and ants, the larvae were placed directly on the floor of the arena, or in the case

of *L. ignota* and *E. dolorosa* the inflorescence of the host plant was placed in the arena.

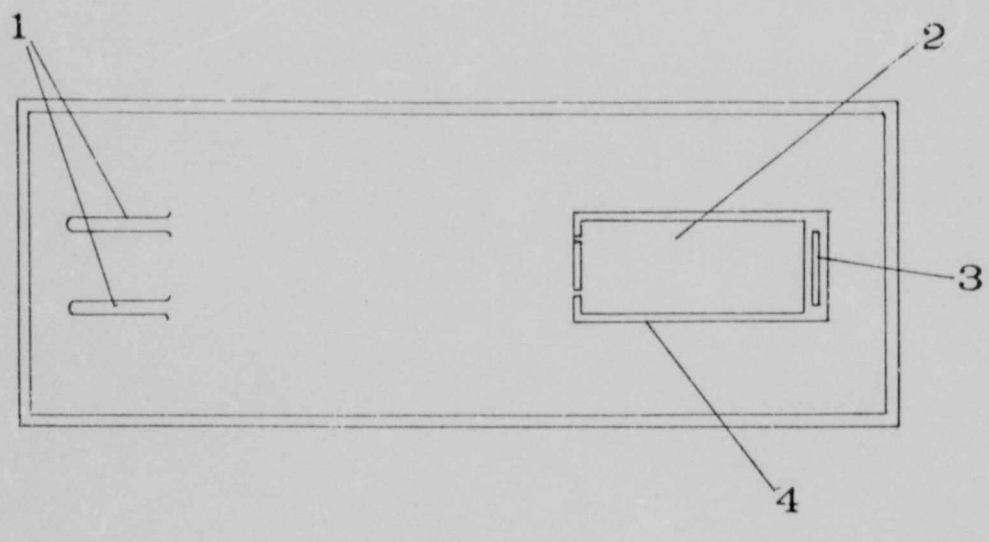


Fig. 19. Plan of formicarium. (1) Test tubes containing sugar water and water respectively; (2) living space of nest; (3) trough for water; (4) plaster of Paris nest.

In the investigation to see whether the presence of the host ants effected egg laying behaviour, 20 adult female lycaenids were captured and placed in containers. Ten females were given only the foodplant to lay on and ten were given foodplant plus host ants. The foodplant was collected from an area where no ants were present.

Before chemical analysis could be performed the pheromones had to be extracted from the bodies of the ants and lycaenid larvae. The solvent used to extract these chemicals was Dichloromethane. The worker ants and lycaenid larvae were killed by chilling. The worker ants were then decapitated and the abdomens removed. The head and abdomen were immediately transferred to separate airtight bottles of the solvent. The honey-gland, tubercles and cuticles were stripped from the chilled lycaenid larvae. The honey-gland or the tubercles with the surrounding cuticle and the cuticle from the thoracic area

were transferred to separate bottles of dichloromethane. For the first association the heads and abdomens of thirty *A. capensis* workers were extracted per ml solvent, while it was found that a single final instar of *A. dentatis* provided enough secretion to give adequate results. For the second association the heads and abdomens of twenty *C. niveosetosus* workers and ten *L. ignota* were used per ml solvent. To obtain brood pheromone whole ant larvae were transferred to a bottle of the solvent. Ten ant larvae were used per ml dichloromethane. All specimens were left for at least 24 hours to allow the maximum amount of pheromone to be extracted out of the tissue by the solvent.

Gas-liquid chromatographic analysis of the extracts were performed on a Packard-417 gas chromatograph equipped with flame ionisation detectors. The glandular products were resolved on a 230cm X 6mm O.D. glass column packed with 20% Carbowax 20M on Anachrom ABS (90/100 mesh). Nitrogen was employed as the carrier gas at a flow rate of 20ml/min. The column temperature was maintained at 80^{o}C for 5 minutes, then programmed at 5^{o}C per minute to a final temperature of 200^{o}C, held for 10 minutes. Carbowax 20M was chosen as the liquid phase, as it approximates the polarity of the suspected sample components. Quantitative analyses were performed using an Autolab Minigrator.

The ant and lycaenid extracts were run alternately on the gas chromatograph so that conditions applying at the time were virtually identical for both. This allowed the chromatograms to be compared directly by superimposing one upon the other over a light box. Comparison of the retention times of components of the extracts as recorded by the Autolab Minigrator confirmed the observations made over the light box.

To aid in the comparison of the chromatograms which were run on different days, when running conditions may have been different, the relative retention times of the peaks were calculated. This was done by selecting a peak common to both ant and lycaenid extracts, as determined above, and using it as a standard to calculate the relative retention times. This peak must be easily identifiable even though the retention times may vary between different days.

$$\text{Relative retention time (RRT)} = \frac{\text{retention time of unknown peak}}{\text{retention time of Std. peak}}$$

Behavioural experiments which involved the use of extracts from

the various lycaenid larvae and ant worker glands were performed as follows. The extracts were obtained in a similar manner to those used for gas chromatographic analysis. The specimens were extracted in airtight bottles of the solvent dichloromethane.

The heads and abdomens of thirty *A. capensis* workers were removed and extracted in separate bottles of dichloromethane. To obtain brood pheromone twenty *A. capensis* larvae extracted per ml solvent. A single final instar larva of *A. dentatis* was extracted per ml solvent. The cuticle from the thoracic area and the tubercles and their surrounding cuticle were extracted in separate bottles of dichloromethane.

Similarly the heads and abdomens of ten *C. niveosetosus* were removed and extracted in dichloromethane. To obtain brood pheromone ten *C. niveosetosus* larvae were extracted per ml solvent. Five third instar *L. ignota* larvae were extracted per ml solvent. The cuticle from the thoracic area and the honey-gland and its surrounding cuticle were extracted in separate bottles of dichloromethane.

For the third investigation two final instar *E. dolorosa* larvae were extracted per ml dichloromethane.

Following the method of Glancey *et al* (1970) corn cob grits (corn crushed small enough for the ants to pick up) were obtained and extracted in dichloromethane using a Sohxlet apparatus to remove any impurities (Vogel, 1948). The corn cob grits were soaked in the various extracts of both ants and lycaenid larvae for 24 hours. The grits were removed from the bottles with tweezers and left for 5 minutes to allow most of the solvent to evaporate, then placed in the arena of the respective formicaria along a line 4cm from the entrance to the nest. Only 2 or 3 grits were placed in the arena at a time so that the responses of the ants to each grit could be recorded in detail. A total of 30 grits in all were used for each extract.

The response of the ants to untreated grits and to grits soaked in dichloromethane for 24 hours, was tested.

4.2 Results

4.2.1 *Aloeides dentatis* (Swierstra) and *Acantholepis capensis* Mayr.

4.2.1.1 Field observations

A. *Aloeides dentatis* (Swierstra)

(I) Eggs and early larval instars

In the study area the foodplant is *Hermania depressa* N.E.Br. (Sterculiaceae). Two eggs are laid next to each other on the under surface of the leaves of the foodplant. The first and second instars eat only the surface of the leaf, leaving patches or short furrows. The third instar starts to feed on the margins of the leaves. During this period the larvae are attended by the ant *Acantholepis capensis* Mayr. The first two instars do not appear to leave the foodplant as they are small enough to be concealed amongst the leaves. The third instar larvae are usually absent from the foodplants during the day as they shelter amongst the ant brood within the nest.

(II) Late larval instars

The third to sixth larval instars shelter during the day in the nest of the ant *Acantholepis capensis*. *A. dentatis* found in the nest were always attended by ants. The larvae tend to congregate together in the nest often with the brood of the ants. The largest number of larvae found in a single *A. capensis* nest was four.

The larvae appear to remain in the ants nest over the winter months when their foodplant is not available. Fifth and sixth instar larvae found in an *A. capensis* nest in July were in a dormant state and remained so for a considerable time. The sixth (final instar) larva finally pupated in October without further feeding. The fifth instar commenced feeding at this time and finally moulted into the final instar in February of the following year. The actual duration of this instar could not be recorded, but 196 days had elapsed between the date of its discovery to the time it finally moulted. The duration of the sixth instar during the summer months is some 42 days, but is obviously much longer during the winter months when diapause takes place

(III) Pupa

The pupae are found in the ants nest attached by their cremastral hooks on the floor of the tunnels. The pupae have always been in side tunnels, away from the main brood chamber. This stage lasts some twenty days.

(IV) Egg laying behaviour of female

During the course of the study 25 females were observed in the field and their behaviour was found to be the same in all instances.

The females would settle in an area and investigate the *H. depressa* plants with their antennae. They would walk for a considerable distance passing their antennae over the ground and over the plants (see fig. 20). One female was watched for 45 minutes in which time she did not take to the wing once. She walked about three metres during which time she investigated numerous plants, but did not lay a single egg. After 45 minutes she came to a *H. depressa* plant near an *A. capensis* nest which was frequented by the ants. The *A. dentatis* female investigated the plant and surrounding area by slowly passing her antennae over the surface. She then climbed on to the leaves of the plant, all the time passing her antennae over the surface. She then curved her abdomen over the edge of the leaf and laid two eggs in quick succession next to each other on the under surface and then flew off. All females observed displayed a similar behaviour pattern to the one described above. There was no actual interaction between the female and the ants and in some



Fig. 20. *Aloeides dentatis* (Swierstra) male walking on the ground.

instances there were no worker ants present on the plant at the time of ovipositing. In these instances ants were observed on the plants before the *A. dentatis* female investigated it and oviposited. It appears that something deposited by the ants may cause the female to lay. Watching the females laying was found to be the easiest way to obtain eggs.

To test the hypothesis that ants were necessary for egg laying to occur, 20 females were captured. Ten females were placed in a container with the foodplant alone, and ten with the foodplant plus ants. The result was that all ten of the females housed with *H. depressa* and the ants laid at least two eggs, while none of those placed with the foodplant alone laid even a single egg. Three of the females outlived the ants placed with them, but continued to lay even though the ants were no longer present.

B. *Acantholepis capensis* Mayr

This is a small shining black monomorphic ant only 3mm long. It nests in the soil, under stones, and the colonies are never very large, usually consisting of only two or three hundred individuals.

During hot dry weather it was difficult to find as it was deep under ground, but after rain, when the ground was still damp, it brought its brood up to the surface and piled it immediately under a stone.

There are usually two or three queens in the nest. The queens are 5mm. long and dull brown in colour. The eggs, larvae and pupae are usually piled together. The cocoons are pale yellow in colour

4.2.1.2 Formicarium observations

A. *Aloeides dentatis* (Swierstra)

(I) Descriptions of early stages

For a full description and duration of the egg, larval and pupal stages see Appendix I. For a photograph of a final instar larva see fig.21.

(II) Interaction between *A. dentatis* and *A. capensis*

In the study of the behaviour of the ants towards the lycaenids it was found that third, fourth, fifth and sixth instar larvae placed in



Fig. 21. Final instar larva of *Aloeides dentatis* (Swierstra).

the arena near the nest were all treated in a simila[illegible]che ants. The larvae were soon dicovered by the ants in the[illegible]ng. The ants ran up to the larvae and begen stroking them with their antennae, concentrating on the area dorsally on the thorax and around the tubercles. The larvae are much larger than the ants which would run all over them (fig. 22). The *A. dentatis* larvae responded by extruding their tubercles, usually both at the same time, which resulted in the ants within a range of 2-4cm becoming alerted. The ants rate of locomotion increased, they made short fast runs with frequent changes of direction, with mandibles held open and had increased antennal movement. The ants within a 2cm range were also alerted, but were also attrcted directly towards the lycaenid larva. After about four hours the *A. dentatis* larvae had usually made their way to the *Acantholepis capensis* nest, accompanied at all times by the ants. The *A. dentatis* larvae followed the same pheromone trails used by the ants which discovered them.

In the nest the *A. dentatis* larvae congregated together and remained motionless most of the time. The ants constantly attended the larvae, either stroking them slowly with their antennae or just standing on or near them. The droppings of the larvae were removed by the ants to the

"refuse pile".

The *A. dentatis* larvae usually left the nest between 1900 and 2100 hours to feed. Often the larvae would leave the nest together, following one behind the other. The *A. capensis* ants in their foraging continually followed the same path to the sugar water placed at the opposite end of the arena to the nest and it was this same route that was followed by the *A. dentatis* larvae when they left the nest. They appeared to be following the odour trails laid by the ants in their foraging. The foodplant was usually placed two thirds of the distance along the arena just to the left of, but touching, the trail to the sugar water.

Once the *A. dentatis* larvae started moving they repeatedly extruded their tubercles, usually both at the same time. This alerted the attendant ants. They would make short fast runs with frequent changes of direction around and over the larvae, with mandibles held open and with increased antennal movement. The focus of attention was the tubercles. Several workers usually ran up to the tubercles and if the long crowning setae were touched by the ants' antennae, they were retracted immediately.

As the *A. dentatis* larvae made their way along the odour trails towards the foodplant the tubercles were extruded at intervals keeping the ants in attendance at a high level of alertness.

During the actual feeding, when the larvae were stationary, the tubercles were not repeatedly extended and the ants in contact with them were not at such a high level of alertness as when the larvae were moving. The number of ants remained relatively constant around the larvae as they fed. The activity of the ants on or close to the larvae was greater when the larvae were feeding than when they were at rest in the nest because of the occasional extrusion of the tubercles. There were usually from 3-10 ants present around each larva.

After feeding the larvae returned to the nest by following the same pheromone trail back. This may be ½ to 2 hours after first leaving the nest. The ants were again kept at a high level of alertness by the extrusion of the tubercles.

If the ants were removed from the formicarium it was found that after one or two days the *A. dentatis* larvae did not return to the nest after feeding in the arena, but tended to shelter amongst the leaves of

the foodplant.

If the *A. dentatis* larvae were disturbed in any way they would respond by extruding their tubercles. Several larvae were deliberately disturbed by touching them with the hairs of a paint brush. This resulted in the larvae extruding their tubercles and causing increased activity in the attendant ants in the vicinity. This behaviour was similar to that exhibited by the ants to the *A. dentatis* larvae leaving the nest to feed.



Fig. 22. Fifth and sixth instar larvae of *Alceides dentatis* (Swierstra) with the ant *Acantholepis capensis* Mayr.

(III) Summary of the reactions of *Acantholepis capensis* to *Alceides dentatis*

1. *A. dentatis* undisturbed in nest (tubercles not extruded):-

   The ants stand on or near the larvae often passing their antennae slowly over the surface of the body, usually concentrating on the prothoracic region and the area around the tubercles.

2. *A. dentatis* disturbed (tubercles repeatedly extruded and retracted):-

   When the tubercles are extruded the ants within a radius of 2-4cm

become alerted. Their rate of locomotion is increased, they make short fast runs with frequent changes of direction, with mandibles held open and increased antennal movement. The ants within a 2cm radius are also alerted, but they are attracted towards the tubercles. If the tubercles were touched by an ant they would be immediately and rapidly retracted.

3. *A. dentatis* moving (tubercles repeatedly extruded and retracted):-

The ants are kept at a high level of alertness. They follow the larvae, running rapidly around and over it as it moves along. The mandibles are held open and there is considerable antennal movement. The focus of attention of the ants appeared to be the tubercles.

4. *A. dentatis* feeding (tubercles occasionally extruded and withdrawn):-

The activity of the ants on or within 3cm of the larvae is greater than when it is at rest in the nest. The activity appears to be kept at this slightly higher level by the occasional extruding of the tubercles.

4.2.1.3 Reaction of the ants to the glandular extracts absorbed on to corn cob grits

The following behavioural responses were obtained from worker ants which discovered the grits.

A. *A. capensis* extracts

(I) Head extract (Table 5)

The grits were placed in the arena (see p. 63) and after 20-30 seconds the worker ants within 2-3.5cm became alerted. Their rate of locomotion increased, they started making short fast runs with frequent changes of direction with mandibles usually open and considerable antennal movement. The ants within 2cm were attracted directly towards the grits. For the first 5 minutes the ants appeared to be repelled from touching the grits. After this period of time the ants attracted to the grits investigated them with their antennae, but after 10-20 seconds would lose interest and wander off, often only to be attracted again and repeat the performance. The ants remained at this high level of activity for some 5-10 minutes.

Table 5. The reactions of *Acantholepis capensis* workers to various glandular extracts absorbed on to corn cob grits

Glandular extracts on grits	n	Reactions of *A. capensis* workers			
		no reaction	Alarm Behaviour	Investigation	Carrying & brood tending behaviour
A. capensis					
Mandibular gland	30		30		
Abdominal extract	30	4		26	
Brood extract	30	1		1	28
A. dentatis					
Thoracic area	30	1		6	23
Tubercles and surrounding area	30	1	29	17'	12'
Dichloromethane	30	30			
Untreated grits	30	30			

n = number of individual grits

' = After alarm behaviour

Foraging ants investigated the grits for about an hour.

(II) Abdominal extract (Table 5)

The grits were placed in the arena (see p. 63) and after 30-60 seconds the worker ants within 2-2.5cm raised their antennae and swept them through the air. With considerable antennal movement the ants would approach the grits. They would investigate the grits with their antennae for as long as five minutes, but then wander off only occasionally returning. The grits were generally investigated by the ants for about 30 minutes after which they were ignored or removed to the refuse pile.

(III) Brood extract (Table 5)

The grits were placed in the arena near the entrance to the nest and after 40-45 seconds the ants within 2cm raised and extended their antennae and started to investigate the air with them. They would approach the grits and investigate them with their antennae. The ants then picked up the grits and carried them into the nest. In the nest the grits were treated as if they were brood. The ants appeared to groom the grits and rubbed them with their antennae. When the colony was disturbed by sharp tapping that caused an alarm reaction, the worker ants picked up the treated grits and ran about with them as they normally did with brood. After about three hours the grits were removed from the nest to the refuse pile.

B. *A. dentatis* extracts

(I) Thoracic area (Table 5)

The response of the ants to the grits soaked in extracts from the thoracic area were usually similar to those obtained with the ant brood extract. As the worker ants passed nearby the grits they would raise and extend their antennae and sweep the air with them. They would then approach the grits and slowly run their antennae over the surface. The ants then (in 77% of the cases (see Table 5)) picked up the grits and carried them to the brood in the nest. In the nest the grits were treated as if they were brood. The ants appeared to groom the grits

Author Henning Stephen Frank
Name of thesis Chemical Communication Between Lycaenid Larvae (lepidoptera: Lycaenidae) And Ants (hymenoptera: Formicidae). 1980

PUBLISHER:
University of the Witwatersrand, Johannesburg
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