DISTRIBUTION, COMPOSITION AND WATER PERMEABILITY OF LOCUST CUTICULAR LIPID

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A thesis presented for the degree
of Doctor of Philosophy in the
University of Glasgow,
Faculty of Science,
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May 1989

Declaration:

I declare that this thesis represents, except where a note is made to the contrary, work carried out by myself. The text was composed by myself.

Victor Shabi Oraha

10 May 1989

To my parents

with love and respect

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Acknowledgements

First, I would sincerely like to thank my supervisor Dr K.H. Lockey for his invaluable supervision, constant encouragement, constructive criticism and excellent example throughout the course of this study.

I also thank Professor R.S. Phillips for making departmental facilities available.

I also wish to thank Professor A.A. Watson for use of V.G. Micromass gas chromatograph-mass spectrometer and Dr A.A. Anderson for the gas chromatograph-mass spectrometer analyses.

I wish to take the opportunity to thank the following people: Dr L. Tetley, Mrs H. Hendry and Mrs M. Mullin for their help with the scanning and transmission electron microscopy; Miss M. Gardner; Mr P. Rickus for their help in printing the electron micrographs; Mrs E. Denton for her help in preparing and labelling figures and plates and Mr J. McColl for advice on the statistical analyses. Thanks are also due to Mr A. McGregor for making the cuticle holders and Mr W. Orr, the departmental Storeman.

My sincere thanks also to the Iraqi government, Ministry of Higher Education for a government scholarship and financial support.

Finally, I would like to thank my family for their understanding, support and encouragement during the whole of my education.

SUMMARY

In this work, the distribution, chemical composition and water permeability of the cuticular lipids of the African migratory locust, *Locusta migratoria migratoriodes* and the desert locust, *Schistocerca gregaria* have been investigated.

Scanning electron microscopy of the wings and terga of the two species showed that the epicuticular lipid formed a liquid coating over the surface. The lipid layer on the fore wing of *L.migratoria migratoriodes* was thinner and more crystalline than that of *S.gregaria*. The tergum of *L.migratoria migratoriodes* had a smooth epicuticular lipid layer while that of *S.gregaria* had a lipid layer with amorphous lipid masses.

Transmission electron microscopy revealed that the wing and tergal cuticle of both species conformed to the basic structural plan of insect cuticle, namely an outer, thin epicuticle and an inner, thick procuticle divided into an outer exocuticle and an inner endocuticle, overlying a single layer of cells, the hypodermis. While no structural differences were observed in the wing cuticle of the two species, their terga differed, particularly in the procuticle. In *L.migratoria migratoriodes*, the procuticle was about 3 times thicker than that of *S.gregaria*, while the endocuticle of the latter possessed well developed lipid lamellae which were only poorly developed in the endocuticle of *L.migratoria migratoriodes*.

Chemical analysis of the cuticular lipids of *L.migratoria migratoriodes* and *S.gregaria* using thin layer chromatography revealed the presence of five major fractions namely, hydrocarbons, esters, triglycerides, free fatty acids and free primary alcohols.

The cuticular lipid of *S.gregaria* contained higher proportions of

hydrocarbons (wings) and triglycerides than the lipid of *L.migratoria migratoriodes*, while the cuticular lipid of the latter had higher proportions of esters, free fatty acids and alcohols.

47% of the free fatty acids, triglyceride fatty acids and ester fatty acids in *L.migratoria migratoriodes* were unsaturated, while only 3% were unsaturated in *S.gregaria*.

The hydrocarbons of the two species, which were saturated and comprised n-alkanes and methylalkanes, consisted of the following classes: n-alkanes (class A), terminally branched monomethylalkanes (class C_2, 3-methylalkanes), internally branched monomethylalkanes (class D) and dimethylalkanes (class E). *S.gregaria* possessed one more class, namely, trimethylalkanes (class F). The hydrocarbon mixture of *S.gregaria* contained a higher proportion of n-alkanes and a lower proportion of methylalkanes than the mixture *L.migratoria migratoriodes*.

The lipid layer on the hind wing of *S.gregaria* was found to be thicker than that of *L.migratoria migratoriodes*.

The intact hind wings and abdominal terga of *S.gregaria* were found to have lower water permeabilities at temperatures 25, 35, 45, 55 and 65^{o}C than those of *L.migratoria migratoriodes*.

Extracted hind wings had 8-times the water permeability of intact wings in both species. While the extraction of abdominal terga doubled water permeability in *L.migratoria migratoriodes* and increased permeability 1.5 times in *S.gregaria*.

The water evaporation from the intact wings and terga of both species was found to rise over a range of 40^{o}C. A gradual rise in water permeability was found to occur between 25 and 45^{o}C and a steep rise between 45 and 65^{o}C. The shape of the water evaporation curves of the two species was similar, though the curve of *S.gregaria*

was shifted to the right of that of *L.migratoria migratoriodes*, indicating a lower water permeability in *S.gregaria* over a range of temperatures.

The effect of amount/unit area, composition and temperature on the water permeability of lipid was investigated by spreading synthetic and natural mixtures of lipids on the extracted hind wings of *S.gregaria* and *L.migratoria migratoriodes*.

Natural mixtures of lipids were found to be more efficient in reducing water permeability than synthetic mixtures. Synthetic and natural mixtures of n-alkanes, alcohols and esters formed layers which were highly impermeable to water, while synthetic mixtures of alkenes and synthetic and natural mixtures of fatty acids and triglycerides formed layers which were permeable to water. Natural mixtures of methylalkanes were only partially efficient in reducing water permeability, while synthetic mixtures of methylalkanes formed highly permeable layers.

Methylalkanes, alkenes and alcohols were found to spread readily over a cuticle surface, while n-alkanes and fatty acids showed only limited spreading. Triglycerides followed by esters showed the lowest spreading characteristics.

Compared with *L.migratoria migratoriodes*, *S.gregaria* has a thicker lipid layer on the hind wings, a cuticular lipid containing a high proportion of n-alkanes and a low proportion of unsaturated fatty acids and terga with well developed endocuticular lipid lamellae. All of these features may be regarded as adaptations to living in arid desert conditions.

CHAPTER ONE

GENERAL INTRODUCTION

Water conservation is a vital mechanism for terrestrial species which live under desiccating conditions and have limited access to free water (Beament, 1961; Ebeling, 1974; Hadley, 1972, 1981). The complex mixture of lipids which occurs in the epicuticle and which forms an efficient water barrier contributes to water conservation by greatly reducing the amount of water lost by evaporation (Ahearn, 1970; Appel *et al.*, 1986; Beament, 1961, 1964; Cooper, 1983; Ebeling, 1974; Edney, 1977; Gilby, 1980; Hadley, 1972, 1980a,b, 1981, 1984; Hadley *et al.*, 1986; Juliano, 1986; Kimura *et al.*, 1985; Locke, 1965; Machin, 1980; Machin & Lampert, 1985; Machin *et al.*, 1985; Toolson, 1978, 1982, 1984; Toolson *et al.*, 1979; Wharton & Richards, 1978). These lipids also protect against entry of microorganisms and chemical sprays, shield against excess ultraviolet radiation and are important in chemical communication (Hadley, 1980a, 1981; Blomquist & Dillwith, 1985).

In plants and arthropods, particularly insects, cuticular lipids occur either as free surface lipid or as bound lipid impregnating the cuticular layers. The chemical composition of lipid components extracted from plant and arthropod cuticles is similar (Hadley, 1981). Scanning electron microscopy has enabled investigators to describe in more detail the form of these surface lipids (Hadley, 1981, 1982; Hendricks & Hadley, 1983). Surface lipids assume different forms in both plants and insects (Hadley, 1981). In both groups, the surface lipid occurs either as a thin film which uniformally covers the cuticle surface often obscuring details of cuticular microstructures (Hadley, 1981, 1982; Hendricks & Hadley, 1983) or as wax deposits in the form of rods, filaments, threads, plates or crusts (Hadley, 1981).

The cuticle of both plants and arthropods is a multilayered structure which overlies a single layer of cells, the hypodermis, to

form the integument (Hadley, 1980a, 1981; Hendricks & Hadley, 1983; Hepburn, 1985; Locke, 1974; Neville, 1975). The cuticle also forms a thin lining to integumentary openings such as stomata in plants and spiracles in arthropods (Hadley, 1981). In plant cuticle, the cutin which is composed of cross-esterified fatty acids (Holloway, 1977) is responsible for the structural integrity of the cuticle, while the chitin-protein complex gives structural integrity to arthropod cuticle (Hadley, 1981, 1986; Hepburn, 1985; Neville, 1975).

Environmental factors, such as temperature and humidity influence the amount, morphology and chemical composition of surface lipids in both plants and arthropods. Changes in these features can affect water relations. One example from plants is the Brussel sprout *Brassica oleracea*. The amount of lipid on the leaves of this plant increases as temperature and humidity decrease. The surface lipid also changes its form from rods, through to tubes, dendrites and finally to ribbons (Baker, 1974). This worker also noted that plants grown at low temperatures have higher proportions of n-alkanes and lower proportions of aldehydes than plants grown at high temperatures when large lipid plates are formed. Similar structural changes occur particularly on the lower surfaces of the leaves of *Clarkia elegans* (Hunt *et al.*, 1976) where lower temperature caused a marked reduction in the proportion of ketones and an increase in the proportion of hydrocarbons. In arthropods the desert tenebrionid beetle, *Eleodes armata*, for example, shows higher quantities of hydrocarbons and higher proportions of long-chain molecules in summer-active beetles than in winter-active beetles. Further, winter-active beetles acclimatized to summer temperatures show higher proportions of long-chain hydrocarbons compared to winter-active controls (Hadley, 1977).

In arthropods, many investigators have demonstrated a marked increase in transpiration following removal of surface lipids by

solvents, detergents and abrasion (Beament, 1945; Ebeling, 1974; Hadley & Quinlan, 1987; Toolson, 1982, 1984; Wigglesworth, 1945). In plants, Schönherr (1976) studied the diffusion of tritiated water across the isolated cuticles from leaves of the Seville orange, *Citrus aurantium* and the pear, *Pyrus communis* before and after the removal of surface lipids with solvents. Schönherr found that the water permeability of the extracted cuticle was determined entirely by the surface lipids. Skoss (1955) also demonstrated the importance of impregnated lipid and cutin in the water permeability of plants.

Despite the detailed investigation of plant and arthropod cuticle (Hadley, 1980a, 1981, 1986; Hepburn, 1985; Locke, 1974; Neville, 1975), there is at present no satisfactory explanation as to how lipids waterproof cuticle.

In the work to be described:

A) The wings and abdominal terga of two species of locust, namely the African migratory locust, *Locusta migratoria migratoriodes* (shortened in the text to *Locusta migratoria*) and the desert locust, *Schistocerca gregaria* have been used as sources of cuticle to investigate:

   1. The distribution and composition of natural cuticular lipid
   2. The effect of amount , composition and temperature on the water permeability of natural and synthetic lipid
   3. The effect of composition on the spreading features and physical characteristics of natural and synthetic lipid.

B) The relationships between the above factors are discussed in the context of the ecology of the two locust species.

CHAPTER TWO

CUTICLE STRUCTURE AND LIPID DISTRIBUTION IN *Locusta migratoria migratoriodes* AND *Schistocerca gregaria*

2.1. INTRODUCTION

Detailed accounts of insect cuticle are given by Hepburn (1985), Locke (1974) and Neville (1975). Insect cuticle consists of a thin, outer layer, the epicuticle and a thicker, inner layer, the procuticle which is usually divided into an outer exocuticle and an inner endocuticle. These two layers overlie a single layer of cells, the hypodermis. Together, the cuticle and the hypodermis form the integument. The integument protects and supports the body and it has contributed significantly to the adaptive success of arthropods (Hadley, 1986; Neville, 1975).

The epicuticle consists of several layers, namely, an outer cement layer, a lipid (or wax) layer, an outer epicuticle and an inner epicuticle (Filshie, 1970a; Hendricks & Hadley, 1983; Hepburn, 1985; Locke, 1974; Neville, 1975; Wigglesworth, 1972).

The cement layer is the outermost layer of the epicuticle and in many species it protects the underlying lipid layer. In *Rhodnius prolixus*, this layer is formed from the secretory products of two types of dermal glands (Wigglesworth, 1933, 1947). The chemical composition of the cement layer is uncertain. Lai-Fook (1972) and Wigglesworth (1975a) suggest that in *Rhodnius prolixus*, cement is a mucopolysaccharide, while Beament (1955) suggests that in *Periplaneta americana* it is a shellac. In *Tenebrio molitor* pupa, the cement layer is thought to consist of a wax stabilized with shellac (Holdgate & Seal, 1956). The honeybee, *Apis mellifera* lacks a cement layer altogether (Locke, 1961).

The lipid layer is composed of chemically unbound lipid and it forms the outermost layer of the epicuticle in those species which lack a cement layer. In *Periplaneta americana* (Kramer & Wigglesworth, 1950) and in *Rhodnius prolixus* (Wigglesworth, 1975a,b, 1985a), the

lipid layer covers and impregnates the outer epicuticle. In species such as *Rhodnius prolixus*, *Schistocerca gregaria*, *Tenebrio molitor* and *Manduca sexta*, the lipid layer also contains protein and tanning agent (Wigglesworth, 1985b). The chemical composition of the lipid layer is discussed in Chapter 3 while its contribution to cuticular permeability is discussed in Chapter 4. In *Tenebrio molitor* pupa (Holdgate & Seal, 1956), the wax layer permeates the overlying cement layer to form a surface bloom, while in *Periplaneta americana*, Beament (1945) suggests that the wax layer contains a compressed monolayer of polar molecules overlying the outer epicuticle.

Below the lipid layer is the outer epicuticle which is a very thin (12-18nm) membrane, highly resistant to acid hydrolysis (Filshie, 1970b; Wigglesworth, 1985b). Chemically, it is thought to be a mixture of lipid and a small amount of protein which hardens due to oxidation and polymerization (Dennell & Malek, 1954; Wigglesworth, 1947, 1975a, 1985b). Locke (1966) refers to the outer epicuticle as the cuticulin layer but Wigglesworth (1970) used the term cuticulin to describe the mixture of tanned lipoproteins which comprises the inner epicuticle. Dennell and Malek (1954) refer to the outer epicuticle as the paraffin layer. Hackman (1986) showed that the outer epicuticle of the larva of the sheep blowfly, *Lucilia cuprina*, is a tough resistant membrane. Examination by Nuclear Magnetic Resonance (NMR), Infra-red (IR) and Ultraviolet absorption (UV) led Hackman to suggest that the membrane consists of high molecular weight cross-linked compounds built up mainly from methylene groups.

The outer epicuticle is the first layer of the new cuticle to be deposited. Locke (1966) showed that in *Calpodes ethlius* it starts as a series of secretions at the tips of the hypodermal microvilli. These secretions then fuse to form a continuous layer, the outer epicuticle. Similarly, Greenstein (1972) found that in the wing of the silkworm,

Hyalophora cecropia, the outer epicuticle appears as patches over the hypodermal microvilli. These patches enlarge and coalesce to form the outer epicuticle. In *Rhodnius prolixus* (Wigglesworth, 1973a), the outer epicuticle starts as a characteristic curved plaque over each hypodermal microvillus. These plaques then extend laterally and fuse to form a continuous outer epicuticle which is about 17nm thick. The outer epicuticle is trilaminar in the larva of *Calpodes ethlius* (Locke, 1966), the nymphs of *Rhodnius prolixus*, *Schistocerca gregaria* (Wigglesworth, 1973a, 1975a, 1985b) and *Leucophaea maderae* (Rinterknecht, 1985) and *Hyalophora cecropia* (Greenstein, 1972).

The inner epicuticle lies below the outer epicuticle. It is much thicker than the outer epicuticle and it forms the bulk of the epicuticle. The inner epicuticle consists of a mixture of lipid bound to protein which is subjected to sclerotization (Wigglesworth, 1947, 1975a,b, 1985a). The bound lipid of the inner epicuticle, which Wigglesworth (1970) suggests consists of hydroxy fatty acids, is stained by the argentaffin reaction (Wigglesworth, 1975a,b, 1985b). Hendricks and Hadley (1983) in *Acheta domesticus* and Locke (1966) in *Calpodes ethlius* refer to the inner epicuticle as the dense homogeneous layer.

In this study, the terms outer epicuticle and inner epicuticle will be used.

The procuticle forms the bulk of the cuticle and it is composed of long, straight chains of chitin (poly-N-acetylglucosamine) held together by covalent bonds and embedded in a protein matrix (Hepburn, 1985; Neville, 1975). It is the chitin-protein complex which gives the cuticle flexibility and strength against cracking and tearing (Hepburn, 1985; Neville, 1975). Electron microscopy and x-ray diffraction have been used to investigate the chitin-protein complex

in the procuticle of insects and other arthropods (Hepburn, 1985; Neville, 1975). The chitin component of the complex occurs as microfibrils which differ in their diameters among different cuticles but which have a constant diameter within any one sample of cuticle. For example, the chitin microfibril diameter in *Schistocerca gregaria* is 27.37A^{o}. A chitin microfibril consists of from 18 to 21 chitin molecules arranged together in two or three rows (Hadley, 1986). The chitin microfibrils are joined to protein molecules at their periphery to form the chitin-protein complex which occurs in the procuticle as sheets. Within each sheet, the chitin microfibrils are parallel to each other and the sheets in their turn, are parallel to each other and to the surface of the cuticle. A collection of sheets forms a lamella, and these lamellae are observed within the exocuticle and the endocuticle by electron microscopy. Sheets within one lamella are arranged in a helix, the microfibrils in each alternating sheet being slightly rotated counterclockwise at a regular angle to those in the underlying sheet. A 180^{o} rotation of chitin-protein sheets forms a lamella. Some sheets may have unidirectional microfibrils only. For example, locusts produce unidirectional sheets during the day and helicoid sheets at night (Neville, 1975).

Insect chitin occurs as three polymorphs, namely, alpha-chitin, beta-chitin and gamma-chitin which differ in their molecular chain orientation (Hepburn, 1985; Neville, 1975). Protein types may also vary in cuticle as well as the protein-chitin ratio and the degree of sclerotization (Hepburn, 1985; Neville, 1975). Neville (1975) describes four types of cuticle. The first type is the solid cuticle, which is found in sclerites and which consists of chitin and beta-extended protein. Type 2 cuticle contains the rubber-like protein, resilin, which is a molecule of coiled polypeptide chains linked together to form a network. Resilin provides elasticity to the cuticle

and it occurs for example in the rubber-like cuticle of the tarsal pads of locusts. The third type of cuticle is the soft cuticle which is found in arthrodial or intersegmental membranes. The proteins of soft cuticle are not sclerotized, so that this type of cuticle is flexible and stretchable. The last type of cuticle is the transitional cuticle which has physical and chemical properties intermediate to those of solid and rubber-like cuticle. Lens cuticle of the compound eye is an example of transitional cuticle.

The procuticle is usually divided into an outer hard region, the pre-ecdysial cuticle or exocuticle in which proteins are subjected to phenolic tanning following impregnation by lipid, and an inner softer region, the post-ecdysial cuticle or endocuticle which remains untanned (Neville, 1975). In *Rhodnius prolixus*, bound lipid occurs in bands in the endocuticle to form the lipid lamellae (Wigglesworth, 1976). In some insect species, for example, the common house cricket *Acheta domesticus* (Hendricks & Hadley, 1983), a mesocuticle occurs between the exocuticle and the endocuticle. The mesocuticle like the exocuticle is impregnated with bound lipid but is untanned (Neville, 1975).

Vertical channels penetrate the various cuticular layers, the largest of these are the ducts of the dermal glands which transport cement to the cuticular surface (Hepburn, 1985; Neville, 1975; Wigglesworth, 1975a). Their openings are often spaced at regular intervals (Hendricks & Hadley, 1983). The other channels, complexes of pore canals are narrower and more numerous than the dermal gland ducts. The pore canals run through the procuticle to the junction of the exocuticle and the epicuticle where they branch into fine wax canals (Locke, 1961) or epicuticular channels (Wigglesworth, 1975a) which perforate the outer epicuticle (Hendricks & Hadley, 1983; Locke,

1961; Wigglesworth, 1975a, 1985a).

From the hypodermis, fine cytoplasmic extensions run through the pore canals and epicuticular channels. In the pore canals, the cytoplasmic extensions are referred to as pore canal filaments or axial filaments (Wigglesworth, 1985a), in the epicuticular channels they are referred to as epicuticular filaments (Filshie, 1970a) or tubular filaments (Wigglesworth, 1985a). In the present study, the terms pore canal, pore canal filament, epicuticular channel and epicuticular filament will be used.

The complex system of pore canals and epicuticular channels and their internal filaments provide the route whereby lipid travels from the hypodermis to the epicuticle (Locke, 1961; Wigglesworth, 1975a). However, it is not known whether the lipid is transmitted within the epicuticular filaments or in the spaces between the filaments and the inner wall of the epicuticular channels (Wigglesworth, 1986). The filaments are also a means whereby the hypodermis controls processes in the cuticle (Wigglesworth, 1945).

A deposition zone, the region between the completely formed cuticle and the hypodermis has also been described in insects. It may be a single layer 1μm in width (Locke, 1961) or it may be composed of several layers (Hendricks & Hadley, 1983). The zone differs from formed cuticle in that it is more darkly staining and has a granular appearance (Neville, 1975).

The hypodermis consists of a single layer of cells underlying the cuticle and it is only seen in the fully developed state when a new cuticle is being laid down (Wigglesworth, 1972). In *Oncopeltus*, a second layer of cells has been observed between the hypodermis and the basement membrane (Neville, 1975). Hypodermal cells interconnect to form a functional syncitium (Caveney, 1976). The main function of the hypodermis is the secretion of the cuticle (Filshie, 1970a;

Greenstein, 1972; Locke, 1961; Wielgus & Gilbert, 1978; Wigglesworth, 1972). It also maintains and repairs the lipid layer (Wigglesworth, 1975a), digests and reabsorbs the endocuticle during moulting and long periods of starvation, and it is also capable of absorbing water (Neville, 1975).

The basement membrane occurs as an internal lining to the hypodermis. Early workers thought that the basement membrane was secreted by the hypodermis but Wigglesworth (1973b) has shown that in *Rhodnius prolixus* it is secreted by blood cells, particularly the plasmocytes.

In the work to be described, the form and distribution of the free epicuticular lipid on the terga and wing membranes of *Locusta migratoria* and *Schistocerca gregaria* are investigated by scanning electron microscopy. The distribution of bound lipid, visualized by the myrcene method (Wigglesworth, 1975a, 1981) and the argentaffin test (Wigglesworth, 1945, 1970, 1975a,b), is investigated in wings and terga by transmission electron microscopy.

2.2. MATERIALS AND METHODS

Fore wings and the third and fourth abdominal terga of *L.migratoria* and *S.gregaria* were used throughout this part of the work.

2.2.1. Scanning electron microscopy (SEM)

The epicuticular lipid layer of wings and terga were first examined by SEM. In both locust species, wings and abdominal terga were dissected from the body, and pieces of each were fixed to clean stubs which were painted with silver paint immediately before fixing. The specimens were left in a fumehood for 10 to 20 minutes to dry and then coated with gold for 10 minutes. As the rising temperature during

coating was likely to affect the superficial lipid layer, the cooling system of the gold coating equipment was kept on during the coating process. Specimens were then examined with a model 500 Philips scanning electron microscope and photographed without delay to minimize charging of the lipid layer by the electron beam.

Solvent extracted wings and terga were also examined. Cuticular specimens were extracted in three volumes of hot chloroform for 15 minutes each volume. In order to expose the surface sculpturing of extracted wing membranes, the wings were frozen in liquid nitrogen before fixing to the silver painted stubs.

2.2.2. Transmission electron microscopy (TEM)

The procedure given by Wigglesworth (1975) for the visualization of bound lipid under TEM was tried at first, but it failed to give satisfactory results. A later procedure by Wigglesworth (1981) however, gave acceptable results and this was used throughout the work. The procedure was as follows:

1. Epicuticular lipid was removed from abdominal terga and wings by refluxing in chloroform for 24 hours.
2. Extracted cuticular specimens were transferred to 10% sucrose solution and cut into small pieces which were then fixed in formol/glutaraldehyde (F/G-paraformaldehyde) for 24 hours at $4^{o}C$.
3. Buffer and fixative were prepared as follows:
   i. A 0.2M cacodylate stock solution was prepared by dissolving 2.14g of sodium cacodylate in 50ml distilled water.
   ii. Equal volumes of the stock solution and a 20% sucrose solution were mixed to give 10% sucrose in 0.1M stock solution.
   iii. The cacodylate buffer was then prepared by heating the sucrose/stock solution at $60^{o}C$ in a water bath and adding 2g

of paraformaldehyde with continual stirring.

iv. The fixative was prepared by adding 2ml of glutaraldehyde containing 0.2g calcium chloride to the cacodylate buffer.

4. Fixed specimens were then transferred in turn to:
   a) 1% osmium tetroxide in cacodylate buffer for 1 hour. (The 1% osmium tetroxide solution was prepared by mixing equal volumes of 2% osmium tetroxide and 0.1M cacodylate buffer).
   b) A solution containing 1% thymol and 10% sucrose and left overnight at 60°C.
   c) 4% myrcene solution for 30 minutes (5ml of myrcene added to 100ml 80% ethanol and the solution gently agitated and filtered).
   d) 1% osmium tetroxide for 45 minutes.
   e) The following series of alcohols for dehydration: 30%, 50%, 70%, 90%, 100% (twice), 10 minutes in each dilution.
   f) Three mixtures of resin dissolved in absolute ethanol for 1 hour each mixture (resin:ethanol mixtures were 25:75, 50:50 and 75:25 v/v).
   g) Pure resin, two changes, 1 hour each change.
5. Specimens were then embedded in Spurr's medium at 60°C for 48 hours.
6. Sections were cut with a diamond knife.

Some sections were examined directly and photographed, while others were double-stained with uranyl acetate and lead citrate. Sections were examined with a model 902 Zeiss transmission electron microscope.

2.2.3. Argentaffin test

This method by Wigglesworth (1945, 1970, 1975a,b) was used in the following way:

1. Extracted wings and terga of both species were treated with 1% silver hydroxide in darkness for 30 minutes. The silver hydroxide was prepared by adding 1ml of freshly prepared 5% silver nitrate solution to 4ml of a 2.5% aqueous solution of Tris (hydroxymethylaminomethane). This gave a solution with pH>9.
2. Specimens were then transferred to 96% ethanol and finally embedded in Spurr's medium. Sections were cut with a diamond knife and examined directly with the model 902 Zeiss transmission electron microscope.

2.3. RESULTS

2.3.1. Scanning electron microscopy (SEM)

Electron micrographs of intact terga and wings of both species of locusts revealed that the epicuticular lipid formed a liquid coating over the cuticular surface. On the terga of *L.migratoria* the lipid formed a smooth layer (Plate 1a) while on the terga of *S.gregaria*, the lipid layer contained amorphous lipid masses (Plate 1b). On intact wings, the epicuticular lipid of *L.migratoria* (Plate 2a) appears thinner and more crystalline than that of *S.gregaria* (Plate 2b). Removal of the epicuticular lipid from terga and wings revealed the surface sculpturing to consist of a series of repeating polygons with slightly raised edges. These polygons which coincide with the outline of each hypodermal cell are more clearly seen in extracted terga (Plates 3a & 3b) than in extracted wings (Plate 4a).

However, freezing the wings in liquid nitrogen after extraction showed up the polygons more clearly (Plate 4b). Small projections occur on the ridges of the tergal polygons (Plates 3a & 3b) and setae are clearly seen on both extracted wings and terga (Plates 3b & 4a). On intact terga and wings, the setae are covered or surrounded by lipid (Plates 1b & 2a). Openings of the dermal gland ducts are also

clearly seen with often a small spherical mass of lipid occluding them (Plate 2b). The electron micrographs of extracted terga showed that these ducts were not regularly distributed (Plates 3a & 3b). Dermal gland duct openings were not observed in extracted wings (Plates 4a & 4b). Neither were the openings of the epicuticular channels, the fine distal extensions of the pore canals which perforate the outer epicuticle, seen in either extracted terga or wings (Plates 3a, 3b, 4a & 4b).

2.3.2. Transmission electron microscopy (TEM)

Electron micrographs of terga and wing membranes showed that the cuticle of the two species conformed to the basic insect plan of an outer, thin epicuticle, an inner thicker procuticle and a hypodermis (Plates 5a,b & 6a,b). In both Plates 5 and 6, two distinct layers can be seen in the epicuticle, these are the outer epicuticle and the inner epicuticle. In both species trilamination of the outer epicuticle is clearly observed, particularly in the electron micrographs of those terga which had been refluxed with chloroform for 24 hours. Plate 7, for example, shows the trilaminar outer epicuticle of the fourth abdominal tergum of *S.gregaria* with the epicuticular channels terminating in the innermost layer of the outer epicuticle. The inner epicuticle is thicker than the outer epicuticle in both wings and terga and its bound lipid is revealed by the presence of granules darkly stained by the argentaffin reaction. This reaction also occurs in the epicuticular channels (Plates 8a & 8b).

The procuticle, with its subdivisions, exocuticle and endocuticle, makes up the bulk of the cuticle (Plates 5a,b & 6a,b). The procuticle is lamellate, the lamellae representing alternate electron lucent and electron dense bands which run parallel to the surface. The lamellae of the exocuticle are thinner than those of

endocuticle. The procuticle of the two species differ. In *L.migratoria* (Plate 5a,b), the procuticle is about three times thicker than that of *S.gregaria* (Plate 6a,b), while bands of bound lipid, the lipid lamellae, of *L.migratoria* are thinner and less obvious than those of *S.gregaria*, particularly in the endocuticle.

Mesocuticle, a region of densely staining cuticle between the exocuticle and endocuticle, was not observed in the cuticle of either species. In all the vertical sections, the first formed endocuticular lamellae are thicker than those near the hypodermis. This is clearly seen in the cuticle of *S.gregaria* (Plate 6a,b). In both species, the pore canals are clearly observed to twist as they traverse the helicoid lamellae of the procuticle (Plates 5a,b & 6a,b). In the exocuticle, the twisting of the pore canals is obvious while in the endocuticle, the pore canals appear as irregular arcs. The pore canals of *L.migratoria* (Plate 5a,b) contain less stained lipid than those of *S.gregaria* (Plates 6a,b), particularly in the exocuticle.

Epicuticular channels which branch from pore canals at the junction of the exocuticle and epicuticle can be seen in Plate 7 where they terminate in the innermost layer of the trilaminar outer epicuticle.

A deposition zone between the formed cuticle and the hypodermis was not observed in the cuticle of the two locust species.

The hypodermis in both species showed no clearly recognisable organelles (Plates 5a & 6b) and a basement membrane was only clearly seen in *L.migratoria* (Plate 5a).

No structural differences were observed in the wing cuticle of the two species. Neither were any differences observed between the single-stained and double-stained sections of wings and terga, except that in double-stained sections the hypodermis appeared more deeply stained, particularly in *S.gregaria* (Plate 6b).

2.4. DISCUSSION

2.4.1. Scanning electron microscopy (SEM)

Scanning electron micrographs showed minor differences between the epicuticular lipid of the two species. Although the epicuticular lipid of both species resembles a liquid coating on both terga and wings (Plates 1 & 2), on the terga of *L.migratoria*, the lipid assumes a smooth layer (Plate 1a) while on those of *S.gregaria* it contains amorphous lipid masses which surround the setae (Plate 1b). The epicuticular lipid on the wing of *L.migratoria* (Plate 2a) was observed to be thinner and more crystalline than that of the wing of *S.gregaria* (Plate 2b). Such liquid films of epicuticular lipid have been described in many arthropod species such as the black widow spider *Latrodectus hesperus* (Hadley, 1982) and the cricket *Acheta domesticus* (Hendricks & Hadley, 1983).

Extraction of the tergal epicuticular lipid clearly showed the surface sculpturing to consist of polygons with raised edges (Plate 3a & 3b). Extraction also revealed the setae more clearly (Plate 3b). Extracted wings showed clear polygons only when frozen in liquid nitrogen (Plate 4B). These polygons which coincide with the outlines of the hypodermal cells have been described in many species, for example *Latrodectus hesperus* (Hadley, 1982) and *Acheta domesticus* (Hendricks & Hadley, 1983).

The openings of dermal gland ducts were not observed in intact terga of either species (Plates 1a & 1b), but they were observed in the intact wing of *S.gregaria* (Plate 2b) where each opening was occluded by a spherical mass of lipid. Duct openings were clearly seen in extracted terga where they have an irregular distribution (Plates 3a & 3b). This is unlike the arrangement in *Acheta domesticus* (Hendricks & Hadley, 1983) where the ducts open regularly on every

third polygon.

Perforations in the outer epicuticle due to epicuticular channels were not observed in either extracted terga or wings. Hendricks and Hadley (1983) identified them as small depressions in the extracted cuticle of *Acheta domesticus*.

2.4.2. Transmission electron microscopy (TEM)

Transmission electron micrographs of extracted wings and terga of the two species show no differences in the structure of the epicuticle but major differences in that of the procuticle. The integument of both *L.migratoria* and *S.gregaria* consists of an outer thin layer, the epicuticle, an inner, thicker layer, the procuticle, and a single layer of cells, the hypodermis (Plates 5 & 6). Thus, the integument of both species conforms to the basic structural plan of insect cuticle (Hadley, 1982; Hendricks & Hadley, 1983; Neville, 1975).

Of the four layers present in the insect epicuticle (Filshie, 1970a; Hendricks & Hadley, 1983; Locke, 1974; Neville, 1975; Wigglesworth, 1972), only the outer epicuticle and inner epicuticle were seen (Plates 5 & 6), as extraction removes the lipid and cement layers.

The outer epicuticle is a thin trilaminar layer in both locust species (Plate 7). Trilamination of the outer epicuticle was first described in *Calpodes ethlius* by Locke (1966) and later in many other species such as *Hyalophora cecropia* (Greenstein, 1972), *Leucophaea maderae* (Rinterknecht, 1985), *Rhodnius prolixus* and *Schistocerca gregaria* (Wigglesworth, 1973a, 1975a, 1985b).

The inner epicuticle of the two locust species makes up the bulk of the epicuticle and its bound lipid was stained by the argentaffin reaction (Plates 8a & 8b). This reaction was also observed in the

outer epicuticle, which appeared as a deeply stained superficial layer, as well as in the epicuticular channels.

The procuticle which makes up the bulk of the cuticle has a lamellate structure (Plates 5 & 6). Two regions were clearly observed in the procuticle, an outer, thinner exocuticle and an inner, thicker endocuticle. The thinner exocuticle appears as tightly packed alternating electron lucent and electron dense lamellae. The endocuticle makes up the bulk of the procuticle and its lamellae are thicker than those of the exocuticle. This arrangement occurs in the cuticle of many other insect species (Hendricks & Hadley, 1983; Neville, 1975).

The tergal procuticle of the two species differ and in *L.migratoria* (Plate 5), the procuticle is about three times thicker than that of *S.gregaria*. However, the lipid lamellae of *S.gregaria* are thicker and more obvious than those of *L.migratoria*. In Chapter 4, it will be seen that even though the tergal cuticle of *S.gregaria* is about 3 times thinner than that of *L.migratoria*, it is less permeable to water than the cuticle of *L.migratoria* and it may be that the well developed lipid lamellae of *S.gregaria* contribute to cuticular waterproofing. Lipid lamellae have been described in the endocuticle of *Rhodnius prolixus* where Wigglesworth (1976) considers them to play a role in the expansion of the cuticle during a blood meal.

Although a mesocuticle was not observed in the vertical sections of the two locust cuticles, it has been described in *Acheta domesticus* by Hendricks and Hadley (1983) where it forms the first five to six thick lamellae directly under the exocuticle. According to these workers, the mesocuticle is intermediate between the hard, tanned exocuticle and the untanned, soft endocuticle.

The cuticle of both locust species contains a network of pore

canals which are clearly seen in the vertical sections (Plates 5 & 6). In insect cuticle, the pore canals arise from the hypodermis, traverse the procuticle and branch at the junction of the exocuticle and the epicuticle to form epicuticular channels which perforate the outer epicuticle (Filshie, 1970a; Hendricks & Hadley, 1983; Locke, 1974; Neville, 1975; Wigglesworth, 1972). In Plate 7, epicuticular channels can be seen to terminate in the innermost layer of the trilaminar outer epicuticle. In the exocuticle of the two locust species, the pore canals appear as twisted ribbons as they pass through the tightly packed helicoid lamellae, while in the endocuticle, they appear as irregular arcs or crescents (Plates 5 & 6) due to thicker lamellae. Such observations have been reported by Hendricks and Hadley (1983) in *Acheta domesticus* and by Neville (1975) in *L.migratoria* and *S.gregaria*. Lipid material was demonstrated in the pore canals and epicuticular channels of the two locust species (Plates 5 & 6) and this supports their role in lipid transport (Locke, 1961; Wigglesworth, 1975a).

Unlike the cuticle of *Calpodes ethlius* (Locke, 1961) and *Acheta domesticus* (Hendricks & Hadley, 1983), the cuticle of the two locust species did not show a deposition zone, although such a zone has been reported in *L.migratoria* (Neville, 1975).

The staining of the hypodermis was variable between the two species and between single and double staining (Plates 5 & 6). No organelles could be identified in the hypodermis of either species and a basement membrane was only seen in *L.migratoria* (Plate 5a).

Plate 1a

Scanning electron micrograph of the intact third abdominal tergum of *Locusta migratoria* showing the form of the epicuticular lipid (Bar = 10μ).

Plate 1b

Scanning electron micrograph of the intact fourth abdominal tergum of *Schistocerca gregaria* showing the form of the epicuticular lipid (Bar = 10μ).

a

b

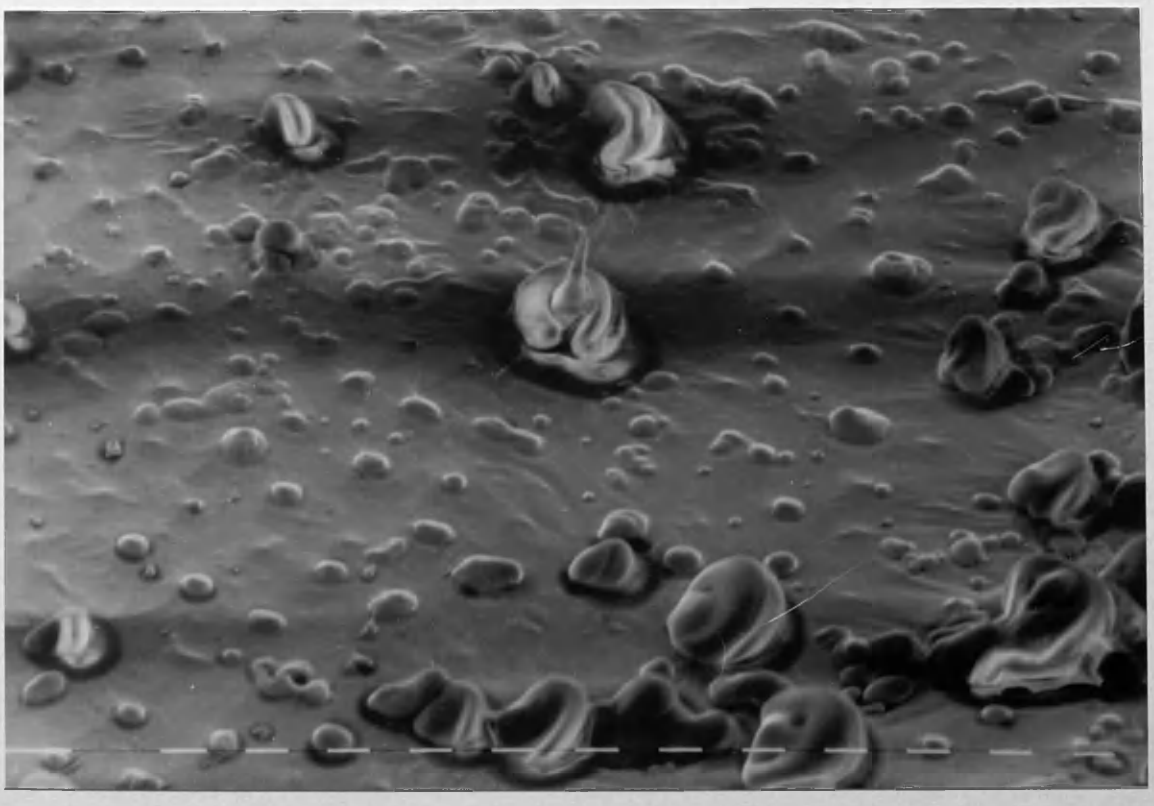
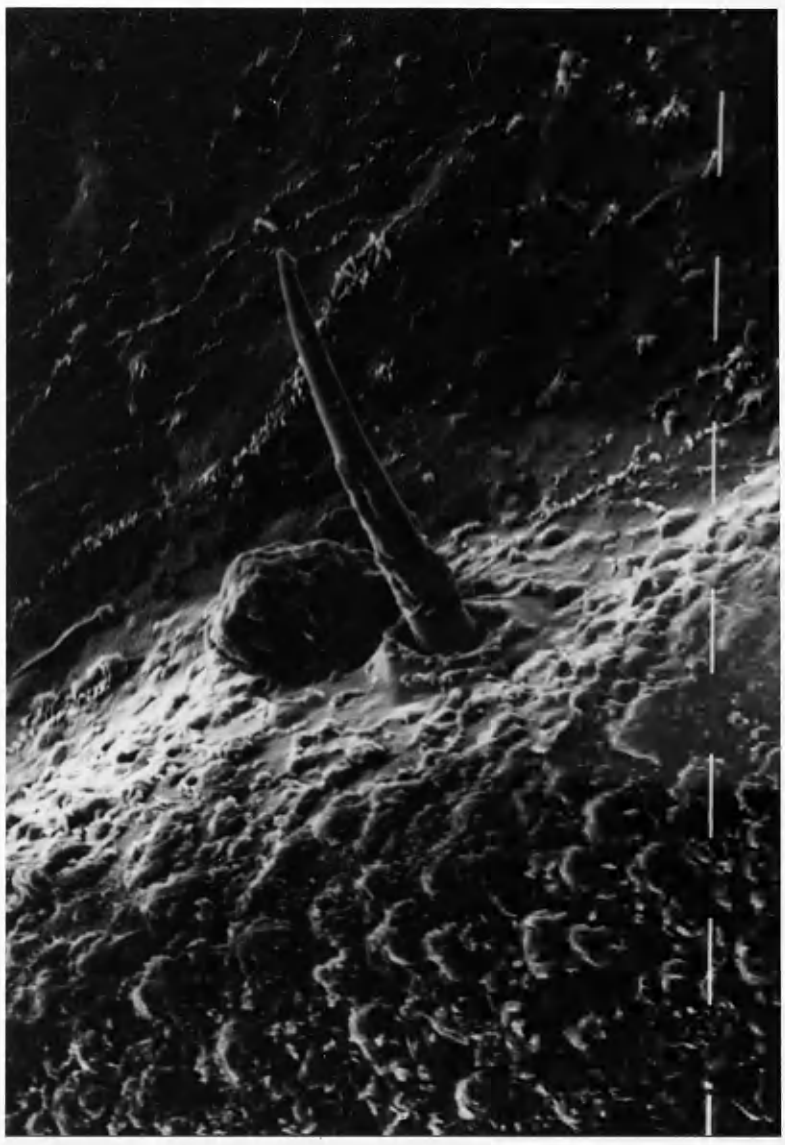


Plate 2a

Scanning electron micrograph of the intact fore wing of *Locusta migratoria* showing the form of the epicuticular lipid (Bar = 10μ).

Plate 2b

Scanning electron micrograph of the intact fore wing of *Schistocera gregaria* showing the form of the epicuticular lipid and spherical masses of lipid occluding the openings of the dermal gland ducts (indicated by arrows). (Bar = 10μ).


a

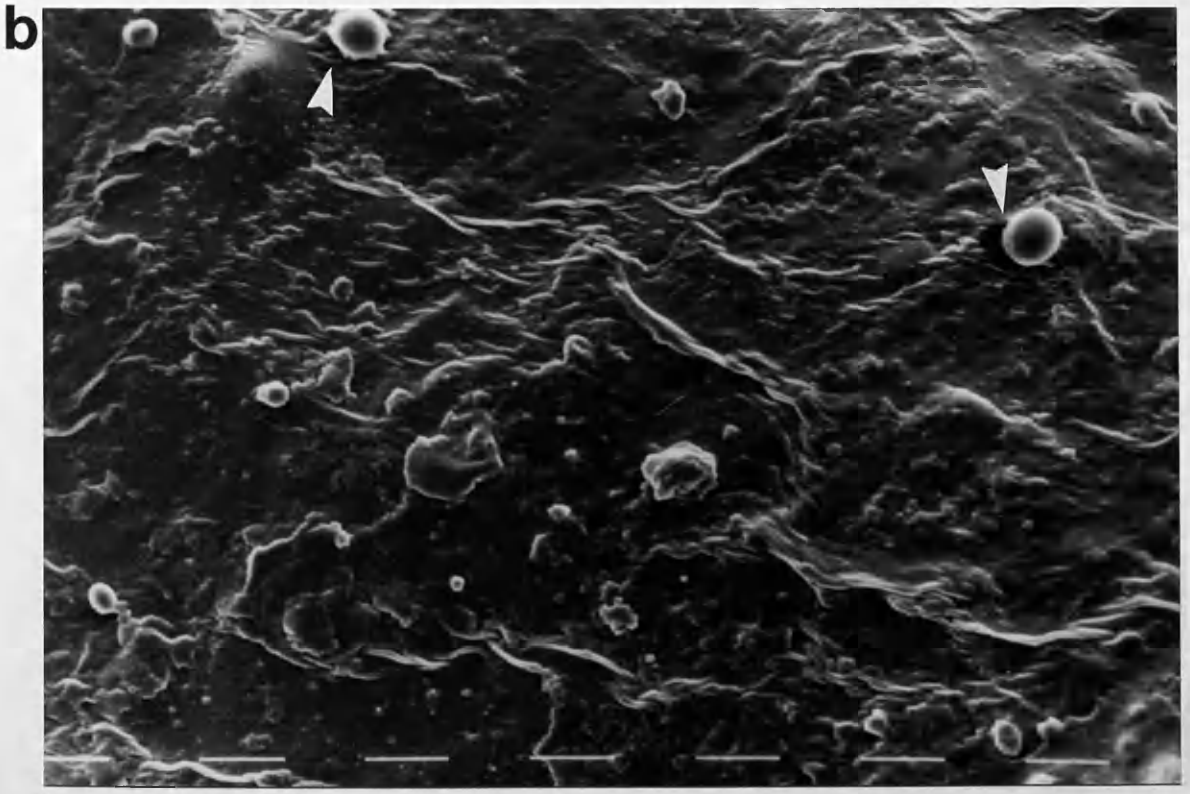

b

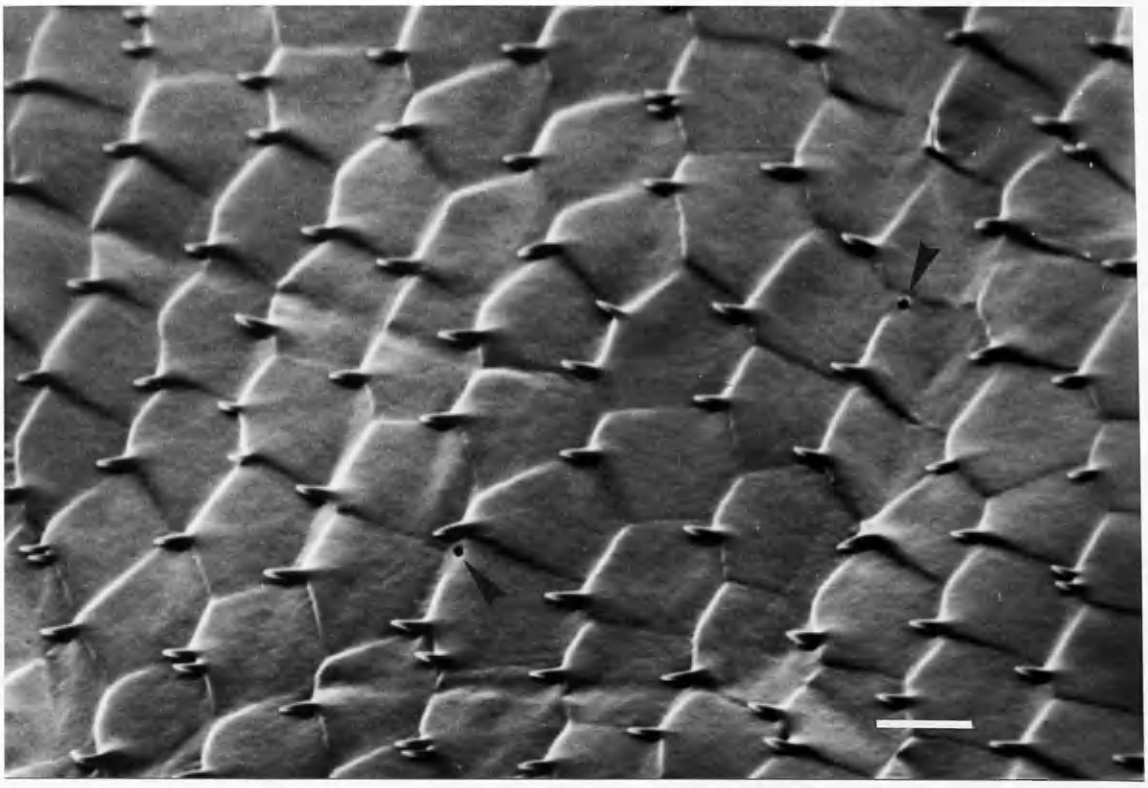
Plate 3a

Scanning electron micrograph of the extracted third abdominal tergum of *Locusta migratoria* showing the surface polygons with projections on their ridges. Openings of the dermal gland ducts indicated by arrows. (Bar = 10μ).

Plate 3b

Scanning electron micrograph of the extracted fourth abdominal tergum of *Schistocerca gregaria* showing the surface polygons with projections on their ridges. (Bar = 10μ).

a



b

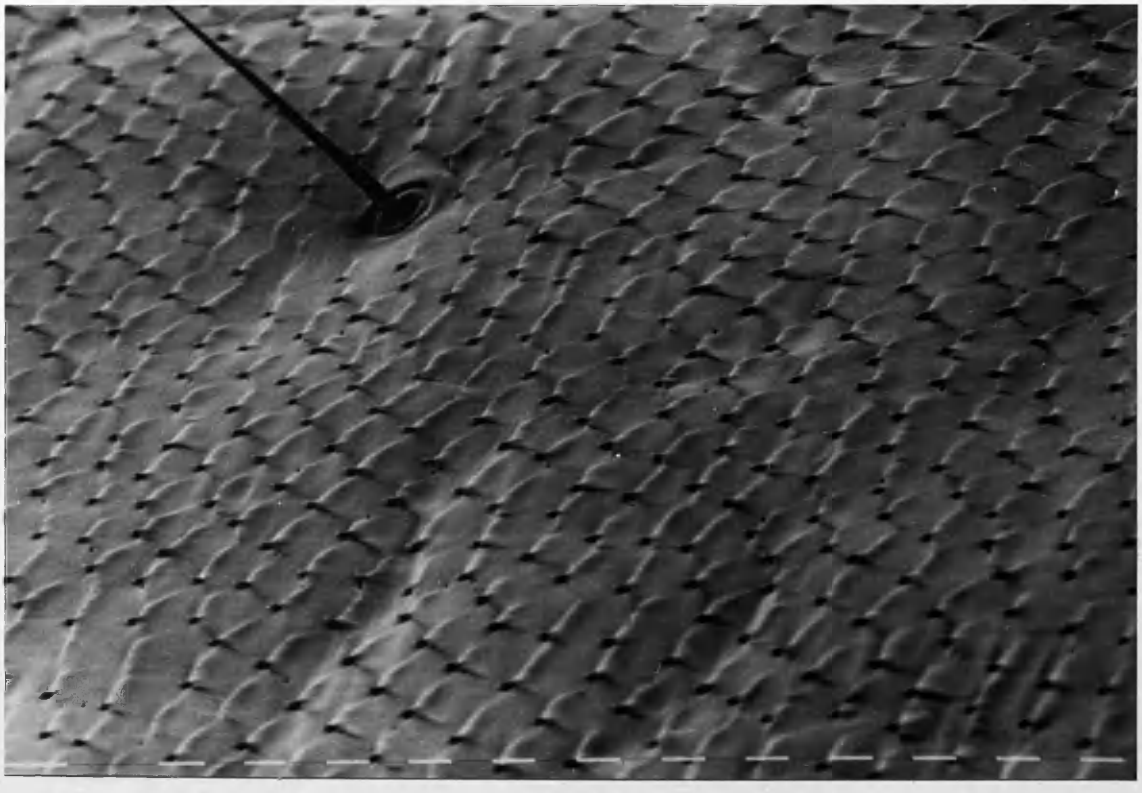


Plate 5

A montage of transmission electron micrographs to show a full vertical section through the extracted third abdominal tergum of *Locusta migratoria*.

a) Section stained according to Wigglesworth (1981)

b) Section double-stained with uranyl acetate and lead citrate.

For a & b (X 8,350)

b.m., basement membrane; endo., endocuticle; exo., exocuticle; hypo., hypodermis; in.epi., inner epicuticle; o.epi., outer epicuticle; p.c., pore canals.

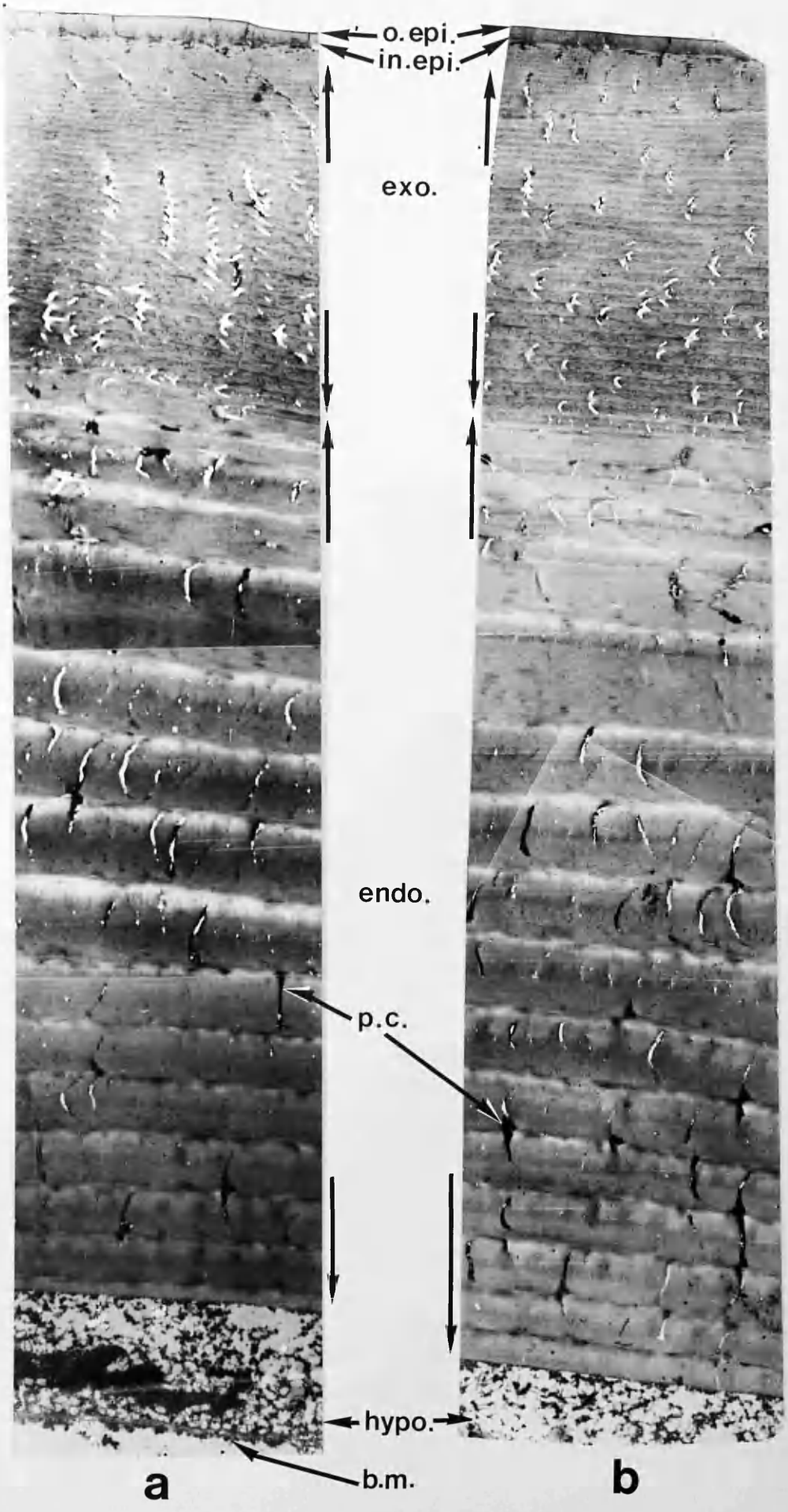

o.epi.
in.epi.
exo.
endo.
p.c.
hypo.
b.m.
a
b
5μ

Plate 6

A transmission electron micrograph of a vertical section through the extracted fourth abdominal tergum of *Schistocerca gregaria*.

a) Section stained according to Wigglesworth (1981)

b) Section double-stained with uranyl acetate and lead citrate.

For a & b (X 7,220)

endo., endocuticle; exo., exocuticle; hypo., hypodermis; in.epi., inner epicuticle; l.l., lipid lamellae; o.epi., outer epicuticle.

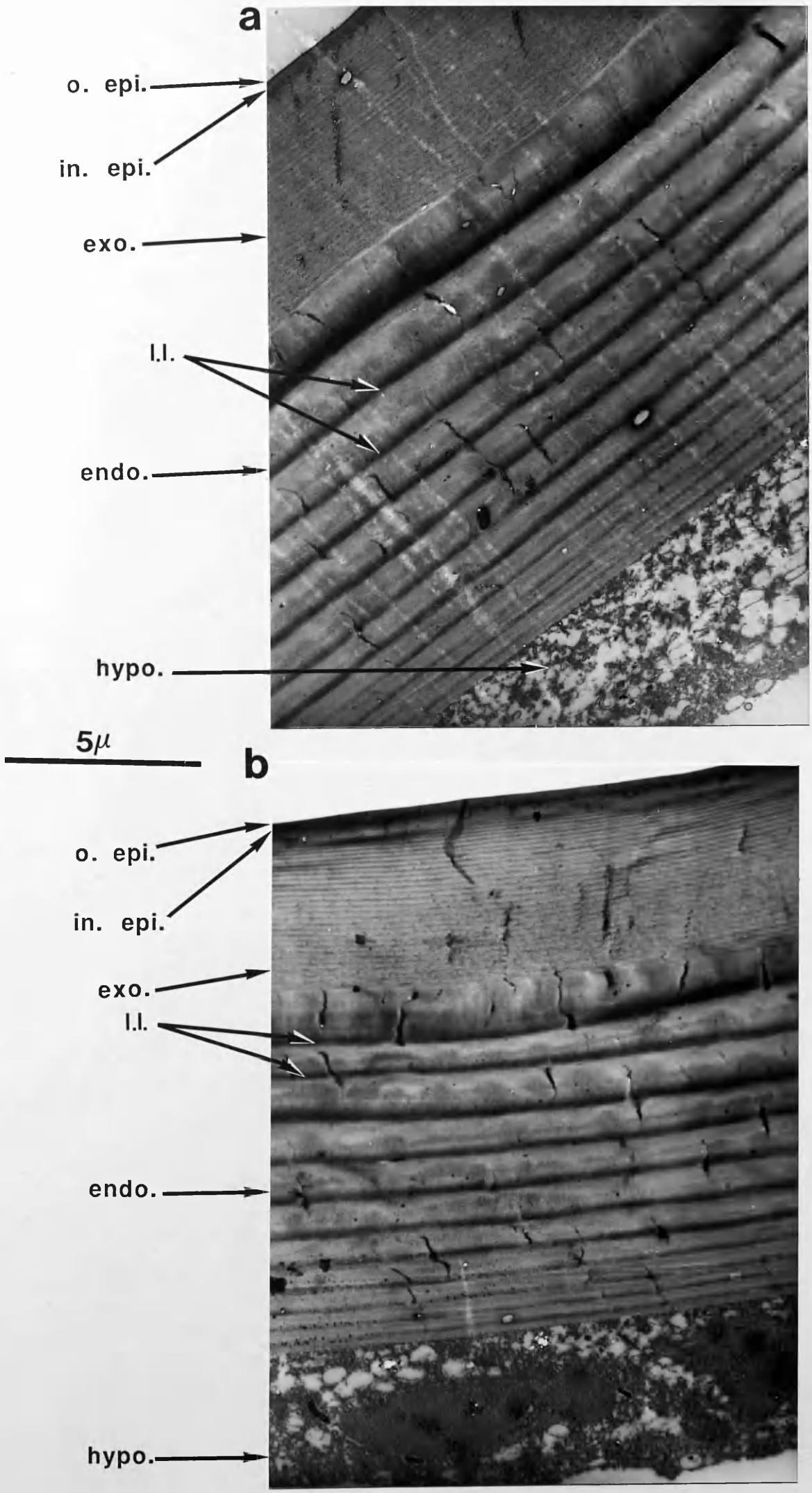

a
o. epi.
in. epi.
exo.
l.l.
endo.
hypo.
5μ
b
o. epi.
in. epi.
exo.
l.l.
endo.
hypo.

Plate 7

Transmission electron micrograph of the outer epicuticle of the fourth abdominal tergum of *Schistocerca gregaria* showing triple lamination and the epicuticular channels terminating in the inner layer of the outer epicuticle.

(X 102,600)

epi.ch., epicuticular channels; in.epi., inner epicuticle; o.epi., outer epicuticle.

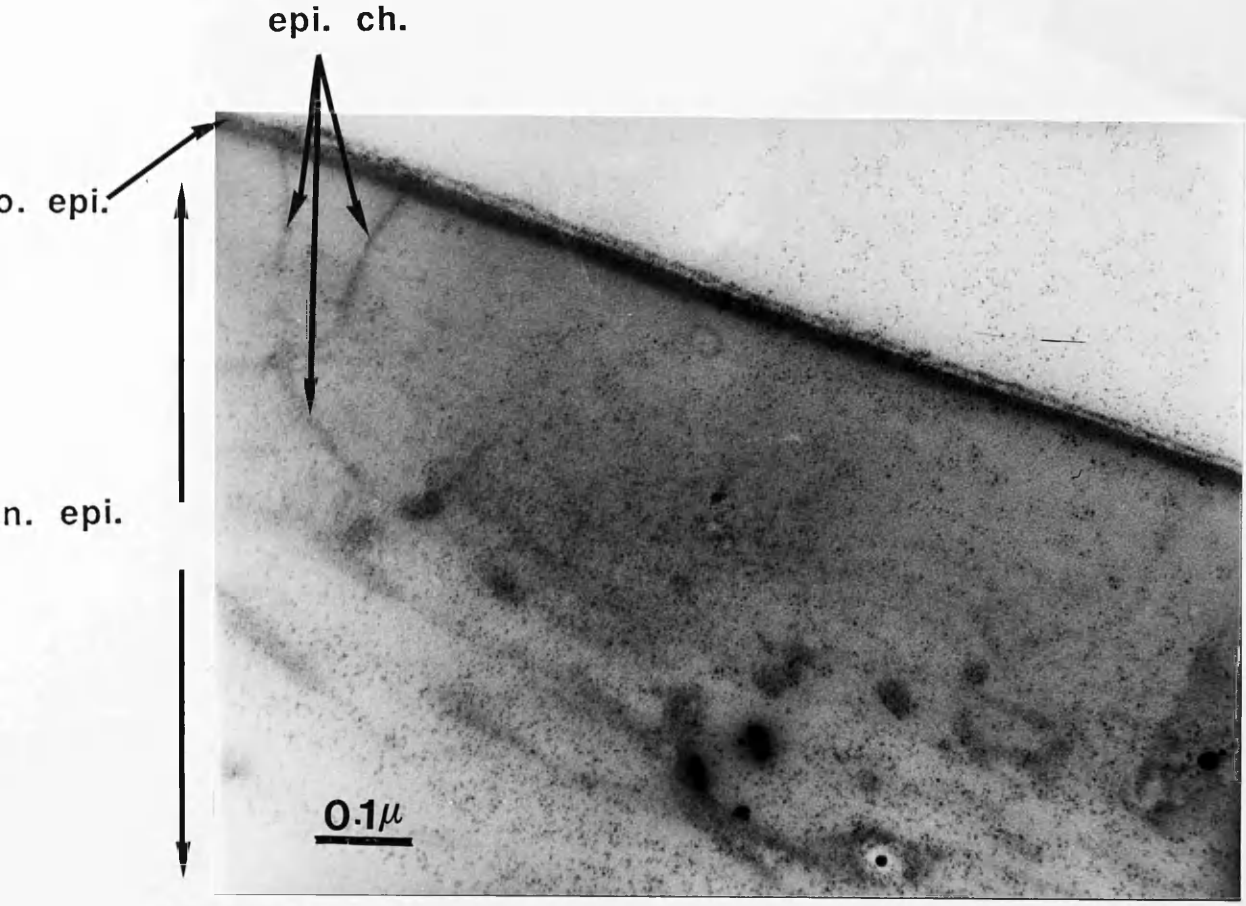

epi. ch.
o. epi.
n. epi.
0.1μ

Plate 8a

Transmission electron micrograph of the epicuticle of the fore wing of *Schistocerca gregaria* showing the argentaffin reaction after extraction with chloroform at 60°C for 24 hours. Silver deposits occur in the inner epicuticle (in.epi.). The outer epicuticle (o.epi.) appears as a darkly stained superficial band. Epicuticular channels (epi.ch.) are lightly stained.

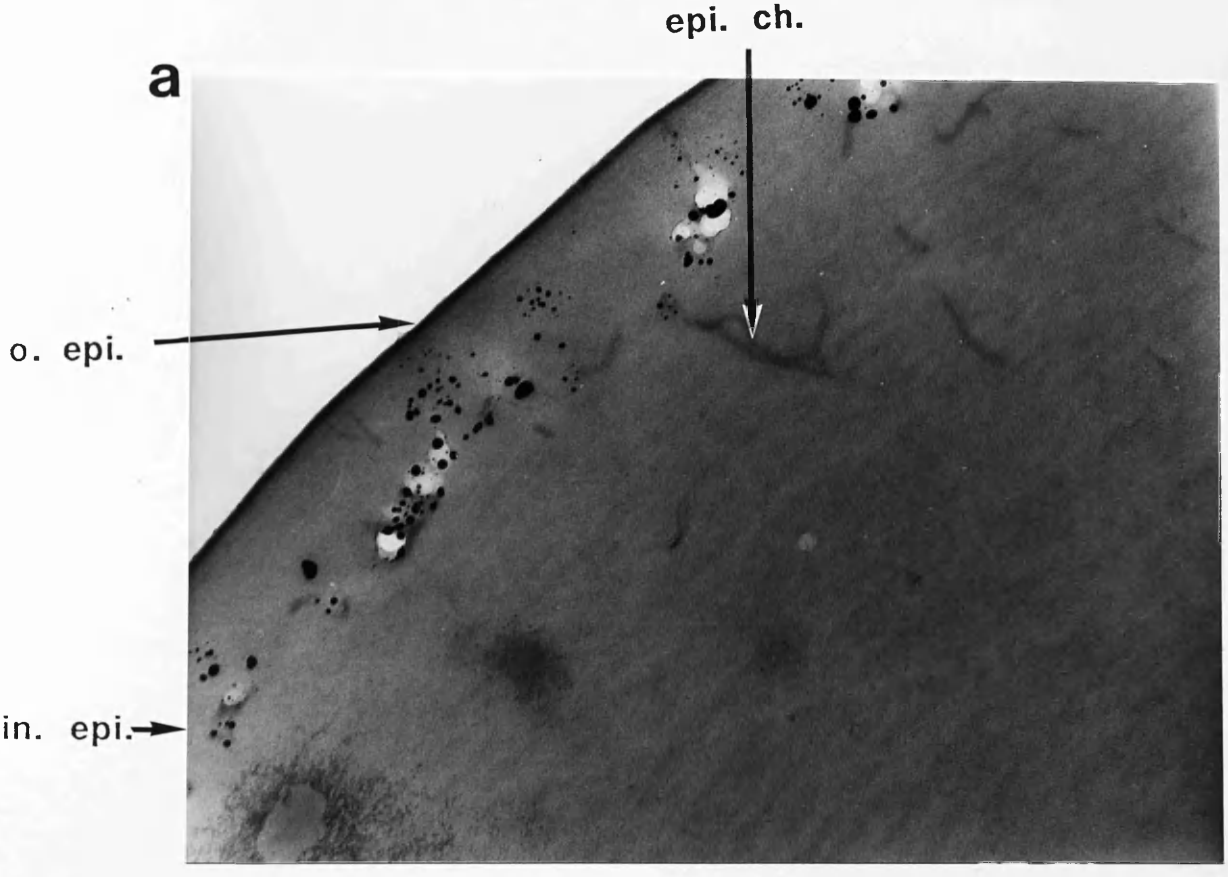
(X 69,730)

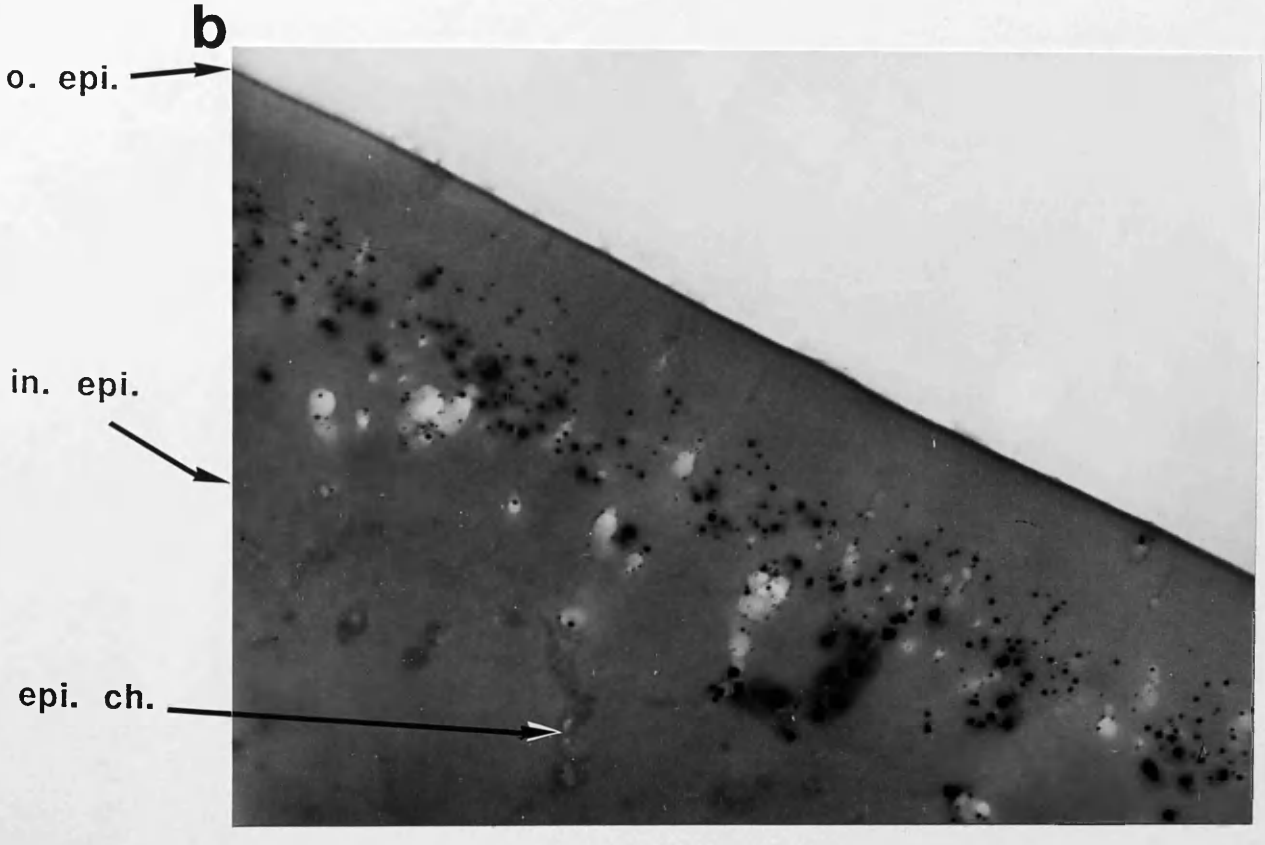
Plate 8b

Transmission electron micrograph of the epicuticle of the fourth abdominal tergum of *Schistocerca gregaria* showing the argentaffin reaction after extraction with chloroform at 60°C for 24 hours. Labels as in Plate 8a.

(X 69,730)

0.5μ





CHAPTER THREE

COMPOSITION OF THE CUTICULAR LIPIDS OF *Locusta migratoria migratoriodes* AND *Schistocerca gregaria*

3.1. INTRODUCTION

3.1.1. Extraction and analysis

A considerable amount of information is now available on the chemical composition of insect cuticular lipids and this information has been reviewed by Blomquist and Dillwith (1985), Blomquist and Jackson (1979) and Lockey (1985a, 1988). The analytical techniques used in insect lipid chemistry were first used in the early 1960's and these techniques have been reviewed by Jackson and Armold (1977), Gilby (1980) and Lockey (1988).

Insect cuticular lipids are usually obtained by extracting whole, non-homogenized bodies or parts of the body, such as wings, elytra or exuviae. Extraction procedures range from agitating the source of lipid in a non-polar solvent, such as pentane for one minute (Castner & Nation, 1986) to refluxing with a polar solvent such as chloroform for one hour (Lockey, 1985b,c). Both methods have disadvantages. In the first, not all of the cuticular lipid is extracted by the non-polar solvent (Hadley, 1978) during the short period of extraction because of the resistant nature of the cement layer (Wigglesworth, 1945) and the sclerotin/lipid mixture (Wigglesworth, 1985b), while in the second method, contamination from the body lipids may occur because of the polar solvent and the length of extraction.

Following extraction, lipids are usually analysed by column chromatography or by thin layer chromatography (TLC). TLC plates impregnated with silver nitrate are often used for separating unsaturated components while molecular sieve is used for separating branched from straight-chain hydrocarbons. Most of the isolated chemical fractions have to be modified before they can be analysed by gas chromatograph (GC). These modifications include the saponification of esters and triglycerides, the methylation of fatty acids and the acetylation of alcohols.

Gas chromatography (GC) is routinely used to identify and to quantify the components of each lipid fraction. The analysis and identification of hydrocarbons is more difficult than that for polar components as hydrocarbons often occur as complex mixtures of n-alkanes, methylalkanes and olefins. High resolution capillary columns such as Support Coated Open Tubular (SCOT) columns and Wall Coated Open Tubular (WCOT) columns have mostly replaced packed columns in the GC analysis of hydrocarbon mixtures. Techniques such as infra-red spectroscopy (IR), nuclear magnetic resonance (NMR) and combined gas chromatography-mass spectrometry (GC-MS) have proved useful in determining hydrocarbon structure. Combined gas chromatographs-mass spectrometers are now usually interfaced to data systems which allow spectral data to be stored and manipulated.

3.1.2. Lipid composition

A. *Hydrocarbons*

The aliphatic hydrocarbons of cuticular lipids are the most commonly studied components and often they are the only components characterized in the cuticular lipid of a species (Blomquist & Dillwith, 1985; Blomquist & Jackson, 1979; Lockey, 1980a, 1985a, 1988). The proportion of hydrocarbons in a cuticular lipid varies between species and between the instars of a species. For example, in the stonefly, *Pteronarcys californica* (Armold *et al.*, 1969), hydrocarbons account for 3 and 12% of the cuticular lipid of the aquatic nymph and the terrestrial adult respectively. In many species, hydrocarbons are the major cuticular component. For example, they account for from 52 to 78% of the cuticular lipid in *Locusta migratoria cinerascens* (Genin *et al.*, 1986), 86% in *Melanoplus differentialis* (Nelson *et al.*, 1984) and >80% in *Schistocerca americana* (Jackson, 1982).

Insect hydrocarbon mixtures often contain a large number of components which may be conveniently grouped in the following classes (Lockey, 1985c):

A - n-Alkanes, B - Unsaturated hydrocarbons, divided into: B_1 - Alkenes, B_2 - Alkadienes and B_3 - Alkatrienes, C - Terminally branched monomethylalkanes divided into: C_1 - 2-Methylalkanes (iso-alkanes) and C_2 - 3-Methylalkanes (anteiso-alkanes), D - Internally branched monomethylalkanes, E - Dimethylalkanes and F - Trimethylalkanes.

(1) n-Alkanes

Normal alkanes occur in the hydrocarbon mixtures of most examined species, those with odd carbon numbers predominating. The proportion of n-alkanes in a hydrocarbon mixture differs not only between species but also between the instars of a species. In the tsetse fly, *Glossina morsitans morsitans*, they are, according to Nelson and Carlson (1986), virtually absent, while in the adult tenebrionid beetle, *Eurychora* species (Lockey, 1985b), n-alkanes account for up to 98.3% of the hydrocarbon mixture.

Short or long homologous series of n-alkanes are found in insect hydrocarbon mixtures. A short series consisting of the odd-numbered n-alkanes, nC_{25} to nC_{33} occur in *Schistocerca americana* (Jackson, 1982), while a long series ranging from nC_{14} to nC_{33} occurs in *Locusta migratoria* (Lockey, 1976).

(2) Unsaturated hydrocarbons (Olefins)

Olefins occur as components of the cuticular hydrocarbons of many species, though their proportions may vary from one species to another. Like saturated hydrocarbons, olefins usually occur as mixtures. These mixtures may comprise alkenes, alkadienes and

alkatrienes though the proportions of such mixtures vary among species. For example, Gilby and Cox (1963) and Jackson (1972) found that in the cockroach, *Periplaneta americana*, the alkadiene, 6,9-heptacosadiene is the most abundant cuticular hydrocarbon, while alkenes occur only in trace amounts. The position of the double bond in olefins has been determined in many insect species. The most common alkenes are 9-enes, with 7-enes and 11-enes occurring less frequently.

(3) Terminally branched monomethylalkanes

Terminally branched monomethylalkanes include two groups, namely, C_1-2-methylalkanes (iso-alkanes) and C_2-3-methylalkanes (anteiso-alkanes). The most abundant 2-methylalkanes have odd carbon numbers, while the most abundant 3-methylalkanes have even carbon numbers.

The occurrence of terminally branched monomethylalkanes in the cuticular lipids of species differs. Both groups may be present, as in *Melanoplus differentialis*, *M.packardii* and *M.sanguinipes* (Nelson *et al.*, 1984), where the 2-methylalkanes range from 27 to 31 carbons and the 3-methylalkanes from 26 to 32 carbons. Alternatively, both groups may be absent as in *Schistocerca americana* (Jackson, 1982), *S.gregaria* (Lockey, 1976) and *S.vaga* (Nelson & Sukkestad, 1975). Species which have only one group of terminally branched monomethylalkanes include *Melanoplus bivittatus*, *M.dawsoni* and *M.femurrubrum* (Jackson, 1981) which have only trace amounts of 2-methylalkanes and *Locusta migratoria cinerascens* (Genin *et al.*, 1986) which has only 3-methylalkanes with both odd and even carbon numbers.

(4) Internally branched monomethylalkanes

Most examined species have internally branched monomethylalkanes in their hydrocarbon mixtures. The proportions of internally branched monomethylalkanes differ between species and between the instars of a

species. For example, they account for only 8% of the hydrocarbon mixture of *Aeropedellus clavatus* (Hadley & Massion, 1985) but for 35% of the hydrocarbon mixture of *Melanoplus femurrubrum* (Jackson, 1981). Internally branched monomethylalkanes may form short or long homologous series comprising members with both odd and even carbon numbers. An example of a short series occurs in the hydrocarbon mixtures of the castes of the termite, *Recticulitermes flavipes* (Howard *et al.*, 1978) where only the 5-methylisomer of C_{25} occurs. A long homologous series is found in three species of the migratory cricket, *Melanoplus*, where the monomethylalkanes range from 26 to 44 carbons in *Melanoplus differentialis* and from 26 to 50 carbons in *M.packardii* and *M.sanguinipes* (Nelson *et al.*, 1984). In *Melanoplus bivittatus*, *M.femurrubrum* and *M.dawsoni* (Jackson, 1981), monomethylalkanes range from 32 to 40 carbons. The adult males and females of *Locusta migratoria cinerascens* (Genin *et al.*, 1986) have a shorter homologous series, ranging from 28 to 38 carbons, than those of *Melanoplus*.

A continuous series of monomethylisomers has been identified in insect species, ranging from isomers with their side chain positioned at carbon 4 to isomers with their side chain positioned at the centre of the alkyl chain. The adult tenebrionid beetle, *Stenocara gracilipes* (Lockey, 1982b) has such a complete series.

(5) Dimethylalkanes

Dimethylalkanes occur in the hydrocarbon mixtures of many species though less frequently than monomethylalkanes. Like other hydrocarbon classes, the proportion of dimethylalkanes varies not only between species but also between the instars of a species. For example, dimethylalkanes occur in only trace amounts in the hydrocarbon mixture of *Melanoplus femurrubrum* and *M.dawsoni* (Jackson, 1981) but account for 46% of the hydrocarbon mixture of *Schistocerca americana* (Jackson,

1982). Dimethylalkanes may form short homologous series, restricted to a few dimethylisomers or long, extensive homologous series. An example of the former is found in the adult beetle, *Attagenus megatoma* (Baker *et al.*, 1979a) which has a homologous series restricted to the dimethylisomers of C_{31} and one dimethylisomer of C_{33}. An example of a long and extensive series occurs in the cricket *Melanoplus packardii* (Nelson *et al.*, 1984) which has a mixture of just under forty dimethylisomers ranging from 29 to 55 carbons.

The position of the methyl branches on the alkyl chain varies among insect dimethylalkanes. The first methyl branch may be positioned at carbon three or at any carbon up to the middle of the chain. In general, the first methyl branch is usually positioned at an odd-numbered carbon in odd numbered dimethylalkanes and at either an odd- or an even-numbered carbon in even numbered dimethylalkanes. Most insect dimethylalkanes have their methyl branches separated by three methylene groups, but dimethylisomers with their branches separated by either 5, 7 or 9 methylene groups have also been found. For example, in female, *Musca domestica* (Nelson *et al.*, 1981), dimethylnonacosane occurs as a mixture of 3,7-, 3,9-, 3,11-, 3,13-, 9,13-, 11,15- and 13,17-dimethylisomers, while dimethyltriacontane occurs as a mixture of 4,10-, 4,12-, 4,14- and 10,14-dimethylisomers. One of the first examples of a dimethylalkane in which the methyl branches were separated by seven methylene groups was identified in *Locusta migratoria* (Lockey, 1976). A further example is found in *Locusta migratoria cinerascens* (Genin *et al.*, 1986) which has a mixture of the 12,20- and 13,21-dimethylisomers of C_{34}. Side chains separated by 9 methylene groups have also been reported in insect species. For example, a mixture of 9,19-, 10,20-, 11,21-, 12,22- and 13,23-dimethylisomers occurs in *Melanoplus packardii* and *M.sanguinipes* (Nelson *et al.*, 1984), while similar, though simpler mixtures occur in

M.differentialis (Nelson *et al.*, 1984), *Locusta migratoria cinerascens* (Genin *et al.*, 1986) and *Schistocerca vaga* (Nelson & Sukkestad, 1975).

(6) Trimethylalkanes

Trimethylalkanes, in varying proportions, have been detected in the hydrocarbon mixtures of several insect species. For example, they account for only 3% of the cuticular hydrocarbon mixture of *Schistocerca vaga* (Nelson & Sukkestad, 1975), but for up to 79% of the hydrocarbon mixture of the ant, *Atta colombica* (Martin & MacConnell, 1970). Most of the trimethylalkanes have even carbon numbers and some species, such as *Schistocerca americana*, have only even-numbered trimethylalkanes (Jackson, 1982). However, both odd- and even-numbered trimethylalkanes occur in the adult tenebrionid beetle, *Cylindrinotus laevioctostriatus* (Lockey, 1981). Trimethylalkanes with even carbon numbers have their first methyl branch positioned at an odd-numbered carbon, while those with odd carbon numbers have their first branch positioned at either an odd- or an even-numbered carbon. Methyl branches are usually separated by three methylene groups. *Cylindrinotus laevioctostriatus* (Lockey, 1981), for example, has a mixture of the 9,13,17-trimethylisomers of C_{31}. Trimethylalkanes in which the methyl branches are separated by either 3, 5 or 7 methylene groups have been detected in female *Glossina pallidipes* and female *Glossina austeni*. For example, *G.pallidipes* has the 11,15,21-trimethylisomers of C_{35} and C_{33} and *G.austeni* has the 5,13,17-trimethylisomers of C_{33} (Nelson & Carlson, 1986).

(7) Hydrocarbon chemotaxonomy

Although cuticular hydrocarbons are of limited value in characterizing plants (Eglinton & Hamilton, 1963, 1967), they have proved to be useful in insects chemotaxonomy (Jackson & Blomquist,

1976; Lockey, 1976). Lockey (1978a,b, 1979, 1980b, 1981, 1982a,b,c, 1984a,b, 1985b,c) has studied the chemotaxonomic value of hydrocarbon composition in family Tenebrionidae, Order Coleoptera. The Tenebrionidae is a large family found in a wide range of habitats including deserts. Lockey's series of papers demonstrates the following:

1. Hydrocarbon composition is species-specific.
2. Closely-related species, such as congenerics, tend to have qualitatively similar hydrocarbon mixtures.
3. Distantly-related species tend to have mixtures which differ both qualitatively and quantitatively.
4. Species belonging to higher levels of classification such as genus and tribe tend to have hydrocarbon mixtures with shared characteristics.

Furthermore, Lockey and Metcalfe (1988) subjected the data obtained from the hydrocarbon compositions of twenty-two species of tenebrionids to statistical analysis comprising Cluster Analysis and Multidimensional Scaling. The former determines how readily data fall into groups, while the latter positions data according to their similarity. These analyses have confirmed in large measure Lockey's observations.

Cuticular hydrocarbon composition or in the case where species are small, cuticular lipid composition has also been used to distinguish sibling species of insect vectors such as the mosquito, *Anopheles* (Carlson & Service, 1979, 1980; Hamilton & Service, 1983; Milligan *et al.*, 1986), the black fly, *Simulium* (Carlson & Walsh, 1981; Phillips *et al.*, 1985) and the sandflies, *Psychodopygus* (Ryan *et al.*, 1986) and *Phlebotomus* (Kamahawi *et al.*, 1987).

The method has also been used to separate species of mole

crickets (Castner & Nation, 1984, 1986), bees (Francis *et al.*, 1985), fruit flies (Carlson & Yocom, 1986) and *Drosophila* (Bartelt *et al.*, 1986).

B. *Esters*

Esters have been found in the cuticular lipids of many species. Their proportions vary between species and between the sexes and the instars of the same species. For example, in the beetle, *Attagenus megatoma*, cuticular esters constitute from 60 to 65% of the larval lipid (Baker, 1978) but only 15% of the adult lipid (Baker *et al.*, 1979a).

Cuticular esters may consist of either primary or secondary alcohols. The former are more common and may occur as a complex mixture of monoesters, diesters and triesters as in the worker honey-bee *Apis mellifera* (Blomquist *et al.*, 1980). The most common primary alcohols are tetracosanol (C24), hexacosanol (C26) and octacosanol (C28). Esters of secondary alcohols have only been reported in the cuticular lipids of genus *Melanoplus*. For example, in *Melanoplus bivittatus*, *M.femurrubrum* and *M.dawsoni*, cuticular esters range from 31 to 41 carbons with the main esters having, in order of abundance, 37>41>39 carbons (Jackson, 1981). In *Melanoplus packardii* and *M.sanguinipes* (Blomquist *et al.*, 1972), cuticular esters range from 37 to 45 carbons with the most abundant ester of both species having 41 carbons.

The constituent acids of esters are mainly saturated and have even carbon numbers ranging from 14 to 28. The most abundant acids are palmitic (16:0*), stearic (18:0), docosanoic (22:0) and tetracosanoic (24:0). However, unsaturated acids when they occur can be in high

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* Carbon number/No. of double bonds

proportions such as in the larva of *Attagenus megatoma* (Baker, 1978). The most common unsaturated acids are palmitoleic (16:1), oleic (18:1) and linoleic (18:2). Methyl-branched acids have been reported in the weevil, *Ceutorrhynchus assimilis* (Richter & Krain, 1980).

Unusual esters have been detected in the cuticular lipid of the diapausing pupa of the noctuid moth, *Manduca sexta*. In this species, the acid fraction of the ester is either acetic acid, acetoacetic acid or 3-hydroxybutyric acid, while the alcohol fraction consists of primary oxoalcohols (Buckner *et al.*, 1984a,b).

C. *Glycerides*

Mono-, di- and triglycerides occur in the cuticular lipids of many insect species though some doubt exists as to whether they are exclusively derived from the cuticular lipid or whether they are contaminants from the body lipid. Like other cuticular components, the proportion of glycerides differ between species and between the instars of a species. In general, the fatty acid composition of glycerides is usually similar to that of the free fatty acid fraction, though their proportions differ.

Triglycerides occur only in low proportions in genus, *Melanoplus*. In *Melanoplus bivittatus*, *M.femurrubrum* and *M.dawsoni*, they account for from 2 to 13% of the cuticular lipid (Jackson, 1981) and for from 1 to 5% of the cuticular lipid of *Melanoplus sanguinipes* and *M.packardii* (Soliday *et al.*, 1974).

D. *Free fatty acids* (Ffa)

Mixtures of straight-chain, saturated and unsaturated free fatty acids occur in the cuticular lipids of many insect species. Free fatty acids (Ffa) differ quantitatively between species as well as between the sexes and the instars of the same species. For example, in the stonefly, *Pteronarcys californica* free fatty acids account for 12 and

49% of the cuticular lipid of the aquatic nymph and the adult respectively (Armold *et al.*, 1969). Free fatty acid mixtures also differ qualitatively and a typical example is the inter-specific differences which occur between four species of dragonfly, namely, *Sympetrum sanguineum*, *S.danae*, *Aeschna grandis* and *A.mixta* (Jacob & Hanssen, 1979). *Sympetrum sanguineum* unlike *S.danae* has fatty acid 16:1 but not fatty acid 23:0, while *Aeschna grandis*, unlike *A.mixta*, has fatty acid 23:0. Both species of *Aeschna* have fatty acid 10:0 which distinguishes them from the two species of *Sympetrum*.

The main cuticular free fatty acids have even carbon numbers which range from 10 to 36. The most abundant saturated free fatty acids are lauric (12:0), myristic (14:0), palmitic (16:0) and stearic acid (18:0). The most abundant unsaturated fatty acids are palmitoleic (16:1), oleic (18:1) and linoleic acid (18:2).

Some saturated and unsaturated free fatty acids with odd carbon numbers have been found in the cuticular lipids of several insect species. For example, all four species of dragonfly mentioned above (Jacob & Hanssen, 1979) have pentadecanoic acid (15:0), while *Sympetrum danae* and *Aeschna mixta* have tricosanoic acid (23:0). Unsaturated fatty acids with odd carbon numbers have been reported by Stránský *et al.* (1973) in the aphid, *Acyrthosiphon pisum* where pentadecenoic acid (15:1) is present in trace amounts.

E. *Free alcohols*

Free primary and secondary alcohols have been detected in the cuticular lipids of many insect species. Like other components of the cuticular lipid, free alcohols vary in their proportions between species as well as between the instars of a species. For example, in the beetle, *Epilachna varivestis*, the free alcohols, which in this species are primary alcohols, of the larval, pupal and adult instars

account for 21.2, 25.4 and 5.4% of the cuticular lipid respectively (Danehower & Bordner, 1984).

Five species of the migratory grasshopper, *Melanoplus* have both primary and secondary alcohols in their cuticular lipid. The free primary alcohols of *Melanoplus packardii* and *M.sanguinipes* form a nearly complete series from C22 to C32. 1-Tetracosanol (C24) and 1-hexacosanol (C26) are the main primary alcohols of the series where members with even carbon numbers predominate. The free secondary alcohols occur in trace amounts only and are of the same range as the ester secondary alcohols (Soliday *et al.*, 1974). Free primary and secondary alcohols account for less than 1% of the cuticular lipid of *Melanoplus bivittatus*, *M.femurrubrum* and *M.dawsoni*. The primary alcohols of the three species range from C22 to C32 with C24 and C26 in the highest proportions. The secondary alcohols have odd carbon numbers ranging from 21 to 27 (Jackson, 1981).

F. *Sterols, aldehydes and ketones*

These lipid fractions represent only minor cuticular components. The most common sterol to occur is cholesterol which is found for example in five species of the migratory grasshopper, *Melanoplus* (Jackson, 1981; Soliday *et al.*, 1974).

Aldehydes have been detected in the cuticular lipid of the cockroach *Periplaneta americana* (Gilby & Cox, 1963) and ketones in the cuticular lipid of the female housefly, *Musca domestica* (Uebel *et al.*, 1978).

In the following work the cuticular lipids of *Locusta migratoria* and *Schistocerca gregaria* have been analysed by thin layer chromatography; gas chromatography and combined gas chromatography-mass spectrometry.

3.2. MATERIALS AND METHODS

3.2.1. Lipid source and extraction

Male and female adult *Locusta migratoria* and *Schistocerca gregaria* were obtained from Bioserv Ltd. Males and females were separated, killed by freezing and their wings removed from their bodies. Separated wings and bodies were used as sources of cuticular lipid. Thus for each species, cuticular lipid from four sources was available for analysis, namely male wing and body, and female wing and body. Throughout the work, organic solvents, chloroform and petroleum spirit (boiling range 60-80^{o}C) of "AnalaR" grade of purity were used after redistillation through a 61cm fractionating column.

Lipids were extracted from wings and bodies by refluxing with chloroform for one hour. Lipid samples were filtered, dried with anhydrous sodium sulphate and evaporated to dryness with a model R1A, type R 20257 Buchi rotavapor. Lipid samples were then transferred to a glass vial and weighed.

3.2.2. Lipid analysis

Cuticular lipid was analysed by thin layer chromatography (TLC) using 20x20cm plates coated with a 2mm thick layer of silica gel 60 (Merck). Lipid samples were co-chromatographed with standard hydrocarbons, esters, triglycerides, free fatty acids and primary alcohols. TLC plates were first developed in petroleum spirit, dried and then developed in a 70:30:1 v/v mixture of petroleum spirit:diethyl ether:glacial acetic acid. Separated fractions were visualized on the TLC plate with iodine vapour. Each fraction was then scraped from the plate into a beaker, dissolved in a small volume of chloroform, filtered, transferred to a weighed glass vial and evaporated to dryness. The weight and the percentage composition of

each fraction was then determined. Repeatable values were difficult to obtain by this method however, and percentage composition values are approximate only. As TLC yields of esters, free fatty acids and free primary alcohols were low, the fractions from male, wing and body were combined with those from female wing and body.

A. *Non-polar lipid - Hydrocarbons*

Hydrocarbon fractions were dissolved in petroleum spirit and purified by column chromatography using a 200x20cm i.d. glass column packed with alumina (Merck, Neutral) and redistilled petroleum spirit as the eluant. Before use, the alumina was activated by heating at 110^{o}C for two hours and then transferred to redistilled petroleum spirit whilst hot. Because of low yields, hydrocarbon fractions from the same lipid source were combined from several TLC plates. Purified hydrocarbons were tested for contaminants by infra-red spectroscopy. Column chromatography, using the above method was also used to obtain samples of hydrocarbons. Yields by this method were much higher than those by TLC and the results were repeatable.

Hydrocarbon fractions were analysed with a model F17 Perkin-Elmer gas chromatograph using a 12m Wall Coated Open Tubular (WCOT) fused silica capillary column (Bonded Phase BP1). Each fraction was analysed by temperature programming from 100 to 300^{o}C at 2^{o}C/min. Helium at a flow rate of 3ml/min was used as the carrier gas. The retention indices (I) of hydrocarbons were calculated from their retention times which were determined by co-chromatographing each mixture with even-numbered reference n-alkanes, ranging from C_{10} to C_{38} and temperature programming from 100 to 300^{o}C at 2^{o}C/min (Ettre, 1964). Unsaturation was tested for by adding bromine dissolved in petroleum spirit to each mixture and noting the disappearance of any component in the subsequent gas chromatographic analysis.

Methylalkanes were separated from straight-chain components by refluxing each hydrocarbon mixture with Linde molecular sieve (type 5A) in iso-octane for 8 hrs (O'Connor *et al.*, 1962). Before use, the molecular sieve was heated to 380°C in a stream of nitrogen for 48 hrs and then transferred to iso-octane whilst hot.

Methylalkanes were identified from their mass spectra which were obtained with a 16F V.G. Micromass gas chromatograph-mass spectrometer, using a 25m bonded phase capillary column similar in type to the one used in GC analyses. The methylalkanes of both species were temperature programmed from 100 to 300°C at 10°C/min and then from 200 to 300°C at 1°C/min. The temperature of the ion source was 230°C and the ionization voltage, 70 eV. Helium at a flow rate of 2ml/min was used as the carrier gas. The mass spectrometer was interfaced to a V.G. Data system 2000 and mass spectral scans (MS scans), ranging from m/z 20 to m/z 650, were taken repetitively at a cycle time of 3.5 sec. MS scans were selected for examination from the total ion count (TIC) chromatogram of each mixture. Background was subtracted by the Data system before MS scans were examined. The mass spectra of methylalkanes were interpreted according to the criteria proposed by McCarthy *et al.* (1968), Nelson *et al.* (1972), Nelson (1978) and Pomonis *et al.* (1978, 1980).

B. *Polar lipid*

The analysis of polar lipid described in this section is based upon the method given by Blomquist *et al.* (1985). Separated polar constituents and their derivatives were analysed with the same gas chromatograph and capillary column as that for hydrocarbons (Section A).

(1) Esters

Ester fractions were saponified with a 5% solution of KOH in

methanol for 30 minutes in a water-bath at 60°C. The ester fatty acids were then methylated by adding to the saponified mixture a 14% solution of borontrifluoride (BF_3) in methanol and heating at 60°C for one hour. Methylation was stopped by adding water to the mixture which was then extracted twice with chloroform. The extracted fatty acid methyl esters and primary alcohols were then separated by thin layer chromatography (TLC), using 5x20cm plates coated with 0.25mm thick layer of silica gel 60 (Merck). TLC plates were developed in a 70:30:1 v/v mixture of petroleum spirit:diethyl ether:glacial acetic acid. Separated fractions of methyl esters and primary alcohols were identified on the TLC plate by co-chromatographing with standard mixtures of methyl esters and alcohols. The methyl esters were removed from the plates, purified by further TLC and then analysed by gas chromatography. The methyl esters were identified from their retention indices which were determined by co-injecting each sample with standard mixtures of n-alkanes ranging from C_{10} to C_{38} and fatty acid methyl esters ranging from C_{10} to C_{22}. GC analyses were carried out by temperature programming from 50 to 300°C at 2°C/min.

The alcohol fractions of the esters were scraped from the TLC plates, dissolved in chloroform, filtered, evaporated to dryness and acetylated by adding a 1:1 v/v mixture of pyridine and acetic anhydride. After 24 hours, the reaction was stopped by adding water and the acetylated alcohols extracted twice with petroleum spirit. The extracted acetylated alcohols were purified by TLC and identified on the TLC plate by co-chromatographing with a standard mixture of acetylated alcohols. The acetylated alcohols were then removed from the TLC plate and analysed by GC. Their retention indices and identity were determined by co-injecting each sample with standard mixtures of n-alkanes and acetylated alcohols.

(2) Triglycerides

The triglyceride fraction was saponified with a 5% solution of KOH in methanol for 30 minutes at 60°C. Fatty acid constituents were then methylated by adding to the saponified mixture a 14% solution of BF_3 in methanol and heating at 60°C for one hour. The reaction was stopped by adding water to the mixture and the resulting fatty acid methyl esters were extracted twice with chloroform and purified by TLC. Methyl ester fractions were identified on TLC plates by co-chromatographing with standard mixtures as described in section 1. The methyl esters were then analysed by GC. They were identified from their retention indices which were determined by co-injecting with the same standard mixtures of n-alkanes and fatty acid methyl esters as in section 1.

(3) Free fatty acids

Free fatty acids were methylated directly with a 14% solution of BF_3 in methanol at 60°C for one hour. The resulting fatty acid methyl esters were treated and identified as in sections 1 and 2.

(4) Free primary alcohols

Free primary alcohols were acetylated and identified as in section 1.

3.3. RESULTS

3.3.1. Lipid composition

TLC analysis of the cuticular lipids of *Locusta migratoria* and *Schistocerca gregaria* revealed the presence of five major fractions, namely, hydrocarbons, esters, triglycerides, free fatty acids and free primary alcohols (Plates 9a & 9b). As an example, the retention factors (R_f) of the lipid fractions of *L.migratoria* female body and *S.gregaria* male wing and their respective standards are given in Table

1a. Examples of the R_f values of the separated polar derivatives and their respective standards are given in Table 1b. Table 2a gives the approximate percentage composition of the lipid fractions after TLC separation. The percentage composition of hydrocarbons obtained from column chromatography of the various lipid sources are given in Table 2b.

A. *Hydrocarbons*

The gas chromatograms of the hydrocarbon mixtures of *L.migratoria* male body, male wing and female wing are given in Figures 1a, 1b, 1c and 1d. For *S.gregaria*, the hydrocarbon mixtures of the male body, female wing and female body are given in Figures 2a, 2b, 2c and 2d. The approximate percentage composition of each mixture obtained by GC peak triangulation is given in Tables 3a and 3b, which also give the identity of the male body hydrocarbons of both species determined by combined gas chromatography-mass spectrometry.

GC analyses showed that the hydrocarbon mixtures of both species comprise long-chain, saturated aliphatic compounds, since unsaturation was not detected by bromination. Figure 3 shows that the hydrocarbon mixtures of *L.migratoria* (Lm) and *S.gregaria* (Sg) contain the following hydrocarbon classes: class A, n-alkanes (Lm 41.4%, average of four sources, Sg 64.9%), class C, terminally branched monomethyl-alkanes (Lm 24.3%, Sg 1.4%), class D, internally branched monomethyl-alkanes (Lm 18.1%, Sg 16.4%), class E, dimethylalkanes (Lm 13%, Sg 14.2%). *S.gregaria* in addition has trimethylalkanes (class F) (1.1%). Table 3a shows that the hydrocarbon mixture of *L.migratoria* is more extensive than that of *S.gregaria*, even though the latter species has trimethylalkanes (Table 3b).

(1) Class A, n-alkanes

n-Alkanes comprise a significant proportion of the hydrocarbons of both species with higher proportions in *S.gregaria* than in *L.migratoria*. In the latter species, n-alkanes range from 30.9% (male wing) to 50.4% (female wing). The proportion of n-alkanes in male and female bodies lies between these values, accounting for 46% in the female and 38.1% in the male (Table 3a). In *S.gregaria*, n-alkanes range from 54.9% (female body) to 77.7% (female wing). n-alkanes from male wings and bodies account for 62.1% and 64.8% respectively (Table 3b).

In both species, n-alkanes form a complete and extensive homologous series from nC_{14} to nC_{36}. In *L.migratoria*, nC_{29} (GC peak, Lm 29) is the most abundant n-alkane in all of the four mixtures (Table 3a, Figure 1b, 1c & 1d). nC_{31} (GC peak, Lm 37) and nC_{27} (GC peak, Lm 20) are the second most abundant n-alkanes in mixtures from the female wing and the female and male body. However, in the male wing of *L.migratoria*, although nC_{27} is the second most abundant n-alkane, nC_{31} is only in low proportions.

In *S.gregaria*, nC_{27} (GC peak, Sg 14) and nC_{29} (GC peak, Sg 19) are the most abundant n-alkanes (Table 3b, Figures 2a, 2c & 2d). nC_{27} is in higher proportions in male and female wings, while nC_{29} is in higher proportions in male and female bodies. nC_{25} (GC peak, Sg 12) is the second most abundant n-alkane in all of the four hydrocarbon mixtures of *S.gregaria*.

(2) Methylalkanes

The proportion of methylalkanes differs within each species as well as between the two species. In *L.migratoria*, methylalkanes account for 61% (56.7% identified & 4.3% unidentified) in the male body mixture, 53.2% (49.6% identified & 3.6% unidentified) in the

female body mixture, 68.6% (67.8% identified & 0.8% unidentified) in the male wing mixture and 48.5% (47.5% identified & 1% unidentified) in the female wing mixture (Table 3a).

In *S.gregaria*, methylalkanes account for 33.8% (33.6% identified & 0.2% unidentified) in the male body mixture, 43.6% (41.4% identified & 2.2% unidentified) in the female body mixture, 36.8% (36.3% identified & 0.5% unidentified) in the male wing mixture and 21.2% (21% identified & 0.2% unidentified) in the female wing mixture (Table 3b).

(a) *Class C, terminally branched monomethylalkanes*

2-Methylalkanes (class C_1) were detected in none of the hydrocarbon mixtures, though 3-methylalkanes (class C_2) were found in all four hydrocarbon mixtures of each species.

L.migratoria has a complete homologous series of 3-methylalkanes ranging from 26 to 32 carbons and containing isomers with both even and odd carbon numbers. 3-Methylnonacosane (GC peak, Lm 32) is the most abundant 3-methylalkane in all four mixtures (average = 14.45%). 3-Methylhentriacontane (GC peak, Lm 40) and 3-methylheptacosane (GC peak, Lm 24) are the second most abundant components. The approximate percentage composition of 3-methylhentriacontane and 3-methylheptacosane are, 5.3% and 4% in the female wing, 4.7% and 3.7% in the female body and 4.3% and 4.2% in the male body (Table 3a). In the hydrocarbon mixture from the male wing however, 3-methylheptacosane is the second most abundant 3-methylalkane (7.5%), while 3-methylhentriacontane occurs in only low proportions (0.6%).

In *S.gregaria*, 3-methylalkanes are found only in low proportions, namely, female wing: 1.5%, male wing: 1.6%, female body: 1.1%, and male body: 1.2% (Table 3b). In the four hydrocarbon mixtures of *S.gregaria*, unlike those of *L.migratoria*, 3-methylalkanes form

incomplete homologous series which range from 28 to 32 carbons and which contain isomers with even carbon numbers only. 3-methylnonacosane (GC peak, Sg 24) is the most abundant 3-methylalkane in all of the four mixtures of *S.gregaria* (approximate average percentage = 1.1%). Although present in only very low proportions, 3-methylheptacosane is the second most abundant 3-methylalkane in the four mixtures (approximate average percentage = 0.3%).

A data system print-out of a mass spectral (MS) scan of 3-methylnonacosane (GC peak, Lm 32), a 3-methylalkane typical of the two species, is given in Figure 4a. This shows a M-15 ion at m/z 407 (C_{29}) and ion doublets at m/z 56/7 (C_4) and m/z 392/3 (C_{28}).

(b) *Class D, internally branched monomethylalkanes*

Both species have mixtures of internally branched monomethylalkanes. In *L.migratoria*, they range from 13.1% (female wing) to 23.1% (male wing) (Table 3a). The internally branched monomethylalkanes of *L.migratoria* form an incomplete homologous series which range from 26 to 35 carbons and which contain isomers with both even and odd carbon numbers. The monomethylisomers of C_{33} (GC peak, Lm 45) and C_{31} (GC peak, Lm 38) are the most abundant monomethylalkanes in the female wing, female body and male body mixtures. The monomethylisomers of C_{29} (GC peak, Lm 30) are the second most abundant compounds in these mixtures. In the male wing mixture of *L.migratoria* however, the monomethylisomers of C_{31} are the most abundant followed by the monomethylisomers of C_{29} and C_{33}.

A data system print-out of a MS scan of GC peak, Lm 38, a typical mixture of monomethylisomers is given in Figure 4b. This shows a molecular ion (M^+) at m/z 450 ($C_{32}H_{66}$) and ion doublets at all even-numbered ion clusters from m/z 140/1 (C_{10}) to m/z 308/9 (C_{22}). This fragmentation pattern is interpreted as being that of a mixture of 9-,

11-, 13- and 15-methylhentriacontane.

In *S.gregaria*, internally branched monomethylalkanes range from 13.3% (female wing) to 21.4% (female body). The monomethylisomers of C_{33} (GC peak, Sg 37) are the most abundant monomethylalkanes in the female wing, female body and male body hydrocarbon mixtures. The monomethylisomers of C_{31} (GC peak, Sg 29) are the second most abundant monomethylalkanes in these mixtures. In the male wing mixture however, the reverse is true. The monomethylalkanes of the four mixtures of *S.gregaria* form homologous series which range from 30 to 36 carbons and which contain isomers with both even and odd carbon numbers.

4- and 5-methylalkanes were detected in both species. However, only 4-methyloctacosane was found in *L.migratoria* (GC peak, Lm 27) and no 4-methylalkanes were found in *S.gegaria*. 5-methylalkanes were similarly limited in that only 5-methylnonacosane (GC peak, Lm 31 & GC peak, Sg 22) was detected in the two species.

(c) *Class E, Dimethylalkanes*

Both species have mixtures of dimethylalkanes. In *L.migratoria*, dimethylalkanes account for 5.5% in the female wing, 11.6% in the male wing, 14.4% in the female body and 20.3% in the male body. The dimethylalkanes of the four mixtures of *L.migratoria* form incomplete homologous series ranging from 27 to 39 carbons and containing isomers with both even and odd carbon numbers. In the four mixtures, the dimethylisomers of C_{35} (GC peak, Lm 53) are the most abundant dimethylalkanes (approximate average percentage = 6.5%) followed by the dimethylisomers of C_{33} (GC peak, Lm 46) (approximate average percentage = 2.3%) (Table 3a).

Terminally branched as well as centrally branched dimethylalkanes occur in the hydrocarbon mixtures of *L.migratoria*. The former are represented by 5, 13-dimethylpentacosane (GC peak, Lm 15) in which the

side chains are separated by 7 methylene groups. All other dimethylalkanes have their side chains centrally positioned and separated by either 3, 7 or 9 methylene groups (Table 3a). Those dimethylalkanes with odd carbon numbers have their first side chain positioned at an odd-numbered carbon of the alkyl chain, while those with even carbon numbers, have their first side chain positioned at either an odd- or even-numbered carbon.

In *S.gregaria*, dimethylalkanes range from 5.9% (female wing) to 18.3% (male wing). In the four mixtures of *S.gregaria*, dimethylalkanes occur as continuous series which range from 31 to 37 carbons and which contain isomers with both even and odd carbon numbers (Table 3b). Dimethyltritriacontane is the most abundant dimethylalkane of the four mixtures (approximate average percentage = 8.8%) followed by dimethylhentriacontane (approximate average percentage = 3.0%).

A data system print-out of a MS scan of GC peak Sg 38, a typical mixture of dimethylisomers, is given in Figure 5a. This MS scan shows a M-15 ion at m/z 477 (C_{34}), ion doublets at m/z 140/1 (C_{10}), 168/9 (C_{12}), 196/7 (C_{14}), 280/1 (C_{20}), 308/9 (C_{22}) and enhanced fragment ions at m/z 211 (C_{15}), m/z 239 (C_{17}), m/z 351 (C_{25}) and m/z 379 (C_{27}) which is interpreted as the fragmentation pattern of 9,13-, 11,15- and 13,17-dimethyltritriacontane.

All of the dimethylalkanes of *S.gregaria* have their side chains centrally positioned and separated by either 3, 5 or 9 methylene groups (Table 3b). As with the dimethylalkanes of *L.migratoria*, odd numbered dimethylalkanes have their first side chain positioned at an odd-numbered carbon, while even numbered dimethylalkanes have their first side chain positioned at either an even- or odd-numbered carbon.

(d) *Class F, Trimethylalkanes*

Trimethylalkanes were detected only in *S.gregaria* where they

occur as a discontinuous homologous series with even carbon numbers only ranging from 34 to 38 (Table 3b). In all of the four mixtures, the trimethylisomers of C_{33} (GC peak, Sg 39) are the most abundant (approximate average percentage = 0.7%) and the trimethylisomers of C_{31} (GC peak, Sg 32) the second most abundant trimethylalkanes (approximate average percentage = 0.3%). The trimethylisomers of C_{35} (GC peak, Sg 47) are in the lowest proportions (approximate average percentage = 0.2%). The homologous series of even-numbered trimethylalkanes of *S.gregaria* have their side chains centrally positioned at odd-numbered carbons. Side chains 1 and 2 are separated by 3 methylene groups and side chains 2 and 3 by 7 methylene groups.

A data system print-out of a MS scan of 11,15,23-trimethylpentatriacontane (GC peak, Sg 47) is given in Figure 5b. This shows ion doublets at m/z 168/9 (C_{12}), m/z 196/7 (C_{14}) and enhanced fragment ions at m/z 239 (C_{17}), m/z 323 (C_{23}), m/z 365 (C_{26}) and m/z 393 (C_{28}).

B. *Esters*

In *L.migratoria*, esters account for 11.4% of the female lipid and for 7.4% of the male lipid, while in *S.gregaria*, they account for 6.7% of the female lipid and for 6.4% of the male lipid. In both species, these proportions are for combined lipid from wings and bodies.

(1) Ester fatty acids

In both species, the fatty acid components of the esters range from 10 to 22 carbons and saturated fatty acids with even carbon numbers predominate. Table 4a gives the chain length and the percentage composition of the ester fatty acids of both species. Although fatty acids 17:0 and 20:1 (carbon number:number of double bonds) were detected in low proportions in *S.gregaria*, they were not found in *L.migratoria*. The major fatty acids of *L.migratoria*, in order of abundance, are 16:0 > 18:0 > 14:0 > 20:0 (Figure 6a) while those of

S.gregaria are 16:0 > 14:0 > 10:0 > 12:0 > 18:0. dI is the difference between the retention index of a compound and that of a n-alkane with the same carbon number and the dI values of the identified methyl esters range from 298 to 317 (average dI = 308) in *L.migratoria* and from 277 to 316 (average dI = 297) in *S.gregaria* (Table 4a).

(2) Ester alcohols

The alcohol constituents of the esters of both species are primary alcohols and they form incomplete homologous series of even and odd carbon numbers which range from 12 to 32 (Table 4b). The total percentage of even-numbered alcohols is higher than that for odd numbered alcohols in both species.

Table 4b gives the chain length and the percentage composition of the ester alcohols of both species. The major alcohols of *L.migratoria*, in order of abundance, are C21 > C14 > C18 > C16 > C22 and those of *S.gregaria*, C16 > C18 > C22 > C17 > C15.

The dI values of the ester alcohols range from 388 to 401 (average = 395) in *L.migratoria* and from 390 to 400 (average dI = 395) in *S.gregaria*. One component in each of the alcohol fractions could not be identified. In *L.migratoria*, the unidentified component has a retention index equal to 2371 and accounts for 8.8% of the alcohol fraction. In *S.gregaria*, the unidentified component has a retention index equal to 1783 and accounts for 5.8% of the alcohol fraction (Table 4b).

C. *Triglycerides*

TLC yields of triglycerides were high in both species. In *L.migratoria*, triglycerides account for 57.1% of the female lipid and for 51.9% of the male lipid. In *S.gregaria*, the proportions are higher, namely, 64.8% of the female lipid and 65.7% of the male lipid.

Triglyceride fatty acids form a more complicated mixture in *L.migratoria* than in *S.gregaria*. Table 5 gives the chain length and the percentage composition of triglyceride fatty acids of both species. In both male and female *L.migratoria*, the fatty acids range from 10 to 22 carbons, while in *S.gregaria*, they range from 10 to 18 carbons (females) and from 14 to 18 carbons (males). Intraspecific differences occur in *S.gregaria*. Thus fatty acid 15:0 is present in males but not in females.

Both saturated and unsaturated fatty acids occur in the triglycerides of *L.migratoria* with the percentage of the former predominating, namely saturated acids, 73.7%, unsaturated acids, 26.4% (average for combined males and females). The triglycerides of *S.gregaria* contain only saturated acids. In both species, fatty acids with even carbon numbers predominate. Thus, the major triglyceride fatty acids of *L.migratoria*, in order of abundance, are 16:0 > 20:1 > 18:0 > 18:2 > 14:0 (males) and 16:0 > 18:0 > 14:0 > 20:1 (peak 12) > 18:2 (peak 9) (females) (Figure 6b), while those of *S.gregaria* are 16:0 > 18:0 > 14:0 (males) and 16:0 > 14:0 > 18:0 (females).

In *L.migratoria*, the dI values for triglyceride fatty acids range from 268 to 316 (males) and from 264 to 316 (females), whereas in *S.gregaria*, they range from 312 to 321 (males) and from 306 to 322 (females) (Table 5).

D. *Free fatty acids*

The free fatty acids of *L.migratoria* account for 4.6% of the female lipid and for 7.4% of the male lipid, while in *S.gregaria*, the proportions are 4.7% of the female lipid and 1.5% of the male lipid. Table 6 gives the chain length and the percentage composition of the free fatty acids of both species.

The free fatty acids of *L.migratoria* range from 10 to 22 carbons

while those of *S.gregaria* range from 10 to 20 carbons. Unlike *S.gregaria*, *L.migratoria* lacks the unsaturated fatty acids 12:1, 17:1, 19:1 and the saturated acid 19:0.

In both species, saturated acids are in higher proportions than unsaturated acids. At the same time, acids with even carbon numbers are in higher proportions than those with odd carbon numbers. Thus in *L.migratoria*, saturated acids account for 83.4% and unsaturated acids for 15.7%, while in *S.gregaria* saturated acids account for 97.5% and unsaturated acids for 2.2%. The most abundant fatty acids of *L.migratoria*, in order of abundance, are 16:0 > 14:0, 15:0 > 18:0 and those of *S.gregaria*, 16:0 > 14:0 > 18.0.

The dI values of the free fatty acids range from 266 to 322 in *L.migratoria* and from 268 to 317 in *S.gregaria* (Table 6).

E. *Free primary alcohols*

In *L.migratoria*, free primary alcohols account for 4% of the female lipid and for 3.7% of the male lipid, while in *S.gregaria*, they account for 5.1% of the female lipid and for 1.7% of the male lipid. Table 7 gives the chain length and the percentage composition of the free primary alcohols of both species.

The free primary alcohols of *L.migratoria* range in chain length from 10 to 34 carbons, while those of *S.gregaria*, range from 10 to 32 carbons. A component in both of the alcohol mixtures, with a retention index equal to 2365 in *L.migratoria* and 2370 in *S.gregaria*, could not be identified.

In both species, even numbered alcohols predominate and the major alcohols of *L.migratoria* in order of abundance are C14 > C18 > C22 > C16 and those of *S.gregaria*, are C15 > C16 > C18 > C22. The odd numbered primary alcohol, 1-nonacosanol (C29) was detected only in *S.gregaria*.

The dI values of the alcohols range from 389 to 401 in *L.migratoria* and from 388 to 402 in *S.gregaria*.

3.4. DISCUSSION

This is the first complete analysis of the cuticular lipid of the African migratory locust, *Locusta migratoria migratoriodes* and the desert locust, *Schistocerca gregaria*. The investigation shows that the lipids of both species consist of five major fractions namely, hydrocarbons, esters, triglycerides, free fatty acids and free primary alcohols. The differences between the lipid composition of *L.migratoria* and *S.gregaria* will be discussed together with the differences between other species of family Acrididae examined by various workers. However, strict comparisons are not always possible because some examinations are incomplete and some are not strictly comparable. The acridid species examined by other workers are subfamily Cyrtacanthacridinae; *Schistocerca americana* (Jackson, 1982); *S.gregaria* (Lockey, 1976); *S.vaga* (Nelson & Sukkestad, 1975), subfamily Oedipodinae; *Locusta migratoria cinerascens* from Sardinia (Genin *et al.*, 1986); *L.migratoria* (Lockey, 1976); subfamily Catantopinae; *Melanoplus sanguinipes* and *M.packardii* (Blomquist *et al.*, 1972; Soliday *et al.*, 1974); *M.bivittatus*, *M.femurrubrum* and *M.dawsoni* (Jackson, 1981); *M.differentialis*, *M.packardii* and *M.sanguinipes* (Nelson *et al.*, 1984), subfamily Gomphocerinae; *Aeropedellus clavatus* (Hadley & Massion, 1985).

A. Hydrocarbons

Both *L.migratoria* and *S.gregaria* have significant proportions of hydrocarbons in their cuticular lipid. Table 2a shows the proportions of hydrocarbons and polar lipid obtained by TLC, while Table 2b shows the hydrocarbon proportions obtained by column chromatography.

Hydrocarbon proportions obtained by TLC are lower than those obtained by column chromatography (Tables 2a & 2b). This is probably due to the small lipid samples analysed on TLC plates and to the loss of material during analysis and extraction. In column chromatography much larger lipid samples can be analysed and loss of material is minimal. The hydrocarbon proportions obtained from column chromatography for *L.migratoria* range from 37.6% (male body) to 53.3% (female wing) and for *S.gregaria* from 36.2% (male body) to 68.3% (female wing). Both *L.migratoria* and *S.gregaria* have higher proportions of hydrocarbons in their wings than in their bodies (Table 2b). Thus, the hydrocarbon proportions obtained from column chromatography range from 40.5% (male wing) to 53.3% (female wing) in *L.migratoria* and from 62.5% (male wing) to 68.3% (female wing) in *S.gregaria*. These values are lower than those given by Lockey (1976) for the wings from mixed sexes of the two species. These are 54% for *L.migratoria* and 76% for *S.gregaria*. Some of the other examined acridid species have higher proportions of hydrocarbons in their cuticular lipid than either *L.migratoria* or *S.gregaria*. These higher proportions range from 52 to 78% in *L.migratoria cinerascens* (Genin *et al.*, 1986) and to 86% in *M.differentialis* (Nelson *et al.*, 1984) (Table 8).

The hydrocarbon mixtures of both *L.migratoria* and *S.gregaria* have the following hydrocarbon classes: n-alkanes (class A), terminally branched monomethylalkanes (class C), internally branched monomethylalkanes (class D) and dimethylalkanes (class E). *S.gregaria* has an additional class, namely, trimethylalkanes (class F).

(1) *n-Alkanes*

Both *L.migratoria* and *S.gregaria* have high proportions of n-alkanes in their hydrocarbon mixtures (Figure 3). In *L.migratoria*, n-alkanes range from 30.9% (male wing) to 50.4% (female wing) of the

hydrocarbon mixture, while in *S.gregaria*, they range from 62.1% (male wing) to 77.7% (female wing) (Table 3b). The value for *L.migratoria* is lower than that given by Lockey (1976) for this species, namely 61% and is the same value of 70% for *S.gregaria*. However, the n-alkane values obtained in the present work for *L.migratoria* are higher than those for *L.migratoria cinerascens* (Genin *et al.*, 1986), where n-alkanes account for from 28.7 to 47% of the hydrocarbon mixtures. Similarly, the values for *S.gregaria* are higher than those for *S.vaga* (35%) (Nelson & Sukkestad, 1975) and *S.americana* (27%) (Jackson, 1982). For comparison, the proportions of n-alkanes in five examined species of genus *Melanoplus* subfamily Catantopinae and in *Aeropedellus clavatus* subfamily Gomphocerinae are given in Figure 7.

The n-alkanes of *L.migratoria* and *S.gregaria* (Figure 3) form a complete and extensive homologous series ranging from nC_{14} to nC_{36}. The main n-alkanes of *L.migratoria*, are in order of abundance, $nC_{29} > nC_{31} > nC_{27} > nC_{30} > nC_{28}$ in the female wing mixture and $nC_{29} > nC_{27} > nC_{28} > nC_{25}$ in the male wing mixture. Those of *S.gregaria* are $nC_{27} > nC_{29} > nC_{25}$ in both male and female wing mixtures. These results are different from those reported by Lockey (1976) who found the n-alkanes of *L.migratoria* to range from nC_{14} to nC_{33} with nC_{29}, nC_{31}, nC_{30}, nC_{27} and nC_{28} as the main components, and those of *S.gregaria* to range from nC_{22} to nC_{33} with nC_{29}, nC_{27} and nC_{25} as the main components. One explanation for these differences may be that in this study a 12m WCOT capillary column with high resolution was used to analyse the hydrocarbon mixtures. Such a column is likely to separate components more efficiently than the packed column used by Lockey (1976). Furthermore, components tend to elute more rapidly from a capillary column than from a packed column so that high molecular weight hydrocarbons are more likely to be detected.

The n-alkane mixtures of *L.migratoria* are similar to those of

L.migratoria cinerascens (Genin *et al.*, 1986) though the latter subspecies has a less extensive series ranging from nC_{22} to nC_{37}. The main n-alkanes in *L.migratoria cinerascens* (Genin *et al.*, 1986), in order of abundance, are $nC_{29} > nC_{31} > nC_{30} > nC_{27} > nC_{28}$ in the male and female gregarious adults [which is similar to the n-alkane composition of *L.migratoria* proposed by Lockey (1976)] and $nC_{31} > nC_{29} > nC_{30} > nC_{27} > nC_{28}$ in the male and female solitary adults. Comparing the n-alkanes of *S.gregaria* with those of other examined species of subfamily Cyrtacanthacridinae namely, *S.vaga* (Nelson & Sukkestad, 1975) and *S.americana* (Jackson, 1982) shows that *S.vaga* and *S.americana* have less extensive series of n-alkanes (Figure 7). The main n-alkanes of *S.vaga* (Nelson & Sukkestad, 1975), in order of abundance, are $nC_{29} > nC_{31} > nC_{25} > nC_{27}$, while those of *S.americana* are $nC_{29} > nC_{27} > nC_{25}$ (Jackson, 1982). The main n-alkanes of *S.americana* (Jackson, 1982) are similar to those of *S.gregaria* (male and female body) of this study.

The n-alkane composition of *L.migratoria* (male wing) and *S.gregaria* (male and female body) are similar to those of *M.sanguinipes*, *M.packardii* (Soliday *et al.*, 1974) and *M.dawsoni* (Jackson, 1981) in that nC_{29} and nC_{27} are the first and second most abundant components respectively. In general, however, the examined species of *Melanoplus* have a less extensive series of n-alkanes. On the other hand, the n-alkane composition of *S.gregaria* (male and female wing) is similar to that of *M.bivittatus*, *M.femurrubrum* (Jackson, 1981) and *Aeropedellus clavatus* (Hadley & Massion, 1985) in that nC_{27} and nC_{29} are the first and second most abundant components, and nC_{25} is one of the major n-alkanes of the three species.

(2) *Terminally branched monomethylalkanes*

2-Methylalkanes were not detected in the hydrocarbon mixtures of

L.migratoria and *S.gregaria*. 2-Methylalkanes are also absent from the hydrocarbon mixtures of *L.migratoria cinerascens* (Genin *et al.*, 1986), *S.vaga* (Nelson & Sukkestad, 1975) and *S.americana* (Jackson, 1982). They are, however, present in the five examined species of *Melanoplus* (subfamily Catantopinae) (Figure 7), where they range from 27 to 31 carbons in *M.sanguinipes* and from 29 to 31 carbons in *M.packardii*. *M.differentialis* (Nelson *et al.*, 1984) has only one 2-methylalkane, namely, 2-methyltriacontane, and this is also true of *M.bivittatus*, *M.femurrubrum* and *M.dawsoni* (Jackson, 1981) where 2-methylhentriacontane occurs in trace amounts. 2-Methylalkanes are absent from *Aeropedellus clavatus* (subfamily Gomphocerinae).

Both *L.migratoria* and *S.gregaria* have 3-methylalkanes (Figure 3). Those of *L.migratoria*, in order of abundance, are 3-methylnonacosane, 3-methylhentriacontane and 3-methylheptacosane (female wing) and 3-methylnonacosane and 3-methylheptacosane (male wing). In *S.gregaria*, 3-methylnonacosane and 3-methylheptacosane are the most abundant components. These results agree with those of Lockey (1976) for *L.migratoria*, but Lockey (1976) failed to detect 3-methylalkanes in the hydrocarbon mixture of *S.gregaria*. The 3-methylalkane composition of *L.migratoria* is similar to those of *L.migratoria cinerascens* (Genin *et al.*, 1986) where 3-methylnonacosane is the major component in the gregarious form and 3-methylhentriacontane, the main component in the solitary form.

In subfamily Cyrtacanthacridinae, *S.gregaria* has 3-methylalkanes unlike *S.vaga* (Nelson & Sukkestad, 1975) and *S.americana* (Jackson, 1982). In subfamily Catantopinae, only three of the examined species of *Melanoplus* have 3-methylalkanes, namely, *M.sanguinipes*, *M.packardii* and *M.differentialis* (Nelson *et al.*, 1984). In *M.sanguinipes* and *M.packardii* (Soliday *et al.*, 1974), trace amounts of 3-

methylheptacosane, 3-methylnonacosane and 3-methylhentriacontane represent class C_2 hydrocarbons. 3-Methylpentatriacontane is the most abundant 3-methylalkane in *Aeropedellus clavatus* (subfamily Gomphocerinae).

(3) *Internally branched monomethylalkanes*

Both *L.migratoria* and *S.gregaria* have monomethylalkanes in their hydrocarbon mixtures (Figure 3). The monomethylalkanes of *L.migratoria* have a longer chain length than those of *S.gregaria*. In *L.migratoria*, the most abundant monomethylalkanes in order of abundance, are the monomethylisomers of C_{33}, C_{31} and C_{29} (female wing) and C_{31}, C_{29} and C_{33} (male wing). In *S.gregaria*, the most abundant monomethylalkanes are the monomethylisomers of C_{31} and C_{33} (female wing) and C_{33} and C_{31} (male wing). In both species, the 9-, 11-, 13- and 15-methylisomers are the most abundant. The monomethylalkanes identified in the present work differ from those reported by Lockey (1976) who detected only the 9-, 13-, 15- and 17-monomethylisomers of C_{33}, C_{31} and C_{35} in *L.migratoria* and the 11-, 13- and 15-monomethylisomers of C_{33} and C_{35} in *S.gregaria*. The monomethylalkanes of *L.migratoria cinerascens* (Genin *et al.*, 1986) have longer chain lengths than those of *L.migratoria* with the 11-, 13-, 15- and 17-monomethylisomers of C_{35}, C_{33} and C_{31} the most abundant monomethylalkanes. In subfamily Cyrtacanthacridinae, the monomethylalkanes of *S.gregaria* have a shorter chain length than those of *S.vaga* (Nelson & Sukkestad, 1975) and *S.americana* (Jackson, 1982). In the latter two species, the most abundant monomethylalkanes are the 13-, 15- and 17-monomethylisomers of C_{35}, C_{33} and C_{37}.

The most abundant monomethylalkanes of *M.sanguinipes*, *M.packardii* (Soliday *et al.*, 1974) are the 11-, 13- and 15-monomethylisomers of C_{31} and C_{33} and those of *M.bivittatus* (Jackson, 1981), the 11-, 13-

and 15-monomethylisomers of C_{35} and C_{33}. In *M.femurrubrum* and *M.dawsoni*, 11-, 13- and 15-monomethylisomers of C_{33} and C_{35} are in the highest proportions (Jackson, 1981). In *Aeropedellus clavatus*, the pentatriacontanes are the most abundant monomethylalkanes, though no isomers are listed by Hadley and Massion (1985).

(4) *Dimethylalkanes*

The dimethylalkanes of *L.migratoria* range from 27 to 39 carbons, with the 13,21-, 11,21- and 13,19-dimethylisomers of C_{35} and C_{33} being the major components. The side chains of these dimethylisomers are separated by either 5, 7 or 9 methylene groups.

The dimethylisomers of *S.gregaria* have shorter chain lengths than those of *L.migratoria* ranging from 31 to 37 carbons (Figure 3). The 9,13-, 11,15- and 13,17-dimethylisomers of C_{33} and C_{31} are the main dimethylalkanes of *S.gregaria*. The side chains of all these dimethylalkanes are separated by 3 methylene groups.

Lockey (1976) detected only the 13,21-dimethylisomer of C_{35} in *L.migratoria* and the 11,18-dimethylisomer of C_{30} in *S.gregaria*. The side chains of these isomers are separated by 7 methylene groups. In the present study, however, all of the C_{30} dimethylisomers were found to have their side chains separated by 9 intervening methylene groups.

The dimethylalkanes of *L.migratoria* are similar to those of *L.migratoria cinerascens* (Genin *et al.*, 1986), though the dimethylalkanes of the latter subspecies have longer chain lengths (Figure 7) and the most abundant dimethylalkanes are the 13,21- and the 13,23-dimethylisomers of C_{35} which have either 7 or 9 methylene groups separating their side chains.

The dimethylalkanes of *S.gregaria* have shorter chain lengths than those of *S.vaga* (Nelson & Sukkestad, 1975) and *S.americana* (Jackson, 1982). The main dimethylalkanes of *S.vaga* (Nelson & Sukkestad, 1975)

are the 9,13-, 11,15-, 13,17- and 15,19-dimethylisomers of C_{35} and those of *S.americana* (Jackson, 1982), the 11,15-, 13,17- and 15,19-dimethylisomers of C_{35}. This is similar to the dimethylalkane mixture of *S.gregaria*, where the pentatriacontanes are a mixture of 11,15-, 13,17- and 15,19-dimethylisomers. The dimethylisomers of C_{33} are abundant in *S.vaga* (Nelson & Sukkestad, 1975) as well as in *S.gregaria* as found in this study. Furthermore, the dimethylisomer mixtures are similar in all three species. Thus *S.gregaria* and *S.vaga* (Nelson & Sukkestad, 1975) have mixtures of 9,13-, 11,15- and 13,17-dimethylisomers and *S.americana* (Jackson, 1982) mixtures of 9,13-, 11,15-, 13,17- and 15,19-dimethylisomers.

When the dimethylalkanes of *L.migratoria* and *S.gregaria* are compared with those of the examined species of *Melanoplus* (Figure 7), similarities become apparent. Soliday *et al.* (1974) in their early work on the hydrocarbons of *M.sanguinipes* and *M.packardii*, identified the most abundant dimethylalkanes as the 9,13-, 11,15-, 13,17- and 15,19-dimethylisomers of C_{33} and C_{35}, all of which have their side chains separated by 3 methylene groups. However, a subsequent re-analysis of the hydrocarbons of these two species and those of *M.differentialis* by Nelson *et al.* (1984) shows the tritriacontanes and the pentatriacontanes to be mixtures of 11,21- and 13,23-dimethylisomers which have 9 intervening methylene groups.

The dimethylalkanes of *M.bivittatus* (Jackson, 1981) consist of 11,21- and 13,23-dimethylisomers which have 9 methylene groups separating their side chains. The main dimethylalkane of *M.bivittatus* is the 11,21-dimethylisomer of C_{33} which is similar to *L.migratoria* though not to *S.gregaria*. In the other examined species of *Melanoplus*, only one dimethylalkane has been detected in *M.femurrubrum* and only two in *M.dawsoni* (Jackson, 1981). According to Hadley and Massion (1985), *Aeropedellus clavatus* (subfamily Gomphocerinae) lacks

dimethylalkanes altogether (Figure 7).

(5) *Trimethylalkanes*

Trimethylalkanes were found only in *S.gregaria*, where they have even carbon numbers ranging from 34 to 38. The 9,13,21-trimethylisomer of C_{33} is the most abundant trimethylalkane. The trimethylalkanes of *S.gregaria* have their first and second side chains separated by 3 methylene groups and their second and third side chains separated by 7 methylene groups.

Lockey (1976) also detected trimethylalkanes in *S.gregaria*, though according to him, they all had odd carbon numbers ranging from 33 to 37. The 9,13,20-trimethylisomer of C_{32} which Lockey lists as the most abundant trimethylalkane has 3 intervening methylene groups between side chains one and two and 6 intervening methylene groups between side chains two and three. Trimethylisomers of this type were not detected in the present work. Furthermore, biochemical studies show that insects synthesize methylalkanes de novo from acetate and propionate units by a decarboxylation-elongation pathway. Such a process cannot synthesize methylalkanes with an even number of methylene groups between the side chains (Lockey, 1988).

The trimethylalkanes of *S.vaga* (Nelson & Sukkestad, 1975) and *S.americana* (Jackson, 1982) also have even carbon number like those of *S.gregaria*. In *S.vaga* (Nelson & Sukkestad, 1975) and *S.americana* (Jackson, 1982), the most abundant trimethylalkanes are the 11,15,19- and 13,17,21-trimethylisomers of C_{35} which have 3 intervening methylene groups between the side chains.

(6) *Hydrocarbon mixtures of family Acrididae*

The foregoing comparison shows that the cuticular hydrocarbons of 12 examined acridid species (Figure 7) share the following

characteristics:

1. All of the hydrocarbons are saturated (though Hadley & Massion [1985] report trace amounts of unidentified unsaturated hydrocarbons in *Aeropedellus clavatus*).
2. The main n-alkanes in order of abundance are $nC_{29} > nC_{31} > nC_{27} > nC_{25}$.
3. The most abundant monomethylalkanes and dimethylalkanes are respectively the monomethylisomers of C_{33}, C_{35} and C_{31} and the dimethylisomers of C_{33} and C_{35}.

The differences between the hydrocarbons of these species include:

1. Chain length range of hydrocarbon classes.
2. The presence of 2-methylalkanes in five species of *Melanoplus* and the absence of 2-methyl- and 3-methylalkanes from *S.vaga* and *S.americana*.
3. The presence of trimethylalkanes in only *S.gregaria*, *S.vaga* and *S.americana*.
4. The absence of dimethylalkanes from *Aeropedellus clavatus*.

Insects synthesize most of their cuticular hydrocarbons in the oenocytes (Diehl, 1975; Nelson, 1969; Wigglesworth, 1970). The enzymes involved in the synthesis are determined by genotype, so the differences in the hydrocarbon composition of the examined acridid species are most likely due to the different genotypes. Diet may also determine the variety of hydrocarbons synthesized by an insect. *L.migratoria* is a graminivorous species which feeds mainly on grasses, while *S.gregaria* is an omnivorous species feeding on a much wider range of food-plants (Uvarov, 1966). This could allow *S.gregaria* to synthesize a more complex mixture of methylalkanes than *L.migratoria*. Furthermore, Blomquist and Jackson (1973) have shown that *Melanoplus sanguinipes* obtains its cuticular n-alkanes mainly from its diet, so

that the differences in n-alkane composition between the acridid species could also be due in part to diet.

(7) *Hydrocarbon chemotaxonomy*

L.migratoria and *S.gregaria* belong to different subfamilies of Acrididae (Uvarov, 1966). They have clearly different chromatogram profiles (Figures 1 & 2), and they can be readily distinguished by their hydrocarbon composition (Figure 3).

The hydrocarbon composition of the twelve examined acridid species, which together represent four subfamilies of Acrididae, are given in Figure 7. Their shared characteristics are listed in Section 6. In subfamily Cyrtacanthacridinae, *S.gregaria*, *S.vaga* and *S.americana* have similar hydrocarbon mixtures, particularly as each species has even numbered trimethylalkanes. However, *S.gregaria* has 3-methylalkanes which *S.vaga* and *S.americana* lack.

Similarities between the hydrocarbon compositions of *L.migratoria* and *L.migratoria cinerascens* (subfamily Oedipodinae) are also obvious. Both species have extensive homologous series of dimethylalkanes comprising mixtures of dimethylisomers with either 7 or 9 intervening methylene groups between their side chains.

In subfamily Catantopinae, the congenerics, *M.sanguinipes* and *M.packardii* (Soliday *et al.*, 1974) have similar chromatogram profiles and except for differences between methylalkane chain length and the position of the methyl branch on odd numbered monomethylalkanes, their hydrocarbon compositions are very similar. This has been verified in a subsequent re-analysis of the methylalkanes of *M.sanguinipes* and *M.packardii* with another congeneric, *M.differentialis* by Nelson *et al.* (1984), even though more components were detected. All three species have 2- and 3-methylalkanes and internally branched monomethyl- and dimethylalkanes (Figure 7). At the same time, *M.differentialis* (Nelson

et al., 1984) can be distinguished from *M.sanguinipes* and *M.packardii* (Soliday *et al.*, 1974) by having C_{33} and C_{35} dimethylisomers with 9 intervening groups between the side chains rather than 7 as in *M.sanguinipes* and *M.packardii*. *M.bivittatus*, *M.femurrubrum* and *M.dawsoni* (Jackson, 1981) also have 2-methylalkanes and internally branched monomethyl- and dimethylalkanes, but they differ from *M.sanguinipes*, *M.packardii* and *M.differentialis* (Nelson *et al.*, 1984) in that they lack 3-methylalkanes. Even so, in all six species of *Melanoplus*, the most common monomethylalkanes occur as 11-,13- & 15-monomethylisomers and the dimethylalkanes are represented by 11,21- and 13,23-dimethylisomers which have 9 methylene groups separating the side chains.

Thus in conclusion, subfamily Cyrtacanthacridinae can be distinguished from the other subfamilies by the presence of trimethylalkanes and subfamily Catantopinae from subfamily Oedipodinae by the presence of a more extensive range of dimethylalkanes in the latter. According to Hadley and Massion (1985), *Aeropedellus clavatus* lacks dimethylalkanes, and this species therefore differs from all other examined acridid species. On this basis subfamily Gomphocerinae can be separated from the other subfamilies.

B. Esters

In *L.migratoria*, esters account for 7.4% and 11.4% of the male and female cuticular lipids respectively, while in *S.gregaria* they account for 6.4% of the male lipid and 6.7% of the female lipid. These proportions are lower than those of the examined species of genus *Melanoplus* (Table 8), where they constitute from 26 to 31% of the cuticular lipid of *M.sanguinipes* and from 18 to 20% of the lipid of *M.packardii* (Blomquist *et al.*, 1972). The values given by Soliday *et al.* (1974), namely, *M.sanguinipes*, 28% and *M.packardii*, 18% fall

within these ranges. Esters account for from 18 to 30% of the cuticular lipids of *M.bivittatus*, *M.femurrubrum* and *M.dawsoni* (Jackson, 1981). One major difference between the esters of *L.migratoria* and *S.gregaria* and those of genus *Melanoplus* is that the esters of *L.migratoria* and *S.gregaria* are composed of primary alcohols, while those of the species of *Melanoplus* so far examined are composed of secondary alcohols (Table 8). Primary alcohols however, do occur in the cuticular esters of *M.sanguinipes* and *M.packardii* though these were not identified by Soliday *et al.* (1974) due to insufficient quantities.

(1) *Ester fatty acids*

The ester fatty acids of *L.migratoria* and *S.gregaria* range in carbon number from 10 to 22. The main fatty acids of *L.migratoria*, in order of abundance are 16:0 > 18:0 > 14:0 > 20:0 (Figure 6a), while those of *S.gregaria* are 16:0 > 14:0 > 10:0 > 12:0 > 18:0. In both species saturated fatty acids with even carbon numbers predominate. Interspecific differences include the presence of the odd-numbered saturated acid 17:0 and the unsaturated acid 20:1 in *S.gregaria*. The fatty acids of the examined species of *Melanoplus* (Table 8) have shorter chain lengths than those of either *L.migratoria* or *S.gregaria* and range from 14 to 22 carbons in *M.sanguinipes* and from 12 to 20 carbons in *M.packardii* (Blomquist *et al.*, 1972). The main fatty acids of *M.sanguinipes*, in order of abundance, are 18:0 > 20:0 > 16:0 and those of *M.packardii* 16:0 > 18:0 > 14:0 > 20:0. The latter mixture is similar to that of *L.migratoria*. Fatty acid 15:0 which is found in both *L.migratoria* and *S.gregaria* occurs in *M.packardii* but not in *M.sanguinipes* (Blomquist *et al.*, 1972). Although fatty acid 19:0 occurs in *M.sanguinipes*, it is absent from *M.packardii* (Blomquist *et al.*, 1972) and it was not detected in either *L.migratoria* or

S.gregaria. Unsaturated fatty acids 20:1, which occurs in *S.gregaria*, and 22:1 which occurs in both *L.migratoria* and *S.gregaria* have not been reported in either *M.sanguinipes* or *M.packardii* (Blomquist *et al.*, 1972). The main fatty acids of *M.bivittatus* are 14:0 > 16:0 > 18:0 (Jackson, 1981).

(C) Triglycerides

Triglycerides are probably true components of the cuticular lipids of *L.migratoria* and *S.gregaria* rather than contaminants from body lipid as they were found in the wing cuticular lipids. However, the high proportion of triglycerides in the combined wing and tergal lipid of both species is probably due to contamination from body lipid because of the long period of extraction with hot chloroform. If this is true, then the triglyceride fatty acid composition detected could reflect that of the fat body triglycerides. In the fat body of *S.gregaria*, palmitic and stearic acids are the only fatty acid components of the triglycerides while in *L.migratoria*, the fatty acid components of the fat body triglycerides are palmitic and oleic acids (Uvarov, 1966). The triglyceride fatty acids of *L.migratoria* range from 10 to 22 carbons. The most abundant acids are in order of abundance 16:0 > 20:1 > 18:0 > 18:2 > 14:0 (male) and 16:0 > 18:0 > 14:0 > 20:1 > 18:2 (female). The main difference between males and females of *L.migratoria* is the degree of unsaturation, with the male having a higher proportion (32.4%) than the female (20.4%). The triglyceride fatty acids of *S.gregaria* range from 14 to 18 carbons (male) and from 10 to 18 carbons (female). Fatty acids 10:0 and 12:0 occur in the female only and 15:0 in the male only. The main fatty acids of the male, in order of abundance, are 16:0 > 18:0 > 14:0 and the female 16:0 > 14:0 > 18:0. Both species have higher proportions of even-numbered fatty acids than odd-numbered acids. In *L.migratoria*

saturated fatty acids are in higher proportions than unsaturated acids while *S.gregaria* has only saturated fatty acids.

The triglyceride fatty acids of *Melanoplus sanguinipes* range from 12 to 24 carbons and from 14 to 18 carbons in *M.packardii* (Soliday *et al.*, 1974). The main fatty acids of *M.sanguinipes*, in order of abundance, are 16:0 > 18:1 > 18:3 > 18:2 and those of *M.packardii*, 18:1 > 16:0 > 18:2 > 18:3. The main differences between the triglyceride fatty acid compositions of *L.migratoria* and *S.gregaria* and those of *M.sanguinipes* and *M.packardii* (Soliday *et al.*, 1974) are that *L.migratoria* and *S.gregaria* have higher proportions of saturated fatty acids, whereas the two species of *Melanoplus* have higher proportions of unsaturated fatty acids. Fatty acid 17:0, which occurs in *L.migratoria* and *S.gregaria* also occurs in *M.sanguinipes* though only in trace amounts and it is absent altogether from *M.packardii* (Soliday *et al.*, 1974).

The triglyceride fatty acids of the other three examined species of *Melanoplus*, namely, *M.bivittatus*, *M.femurrubrum* and *M.dawsoni* (Jackson, 1981) range from 14 to 18 carbons with 18:1 > 16:0 > 18:2 > 18:0 the main fatty acids. In these three species, unsaturated fatty acids are in higher proportions than saturated acids.

D. Free fatty acids

The free fatty acids of *L.migratoria* range from 10 to 22 carbons, while those of *S.gregaria* range from 10 to 20 carbons. The main fatty acids of *L.migratoria*, in order of abundance, are 16:0 > 14:0 > 15:0 > 18:0, while those of *S.gregaria*, 16:0 > 14:0 > 18:0. Fatty acids 12:1, 17:1, 19:1 and 19:0 occur in *S.gregaria* though not in *L.migratoria*. Conversely, fatty acids 21:3, 22:1 and 22:0 occur in *L.migratoria* but not in *S.gregaria*.

The free fatty acids of two species of *Melanoplus*, namely,

M.sanguinipes and *M.packardii* (Soliday *et al.*, 1974) range from 12 to 22 carbons in *M.sanguinipes* and from 12 to 18 carbons in *M.packardii*. The main fatty acids of both species are, in order of abundance, 18:3 > 18:2 > 18:1 > 16:0. Thus *M.sanguinipes* and *M.packardii* have a higher proportion of unsaturated fatty acids (76% in the former and 81% in the latter) (Soliday *et al.*, 1974) than either *L.migratoria* (15.7%) or *S.gregaria* (2.2%) (Table 6). Fatty acids 12:1, 17:1, 19:1, 19:0 which occur in *S.gregaria* and 21:3, 22:1 which occur in *L.migratoria* are absent from *M.sanguinipes* and *M.packardii* (Soliday *et al.*, 1974).

The free fatty acids of *M.bivittatus* and *M.femurrubrum* range from 14 to 18 carbons with 18:1 > 16:0 > 18:2 > 18:0 as the main fatty acids in order of abundance, while the main fatty acids of *M.dawsoni*, in order of abundance are, 18:1 > 18:2 > 16:0 > 18:3 (Jackson, 1981). In these three species unsaturated fatty acids are in higher proportions than saturated acids.

In some early work, Thompson and Barlow (1970) report that the free fatty acids of *S.gregaria* range from 14 to 18 carbons and that the main acids, in order of abundance, are 18:1 > 16:0 > 18:2 > 18:3. They also report that unsaturated fatty acids are in higher proportions than saturated acids. These results differ from those of the present work which has shown that the fatty acids of this species have chain lengths ranging from C_{10} to C_{20} and that saturated acids are in higher proportions than unsaturated acids. Thompson and Barlow (1970) failed to detect saturated acids 15:0 and 17:0 and unsaturated acids 12:1 and 17:1.

E. Free primary alcohols

The free primary alcohols of *L.migratoria* range from 12 to 34 carbons and those of *S.gregaria* from 12 to 32 carbons. The main alcohols of *L.migratoria*, in order of abundance, are C14 > C18 > C22 >

C16 and those of *S.gregaria*, C15 > C16 > C18 > C22. This free alcohol composition contrasts with those of *Melanoplus sanguinipes*, *M.packardii* (Soliday *et al.*, 1974) and those of *M.bivittatus*, *M.femurrubrum* and *M.dawsoni* (Jackson, 1981) where the free primary alcohols have shorter chain lengths ranging from C22 to C32 and where C24 > C26 are the most abundant alcohols.

Figure 1

Gas chromatograms of the hydrocarbons of *Locusta migratoria*.

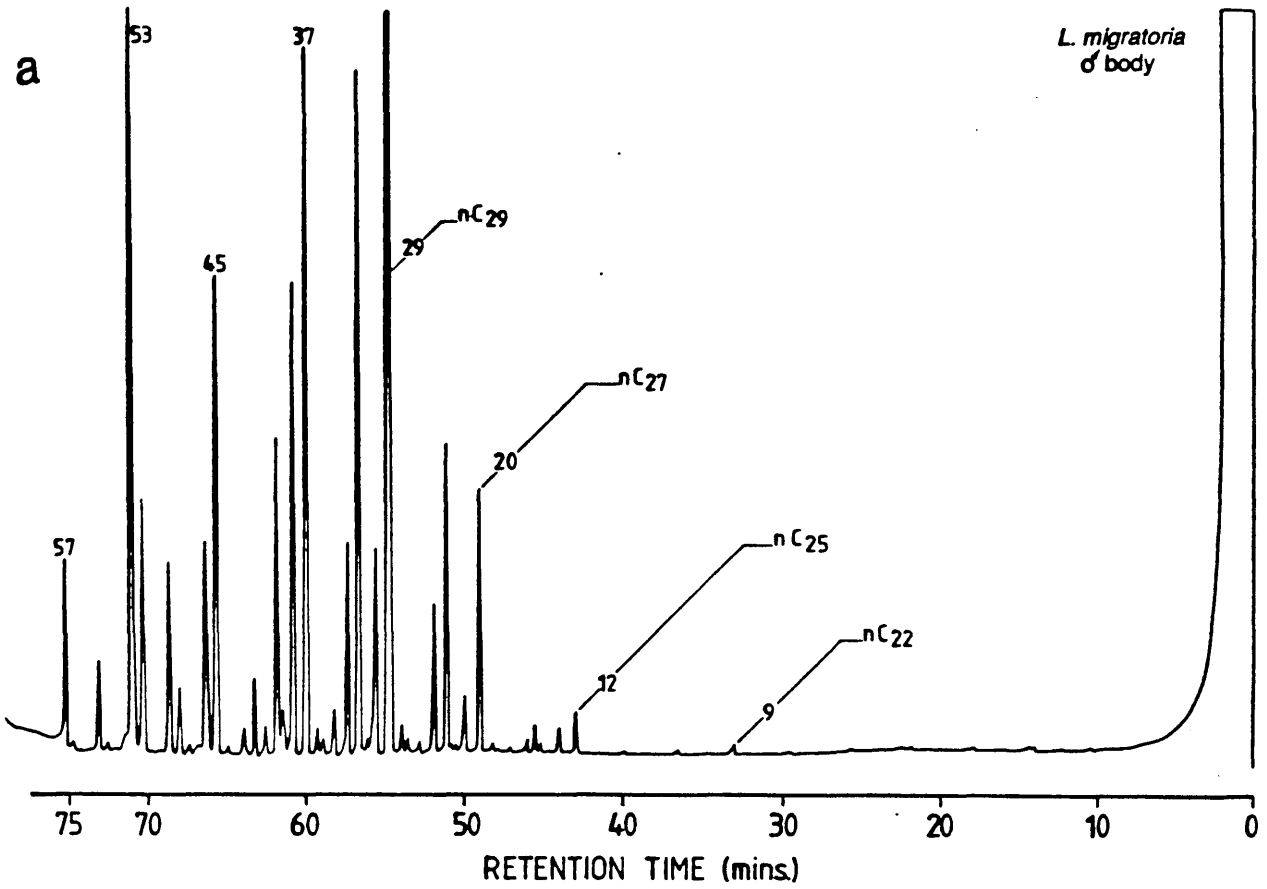
a) Male body whole mixture

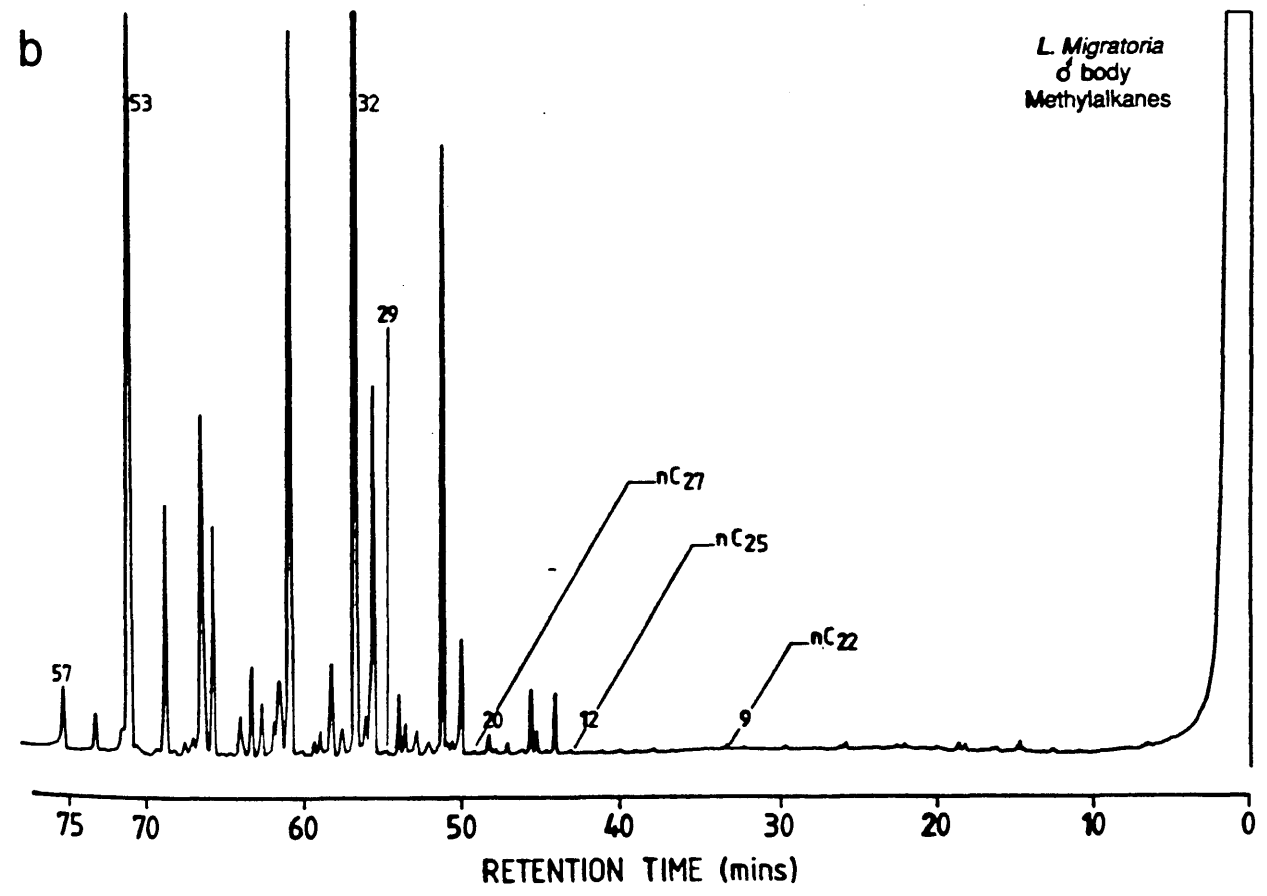
b) Male body methylalkanes

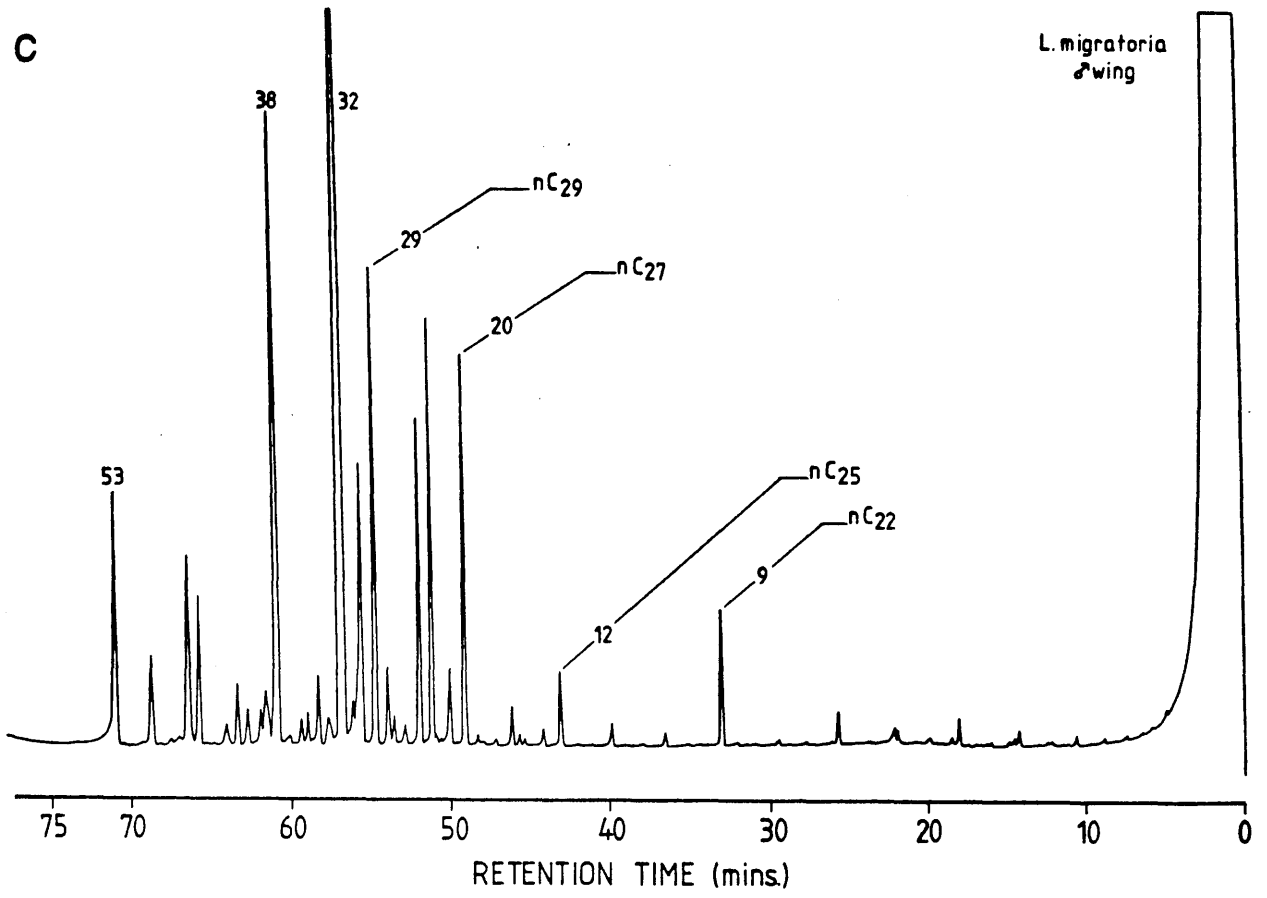
c) Male wing whole mixture

d) Female wing whole mixture

GC analyses on a 12m WCOT fused silica capillary column (bonded phase BP1) temperature programmed from 100 to 300^{o}C at 2^{o}C/minute Carrier gas: helium at 3ml/minute


a
L. migratoria
♂ body
53
37
45
29
nC29
nC27
20
57
nC25
12
nC22
9
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)


b
L. Migratoria
♂ body
Methylalkanes
53
32
29
nC27
nC25
57
nC22
20
12
9
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins)


c
L. migratoria
♂ wing
38
32
nC29
29
nC27
20
53
nC25
nC22
12
9
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)

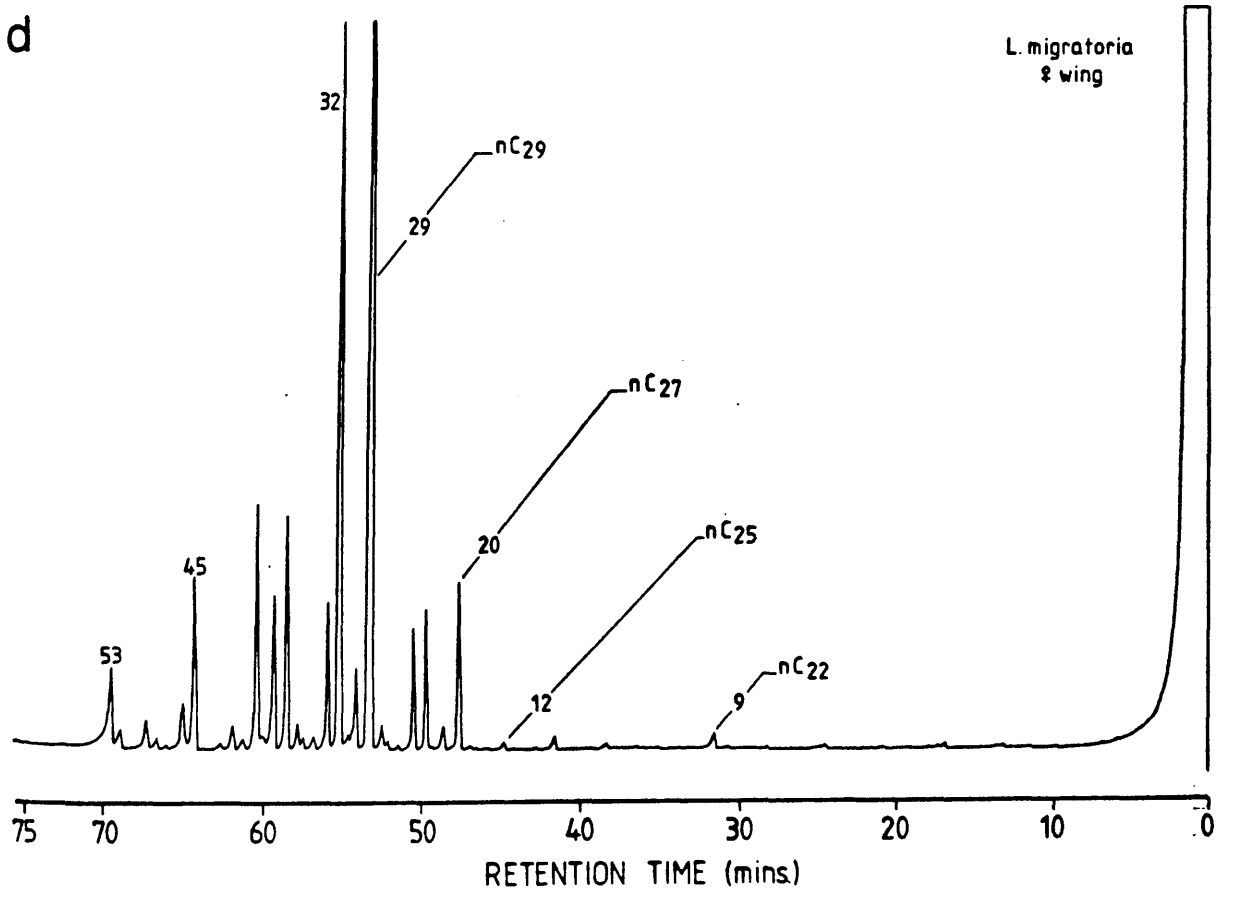

d
L. migratoria
♀ wing
32
nC29
29
nC27
20
nC25
45
53
12
nC22
9
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)

Figure 2

Gas chromatograms of the hydrocarbons of *Schistocerca gregaria*

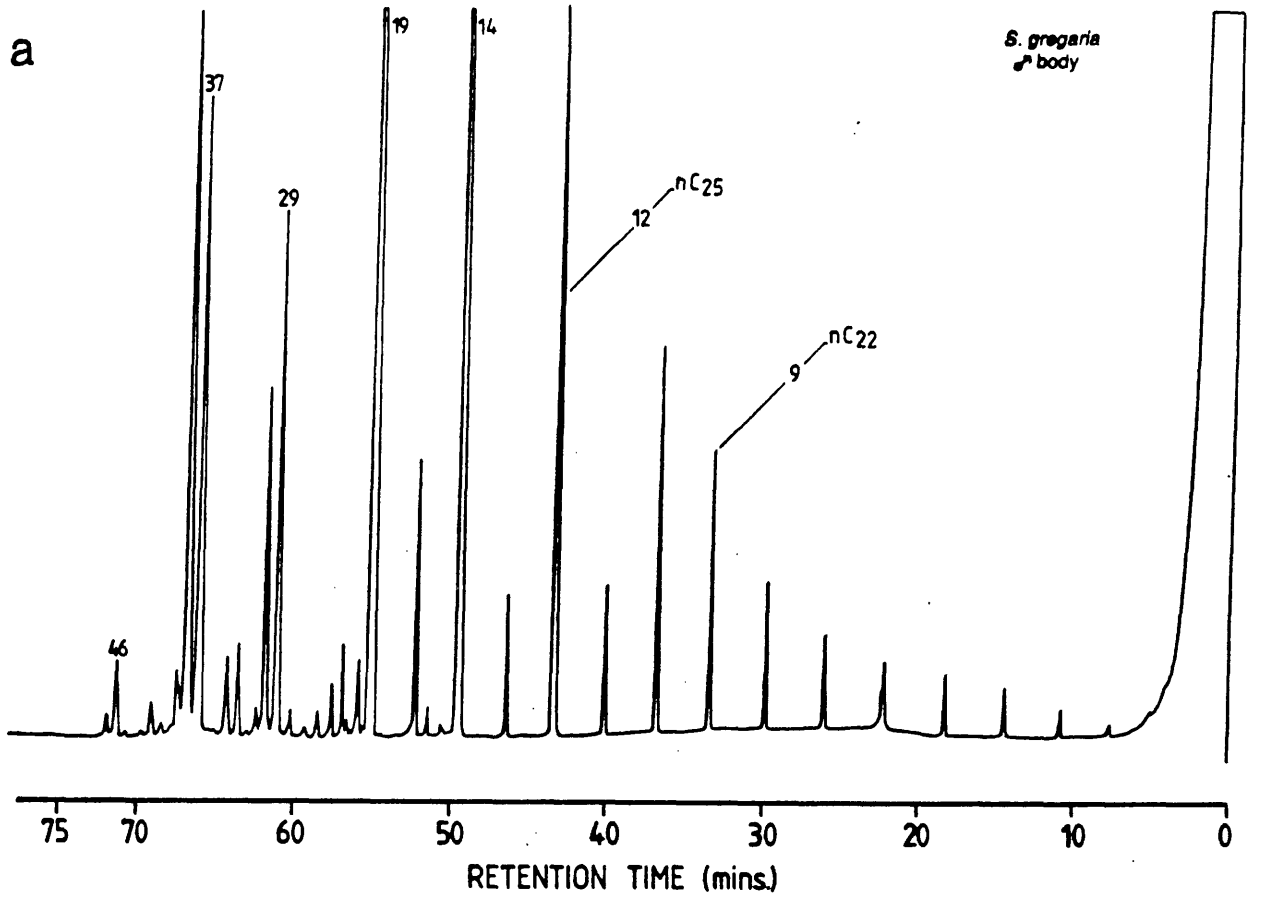
a) Male body whole mixture

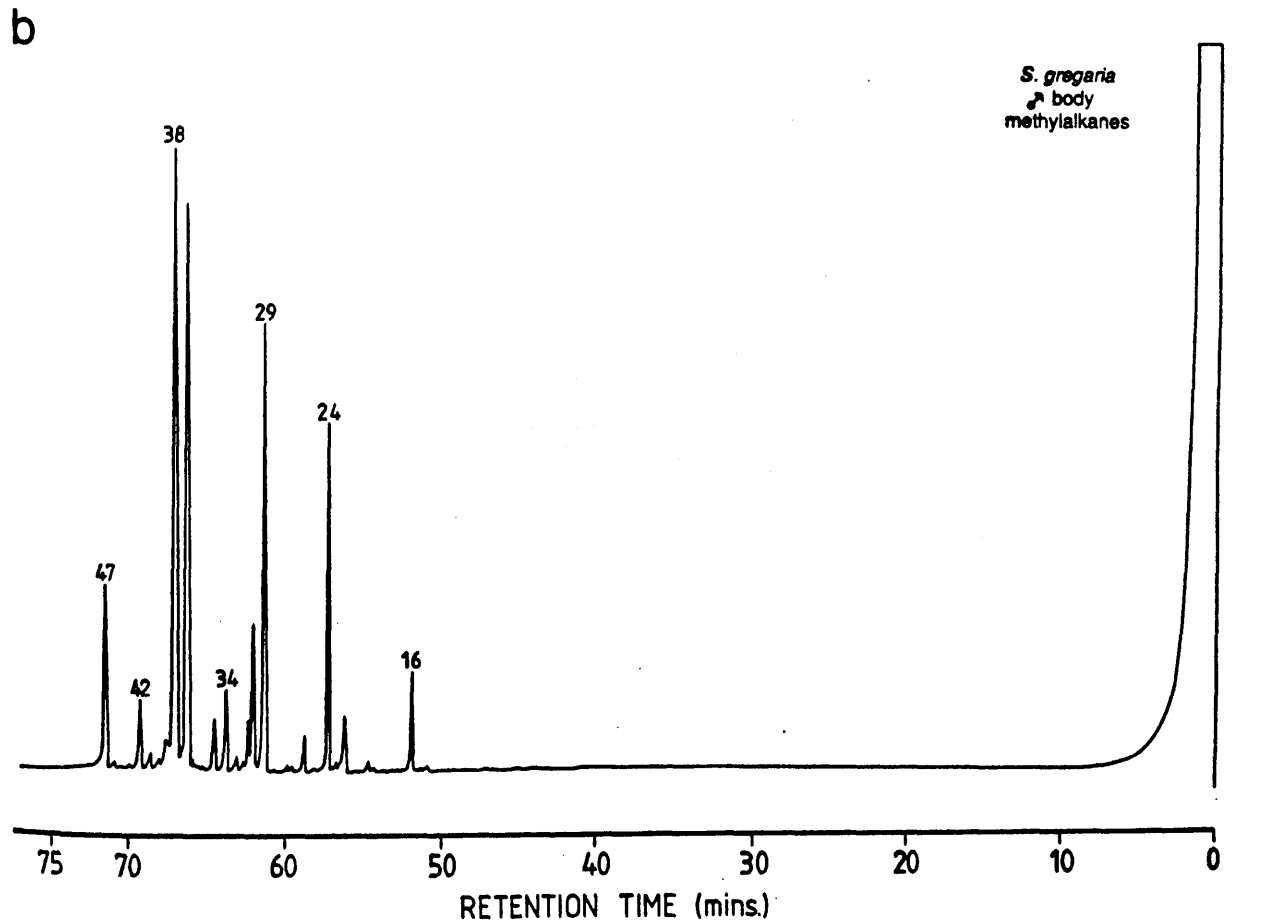
b) Male body methylalkanes

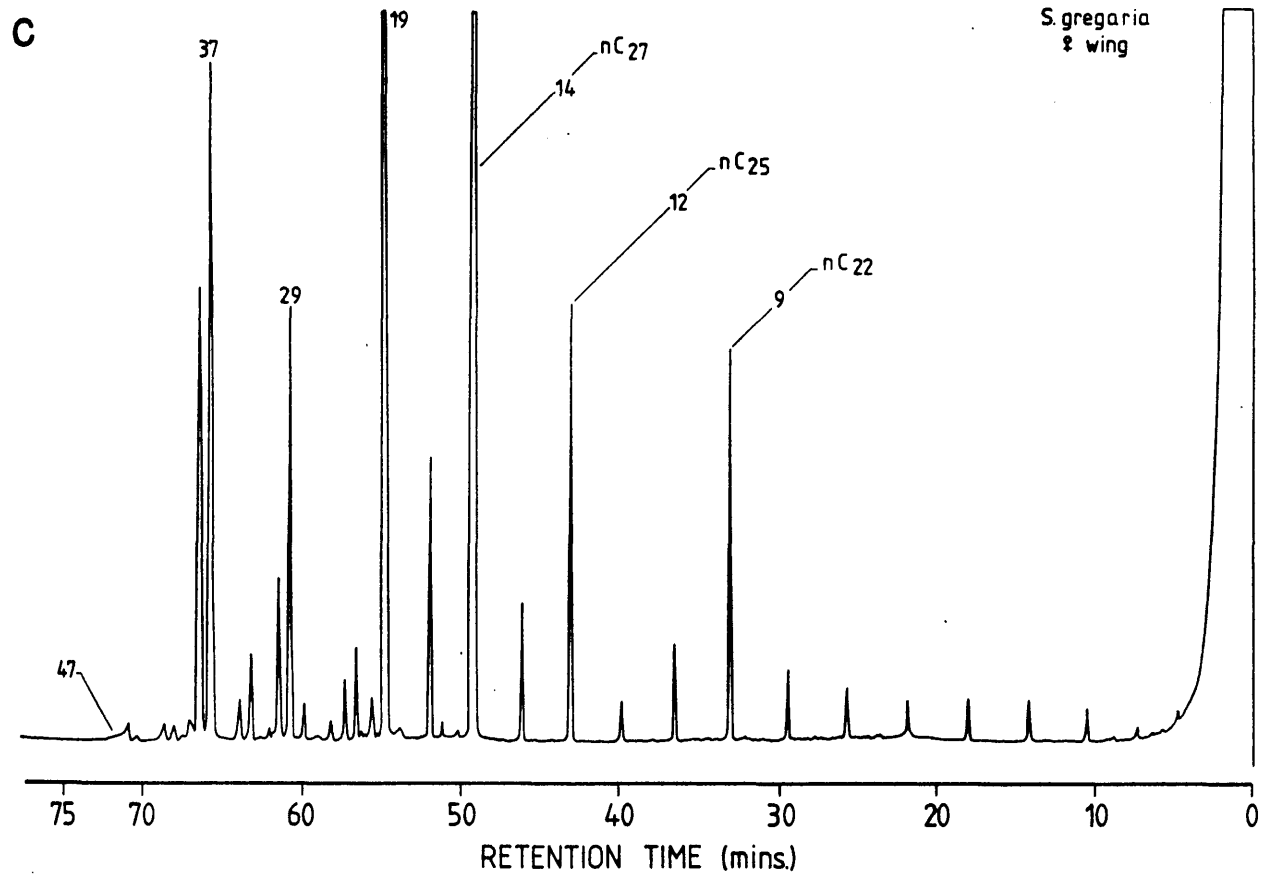
c) Female wing whole mixture

d) Female body whole mixture

GC analyses on a 12m WCOT fused silica capillary column (bonded phase BP1) temperature programmed from 100 to 300^{o}C at 2^{o}C/minute Carrier gas: helium at 3ml/minute


a
S. gregaria
♂ body
37
19
14
29
nC25
12
nC22
9
46
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)


b
S. gregaria
♂ body
methylalkanes
38
29
24
47
42
34
16
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)


c
S. gregaria
♀ wing
37
19
nC27
14
nC25
12
nC22
9
29
47
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)

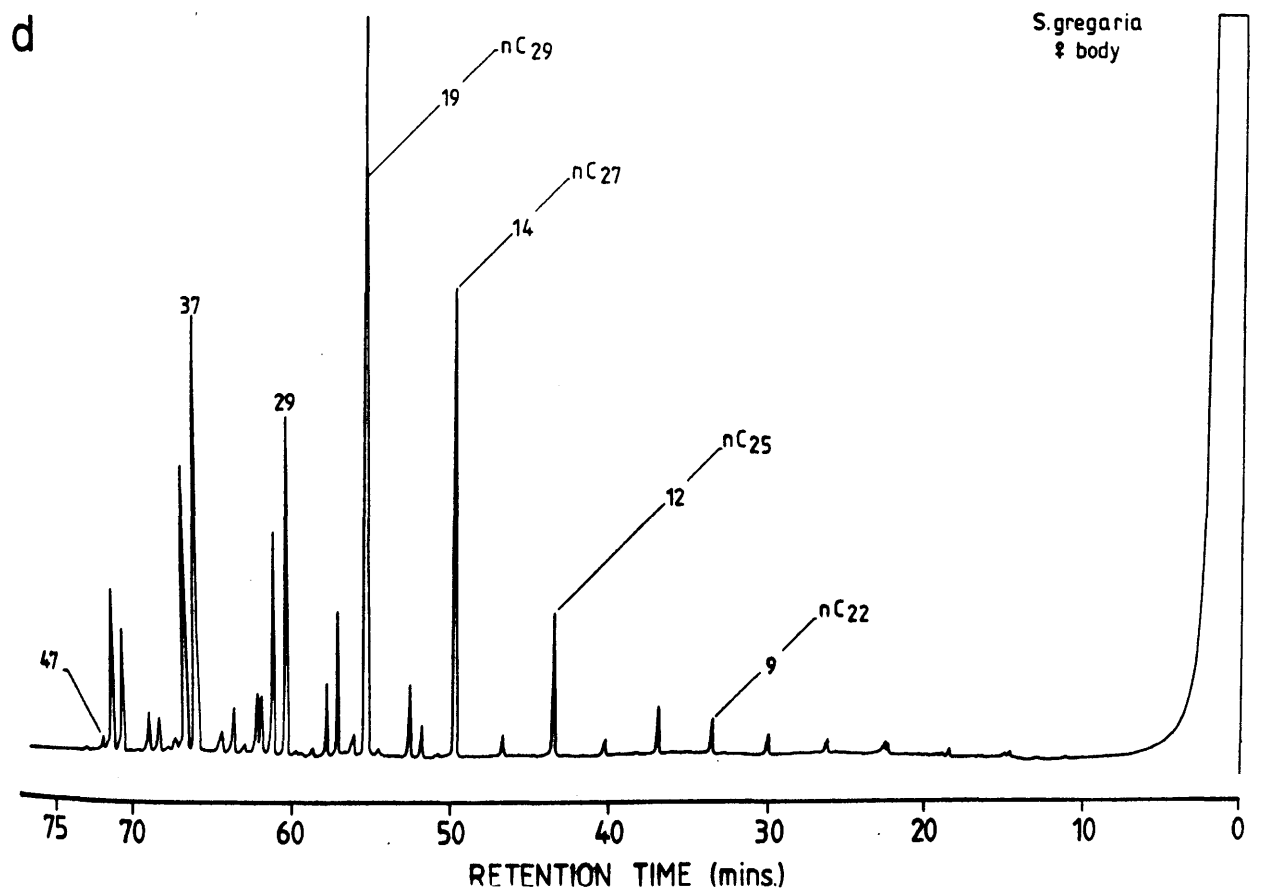

d
S. gregaria
♀ body
nC29
19
nC27
14
37
29
nC25
12
nC22
9
47
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)

Figure 3

The hydrocarbon composition of *Locusta migratoria* and *Schistocerca gregaria* namely, male body mixture

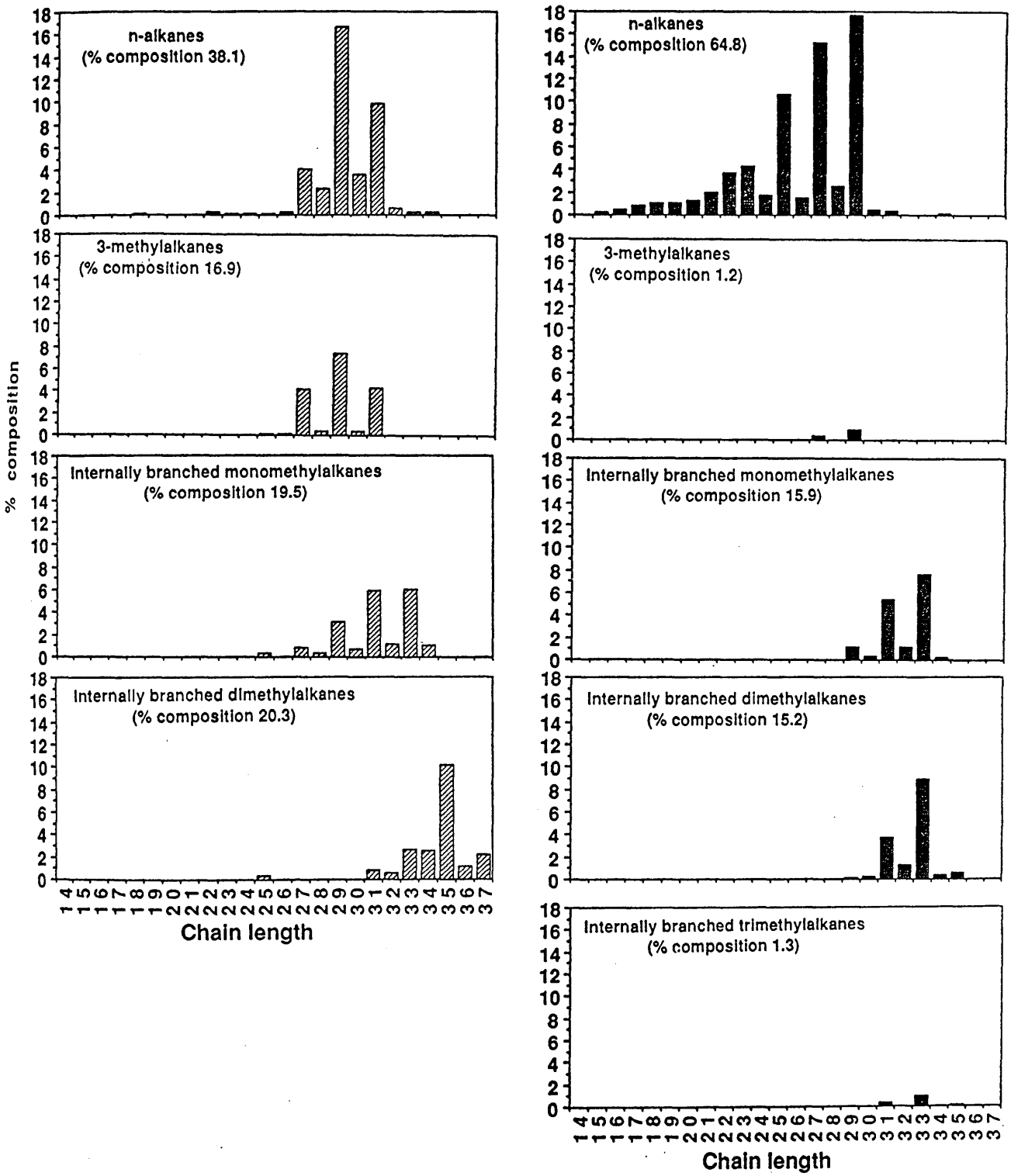

n-alkanes
(% composition 38.1)
3-methylalkanes
(% composition 16.9)
Internally branched monomethylalkanes
(% composition 19.5)
Internally branched dimethylalkanes
(% composition 20.3)
n-alkanes
(% composition 64.8)
3-methylalkanes
(% composition 1.2)
Internally branched monomethylalkanes
(% composition 15.9)
Internally branched dimethylalkanes
(% composition 15.2)
Internally branched trimethylalkanes
(% composition 1.3)
% composition
Chain length
Chain length

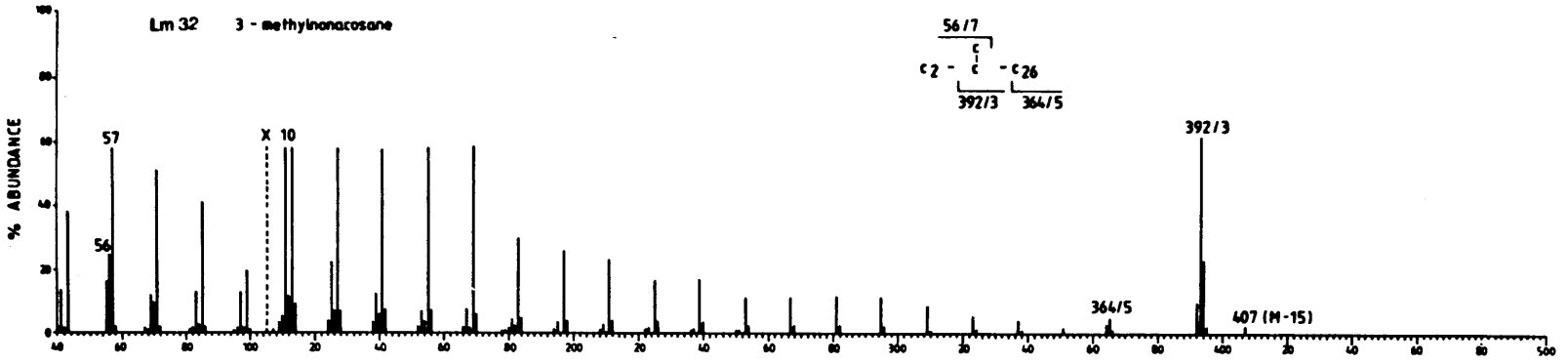
Figure 4

Locusta migratoria: mass spectral scans of

a) GC peak Lm 32, 3-methylnoncosane

b) GC peak Lm 38, 9-,11-,13- & 15-methylhentriacontane

a


Lm 32 3 - methylnonacosane
% ABUNDANCE
57
56
X 10
56/7
C
C2 - C - C26
392/3 364/5
392/3
364/5
407 (M-15)

b

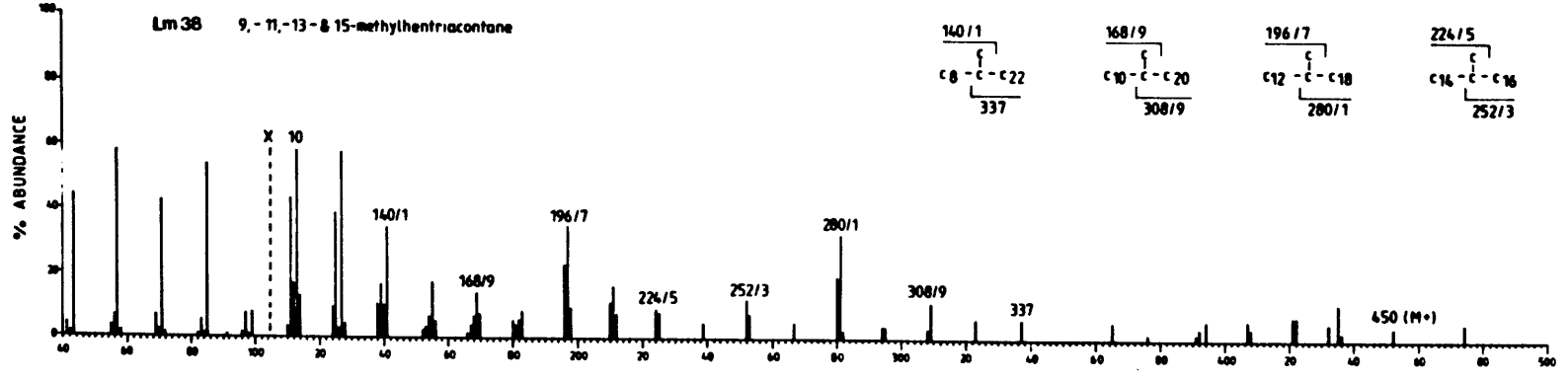
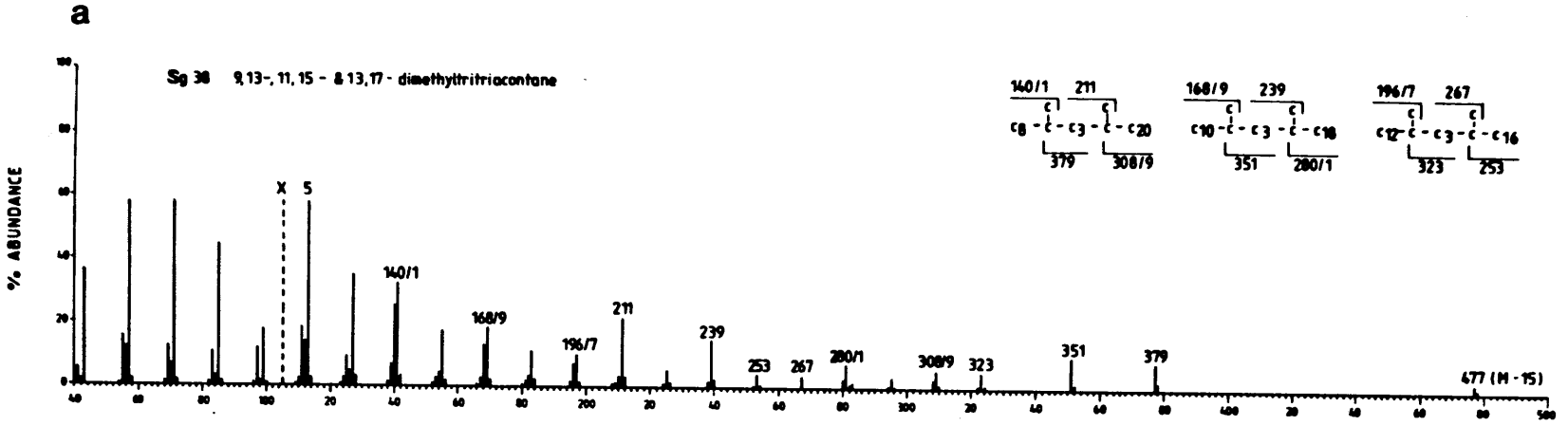

Lm 38 9, - 11, -13 - & 15-methylhentriacontane
% ABUNDANCE
X 10
140/1
168/9
196/7
224/5
252/3
280/1
308/9
337
450 (M+)
140/1
C
C8 - C - C22
337
168/9
C
C10 - C - C20
308/9
196/7
C
C12 - C - C18
280/1
224/5
C
C14 - C - C16
252/3

Figure 5

Schistocerca gregaria: mass spectral scans of

a) GC peak Sg 38, 9,13-,11,15- & 13,17-dimethyltritriacontane

b) GC peak Sg 47, 11,15,23-trimethylpentatriacontane


a
Sg 38 9,13-, 11,15 - & 13,17 - dimethyltritriacontane
% ABUNDANCE
X 5
140/1
168/9
196/7
211
239
253
267
280/1
308/9
323
351
379
477 (M-15)
140/1 211
C8 - C - C3 - C - C20
379 308/9
168/9 239
C10 - C - C3 - C - C18
351 280/1
196/7 267
C12 - C - C3 - C - C16
323 253

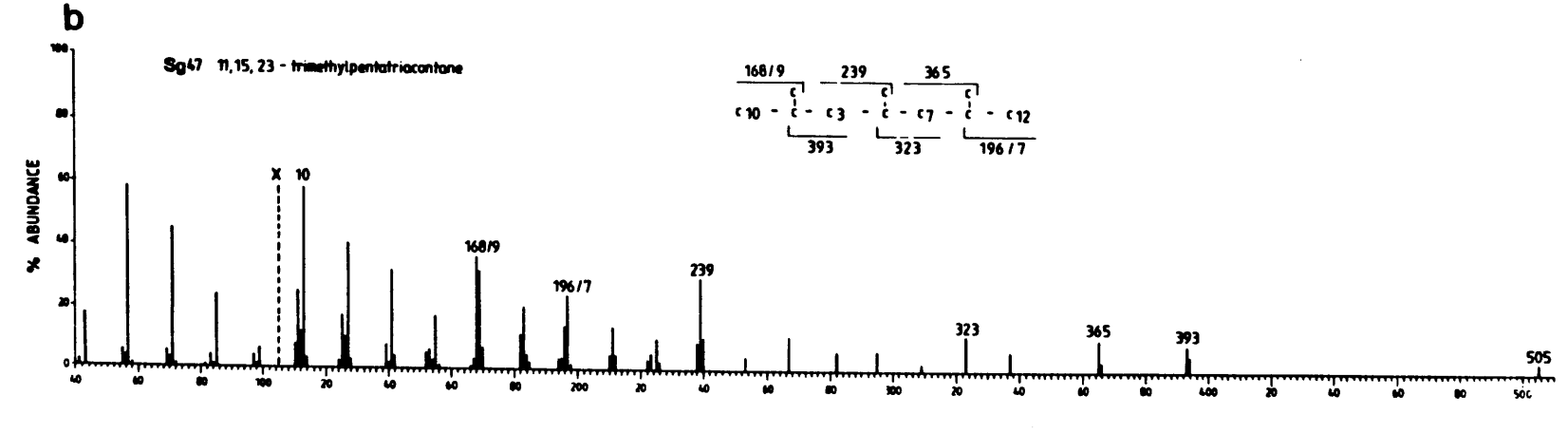

b
Sg47 11,15,23 - trimethylpentatriacontane
% ABUNDANCE
X 10
168/9
196/7
239
323
365
393
505
168/9 239 365
C10 - C - C3 - C - C7 - C - C12
393 323 196/7

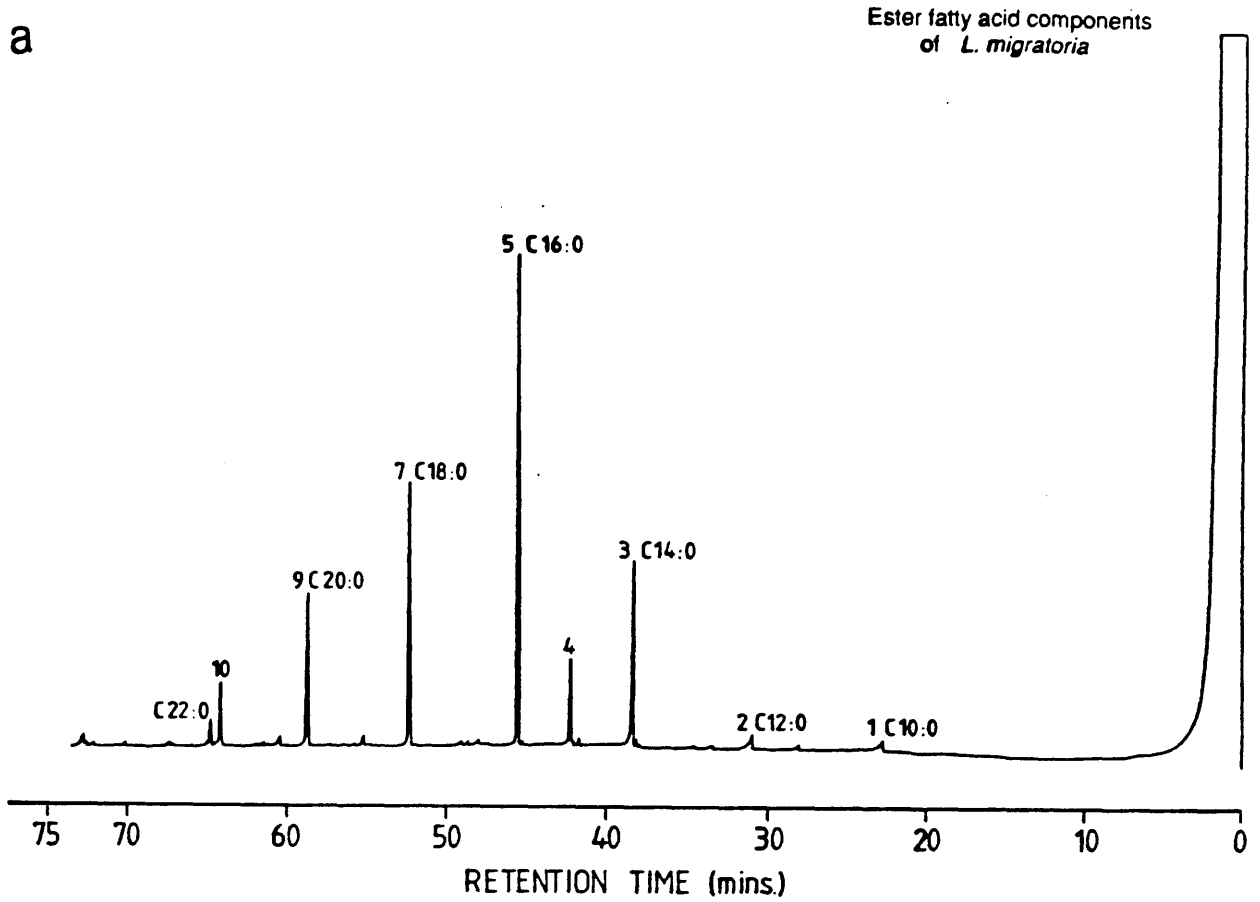
Figure 6

Gas chromatograms of

a) Ester fatty acids (methylated) from mixed males and females of *Locusta migratoria*

b) Triglyceride fatty acids (methylated) from females of *Locusta migratoria*

a


Ester fatty acid components
of L. migratoria
5 C16:0
7 C18:0
9 C20:0
3 C14:0
10
C22:0
4
2 C12:0
1 C10:0
75
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)

b

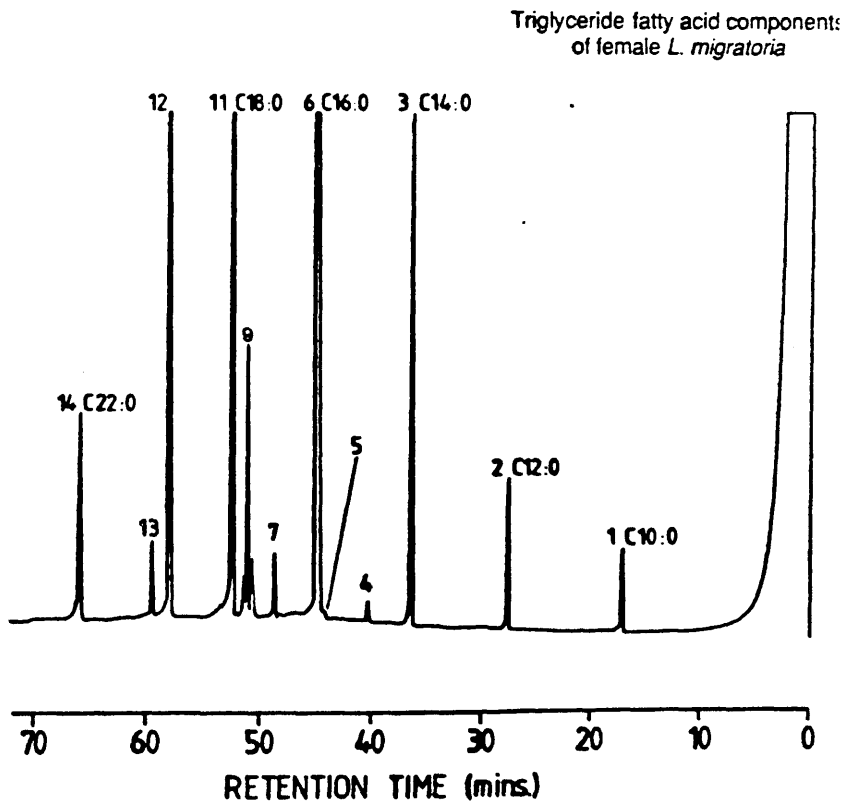

Triglyceride fatty acid components
of female L. migratoria
12
11 C18:0
6 C16:0
3 C14:0
9
14 C22:0
5
2 C12:0
13
7
1 C10:0
4
70
60
50
40
30
20
10
0
RETENTION TIME (mins.)

Figure 7

Hydrocarbons of acridid species

Sg, *Schistocerca gregaria**

Sv, *Schistocerca vaga* (Nelson & Sukkestad, 1975)

Sa, *Schistocerca americana* (Jackson, 1982)

Ms, *Melanoplus sanguinipes* } (Soliday *et al.*, 1974)

Mp, *Melanoplus packardii* }

Ms, *Melanoplus sanguinipes* }

Mp, *Melanoplus packardii* } (Nelson *et al.*, 1984)

Mdiff, *Melanoplus differentialis* }

Lm, *Locusta migratoria**

Lmc, *Locusta migratoria cinerascens* (Genin *et al.*, 1986)

Mb, *Melanoplus bivittatus* }

Mf, *Melanoplus femurrubrum* } (Jackson, 1981)

Mdaw, *Melanoplus dawsoni* }

Ac, *Aeropedellus clavatus* (Hadley & Massion, 1985)

* This study, minimum and maximum amounts from the four lipid sources

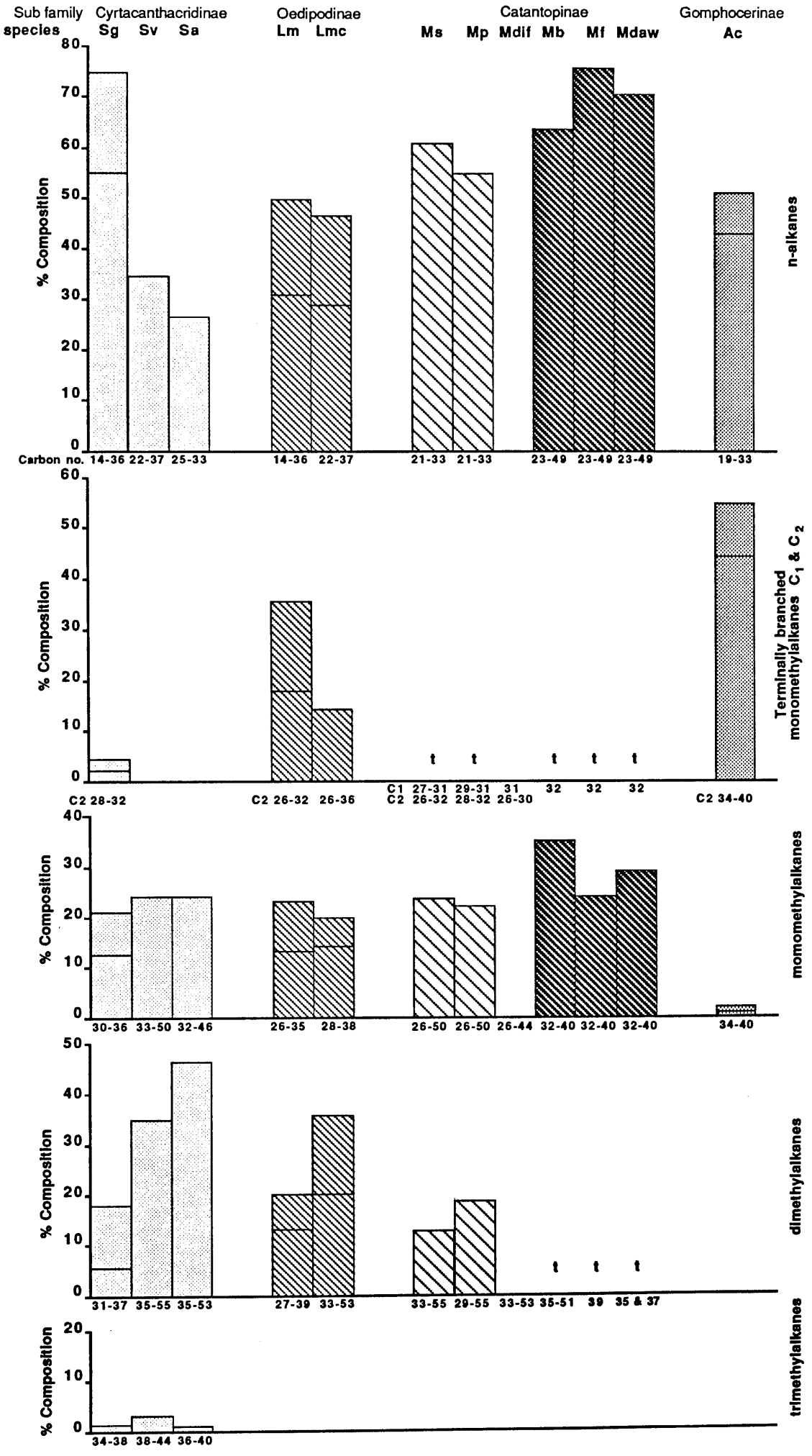

Sub family
species
Cyrtacanthacridinae
Sg
Sv
Sa
Oedipodinae
Lm
Lmc
Catantopinae
Ms
Mp
Mdlf
Mb
Mf
Mdaw
Gomphocerinae
Ac
% Composition
n-alkanes
Carbon no. 14-36 22-37 25-33
14-36 22-37
21-33 21-33
23-49 23-49 23-49
19-33
Terminally branched monomethylalkanes C1 & C2
C2 28-32
C2 26-32 26-36
C1 27-31 29-31 31 32 32 32
C2 26-32 28-32 26-30
C2 34-40
momomethylalkanes
30-36 33-50 32-46
26-35 28-38
26-50 26-50 26-44 32-40 32-40 32-40
34-40
dimethylalkanes
31-37 35-55 35-53
27-39 33-53
33-55 29-55 33-53 35-51 39 35 & 37
trimethylalkanes
34-38 38-44 36-40

Table 1a: Retention factors (R_f) of lipid fractions and standards (in brackets) after thin layer chromatography (TLC)

Species	Sex	Source of lipid	Hydrocarbons	Esters	Triglycerides	Free fatty acids	Alcohols
L.migratoria	Female	Body	84 (82)	50 (49.5)	34 (35)	16 (20)	6 (13)
S.gregaria	Male	Wing	89 (85)	44 (46)	27 (29)	12.5 (17.5)	6 (13))

Table 1b: Retention factors (R_f) of polar derivatives and standards (in brackets) following saponification, methylation and acetylation

Species	Sex	Source of lipid	Esters		Triglycerides	Free fatty acids	Primary alcohols
			Methyl acids	Acetylated alcohols	Methyl acids	Methyl acids	Acetylated alcohols
L.migratoria	Female	Wing & body combined	62.5 (62.5)	54 (54)	52.5 (55.5)	48 (48)	57 (53)
S.gregaria	Male	Wing & body combined	59 (58)	47.5 (48)	55 (53.5)	71 (72)	51 (52.5)

Table 2a: Approximate percentage composition (%) (mean of three replicates) of lipid fractions after thin layer chromatography (TLC)

Species	Sex	Source of lipid	Hc %	E %	Tri. %	Ffa %	Alc. %
L.migratoria	Female	Wing & body combined	22.9	11.4	57.1	4.6	4.0
L.migratoria	Male	Wing & body combined	29.6	7.4	51.9	7.4	3.7
S.gregaria	Female	Wing & body combined	18.7	6.7	64.8	4.7	5.1
S.gregaria	Male	Wing & body combined	24.7	6.4	65.7	1.5	1.7

Alc, alcohols; E, esters; Ffa, free fatty acids;
Hc, hydrocarbons; Tri, triglycerides

Table 2b: Approximate percentage composition (%) (mean of three replicates) of hydrocarbons after column chromatography

Species	Sex	Source of lipid	Hydrocarbons %
L.migratoria	Female	Wing	53.3
" "	"	Body	50.7
" "	Male	Wing	40.5
" "	"	Body	37.6
S.gregaria	Female	Wing	68.3
" "	"	Body	36.7
" "	Male	Wing	62.5
" "	"	Body	36.2

Table 3a: Hydrocarbon composition of L.migratoria.
The values for percentage composition (% comp.) and retention indices (I) the mean of three replicates. t = trace (< 0.1%)
FW = Female wing; MW = Male wing; FB = Female body; MB = Male body

GC Peak No.	Average Retention Index (I)	Average dI	Carbon No.	% Composition				L M B Hc		L M B - diagnostic fragment ions of methylalkanes m/z
				FW	MW	FB	MB	GC	GC-MS	
1	1400		14	t	t	-	t	n-tetradecane		
2	1496		15	t	0.1	-	t	n-pentadecane		
3	1600		16	t	0.3	t	t	n-hexadecane		
4	1701		17	0.1	0.4	-	t	n-heptadecane		
5	1800		18	0.2	0.6	t	0.1	n-octadecane		
6	1902		19	t	0.5	-	t	n-nonadecane		
7	2000		20	0.1	0.7	t	t	n-eicosane		
8	2104		21	t	0.1	-	t	n-heneicosane		
9	2200		22	0.5	2.9	t	0.2	n-docosane		
10	2301		23	t	0.3	t	0.1	n-tricosane		
11	2400		24	0.1	0.5	t	0.1	n-tetracosane		
12	2500		25	0.4	1.5	0.3	0.1	n-pentacosane		
13	2536	64	26	t	0.4	t	0.4		11- & 13-methylpentacosane	168/9, 196/7, 224/5, 351 (M-15)
14	2573	27	26	t	0.1	t	0.1		3-methylpentacosane	56/7, 308/9, 336/7, 351 (M-15)
15	2583	117	27	t	0.1	t	0.3		5,13-dimethylpentacosane	84/5, 196/7, 211, 323
16	2600		26	0.2	0.8	0.2	0.2	n-hexacosane		
17	2635	65		t	0.1	t	0.1			
18	2663	37		t	t	t	t			
19	2673	27	27	0.1	0.2	0.1	0.1		3-methylhexacosane	56/7, 351
20	2699		27	5.0	6.9	5.0	4.0	n-heptacosane		
21	2733	67	28	0.7	1.3	0.7	0.8		11- & 13-methylheptacosane	168/9, 196/7, 224/5, 252/3
22	2746	54		t	t	t	0.1			
23	2757	43		t	t	t	0.1			
24	2773	27	28	4.0	7.5	3.7	4.2		3-methylheptacosane	56/7, 336/7, 365, 379 (M-15)
25	2800		28	3.5	5.6	1.9	2.3	n-octacosane		
26	2835	65	29	0.2	0.4	0.2	0.2		10-,11-,12-,13- & 14-methyloctacosane	154/5, 168/9, 182/3, 196/7, 210/1, 224/5, 238/9, 252/3, 266/7, 280/1, 393 (M-15)
27	2855	45	29	0.2	0.4	0.2	0.2		4-methyloctacosane	70/1, 336/7, 365, 393 (M-15)
28	2872	28	29	0.5	1.3	0.4	0.4		3-methyloctacosane	56/7, 350/1, 379, 393 (M-15)
29	2900		29	29.6	8.0	21.7	16.6	n-nonacosane		
30	2933	67	30	2.1	4.7	2.3	2.9		9-,11- & 13-methylnonacosane	140/1, 168/9, 196/7, 224/5, 252/3, 280/1, 308/9, 407 (M-15)
31	2953	47	30	0.1	0.5	0.2	0.2		5-methylnonacosane	84/5, 336/7, 365
32	2972	28	30	18.3	23.0	9.1	7.4		3-methylnonacosane	56/7, 364/5, 392/3, 407 (M-15)
33	3000		30	4.0	0.7	3.8	3.6	n-triacontane		
34	3025	75	31	0.5	1.2	0.4	0.7		11-,12-,13-,14- & 15-methyltriacontane	168/9, 182/3, 196/7, 210/1, 224/5, 238/9, 266/7, 280/1, 294/5, 421 (M-15)
35	3053	47		0.3	0.5	0.2	0.2			
36	3066	34	31	0.7	0.4	0.4	0.4		3-methyltriacontane	56/7, 379, 408
37	3100		31	6.0	0.2	11.7	9.8	n-hentriacontane		
38	3126	74	32	3.9	10.6	4.9	5.9		9-,11-,13- & 15-methylhentriacontane	140/1, 168/9, 196/7, 224/5, 252/3, 280/1, 308/9, 336/7, 450 (M+)
39	3151	49	33	0.3	1.2	0.6	0.8		9,13-,11,15- & 13,17-dimethylhentriacontane	140/1, 168/9, 196/7, 211, 224/5, 238/9, 253, 267, 295, 323, 350, 449 (M-15)
40	3165	35	32	5.3	0.6	4.7	4.3		3-methylhentriacontane	56/7, 393, 421

GC Peak No.	Average Retention Index (I)	Average dI	Carbon No.	% Composition				L M B Hc		L M B - diagnostic fragment ions of methylalkanes m/z
				FW	MW	FB	MB	GC	GC-MS	
41	3200		32	0.5	0.7	0.8	0.6	n-dotriacontane		
42	3227	73	33	0.7	1.0	0.8	0.2		12-,13- & 14-methyldotriacontane	182/3, 196/7, 210/1, 280/1, 294/5, 308/9, 449 (M-15)
43	3252	148	34	0.3	0.6	0.5	0.6		11,19- & 12,20-dimethyldotriacontane	168/9, 182/3, 196/7, 210/1, 295, 309, 323, 337, 463 (M-15)
44	3300		33	t	t	0.3	0.2	n-tritriacontane		
45	3327	73	34	4.2	2.6	6.1	6.0		11-,13- & 15-methyltritriacontane	168/9, 196/7, 224/5, 280/1, 308/9, 336/7, 463 (M-15)
46	3356	144	35	1.3	3.3	2.1	2.6		11,21- & 13,19-dimethyltritriacontane	168/9, 196/7, 224/5, 295, 323, 351, 477 (M-15)
47	3382	118		t	0.2	t	0.1			
48	3400		34	0.2	0.1	0.3	0.2	n-tetratriacontane		
49	3426	74	35	0.5	t	1.0	1.0		11-,12-,13-,14-,15-,16- & 17-methyltetratriacontane	168/9, 182/3, 196/7, 210/1, 224/5, 238/9, 252/3, 266/7, 280/1 294/5, 308/9, 323/3, 336/7, 350/1, 492 (M+)
50	3454	146	36	1.0	1.6	2.1	2.5		12,20- & 13,21-dimethyltetratriacontane	182/3, 196/7, 210/1, 224/5, 309, 323, 337, 351, 491 (M-15)
51	3500		35	t	t	t	t	n-pentatriacontane		
52	3528	72		0.7	t	3.4	3.6			
53	3556	144	37	2.6	4.8	8.3	10.2		13,21-dimethylpentatriacontane	196/7, 224/5, 323, 351, 505 (M-15)
54	3600		36	t	t	t	t	n-hexatriacontane		
55	3654	146	38	t	t	0.4	1.1		13,21- & 14,22-dimethylhexatriacontane	196/7, 210/1, 224/5, 238/9, 337, 351, 365, 533 (M-15)
56	3725	75		-	-	t	0.1			
57	3744	56	39	-	-	0.4	2.2		13,23-dimethylheptatriacontane	196/7, 224/5, 351, 379, 533 (M-15)
			Hc %	98.9	99.5	99.2	99.1			
			n-alkanes %	50.4	30.9	46.0	38.1			
			methylalkanes %	48.6	68.6	53.2	61.0			
			3-methylalkanes %	28.9	33.1	18.4	16.9			
			monomethylalkanes %	13.1	23.1	16.8	19.5			
			dimethylalkanes %	5.5	11.6	14.4	20.3			

Table 3b: Hydrocarbon composition of S.gregaria.
The values for percentage composition (% comp.) and retention indices (I) the mean of three replicates. t = trace (< 0.1%)
FW = Female wing; MW = Male wing; FB = Female body; MB = Male body

GC Peak No.	Average Retention Index (I)	Average dI	Carbon No.	% Composition				Sg M B Hc		Sg M B - diagnostic fragment ions of methylalkanes m/z
				FW	MW	FB	MB	GC	GC-MS	
1	1400		14	t	t	t	t	n-tetradecane		
2	1496		15	t	0.1	0.1	0.2	n-pentadecane		
3	1600		16	0.4	0.3	0.6	0.5	n-hexadecane		
4	1697		17	0.7	0.3	0.9	0.8	n-heptadecane		
5	1800		18	0.7	0.4	0.8	1.0	n-octadecane		
6	1899		19	0.6	0.4	0.7	1.0	n-nonadecane		
7	2000		20	0.8	0.6	0.8	1.3	n-eicosane		
8	2101		21	1.1	0.8	1.2	2.0	n-heneicosane		
9	2200		22	6.3	2.9	2.9	3.7	n-docosane		
10	2300		23	1.5	1.7	3.1	4.3	n-tricosane		
11	2400		24	0.6	0.7	1.1	1.7	n-tetracosane		
12	2501		25	6.7	8.0	8.9	10.6	n-pentacosane		
13	2600		26	2.0	1.9	1.4	1.5	n-hexacosane		
14	2701		27	26.3	27.2	14.0	15.2	n-heptacosane		
15	2737		26	0.1	0.4	0.2	0.2			
16	2772	28	28	0.3	0.3	0.1	0.3		3-methylheptacosane	56/7, 365
17	2800		28	3.9	4.1	2.8	2.5	n-octacosane		
18	2873			0.1	t	t	t			
19	2900		29	25.0	11.4	14.9	17.6	n-nonacosane		
20	2924			t	0.1	t	t			
21	2933	67	30	0.7	1.0	0.8	1.1		9-,11- & 13-methylnonacosane	140/1, 168/9, 196/7, 224/5, 252/3, 280/1, 308/9, 407 (M-15)
22	2952	48	30	t	t	t	t		5-methylnonacosane (Estimate)	84/5, 365
23	2961	139	31	t	t	t	0.1		11,15- & 13,17-dimethylnonacosane	168/9, 196/7, 211, 225, 239, 267, 295, 393
24	2971	29	30	1.2	1.3	0.9	0.9		3-methylnonacosane	56/7, 392/3, 407 (M-15)
25	3000		30	0.7	0.3	0.4	0.5	n-triacontane		
26	3032	68	31	0.3	0.6	0.5	0.4		9-,10-,11-,12-,13-,14- & 15-methyltriacontane	154/5, 168/9, 182/3, 196/7, 210/1, 224/5, 252/3, 266/7, 208/1, 308/9, 421 (M-15)
27	3058	142	32	t	0.3	0.2	0.2		8,18- & 10,20-dimethyltriacontane	126/7, 154/5, 168/9, 281, 308/9, 323, 351
28	3101		31	0.4	0.1	0.2	0.3	n-hentriacontane		
29	3131	69	32	5.1	6.8	7.1	5.4		11-,13-methylhentriacontane	168/9, 196/7, 280/1, 308/9, 435 (M-15)
30	3161	139	33	1.7	3.1	3.6	3.7		9,13-,11,15- & 13,17-dimethylhentriacontane	140/1, 168/9, 196/7, 211, 224/5, 239, 267, 252/3, 280/1, 295, 323, 351, 449 (M-15)
31	3168	32	32	t	t	0.1	t		3-methylhentriacontane	56/7, 393, 421
32	3183	217	34	0.1	0.4	0.2	0.3		11,15,19-trimethylhentriacontane	168/9, 196/7, 239, 267, 309, 337
33	3200		32	t	0.2	t	t	n-dotriacontane		
34	3228	72	33	0.9	1.6	1.4	1.2		10-,11-,12-,13- & 15-methyldotriacontane	168/9, 182/3, 196/7, 266/7, 294/5, 308/9, 322/3, 449 (M-15)
35	3261	139	34	0.4	1.1	1.5	1.3		9,19-,10,20- & 11,21-dimethyldotriacontane	140/1, 154/5, 182/3, 196/7, 210/1, 295, 337, 351, 365, 463 (M-15)

GC Peak No.	Average Retention Index (I)	Average dI	Carbon No.	% Composition				Sg M B Hc		Sg M B - diagnostic fragment ions of methylalkanes m/z
				FW	MW	FB	MB	GC	GC-MS	
36	3302		33	t	0.3	t	t	n-tritriacontane		
37	3333	67	34	6.2	5.0	11.0	7.6		9-,11-,13- & 15-methyltritriacontane	140/1, 168/9, 196/7, 224/5, 252/3, 280/1, 308/9, 336/7, 464 (M-15)
38	3363	137	35	3.5	11.6	11.2	8.9		9,13-,11,15- & 13,17-dimethyltritriacontane	140/1, 168/9, 196/7, 211, 239, 252/3, 267, 280/1, 308/9, 323, 351, 379
39	3385	215	36	0.2	0.6	1.0	0.9		9,13,21-trimethyltritriacontane	140/1, 196/7, 211, 323, 337, 393, 491 (M-15)
40	3400		34	t	0.3	0.1	0.1	n-tetratriacontane		
41	3426	74	35	0.1	0.1	0.4	0.2		11-,12-methyltetratriacontane	168/9, 182/3, 336/7, 351
42	3455	145	36	0.1	0.8	0.9	0.4		10,20- & 12,22-dimethyltetratriacontane	154/5, 182/3, 196/7, 224/5, 309, 337, 351, 379
43	3482			t	t	0.1	t			
44	3499		35	t	0.1	t	t	n-pentatriacontane		
45	3527	73	36	t	t	0.2	t		11-,13- & 15-methylpentatriacontane	168/9, 196/7, 224/5, 309, 336/7, 365
46	3555	145	37	0.2	1.4	1.9	0.6		11,15-,13,17- & 15,19-dimethylpentatriacontane	168/9, 196/7, 224/5, 239, 252/3, 267, 295, 308/9, 323, 351, 379
47	3583	217	38	t	0.3	0.3	0.1		11,15,23-trimethylpentatriacontane	168/9, 196/7, 239, 323, 365, 393
48	3600		36	-	-	t	t	n-hexatriacontane		

Hc %	98.9	98.9	98.5	98.6
n-alkanes %	77.7	62.1	54.9	64.8
methylalkanes %	21.2	36.8	43.6	33.8
3-methylalkanes %	1.5	1.6	1.1	1.2
monomethylalkanes %	13.3	15.1	21.4	15.9
dimethylalkanes %	5.9	18.3	17.4	15.2
trimethylalkanes %	0.3	1.3	1.5	1.3

Table 4: Cuticular esters of *L.migratoria* and *S.gregaria*

4a. Composition of constituent fatty acids. The values for percentage composition (% comp.) and retention indices (I) the mean of three replicates
M - Male, F - Female

GC Peak No.	Fatty acid	*L.migratoria* (M & F)			*S.gregaria* (M & F)		
		% comp.	I	dI	% comp.	I	dI
1	10:0*	0.5	1309	309	12.3	1310	310
2	12:0	1.1	1509	309	11.8	1511	311
3	14:0	14.4	1713	313	21.1	1715	315
4	15:0	6.5	1817	317	0.6	1816	316
5	16:0	31.2	1911	311	28.2	1915	315
6	17:0	-	-	-	0.4	2014	314
7	18:0	22.5	2114	314	11.6	2113	313
8	20:1	-	-	-	0.2	2277	277
9	20:0	14.0	2316	316	7.1	2314	314
10	22:1	5.3	2498	298	0.4	2495	295
11	22:0	3.4	2515	315	2.4	2515	315
	Unsaturation	5.3%			0.6%		
	Saturation	93.6%			95.5%		
	Total	98.9%			96.1%		

* Carbon number: number of double bonds.

4b: Composition of constituent primary alcohols.
The values of percentage composition (%) and retention indices (I) the mean of three replicates
U - unidentified

GC Peak No.	Primary alcohol	*L.migratoria* (M & F)			*S.gregaria* (M & F)		
		% comp.	I	dI	% comp.	I	dI
1	12	2.5	1588	388	1.1	1590	390
2	U	-	-	-	5.8	1783	
3	14	12.2	1791	391	4.7	1792	392
4	15	3.5	1892	392	6.3	1894	394
5	16	10.7	1992	392	19.3	1992	392
6	17	3.9	2092	392	10.4	2093	393
7	18	11.6	2195	395	19.2	2195	395
8	U	8.8	2371		-	-	-
9	20	5.0	2394	394	8.0	2394	394
10	21	17.4	2492	392	3.2	2498	398
11	22	10.4	2594	394	16.2	2594	394
12	23	1.6	2697	397	-	-	-
13	24	3.9	2800	400	0.9	2800	400
14	25	2.3	2901	401	-	-	-
15	26	3.2	3000	400	1.3	3000	400
16	28	1.0	3200	400	1.3	3200	400
17	30	0.3	3400	400	0.8	3400	400
18	32	1.7	3596	396	1.4	3600	400
	even-numbered		62.5%		74.2%		
	odd-numbered		28.7%		19.9%		
	Total		91.2%		94.1%		

Table 5: Triglyceride fatty acids of *L.migratoria* and *S.gregaria*
The values for percentage composition (% comp.) and retention indices (I) the mean of three replicates
t - trace (< 0.1%), M - Male, F - Female

GC Peak No.	Fatty acid	*L.migratoria* M			*L.migratoria* F			*S.gregaria* M			*S.gregaria* F		
		% comp.	I	dI	% comp.	I	dI	% comp.	I	dI	% comp.	I	dI
1	10:0*	0.1	1306	306	2.5	1305	305	-	-	-	6.5	1306	306
2	12:0	0.3	1511	311	3.3	1511	311	-	-	-	3.0	1513	313
3	14:0	6.6	1710	310	14.2	1711	311	11.5	1713	313	12.8	1711	311
4	15:0	0.2	1814	314	0.6	1820	320	5.4	1815	315	-	-	-
5	16:1	0.6	1881	281	0.1	1883	283	-	-	-	-	-	-
6	16:0	44.3	1912	312	37.1	1913	313	66.5	1912	312	68.6	1914	314
7	17:0	0.5	2016	316	2.2	2016	316	0.4	2021	321	t	2022	322
8	18:3	1.5	2068	268	1.5	2064	264	-	-	-	-	-	-
9	18:2	13.6	2076	276	6.4	2075	275	-	-	-	-	-	-
10	18:1	0.5	2084	284	0.9	2088	288	-	-	-	-	-	-
11	18:0	14.9	2112	312	14.5	2109	309	16.2	2114	314	9.1	2114	314
12	20:1	16.2	2278	278	11.5	2277	277	-	-	-	-	-	-
13	20:0	0.5	2315	315	1.3	2314	314	-	-	-	-	-	-
14	22:0	0.2	2515	315	3.9	2509	309	-	-	-	-	-	-
Unsaturation		32.4%			20.4%								
Saturation		67.6%			79.8%			100%			100%		
Total		100%			100%			100%			100%		

Table 6: Free fatty acids of *L.migratoria* and *S.gregaria*
The values for percentage composition (% comp.) and retention indices (I) the mean of three replicates
t = trace (<0.1%), M - Male, F - Female

GC Peak No.	Fatty acid	*L.migratoria* (M & F)			*S.gregaria* (M & F)		
		% comp.	I	dI	% comp.	I	dI
1	10:0*	0.9	1307	307	3.7	1310	310
2	12:1	-	-	-	0.4	1492	292
3	12:0	7.0	1508	308	4.9	1510	310
4	14:1	0.4	1693	293	0.1	1691	291
5	14:0	14.3	1710	310	12.0	1709	309
6	15:0	14.3	1822	322	0.5	1811	311
7	16:1	1.0	1884	284	0.3	1883	283
8	16:0	25.3	1914	314	64.0	1917	317
9	17:1	-	-	-	1.2	1981	281
10	17:0	0.7	2011	311	0.9	2012	312
11	18:3	0.6	2066	266	0.1	2068	268
12	18:2	3.2	2080	280	t	2076	276
13	18:1	0.2	2089	289	0.1	2085	285
14	18:0	13.5	2111	311	11.5	2115	315
15	19:1	-	-	-	t	2182	282
16	19:0	-	-	-	t	2213	313
17	20:1	0.8	2279	279	t	2278	278
18	20:0	4.6	2312	312	t	2313	313
19	21:3	3.8	2367	267	-	-	-
20	22:1	5.7	2498	298	-	-	-
21	22:0	2.8	2515	315	-	-	-
Unsaturation		15.7%			2.2%		
Saturation		83.4%			97.5%		
Total		99.1%			99.7%		

* Carbon number: number of double bonds.

Table 7: Free primary alcohols of *L.migratoria* and *S.gregaria*
The values for percentage composition (% comp.) and retention indices (I) the mean of three replicates
U - unidentified, M - Male, F - Female

GC Peak No.	Primary alcohol	*L.migratoria* (M & F)			*S.gregaria* (M & F)		
		% comp.	I	dI	% comp.	I	dI
1	12	1.7	1590	390	1.7	1588	388
2	14	16.0	1790	390	4.4	1789	389
3	15	6.3	1892	392	18.8	1893	393
4	16	12.3	1992	392	13.8	1995	395
5	17	5.0	2089	389	4.4	2092	392
6	18	14.6	2192	392	12.6	2195	395
7	19	0.8	2294	394	0.6	2301	401
8	U	1.6	2365		9.8	2370	
9	20	5.5	2394	394	4.1	2394	394
10	21	4.2	2489	389	4.3	2502	402
11	22	12.9	2597	397	8.1	2594	394
12	23	1.0	2698	398	1.1	2700	400
13	24	3.7	2800	400	2.8	2800	400
14	25	1.0	2895	395	1.6	2903	403
15	26	3.6	3000	400	3.2	3000	400
16	27	0.7	3101	401	1.8	3096	396
17	28	2.3	3200	400	2.6	3200	400
18	29	-	-	-	0.9	3290	390
19	30	2.9	3400	400	1.6	3400	400
20	32	2.8	3600	400	1.7	3600	400
21	34	1.4	3800	400	-	-	-
Even-numbered		79.7%			56.6%		
Odd-numbered		19.0%			33.5%		
Total		98.7%			90.1%		

Table 8: Cuticular lipid components of examined species of family Acrididae

Cuticular components	Subfamily	Cyrtacanthacridinae			Oedipodinae		Catantopinae					
	Species	Sg	Sv	Sa	Lm	Lmc	Ms	Mp	Mdif	Mb	Mf	Mdaw
Hydrocarbons	% composition	36.2-68.3	68	> 80	37.6-53.3	52-78	60	68	86	61-73	61-73	61-73
Esters	% composition	6.4-6.7			7.4-11.4		26-31(28)$^{+}$	18-20(18)$^{+}$		18-30	18-30	18-30
	Fatty acid chain length	10-22			10-22		14-22	12-20		14-18		
	Alcohol chain length	12-32			10-32		21-27	21-27		21-27	21-27	21-27
Triglycerides	% composition	64.8-65.7			51.9-57.1		1-5 *	1-5 *		2-13*	2-13*	2-13*
	Fatty acid chain length	10-18			10-22		12-24	14-18		14-18	14-18	14-18
Free fatty acids	% composition	1.5-4.7			4.6-7.4		*	*		*	*	*
	Fatty acid chain length	10-20			10-22		12-22	12-18		14-18	14-18	14-18
Primary alcohols	% composition	1.7-5.1			3.7-4		*	*		*	*	*
	Alocohol chain length	12-32			12-34		22-32	22-32		22-32	22-32	22-32
Secondary alcohols	% composition						*	*		*	*	*
	Alcohol chain length						21-27	21-27		21-27	21-27	21-27

* Comprises the percentage composition for triglycerides, free fatty acids, free primary alcohols and free secondary alcohols

Sg, Schistocerca gregaria
Sv, Schistocerca vaga (Nelson & Sukkestad, 1975)
Sa, Schistocerca americana (Jackson, 1982)

Lm, Locusta migratoria
Lmc, Locusta migratoria cinerascens (Genin et al., 1986)

Ms, Melanoplus sanguinipes
Mp, Melanoplus packardii
Mdiff, Melanoplus differentialis
} (Nelson et al., 1984)

Mb, Melanoplus bivittatus
Mf, Melanoplus femurrubrum
Mdaw, Melanoplus dawsoni
} (Jackson, 1981)

Ms, Melanoplus sanguinipes
Mp, Melanoplus packardii
} (Blomquist et al., 1972; +Soliday et al., 1974)

Plate 9

TLC analysis of the cuticular lipids of

a) *Locusta migratoria*

b) *Schistocerca gregaria*

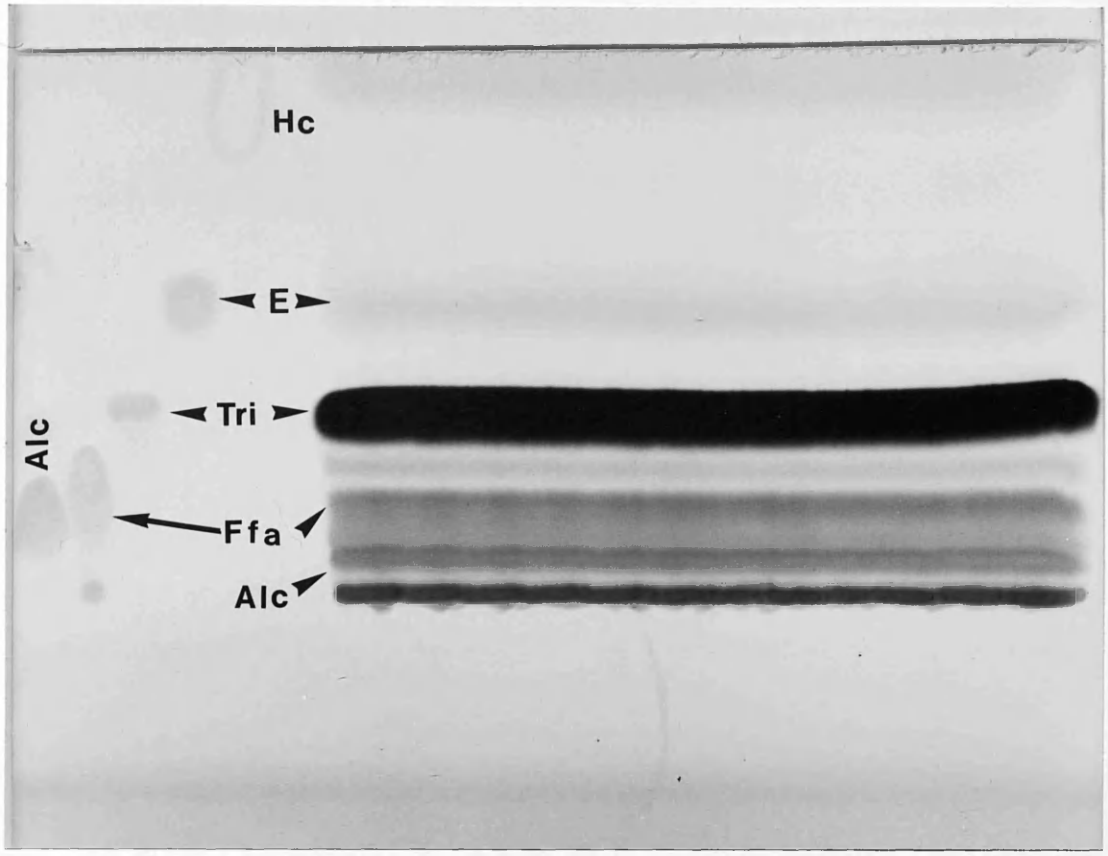
TLC analysis on a 20 x 20cm plate coated with a 2mm thick layer of silica gel. Plate developed in petroleum spirit followed by a 70:30:1 v/v mixture of petroleum spirit, diethyl ether and glacial acetic acid.

Alc, alcohols; E, esters; Ffa, free fatty acids; Hc, hydrocarbons, Tri, triglycerides.

Standards on left, analysed cuticular lipid on right.

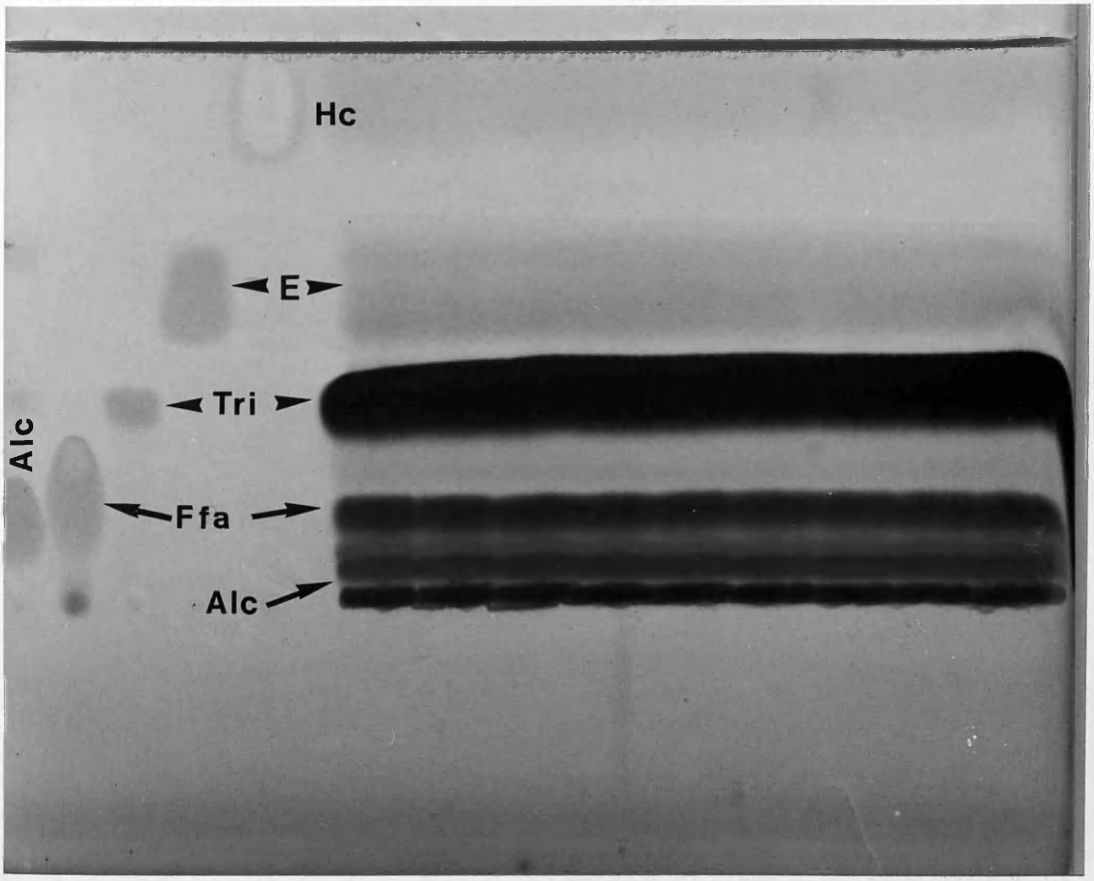
L. migratoria

a



S. gregaria

b



CHAPTER FOUR

THE EFFECT OF AMOUNT, COMPOSITION AND TEMPERATURE ON THE WATER PERMEABILITY OF LIPIDS

4.1. INTRODUCTION

As mentioned in chapter one, cuticular surface lipids perform many functions, yet the establishment of a waterproof barrier is the most critical one. Water loss in insects occurs through several parallel pathways, the cuticle, the tracheal system and the oral and anal openings (Cooper, 1983). However, the cuticle is the primary pathway because of the large surface area to body volume ratio (Ebeling, 1974). The tracheal system has also been reported to be an important site of water loss (Mellanby, 1932). On the basis of Mellanby's results, Gunn (1933) related the high rates of water loss that occurred at temperatures above 30°C in *P.americana* to increased ventilation of the tracheal system. Indeed, in *L.migratoria migratoriodes*, ventilation of the tracheal system is the main method of controlling water loss through the spiracles (Loveridge, 1968a).

Ramsay (1935a,b) found as Gunn (1933) had reported previously, that in the cockroaches *Blatella germanica* and *P.americana* cuticular transpiration was low at normal ambient temperatures and that there was a slow and gradual increase in transpiration until about 30°C when it rose rapidly. Ramsay (1935b) established that a surface layer of lipid on the cuticle of *P.americana* was responsible for its low water permeability and he suggested that the lipid underwent a phase change around 30°C which caused its water permeability to increase. Ramsay called this, the critical temperature. He found that a film of cuticular lipid covering a water droplet on the surface of *P.americana* also showed a critical temperature, the film preventing evaporation of the droplet below but not above 30°C. Similarly, a transition temperature was reported by Loveridge (1968b) in *L.migratoria migratoriodes*, where the transpiratory water loss in dry air was 0.71mg/cm^2/hr, which increased gradually until the temperature was

between 46 and 48°C when an abrupt increase was observed.

Early studies of the epicuticular lipid as a water barrier in insect cuticle include those of Kühnelt (1928, 1939 - quoted by Ebeling, 1974) who demonstrated the lipid nature of this barrier and who also showed that its disruption caused abnormal water loss. The latter observation was confirmed by Wigglesworth (1945) who reported that in the fifth instar nymph of the cockroach, *P.americana*, the rate of water loss was only 0.2mg/hr at 30°C compared to 34.6mg/hr of water under the same conditions after lipid extraction with chloroform at 50°C. Similarly, Toolson (1982, 1984) recorded that after lipid extraction there was a 13-fold increase in cuticular permeability in adult *Drosophila pseudoobscura* and a 79% increase in the cicada *Tibicen dealbatus*. Hadley and Quinlan (1987) similarly observed a 9-fold increase in the permeability of the pleural cuticle of the scorpion, *Hadrurus arizonensis* following hexane extraction of the surface lipids.

With regard to the effect of temperature on cuticular water loss, Wigglesworth (1945) found that cuticular transpiration in most of the species he examined was low at normal ambient temperatures and that transpiration increased slowly and gradually with rise in temperature until a critical temperature was reached when transpiration increased rapidly. He found that the critical temperature was often above the lethal temperature of the insect, that it was species-specific, and that it varied widely among species and among instars of the same species. For example, he found the critical temperature was around 30°C in the larvae of the sawfly *Nematus rubesii*, in the caterpillar of *Pieris brassicae* and in the cockroach *Blatella germanica*, while in the larvae of *Tenebrio molitor* and nymphs of *Rhodnius prolixus* which inhabit dry habitats, the critical temperature was 20 to 30°C higher. Similar results were obtained by Lees (1947) with several species of

ticks.

Experiments with finely divided powders played an important part in demonstrating the function of the protective lipid. For example, Alexander *et al.* (1944) showed that abrasive and sorptive powders caused a breakdown in the water resistance of a lipid film spread on a celluloid membrane, thereby increasing water transpiration. Alexander *et al.* (1944) suggested that dusts used in their experiments caused submicroscopic holes in the lipid film by removing it in some way from the celluloid to which it was probably not strongly attached. Wigglesworth (1945) found that inert dusts caused desiccation of insects which he showed was caused by dust getting between the moving surfaces of the cuticle and abrading the lipid layer, thus leading to a great increase in transpiration through the cuticle. Moreover, through the action of detergents and solvents on insect cuticle, Wigglesworth (1945) confirmed the lipid nature of the water barrier in the cuticle and further proposed that water loss in insects was regulated by a thin layer of oriented lipid on the outer surface of the cuticle. At the critical temperature, Wigglesworth (1945) suggested that the molecular packing of the lipid was loosened and this allowed more water to pass through. Wigglesworth (1947, 1948) also studied the deposition of the epicuticle in *Rhodnius prolixus* and *Tenebrio molitor* and confirmed the presence of a thin superficial layer of lipid. Blackith (1961) investigated the superficial lipid layer in the hatchlings of *L.migratoria*, *Nomadacris septemfasciata* (Serv.) and *S.gregaria* and found that transpiration rates through intact and abraded cuticle was lower in *S.gregaria* than in *Nomadacris* and *L.migratoria*. Ebeling (1961) studied the effect of sorptive powders on beeswax which possesses both amorphous and crystalline fractions. He proposed that the molecules at the surface of a wax

film, which are amenable to adsorption, are removed by powder and that those below take their place. Thus a progressive displacement of molecules from the amorphous wax fraction occurs in this manner down through the film eventually forming minute pathways in an otherwise crystalline matrix.

Much basic information on the water permeability of insect lipid layers was obtained by Alexander *et al.* (1944) in experiments with artificially waxed celluloid membranes. In these experiments, Alexander and his co-workers found that a layer 0.02μm thick of beeswax, a highly polar lipid (Tulloch, 1971; Blomquist *et al.*, 1980) conveys maximum impermeability to a membrane, whereas a layer of non-polar paraffin wax of similar thickness is permeable to water, though impermeability increases with wax thickness. Alexander and co-workers suggested that in beeswax there is orientation and close molecular packing of the polar groups while in paraffin wax which lacks polar groups, there is random crystallisation. Alexander and his co-workers investigated the waterproofing properties of a wide range of lipids and they found that the rate of evaporation from celluloid was considerably reduced when extremely thin films of various lipids were applied to the celluloid, and that naturally-occurring lipids formed the most impermeable films. They concluded from this part of their work that a waterproofing process involving a thin layer of lipid could well occur in living systems such as at the surface of an insect or a plant. Further, Grncarevic and Radler (1967) demonstrated the effect of isolated and purified fractions of cuticular wax of grape applied to artificial plastic membranes. They found that hydrocarbons, long chain alcohols, long chain aldehydes and to some extent esters were effective in reducing water evaporation while long chain fatty acids like C22:0 and C26:0 exert only minor effect on water evaporation. For comparison, Grncarevic and Radler (1967) applied

paraffin wax and found it effective in reducing water evaporation.

Beament (1945) studied the waterproofing properties of isolated cuticular lipids spread on extracted *Pieris* wing and reported similar observations to those of Wigglesworth (1945). Beament (1945) found that isolated lipids had waterproofing characteristics similar to those found in insect cuticle such as a critical temperature and an increase in water permeability after treatment with detergents or emulsifiers. Beament's work on isolated cuticular lipids confirmed many of Wigglesworth's earlier observations on intact cuticle and he extended one of Wigglesworth's ideas by proposing that the cuticular lipid layer consisted of an inner monolayer of tightly packed polar molecules, which were vertically oriented by the attraction forces at the cuticular surface, and several outer layers in which molecular alignment progressively decreased. Beament considered the inner monolayer as the main cuticular water barrier and he suggested that at the critical temperature, which he re-named transition point, the monolayer molecules underwent a transition (Müller, 1932; Piper *et al.*, 1931), at which there was an abrupt increase in inter-molecular spacing and a consequent sharp and substantial increase in water permeability. However, Holdgate and Seal (1956) did not find a transition temperature in *Tenebrio molitor* pupa. On the contrary, their experimental data gave exponential curves which led Holdgate and Seal to question the validity of the transition temperature. Mead-Briggs (1956) reached similar conclusion from his experiments on several species of insects and he emphasized the inconclusive nature of all results at that time. Oloffs and Scudder (1966) supported the conclusions of Holdgate and Seal (1956) and agreed that there was no transition point and that evaporation-temperature curves were exponential. Re-investigating the subject, Beament (1958, 1959)

confirmed the presence of a sudden and substantial increase in transpiration at a transition temperature which was about 29.7°C in the fifth instar nymph of the cockroach, *P.americana* and 32.7°C in a film of its lipid covering a water droplet. Beament (1959) listed the transition temperature for several species, for example 48°C for *S.gregaria*. He re-stated his monolayer hypothesis and suggested that at transition the monolayer lost molecular organisation which caused it to become more permeable to water. Later on, Beament (1964) proposed a very detailed model for the lipid monolayer and suggested that the long chain molecules of the monolayer stood at an angle of 24.5°C to the perpendicular from the cuticle and that they were closely packed so that there were no spaces between them through which water could escape. As well as adsorptive forces at the cuticular surface, the monolayer molecules were stabilized by Van der Waals forces which cross-linked the molecules. At transition temperature, Beament proposed that the Van der Waals forces were overcome and that the molecules become thermally agitated, vibrating about a mean vertical position, so that spaces appeared between them and water was able to escape.

There is much evidence against the monolayer model proposed by Beament. For example, Sebba and Briscoe (1940) showed that although there was a 99% decrease in water evaporation through a monolayer of C20 alcohol, a high optimum lateral pressure had to be applied to the monolayer. Further, Langmuir and Schaeffer (1943) emphasized the importance of using pure samples to prepare impermeable monolayers and they noted that only very small amounts of impurities in a monolayer of C16 alcohol reduced its resistance to water evaporation by 50%. Beament's hypothesis, which is based upon his work with *P.americana*, is also inconsistent with the composition of the cuticular lipid of this species since Gilby and Cox (1963) found that the extractable

cuticular lipid consisted mostly of non-polar hydrocarbons (75 to 77%) with small proportions of free fatty acids (7 to 11%), aldehydes (8 to 9%) and esters (3 to 5%). Gilby and Cox doubted if such a high percentage of non-polar lipid could form an oriented monolayer at the cuticular surface. Furthermore, alcohols which Beament postulated formed the inner monolayer were not detected by Gilby and Cox in the cuticular lipid of *P.americana*.

Several other workers have criticised Beament's monolayer hypothesis (Lockey, 1976; Machin, 1980; Toolson *et al.*, 1979; Wigglesworth, 1985b). Lockey (1976) using a surface film balance, examined freshly extracted cockroach lipid spread on the surface of water. He demonstrated that a tightly packed monolayer was absent at the lipid/water interface and that the constituent molecules of the film were only weakly attracted to the water surface. Machin (1980) proposed that a lipid layer only 13% thicker would be just as impermeable to water as one with a monolayer. Toolson *et al.* (1979) who used electron paramagnetic resonance spectroscopy to study the cuticle of the scorpion, *Centruroides sculpturatus* found no evidence for any preferred orientation in the epicuticle. Furthermore, Wigglesworth (1985b) showed that in *P.americana* and several other species, the inner region of the lipid layer at the surface of the outer epicuticle consists of a thin resistant layer of lipid and tanned protein.

All the evidence mentioned above indicates that the water barrier in the cuticle is unlikely to be a tightly-packed monolayer of polar lipid molecules. Further evidence supporting this view comes from the heterogeneous composition of cuticular lipids. Machin (1980) proposed that the free cuticular lipid has no molecular orientation and that the water barrier in the cuticle consisted of both the bound lipid of

the inner epicuticle and the free non-oriented lipid covering it. Most of the latter he suggested, permeated the outer epicuticle and only a thin layer of free lipid covered the cuticular surface.

Many workers believe that transition temperature, a term coined by Beament and described by him as a sudden and irreversible increase in the water permeability of cuticle, is an artifact (Toolson, 1978; Gilby, 1980). However, Machin (1980), using Toolson's mathematical model, confirmed that discontinuities did occur when cuticular permeability was plotted against the reciprocal of absolute temperature. Research on the transition temperature and permeability changes has been reviewed in detail by Gilby (1980) who concluded that the physical relations between evaporation and temperature in the insect are so complex that virtually all the published experimental work is devoid of significance. Edney (1977) has provided a detailed critical analysis of published research on this problem. He likewise stresses the complexities of the physics involved, but his conclusions are less pessimistic and he finds much of the published work informative. He agrees that at high temperatures there is an increased loss of water by transpiration in excess of that caused by increased vapour pressure deficiency, and that there is indeed a progressive increase in permeability (a progressive reduction of cuticle resistance at temperatures above the transition range).

Wigglesworth (1986) re-examined the effect of temperature on cuticular permeability by observing the effervescence which occurs when an insect is immersed in a mixture of liquid paraffin and butanol. He confirmed once again that the waterproofing barrier in the cuticle is superficial. He also noted that this barrier varies greatly in permeability in different areas of cuticle, and that it is rapidly permeable in the newly moulted insect, but becomes far more resistant as the cuticle tans. In addition, he found that effervescence

increased rapidly over a temperature range which coincided with that over which the instar's cuticular transpiration in dry air increased rapidly.

Abrasion of insect cuticle exposes the argentaffin substance to access by ammonical silver and increases water loss by transpiration (Wigglesworth, 1945). In addition, in some insect species the waterproofing wax is associated with argentaffin material, presumably polyphenols involved in the cross-linking of proteins to form sclerotin (Wigglesworth, 1945, 1985b). These two observations led Wigglesworth (1986) to carry out a histochemical test on cuticle by exposing intact insects to ammonical silver nitrate over a range of temperatures. In doing so, he anticipated that the effect of temperature on access of ammonical silver might parallel the effect of temperature on water transpiration, since the rise in temperature increases the access of ammonical silver to argentaffin component. Wigglesworth's work clearly confirms that increasing temperature causes an increase in the water permeability of the lipid layer. He found however that the increase in permeability occurred over a range of temperature rather than at a precise temperature as proposed by Beament (1945, 1958, 1959). This temperature range varies with species, for example, the range is 8°C in the nymph of *Rhodnius prolixus*, 20°C in the pupa of *Pieris brassica* and 40°C in *S.gregaria* nymphs.

In general, investigators in the field of cuticular permeability agree that the waterproofing of cuticle depends mainly on the epicuticular lipid and that water loss increases with increasing temperature. Disagreement exists about the way in which water loss increases as temperature rises. In addition to Beament's monolayer transition hypothesis, alternative explanations of the critical

temperature phenomenon have been proposed by other investigators. For example, Hurst (1950) suggested that at the critical temperature, there was a change in the form of the lipid above the inner monolayer from an orthorhombic to hexagonal crystal system. Locke (1965) following the work of Luzatti and Husson (1962) proposed another model based on electron micrographs showing wax canal filaments penetrating the epicuticle. He believed these filaments to be lipid water liquid crystals in the middle phase which at the critical temperature changed from middle phase to either complex hexagonal or reversed middle phase. An alternative explanation of critical temperature by Davis (1974) is based on the transition phenomenon in lipids. This is well known in many phospholipids and sterol esters where the hydrocarbon chains are in a highly mobile condition at the transition temperature. In this hypothesis, Davis suggests that at temperatures below the critical temperature lipid molecules could possibly be arranged so that low permeability results. This special arrangement is probably dictated by the molecular arrangement of lipoproteins in the cuticulin layer. At the critical temperature, Davis suggests that certain lipids would change phase from crystal to liquid crystal, and that the hydrocarbon chains would be in a highly mobile condition. The polar moieties of the lipids would not move vertically because of the strong binding to the lipoprotein substrate and because of the possible intermolecular bonds between polar moieties of neighbouring molecules. But any abrupt increase in space between the hydrocarbon chains at the critical temperature would alter the packing of many other lipids in the vicinity. As a result the special arrangement in all of the lipid would be altered, thus allowing water to escape.

A similar explanation was proposed by Toolson *et al.* (1979) who observed a rapid increase in mobility of the hydrocarbon chains of the epicuticular lipids of the scorpion *Centruroides sculpturatus* from

22°C to 35°C, a temperature range which coincides with transition in this species. Moreover, Wigglesworth (1986) has noted that with increasing temperature many solid lipids assume a liquid crystalline or mesophase which results in higher water permeability.

All of the foregoing evidence clearly indicates that the principal barrier in the cuticle to water efflux is the lipid layer and/or the impregnating lipid associated with the epicuticle. There is also some experimental evidence however which suggests that the procuticle and the hypodermis contribute to the low rates of transpiration exhibited by most terrestrial arthropods. Berridge (1970) proposed that the apical plasma membrane of hypodermal cells may regulate the movement of water and thus supplement the resistance provided by the cuticle. The idea of a hypodermal barrier is an attractive hypothesis, since it may be the site of the water pump proposed by Winston (1967) and Winston and Beament (1969). Such a pump, these workers believed, removed water from the cuticle, thus reducing transpiration by decreasing the activity gradient from cuticle to air. Further, Winston (1967) and Winston and Beament (1969), working on isolated cuticular discs excised from cockroaches and locusts, found that the level of water activity in the cuticle was substantially lower than the water activity of the haemolymph in both species. These results were used as evidence for the view that hypodermal cells continually expend energy to regulate the water balance of the cuticle. However, in *P.americana*, hypodermal permeability has been shown to be insignificant by Machin *et al.* (1985). Machin and Lampert (1985) instead proposed a model based on two cuticular layers which they distinguished as endocuticle and combined exocuticle and epicuticle and which differ in their permeability and water affinity.

Treherne and Willmer (1975) showed that hormones from the brain and corpus cardiacum could regulate rates of cuticular transpiration in *P.americana*. They found that decapitation increased cuticular transpiration significantly. Noble-Nesbitt and Al-Shukur (1987) confirmed these results in the same species but they emphasised the importance of the insect's physiological state and the environmental conditions at the time of experimentation. However, Noble-Nesbitt and Al-Shukur (1988a,b) demonstrated a more complicated neuroendocrine regulation of integumentary water loss in *P.americana*. This includes the brain as the main centre and the terminal abdominal ganglion (TAG) as an auxiliary centre. The two authors reported the presence of two factors in the brain of *P.americana*, a water-loss promoting factor and a water-loss restricting factor. The former factor is released in fully hydrated cockroaches causing a high cuticular transpiratory rates, while the latter factor is released after desiccation, thus resulting in a reduction in cuticular transpiration. Similar factors are present in TAG. The promoting factor in the brain activates its corresponding in the TAG and the latter acts by stimulating the release of the former resulting in more water loss. The restricting factor in the TAG is independent of the brain centre and acts even in its absence.

Despite the importance of lipids in cuticular permeability, the effect of lipid composition and lipid morphology on water permeability have been studied in only a few insect species. With regard to lipid composition, Armold *et al.* (1969) found few qualitative but significant quantitative differences between the cuticular lipids of the last instar larvae (naiads) and the adults of the big stonefly *Pteronarcys californica* (Newport). The naiad has a low melting point lipid in which triglycerides (78%) comprise the main fraction, while adults have a higher melting point lipid in which free fatty acids

(49%), sterols (18%) and hydrocarbons (12%) form the main fractions. Furthermore, adults have nearly twice as much extractable lipid as naiads. Armold and his co-workers suggest that the aquatic larva requires less cuticular lipid to prevent water loss than the terrestrial adult with its need for water conservation. Goodrich (1970) also found significant qualitative and quantitative differences between the cuticular lipids of the puparia and adults of the sheep blowfly *Lucilia cuprina* (Wied). The habitats occupied by pupae and adults are very different and Goodrich suggests that cuticular lipids may vary with the environmental need for water conservation.

In their study of *Sarcophaga bullata*, Armold and Regnier (1975) found that the cuticular hydrocarbons varied both quantitatively and qualitatively throughout the life cycle. Their observations revealed two periods of rapid accumulation of cuticular hydrocarbons. The first occurs during days 4 to 13 when the insect is in the final period of the third larval instar and the initial period of the pupal stage and the second, during days 19 to 23 which is followed by adult emergence. The quantity of cuticular hydrocarbons correlates well with the water conservation needs of this insect. The first, second and part of the third larval instar are spent in decaying flesh. This provides a very moist environment and these larvae have relatively low proportions of hydrocarbons in their cuticular lipid which seems reasonable if the hydrocarbon proportion of cuticular lipid determines the rate at which water is lost to the environment. The moist environment occupied by such larvae would not require a highly impermeable cuticle since water is readily available. During the latter period of the third larval instar, the insect ceases feeding, leaves the rotting flesh and burrows into the soil. Cuticular hydrocarbons are synthesized during the latter half of the third larval instar and at a higher rate during

pupation. These additional hydrocarbons will provide some protection from desiccation for the larvae after leaving the flesh and will probably provide good protection for the pupae in soil. After emergence, the adult fly is exposed to the terrestrial environment and the presence of a high proportion of cuticular hydrocarbons argues that cuticular transpiration should be low. Moreover, Armold and Regnier's observations that contact with water, prevents larval pupation and results in a decrease in the quantity of hydrocarbons synthesized, correlates hydrocarbon biosynthesis and pupation. Since quantitative changes in the hydrocarbon mixture accompanies pupation and since pupation is controlled by the moulting hormone, ecdysone, Armold and Regnier argue that ecdysone may also control the rate of hydrocarbon biosynthesis though not the quantitative characteristic of the hydrocarbon mixture.

Changes in the morphology of epicuticular lipids during development have been reported in a few instances. For example, Slifer (1946) found that the onset of diapause in the egg of *Melanoplus differentialis* was associated with the deposition of a waxy layer over the surface of the hydropyle of the egg. She suggested that the wax layer played a role in the maintenance of diapause by preventing the uptake of water since diapause could be terminated if the wax layer was removed by immersing the eggs in xylol. Lees (1955) reported that the overwintering (diapause) egg of the mite, *Pterobia latens*, has a thicker wax layer than the summer (non-diapausing) egg and is therefore better able to withstand desiccation.

In *Manduca sexta*, Bell *et al.* (1975) using light microscopy and scanning electron microscopy, observed distinct morphological differences in the epicuticular surfaces of non-diapausing and diapausing pupae. Their observations showed that the cuticular surfaces of non-diapausing pupae were smooth, shiny and translucent,

whereas the surface of diapausing pupae were coarse and opaque. In addition, they established that diapausing pupae secreted over 3 times as much surface wax and took over 2 to 3 times longer than non-diapausing pupae to deposit the wax. They suggested that the extra thickness of the wax layer protected diapausing pupae from desiccation during diapause and found that increased wax secretion was related to hormonal changes accompanying entry into diapause. The lipid constituents of the diapausing pupae of the tobacco hornworm, *Manduca sexta* have been analysed and found to contain highly polar cuticular lipid consisting of long chain oxoalcohol esters, oxoaldehydes, oxoalcohols and 4% hydrocarbons (Buckner *et al.*, 1984a,b; Coudron & Nelson, 1981). Buckner and his co-workers suggest that the relatively polar lipids that constitute the major components of the surface wax of pupae that overwinter in soil provide a unique means of protection against desiccation and attack by soil organisms. Earlier, Coudron and Nelson (1981) in their study reported a 4 to 5-fold increase in the amount of hydrocarbons in the cuticular surface lipids of the diapausing pupae, which led them to suggest that during diapause the increase in hydrocarbon production parallels the total cuticular surface production.

Similar results to those of Bell *et al.* (1975) were obtained by Hegdekar (1979) who studied the epicuticular wax of diapausing and non-diapausing pupae of the Bertha Armyworm, *Mamestra configurata*. He found that diapausing pupae when compared with the non-diapausing pupa had twice the amount of wax, double the wax secretion period and 3-times the amount of hydrocarbons. Hegdekar proposed that the thicker wax layer in general, and the higher proportion of hydrocarbons in particular, protected diapausing pupae from desiccation during diapause.

However, Coudron and Nelson (1978) working on the tobacco budworm, *Heliothis virescens*, found no difference in the quantity of cuticular wax between diapausing and non-diapausing pupae.

A correlation between potential transpiration and the proportion of hydrocarbons has been noted by Hadley (1980b) in the nymphs of the desert cicada, *Diceroprocta apache*. These nymphs which live underground, where moderate temperature and moisture conditions prevail, contained only 10.9μg of hydrocarbons per exuvium whereas the adults which are active at midday when air temperature approaches 50°C, had 57.8μg of hydrocarbons per adult.

Substantial changes in cuticular lipids such as hardness, thickness and composition during development have been observed in several species. For example, the larval instars of the black carpet beetle, *Attagenus megatoma* (Baker *et al.*, 1979a), and the Mexican bean beetle, *Epilachna varivestis* (Danehower & Bordner, 1984) have hard crystalline cuticular lipids with high proportions of esters. This composition changes in the adult to one in which hydrocarbons predominate, thus there is a shift from a hard, abrasion-resistant wax in the larva to a softer cuticular wax in adult. On the other hand, Baker *et al.* (1979b) found in the cigarette beetle, *Lasioderma serricorne*, that triglycerides are the major components of the larval surface lipids, and that the proportion of hydrocarbons increased by 14% to become the predominant lipid component in the adult. Baker *et al.* (1979b) suggest that the differences in lipid composition between larval and adult stages of *A.megatoma* and *L.serricorne* provide evidence for a rather complex control mechanism regulating the synthesis of wax components and/or the transport of wax components to the cuticle surface.

Physical environmental factors such as temperature and humidity can induce changes in the structure and composition of epicuticular

lipids. However, little is known about the changes in the polar components of lipids and most of the available evidence concerns the hydrocarbon components. For example, in the desert beetle, *Eleodes armata* summer-active adults not only have a higher proportion of hydrocarbons than winter-active adults but they also have a higher percentage of long chain hydrocarbons (Hadley, 1977). Hadley also found that acclimatization of winter-active adults to 35°C for 5- and 10-week periods resulted in an increase in high molecular weight dimethylalkanes when compared with controls. Hadley suggests that summer-active adults are at a higher risk of desiccation than winter-active adults, and that the cuticle of the earlier group is made more waterproof by the higher quantities of hydrocarbons and long chain dimethylalkanes. Similarly, Toolson (1982) who studied the effect of increased rearing temperature on the transcuticular water loss and epicuticular hydrocarbon composition of the egg, larva and pupa of the dipteran, *Drosophila pseudoobscura*, reported that a pupa raised from an egg at 17°C exhibited significantly higher cuticular permeabilities than one raised at 24°C. In addition, he found that high rates of transcuticular water loss were correlated with higher proportions of relatively short chain methylalkanes and alkadienes and that lower cuticular permeabilities were associated with longer chain methylalkanes and alkadienes.

Hadley and Schultz (1987) also found that the highest water loss rates were exhibited by *Cicindela oregona*, a species active in spring and autumn in Arizona, while the lowest water loss rates were exhibited by *C.obsoleta*, a summer-active species that inhabits dry grasslands. The cuticular lipid of the latter species contained the greatest amount of hydrocarbons per unit area and all of the hydrocarbons were saturated with methylalkanes accounting for about

60% of the hydrocarbon fraction.

The cuticular permeability of two vejovid scorpions from Arizona, *Hadrurus arizonensis* and *Uroctonus apacheanus* may also be related to environmental conditions. For example, Toolson and Hadley (1977) found that the desert-living *Hadrurus arizonensis* when compared with its mountain-living counterpart, *Uroctonus apacheanus* has lower cuticular transpiration over the temperature range 20 to 70°C but more cuticular hydrocarbons per gram body weight and a higher percentage of long chain hydrocarbons and fatty acids. In another desert scorpion, *Centruroides sculpturatus*, a decrease in transcuticular water loss during summer months is accompanied by an increase in the proportion of long-chain methylalkanes (Toolson & Hadley, 1979). Hadley *et al.* (1981) also reported lower cuticular transpiration rates in the epigean wolf spider, *Lycosa* species, which lives on the hot non-vegetated lava flows on the Island of Hawaii, than *Lycosa howarthii* which lives in the deep cave zone of the lava tube caves. Hadley and his co-workers suggest that part of the low transpiration rates in *Lycosa* species results from the high surface densities of cuticular lipid and cuticular hydrocarbons.

However, there are exceptions to this general pattern. For example, Hadley (1978) found no correlation between either total cuticular lipid or cuticular hydrocarbons and permeability in five species of desert tenebrionid beetles. Furthermore, Toolson (1984), in the cicada, *Tibicen dealbatus*, reports a negative correlation between cuticular permeability and long chain n-alkanes and methylalkanes.

Humidity may also induce changes in the structure and composition of epicuticular lipids and this relationship is clearly observed in species that exhibit wax blooms such as desert tenebrionid beetles (Hadley, 1979; McClain *et al.*, 1985).

From the foregoing evidence and in particular from the membrane

work of Alexander *et al.* (1944) and Grncarevic and Radler (1967), it seems reasonable to conclude that hydrocarbons, particularly n-alkanes, contribute to cuticular impermeability. However, the role of methylalkanes and alkadienes in cuticular impermeability is unclear in spite of the work by Hadley (1977) and Toolson (1982) who investigated the contribution of these components to cuticle impermeability. These components, unlike n-alkanes, have either a methyl side chain or a double bond which prevent close molecular packing, so that their presence in a lipid increases its water permeability (Hadley, 1980a; Lockey, 1988).

Experiments with plasma membranes and artificial bilayers indicate that long-chain saturated molecules decrease permeability, while branched and unsaturated molecules increase it (de Gier *et al.*, 1968; Taylor *et al.*, 1975). Further, in lipid bilayers containing a high proportion of unsaturated acids, cholesterol reduces fluidity and permeability by restricting the mobility of the hydrocarbon chain (Ladbrooke *et al.*, 1968). These experiments also shed some light on the relation between permeability and the degree of unsaturation.

Lockey (1988) in his review suggests that the complex hydrocarbon mixtures in insect cuticular lipids not only contribute to cuticular waterproofing but they also provide a matrix for the polar fractions since these hydrocarbon mixtures are likely to remain fluid or semifluid over a range of temperature. In this way, he suggests that hydrocarbon composition may control lipid viscosity which could be an important factor in lipid secretion through the system of pore canals and epicuticular channels. Further, Lockey (1988) suggests that the hydrocarbon components of cuticular lipid may also play a role in lipid spreading which would contribute to the integrity of the lipid layer.

In the work to be described, synthetic lipids and the components of natural lipids have been spread singly and as mixtures on the extracted hind wings of *L.migratoria* and *S.gregaria* and their effect on the water permeability of the wings investigated. The aim of the work was to investigate how:

1. Lipid amount/unit area, lipid composition and temperature affect water permeability of hind wings.
2. Composition affects the spreading and physical characteristics of a lipid.

4.2. MATERIALS AND METHODS

Hind wings and the third and fourth abdominal terga of *L.migratoria* and *S.gregaria* were used as a source of cuticle throughout this part of work. Wings were stretched on cork sheets for 24 hours before use. Following stretching, the wings were used either untreated or they were extracted with hot chloroform for 15 minutes three times. Similarly, abdominal terga were used directly after dissection either untreated or extracted.

A. Apparatus

Two similar circular holders were used, a larger holder for hind wings and a smaller one for abdominal terga. The hind wing holder (Figure 8) was 40mm in diameter and consisted of an upper aluminium plate, 4mm thick (Figure 8a) and a lower perspex disc 10mm deep (Figure 8b,c) which was covered on the upper surface by an aluminium plate 4mm thick (Figure 8b). A hole 10mm in diameter was drilled through the upper plate and through the lower disc to a depth of 12mm (Figure 8b). The lower disc had two glass side-arms which were used for filling the central well with water and which were covered with plastic caps to prevent water evaporation during an experiment. Wings were positioned over the central well and clamped between the upper

plate and a 14mm diameter O-ring on the lower disc by three screws.

The abdominal tergum holder was 18mm in diameter and consisted of an upper aluminium plate 4mm thick and a lower disc 9mm deep covered on the upper surface by an aluminium plate 4mm thick. A hole 4mm in diameter was drilled through the upper plate and through the lower disc to a depth of 10mm. Two glass side-arms were connected to the central well of the disc. Terga were clamped between the upper plate and a 5mm diameter O-ring on the lower disc by three screws. Here also the side-arms were used to fill the central well with water.

Each type of holder was suspended in a glass drying chamber from a trapeze resting on the pan of a model F15 Mettler balance (Figure 9). The chamber had a tight fitting rubber bung and sat in a thermostatic water-bath which had a cooling coil. The air in the glass chamber was kept dry by means of phosphorous pentoxide (P_2O_5) which was changed at the beginning of each experiment. During an experiment, air in the chamber was continuously replaced with pre-dried and temperature controlled air at the rate of 300ml/minute. The air temperature in the chamber was monitored with a RS digital thermometer. The water-bath was covered with polythene sheeting to minimize water evaporation and to prevent water vapour entering the drying chamber. The whole apparatus was covered with plastic sheeting to ensure stable balance readings.

B. Experimental procedure

Before use, each holder was tested under standard experimental conditions for leaks by clamping a thin sheet of rubber between the upper plate and the O-ring of the holder. The holder was filled with water by way of the side-arms and suspended in the drying chamber (Figure 9). The weight of the holder was recorded before and after one hour. This process was repeated several times. No differences were

observed between the first and second weighings.

C. Experimental procedure using cuticle

1. Wings or abdominal terga were clamped between the upper plate and the O-ring of the holder.
2. The holder was filled with water through a side-arm. The side-arms were capped and the holder was suspended from the trapeze in the drying chamber.
3. The holder was weighed and the air circulation was started.
4. After one hour, the air circulation was stopped and the holder was weighed again. The difference in weight, which represents the amount of water passing through the cuticle, was then divided by the area of the cuticle over which evaporation occurred and expressed as $mg/cm^2/hr$.
5. Evaporation rates through the intact and extracted wings and abdominal terga of *L.migratoria* and *S.gregaria* were determined in triplicate.
6. Experiments were carried out initially at 25^oC.
7. Natural lipid and synthetic mixtures of lipid were spread over extracted cuticle from a 0.1ml Agla microsyringe. 0.06ml of solution was added to the cuticle in all experiments in a standard pattern of drops to ensure uniform coverage of cuticular surface.

D. Experiments

1. Lipid amount/unit area

a) *Natural lipid*

In this calculation, the amount of natural lipid/unit area of hind wing was determined for *L.migratoria* and *S.gregaria*. The lipid was assumed to be uniformally distributed over the cuticular surface

and the amount of lipid extracted from one side of a hind wing was divided by the surface area of that side of the wing and the results expressed as amount/unit area (mg/cm^2). Hind wing area was determined by tracing the outlines of 25 stretched wings of each species with a mouse on a digitising tablet connected to a microcomputer. The apparatus was calibrated using centimetre graph paper. This method was designed by Dr D. Neil (Zoology Department, University of Glasgow). Lipid was extracted from 10 wings of each species weighed and the average amount of lipid per one side of wing calculated. The amount/unit area for each species were found to be $0.07 mg/cm^2$ (*L.migratoria*) and $0.12\ mg/cm^2$ (*S.gregaria*).

b) *Synthetic lipid*

The amount of synthetic lipid that should be applied to the one centimetre circle of extracted hind wing to achieve the same amount/unit area as natural lipid was calculated as follows:

Area of circle in holder = $\pi\ r^2 = 3.1416 \times (0.5)^2 = 0.785\ cm^2$

amount of lipid to be applied:

i) *L.migratoria*

$0.00007_g \times 0.785 = 0.055$ mg

ii) *S.gregaria*

$0.00012_g \times 0.785 = 0.094$ mg

2. The effect of lipid amount (mg/cm^2) on water permeability

In preliminary experiments, synthetic lipids and the components of natural lipids isolated by TLC were applied to extracted hind wings in amounts similar to the naturally-occurring lipid. However, synthetic lipids and the isolated natural components proved to be considerably more permeable to water than the lipid on the intact wing so that in subsequent experiments the former were investigated in greater amounts.

In the first series of experiments, each lipid component was applied individually in different amounts (mg/cm^2) to the extracted hind wings of *L.migratoria* and *S.gregaria*. Both synthetic lipids and the components of natural lipids were investigated. The following short-chain synthetic lipids were investigated:

1) n-Alkanes (n-octacosane & n-eicosane) applied in the following amounts: 0.13, 0.25, 0.38, 0.51, 0.64, 0.76 & 0.89 mg/cm^2; 2) Methylalkanes (2-methylheptadecane & 2-methylnonadecane); 3) Alkenes (n-heptadecene & n-nonadecene); 4) free fatty acids (stearic acid & arachidic acid); 5) primary alcohols (1-octadecanol & 1-eicosanol); 6) triglycerides (tristearin & triarachidin) and 7) esters (stearic acid stearyl ester). Components (2) to (7) were applied in the following amounts: 0.13, 0.38 and 0.64 mg/cm^2.

3. Effect of lipid composition on water permeability

In this series of experiments, lipid mixtures containing two or more components were applied to the extracted wings of *L.migratoria* and *S.gregaria* in standard amounts, namely 0.38 mg/cm^2 for *L.migratoria* and 0.76 mg/cm^2 for *S.gregaria*. These amounts were chosen as they gave the lowest water permeabilities when n-alkanes were investigated individually. They are, however, six times greater than the amount of lipid occurring naturally on the wings of the two species.

The synthetic components listed above were investigated first as mixtures of n-alkanes and methylalkanes in three ratios, namely 2:1, 1:1 and 1:2 (v/v) respectively. A third component (alkenes) was then added to the 1:1 (v/v) ratio of this mixture to form three further mixtures of similar ratios. This process was repeated for each of the

components listed in section 2 above. The isolated components of the natural lipid of *L.migratoria* and *S.gregaria*, after separation by TLC, were applied to hind wings in the same ratios as the synthetic lipid.

4. Effect of temperature on water permeability of lipid

In this series of experiments, the evaporation rate through the intact wings of *L.migratoria* and *S.gregaria* was measured at 25°C, 35°C, 45°C, 55°C and 65°C in order to investigate the effect of temperature on the water permeability of cuticular lipid. The evaporation rate through the extracted wings of *L.migratoria* and *S.gregaria* to which natural lipid had been re-applied was also investigated at the above temperatures. Lipid amounts (mg/cm^2) were the same as those given in section 3 above. Further, the evaporation rate through the extracted wings of *L.migratoria* and *S.gregaria* was measured at 25°C.

The evaporation rate through the intact terga of *L.migratoria* and *S.gregaria* was also measured at the above temperatures. The evaporation rate through the extracted terga of *L.migratoria* and *S.gregaria* was also measured at 25°C. The approximate surface area of 10 terga of each species was determined by the same method as that used for wings.

5. Effect of composition on the spreading and physical characteristics of lipid

In this series of experiments, the way in which composition affects the spreading and physical characterstics of a lipid was investigated. Mixtures of synthetic lipid comprising n-alkanes, methylalkanes, alkenes and esters were investigated as well as the isolated components of the natural lipid of *S.gregaria*.

In these experiments one drop of a lipid solution (0.1 mg/ml) was added to the extracted hind wings of *S.gregaria* by means of a

microsyringe. The solution was dried with a hot air blower immediately after application. The hind wings were then coated with gold as in section 2.2.1. of chapter 2 and examined with a Philips 500 scanning electron microscope.

4.3. RESULTS

A. Lipid amount/unit area

In *L.migratoria*, the amount of lipid on one wing was 0.00081g, while in *S.gregaria*, it was 0.00175g. In each species, this amount was halved for the wing has two sides. Thus the amount of lipid on one side of a wing was 0.00041g for *L.migratoria* and 0.00088g for *S.gregaria*. These figures clearly show that *S.gregaria* has slightly more than double the amount of lipid that *L.migratoria* has.

However, *S.gregaria* has a greater hind wing surface area than that of *L.migratoria*, the average surface area being 7.3cm^2 for *S.gregaria* and 6.2cm^2 for *L.migratoria*. Thus, when the amount of lipid on one side of a wing is divided by the surface area the results are 0.00012g/cm^2 (0.12mg/cm^2) for *S.gregaria* and 0.00007g/cm^2 (0.07mg/cm^2) for *L.migratoria*. Expressing these figures in terms of the area of wing in a holder (0.785cm^2) gives values equal to 0.055mg for *L.migratoria* and 0.094mg for *S.gregaria*.

Application of 0.13mg/cm^2 of synthetic lipid or extracted natural lipid to the extracted wings of *S.gregaria* and *L.migratoria* did not reduce permeability to that of the naturally-occurring lipid on an intact wing and in subsequent experiments greater amounts of synthetic and isolated natural lipid were applied.

The abdominal terga of *S.gregaria* have a greater surface area than those of *L.migratoria*, the average surface area being 0.82cm^2 for *S.gregaria* and 0.7cm^2 for *L.migratoria*.

1. Effect of lipid amount (mg/cm^2) on water permeability

The effect of different amounts of synthetic and natural lipid components on the water permeability of extracted hind wings is shown in Table 9, where

$$\% \text{ Permeability} = \frac{\text{evaporation rate of extracted wing and lipid}}{\text{evaporation rate of extracted wing alone}} \times 100$$

Figure 10a shows the effect of synthetic lipid components in different amounts, namely $0.13mg/cm^2$, $0.38mg/cm^2$ and $0.64mg/cm^2$, on the water permeability of the extracted hind wings of both species. The effect of the isolated components of natural lipids at these three values is shown in Figure 10b. In both Figures, the water permeability of intact and extracted hind wings is given for comparison.

(a) n-alkanes

$0.13mg/cm^2$ of the synthetic n-alkane mixture reduced the percentage permeability of the extracted wing to 51% in *L.migratoria* and to 85% in *S.gregaria* (Figure 10a). The isolated mixture of n-alkanes from the natural lipid of the two species in the same amounts, reduced percentage permeability to 45% in *L.migratoria* and to 82% in *S.gregaria*. Increasing the amount of synthetic n-alkanes on an extracted hind wing to $0.38mg/cm^2$ reduced percentage permeability to 10% in *L.migratoria* and to 33% in *S.gregaria*. Corresponding values for the natural n-alkane mixtures were 9% for *L.migratoria* and 25% for *S.gregaria*. When the amount of synthetic n-alkanes was increased further to $0.64mg/cm^2$, percentage permeability remained at 10% in *L.migratoria* but was reduced to 16% in *S.gregaria*. For the natural n-alkane mixture, percentage permeability of the extracted hind wing was reduced to approximately 9% in both species.

An interesting difference between the wings of the two species was observed in that $0.38mg/cm^2$ of n-alkanes on *L.migratoria* wing had

similar permeability when present as 0.76mg/cm^2 on *S.gregaria* wing (Table 9).

(b) Methylalkanes

0.13mg/cm^2 of the synthetic methylalkane mixture reduced the percentage permeability of the hind wings of the two species by less than 0.5%, while the isolated natural mixtures of methylalkanes at 0.13mg/cm^2 reduced percentage permeability to 78% in both *L.migratoria* and *S.gregaria*. At 0.38mg/cm^2, the synthetic methylalkane mixture reduced percentage permeability of the wings to 52% and to 65% in *L.migratoria* and *S.gregaria* respectively. The natural methylalkane mixtures when applied in these amounts caused a slight reduction of 1 to 5% in percentage permeability in the two species. At 0.64mg/cm^2, a further reduction in percentage permeability was observed with both the synthetic and the natural mixtures (Table 9).

In both species, a decrease in water permeability was observed as the amount of applied methylalkane mixture was increased.

(c) Alkenes

At 0.13mg/cm^2, the synthetic alkene mixture was more effective in reducing the water permeability of extracted wings than the mixture of synthetic methylalkanes, in that they reduced the percentage permeability of *L.migratoria* wings to 91% and *S.gregaria* wings to 89% (Figure 10a). At 0.38mg/cm^2, percentage permeability was reduced to 77% in *L.migratoria* and to 79% in *S.gregaria*. A further reduction was observed at 0.64mg/cm^2. Only slight differences were observed between the two species in the effect of alkenes on the water permeability of the wings. No natural alkenes were tested since unsaturation was not detected in the cuticular hydrocarbons of the two species.

(d) Alcohols

0.13mg/cm^2 of the synthetic primary alcohol mixture reduced the percentage permeability of hind wings to 57% in both species. The corresponding values for the isolated primary natural mixtures of alcohols of the two species in the same amount were 50% for *L.migratoria* wings and 52% for *S.gregaria* wings (Figure 10b). At 0.38mg/cm^2, a further reduction in the percentage permeability of the wings of both species was observed with both the synthetic mixture and the isolated natural mixture. 0.64mg/cm^2 of the synthetic mixture caused a further decrease in percentage permeability in both species.

In general, *L.migratoria* wings had lower water permeabilities with increasing amounts of primary alcohols than those of *S.gregaria*.

(e) Esters

0.13mg/cm^2 of the synthetic ester reduced the percentage permeability of *L.migratoria* and *S.gregaria* wings to 59% and 67% respectively, while at 0.13mg/cm^2 the isolated natural mixture of esters of the two species reduced percentage permeability to 55% in *L.migratoria* and to 60% in *S.gregaria* (Table 9). Similar results to those obtained with alcohols were observed in both species at 0.38mg/cm^2 and 0.64mg/cm^2 with both the synthetic and natural mixtures of esters.

(f) Free fatty acids

At 0.13mg/cm^2, the free synthetic fatty acid mixture had little effect and percentage permeability was reduced to only 98% in *L.migratoria* and to 91% in *S.gregaria*. The isolated natural mixtures of free fatty acids of the two species in the same amount caused a 5% decrease in percentage permeability in *L.migratoria* and a 10% decrease in *S.gregaria* (Figure 10b). With the synthetic mixture at 0.38mg/cm^2, percentage permeability was reduced to 95% in *L.migratoria* and to 87%

in *S.gregaria*. Corresponding values for the natural mixtures of free fatty acids at 0.38mg/cm^2 were 88% for *L.migratoria* and 76% for *S.gregaria*. A further decrease in percentage permeability was observed at 0.64mg/cm^2 with both the synthetic and natural mixtures. In general, lower water permeabilities were observed when the free fatty acid mixtures were spread on *S.gregaria* wing than when spread on the wing of *L.migratoria*.

(g) Triglycerides

Triglycerides were not very effective in reducing percentage permeability and like other lipid components, the isolated natural mixtures of the two species were more effective than the synthetic mixtures. Triglycerides reduced water permeability more when spread on the hind wings of *L.migratoria* than when on the wings of *S.gregaria* particularly at the higher amount/unit area values (Table 9).

The overall results from these experiments indicate that for all lipids as the amount/unit area increases so permeability decreases and that the most effective lipids at reducing the water permeability of the hind wings are n-alkanes, alcohols and esters.

Statistical analyses were applied to the results within each species, and within synthetic and natural mixtures. Two-way analysis of variance for the amount/unit area experiments was used. Applying this test to the results of synthetic mixtures spread on the wing of *L.migratoria* indicated a highly significant interaction between the lipid components and the amount of lipid applied, where the F-value was 11.11, for 12,42 degrees of freedom (d.f.) ($p < 0.001$). With the natural mixtures of this species, the F-value was 24.42, for 10,36 d.f. ($p < 0.001$), again revealing a highly significant interaction between the lipid components and the amount of lipid applied. This was also true for the *S.gregaria* results where a highly significant

interaction was observed for both the synthetic and natural mixtures. The F-value for the synthetic mixtures was 10.36, for 12,42 d.f. ($p < 0.001$) and 82.27 for 10,36 d.f. ($p < 0.001$) for the natural mixtures.

2. Effect of lipid composition on water permeability

Table 10 shows the effect of lipid composition on the water permeability of extracted wings. The lipid coverings in these experiments were 0.38mg/cm^2 for *L.migratoria* and 0.76mg/cm^2 for *S.gregaria*. Figure 11a shows the effect of synthetic mixtures on the water permeability of the wings of the two species, and Figure 11b the effect of mixtures of the isolated components of the natural lipids on water permeability.

As in the previous amount/unit area experiments, n-alkanes, alcohols and esters proved to be the most impermeable to water. For example, when the ratio of n-alkanes predominate in a mixture of n-alkanes and methylalkanes, this caused a 64% reduction in the percentage permeability of the wing of *L.migratoria* and a 60% reduction in that of *S.gregaria* (Figure 11a). Similarly, the isolated natural mixture of n-alkanes and methylalkanes reduced percentage permeability to 32% in *L.migratoria* and to 36% in *S.gregaria*. However, percentage permeability increased to 68% in *L.migratoria* and to 67% in *S.gregaria* when equal mixtures of synthetic n-alkanes and methylalkanes formed the layer. With the natural mixture of n-alkanes and methylalkanes percentage permeability values were 60% in *L.migratoria* and 52% in *S.gregaria* (Figure 11a). Further increases in percentage permeability up to 81% in *L.migratoria* and 86% in *S.gregaria* were observed when methylalkanes predominated in the synthetic mixtures. Similar mixtures of natural n-alkanes and methylalkanes showed permeability values of 67% in *L.migratoria* and 74% in *S.gregaria*. What is true for the mixture of n-alkanes and

methylalkanes also applies to the synthetic mixture of n-alkanes and alkenes and to the mixture of n-alkanes, methylalkanes and alkenes, where lower permeability values were obtained when n-alkanes were in higher ratios (Table 10). With less effective lipids such as free fatty acids and triglycerides, increases in their proportions in a mixture caused corresponding increases in percentage permeability. With these two components, higher water permeability values were observed when they were spread on the wing of *L.migratoria* than on that of *S.gregaria*.

One interesting point concerning alcohols and esters is that when their percentage is over 33% of a mixture, percentage permeability increases. This is true for both species with *L.migratoria* having higher permeability values than *S.gregaria*. Throughout this part of the work, it was clear that mixtures of the isolated components of natural lipids formed more effective water barriers than synthetic mixtures.

The results of the composition experiments were subjected to the following statistical analyses:

1) One-way analysis of variance (test one)
2) Scheffë's multiple comparison between the means (method one). This method was used to show up detailed differences and is indicated by the following formula which gives the confidence interval for $\mu_i - \mu_k$, where μ_i and μ_k are the means of the ith and kth groups (ratios of the sample).

$$\mu_i - \mu_k \; E(\bar{x}_i - \bar{x}_k) \pm \sqrt{r-1 \; F\,[r-1,\; r(n-1);\; 0.05]} \sqrt{Sp^2 \times 2/n}$$

r, number of samples (ratios, 2:1, 1:1, 1:2)

n, number of replicates (three)

F[r-1, r(n-1); 0.05] is F-value from table

S^2p is the standard deviation of the sample and is calculated as

$$S^2p = 1/3 \sum_{i=1}^{3} S_i^2 = 1/3 \left[(S.D._1)^2 + (S.D._2)^2 + (S.D._3)^2 \right]$$

Test one and method one were applied to the results of both synthetic and natural mixtures.

(A) Synthetic mixtures

(1) *n-Alkane (nA)/methylalkane (Ma) mixture*

Test one indicated that the results within each species showed a clear significant difference between the 2:1, 1:1 and 1:2 proportions of n-alkanes (nA) and methylalkanes (Ma). F-values were 18.0 for 2,6 d.f. ($p < 0.01$) for the *L.migratoria* results and 23.39 for 2,6 d.f. ($p < 0.01$) for the *S.gregaria* results. Applying method one to the results of each species individually revealed that when the proportion of methylalkanes increased from 33% to 50%, there was a significant increase in permeability. Increasing the proportion of methylalkanes from 50% to 66% had no significant effect on permeability. In both species, method one is summarized by $\mu_1 < (\mu_2, \mu_3)$.

(2) *nA/alkene (Alk) mixture*

Test one indicated a significant difference in the results of both species. The F-value was 7.27 for 2,6 d.f. ($p < 0.05$) in *L.migratoria* and 5.74 for 2,6 d.f. ($p < 0.05$) in *S.gregaria*. In both species, method one revealed no difference in permeability when alkenes increased from 33% to 50% and from 50% to 66%, since permeability was quite high even when alkenes accounted for only 33% of the mixture. There was however a significant difference between the 33% and 66% proportions of alkenes. Method one is summarized by $\mu_1 < \mu_3$.

(3) *nA & Ma/Alk [Hydrocarbon (Hc)] mixture*

In both species a highly significant difference was observed between the results of the three ratios of this mixture (test one), with an F-value equal to 69.13 ($p < 0.001$) for *L.migratoria* and 56.57 ($p < 0.001$) for *S.gregaria*. Method one indicated that water permeability is high when alkenes account for only 33% of the mixture and that permeability increases as their proportions increase. Method one is summarized by $\mu_1 < \mu_2 < \mu_3$.

(4) *Hc/alcohol (Alc) mixture*

Test one showed a significant difference in the results of this mixture for the wings of both species, with the F-value equal to 45.52 ($p < 0.001$) for *L.migratoria* and 150.34 ($p < 0.001$) for *S.gregaria*. Method one indicated that there was a significant difference in permeability when alcohols were increased from 33% to 50% of the mixture, while there was no significant difference between the 50% and 66% proportions for both species. This means $\mu_1 < (\mu_2, \mu_3)$.

(5) *Hc & Alc/free fatty acids (FfA) mixture*

Test one revealed a clear significant difference between the permeabilities of the three ratios of the mixture containing free fatty acids in both species. The F-value equals 410.31 ($p < 0.001$) in *L.migratoria* and 65.57 ($p < 0.001$) in *S.gregaria*. Method one indicates a clear proportional relation between the percentage of free fatty acids in a mixture and its water permeability. As the proportions of free fatty acids increased so did the water permeability ($\mu_1 < \mu_2 < \mu_3$).

(6) *Hc, Alc & FfA/triglyceride (Tri) mixture*

Applying test one to this mixture also revealed a significant difference between the permeability results of the three mixtures

containing triglycerides, where F-value equals 205.82 ($p < 0.001$) in *L.migratoria* and 41.18 ($p < 0.001$) in *S.gregaria*. In *L.migratoria*, method one revealed a significant difference between the water permeability of increasing proportions of triglycerides, where $\mu_1 < \mu_2 < \mu_3$. In *S.gregaria*, method one showed no significant difference between the 33% and the 50% triglyceride proportions, though a significant difference occurred when proportions of triglycerides increased from 50% to 66%, $(\mu_1, \mu_2) < \mu_3$.

(7) *Hc, Alc, FfA & Tri/ester (E) mixture*

Test one showed a significant difference between the results from this mixture, where F-value is 65.99 ($p < 0.001$) in *L.migratoria* and 7.40 ($p < 0.05$) in *S.gregaria*. However, in the *L.migratoria* results, method one indicated a clear significant difference between the three proportions of esters where $\mu_1 < \mu_2 < \mu_3$. In *S.gregaria*, method one indicated no significant difference in permeability when ester proportions increased from 33% to 50% and from 50% to 66% $(\mu_1 < \mu_3)$.

B. Mixtures of the isolated components of natural lipids

(1) *nA/Ma [Hydrocarbon (Hc)] mixture*

Test one on the results from this mixture revealed a highly significant difference in both species. In *L.migratoria*, the F-value was 87.57 for 2,6 d.f. ($p < 0.001$) and in *S.gregaria* 209.55 for 2,6 d.f. ($p < 0.001$). However, in *L.migratoria*, method one showed no significant difference in water permeability when the proportion of methylalkanes increased from 50% to 66%, $\mu_1 < (\mu_2, \mu_3)$. In *S.gregaria*, an increase in the proportion of methylalkanes was accompanied by an increase in water permeability, $\mu_1 < \mu_2 < \mu_3$.

(2) *Hc/Alc mixture*

Test one and method one revealed the same pattern in both

species. Highly significant differences were found within each species with test one, with the F-value equal to 66.20 ($p < 0.001$) in *L.migratoria* and 42.57 ($p < 0.001$) in *S.gregaria*. With method one, the results of both species indicated an increase in water permeability as the alcohol proportion increased in the mixture, $\mu_1 < \mu_2 < \mu_3$.

(3) *Hc & Alc/FfA mixture*

Test one showed a highly significant difference in the *L.migratoria* results, where the F-value equals 71.09 ($p < 0.001$), and a significant difference in the *S.gregaria* results where the F-value equals 19.30 ($p < 0.01$). In *L.migratoria*, method one showed that a significant increase in water permeability occurred as the free fatty acid proportion increased ($\mu_1 < \mu_2 < \mu_3$), while in *S.gregaria*, there was no significant difference in the water permeability of mixtures containing 50% and 66% fatty acids. This means $\mu_1 < (\mu_2, \mu_3)$.

(4) *Hc, Alc & FfA/Tri mixture*

Test one showed a highly significant difference between the permeability results within each species. The F-value was 58.96 ($p < 0.001$) in *L.migratoria* and 46.73 ($p < 0.001$) in *S.gregaria*. Method one revealed a difference between the results of the two species. Clear significant differences between water permeability occurred in the three mixtures on *L.migratoria* wing ($\mu_1 < \mu_2 < \mu_3$), while no significant differences occurred between the water permeability of the mixtures containing 33% and 50% triglycerides on *S.gregaria* wing [$(\mu_1, \mu_2) < \mu_3$].

(5) *Hc, Alc, FfA & Tri/E mixture*

The same pattern was observed in the two species with both the test and the method. A highly significant difference was revealed by test one, where the F-value equals 346.43 ($p < 0.001$) in *L.migratoria*

and 99.48 ($p < 0.001$) in *S.gregaria*. With method one a clear increase was observed in permeability as the ester proportion increased ($\mu_1 < \mu_2 < \mu_3$).

3. Effect of temperature on water permeability of natural lipid

(A) *Hind wings*

In *L.migratoria*, the water permeability of the intact hind wing was 2.5 mg/cm^2/hr, while in *S.gregaria* it was 2.4 mg/cm^2/hr. Extraction of epicuticular lipid increased water permeability to 19.2 mg/cm^2/hr in the wing of *L.migratoria* and to 19.1 mg/cm^2/hr in the wing of *S.gregaria*. This means a nearly 8-fold increase in the water permeability of the wings in both species. The above figures were recorded at 25°C, which was the first temperature of this experiment. Other temperatures used were 35°C, 45°C, 55°C and 65°C. Water permeability of the intact wings of *L.migratoria* and *S.gregaria* showed a gradual increase from 25°C to 45°C (Figure 12). At the latter temperature, water permeability through the intact wing was 10.2 mg/cm^2/hr for *L.migratoria* and 7.0 mg/cm^2/hr for *S.gregaria*. At 55°C, a steep increase (nearly twice that at 45°C) was observed in the water permeability of the intact wings of *L.migratoria* and *S.gregaria*. At 65°C, still higher water permeabilities were observed in the intact wings of both species, namely 44.2 mg/cm^2/hr in *L.migratoria* and 40.8 mg/cm^2/hr in *S.gregaria*. Figure 12 clearly shows that the intact wing of *L.migratoria* has a higher water permeability than that of *S.gregaria* at all of the temperatures investigated and that water evaporation occurred over a range of 40°C with a gradual rise in permeability from 25°C to 45°C and a steep rise from 45°C to 65°C.

A similar pattern to that of intact wings was observed when isolated components of natural lipids were re-applied to the extracted

wings of *L.migratoria* and *S.gregaria* (Figure 12). 0.38 mg/cm^2 of lipid was applied to the extracted wing of *L.migratoria* and 0.76 mg/cm^2 to that of *S.gregaria*. At all temperatures, water permeabilities for both species were much higher than those of intact wings. For example, at 25^{o}C the water permeability of re-applied lipid on *L.migratoria* wing was 3.7 mg/cm^2/hr and that for *S. gregaria*, 3.6 mg/cm^2/hr. At 45^{o}C, water permeability increased to 13.5 mg/cm^2/hr and to 10.6 mg/cm^2/hr for *L.migratoria* and *S.gregaria* respectively. A nearly 2-fold increase in water permeability was observed at 55^{o}C in the wings of both species, while at 65^{o}C, water permeability was 87.5 mg/cm^2/hr for *L.migratoria* and 85.2 mg/cm^2/hr for *S.gregaria*. Figure 12 also shows that the wings of *L.migratoria* again have a higher water permeability than those of *S.gregaria*. Here also, the isolated components of natural lipids spread on extracted wings show water evaporation rising over 40^{o}C, with a gradual rise from 25^{o}C to 45^{o}C and a steep rise from 45^{o}C to 65^{o}C.

(B) *Abdominal terga*

The water permeability of the intact abdominal tergum of *L.migratoria* was 17 mg/cm^2/hr and that of *S.gregaria* 10.5 mg/cm^2/hr. Extraction of the epicuticular lipid increased water permeability to 31.25 mg/cm^2/hr in *L.migratoria* and to 15 mg/cm^2/hr in *S.gregaria*. This is nearly a 2-fold increase in permeability of the tergum of *L.migratoria* and a 1.5 fold increase in *S.gregaria*. Like the hind wings, the intact terga of *L.migratoria* and *S.gregaria* showed a gradual increase in water permeability as the temperature increased from 25^{o}C to 45^{o}C. At the latter temperature, the water permeability of *L.migratoria* tergum was 31m g/cm^2/hr and 25.5 mg/cm^2/hr for *S.gregaria* tergum. Here also a steep increase in water permeability was observed between 45^{o}C and 65^{o}C with a nearly 2-fold increase at

55°C and a much higher increase at 65°C namely 103.75 mg/cm^2/hr for the tergum of *L.migratoria* and 98.5 mg/cm^2/hr for the tergum of *S.gregaria*.

4. Effect of composition on the spreading & physical characteristics of lipid

The results of these experiments shed some light on the way in which composition affects the spreading and physical form that a layer of lipid assumes on the cuticular surface. The results also show up differences between synthetic and natural components. Of all the lipid components examined individually, synthetic and natural mixtures of methylalkanes and synthetic mixtures of alkenes have the best spreading characteristics. Plates 10a and 10b show how efficiently one drop of a standard solution of synthetic methylalkane mixture and one drop of a standard solution of synthetic alkene mixture covered over the cuticular surface. Both mixtures seem to assume the same physical form on the cuticular surface. A similar layer is formed by one drop of a standard solution of the natural methylalkane mixture of *S.gregaria* (Plate 11a). The isolated natural alcohol mixture of *S.gregaria* also forms a nearly complete layer over the cuticular surface (Plate 11b). Plates 12a and 12b show the difference between the layer formed by the synthetic n-alkane mixture and the isolated mixture of natural n-alkanes of *S.gregaria*. Both show limited spreading but the latter has a crystalline appearance. Natural n-alkanes (Plate 12b) and natural free fatty acids (Plate 13) however show only limited spreading and the layers they form have a crystalline appearance. Natural esters spread less well than natural free fatty acids and natural n-alkanes and the layers they form have a hard crystalline appearance (Plate 14a). Natural triglycerides spread the least of all examined components (Plate 14b). Like esters,

triglycerides also assume a crystalline appearance.

What is true for individual components, is also true when these components are mixed together. For example, the layer formed by one drop of a standard solution of mixture of the 1:1 (v/v) synthetic n-alkanes and methylalkanes is shown in Plate 15a. For comparison, a layer formed by one drop of a standard solution of the natural mixture of n-alkanes and methylalkanes is shown in Plate 15b. It seems clear that the spreading of n-alkanes is enhanced by the addition of methylalkanes. Another example is that of the layer formed by one drop of a standard solution of the synthetic mixture of 1:1:1:1 (v/v) n-alkanes, methylalkanes, alkenes and esters which clearly shows reduced spreading (Plate 16). A high percentage of esters appears to inhibit the spreading of the hydrocarbons, and gives the layer a hard crystalline appearance. For comparison, scanning electron micrographs of intact and extracted hind wings are given in Plates 17a and 17b.

4.4. DISCUSSION

A greater amount of lipid was extracted from a hind wing of *S.gregaria* (1.75mg) than from a wing of *L.migratoria* (0.81mg). Thus the amount of lipid on one side of a wing was greater in *S.gregaria* (0.88mg) than in *L.migratoria* (0.41mg).

S.gregaria has a greater hind wing surface area (7.3cm^2) compared with *L.migratoria* (6.2cm^2), but the water permeability of both intact wings is nearly equal. In *S.gregaria* the water permeability of the intact wing is 2.4 mg/cm^2/hr, while in *L.migratoria* it is 2.5mg/cm^2/hr. In *S.gregaria*, the greater wing surface area but similar water permeability to that of *L.migratoria* may be explained by the presence of a greater amount of lipid on the wing. The occurrence of large amounts of cuticular lipid and a need for water conservation have been noted by Bell *et al.* (1975) in the tobacco hornworm, *Manduca*

sexta, by Goodrich (1970) in the sheep blowfly, *Lucilia cuprina* and by Lees (1955) in the mite *Pterobius latens*.

The greater amount of lipid on the hind wing of *S.gregaria* is accompanied by a higher proportion of n-alkanes. In *S.gregaria*, n-alkanes account for 69.9% (average value, male & female wings) of the hydrocarbon mixture, compared with 40.7% in *L.migratoria* (average value, male & female wings). A greater amount of lipid with a high proportion of hydrocarbons as a means of reducing cuticular transpiration have been reported in many species, such as the stonefly, *Pteronarcys californica* (Armold *et al.*, 1969), *Sacrophaga bullata* (Armold & Regnier, 1975), *Manduca sexta* (Coudron & Nelson, 1981), *Diaceroprocta apache* (Hadley, 1980b) and the Bertha Armyworm, *Mamestra configurata* (Hegdekar, 1979).

The greater amount of lipid on the hind wing of *S.gregaria* with a high proportion of n-alkanes may explain the low water permeability when compared with that of *L.migratoria*. This is particularly true at the higher temperatures investigated (Figure 12).

The tergum of *S.gregaria* has a greater surface area ($0.82cm^2$) than that of *L.migratoria* ($0.7cm^2$). However, water permeability is lower in the intact tergum of *S.gregaria* (10.5 $mg/cm^2/hr$) than in the tergum of *L.migratoria* (17.0 $mg/cm^2/hr$). Furthermore, n-alkanes account for a higher proportion of tergal lipid in *S.gregaria* (59.9% average value, male and female terga) than in *L.migratoria* (42.1% average value, male and female terga). The tergum of *S.gregaria* also possesses well developed lipid lamellae in the endocuticle unlike the endocuticle of *L.migratoria* (Section 3.3.2). So the lower water permeability of the abdominal tergum of *S.gregaria* compared with the *L.migratoria* values at all of the investigated temperatures (Figure 13) may be explained by the presence of a high proportion of n-alkanes

accompanied by well developed endocuticular lipid lamellae.

These relatively low permeability values of *S.gregaria*, the desert locust, may be related to the arid environment in which it lives compared with the semi-arid environment of *L.migratoria* (Uvarov, 1977).

Extraction of the epicuticular lipid from a hind wing increases water permeability up to 8 times in both species which indicates that the lipid contributes significantly to waterproofing. The waterproofing function of cuticular lipid is well known and has been investigated in many arthropod species including insects, as well as in plants. Arthropod species investigated include for example, *P.americana* (Beament, 1945; Wigglesworth, 1945), the scorpion, *Hadrurus arizonensis* (Hadley & Quinlan, 1987), *Drosophila pseudoobscura* (Toolson, 1982), *Tibicen dealbatus* (Toolson, 1984). Plants investigated include the Seville orange, *Citrus aurantium* (Schönherr, 1976).

Extraction of the epicuticular lipid of terga nearly doubles water permeability in *L.migratoria* (31.25 mg/cm^2/hr) and increases it to nearly 1.5 times in *S.gregaria* (15 mg/cm^2/hr). However, bound lipid such as the endocuticular lipid lamellae in the terga of *S.gregaria* is not removed by chloroform extraction and its contribution to the water impermeability of the tergum is indicated by the lower tergal permeability values observed in *S.gregaria* when compared with those of *L.migratoria*.

This study has shown that as lipid amount/unit area increases, so water permeability decreases. This observation is confirmed by statistical analysis (two-way analysis of variance) which shows that the water permeability values of the different amounts of lipid applied to the wings of the two species are highly significant. This applies to both synthetic as well as to the isolated components of

natural lipid. The most effective waterproofing lipids in order of impermeability were n-alkanes, alcohols and esters. This is true for both synthetic and the natural components. Less effective lipids in order of impermeability were synthetic alkenes, synthetic methylalkanes, synthetic and natural free fatty acids and synthetic and natural triglycerides.

Experiments on plasma membranes and artificial bilayers indicate that while long-chain saturated molecules decrease permeability, branched and unsaturated molecules increase it (de Gier *et al.*, 1968; Taylor *et al.*, 1975). This also seems true in the present study where n-alkanes were the most efficient components at reducing water permeability. However, the different proportion of n-alkanes in the wing lipids of *L.migratoria* and *S.gregaria* may explain the difference in their waterproofing efficiency when applied in increasing amounts to the wings of the two species. n-Alkanes not only form an efficient water barrier because they are saturated components, but they also give thermal stability to the barrier. Thus n-alkanes with a chain length ranging from 23 to 35 carbons have a melting point range from 47.6 to 75^{o}C (Hadley, 1980a). This study has shown that *L.migratoria* and *S.gregaria* have extensive homologous series of n-alkanes ranging from nC_{14} to nC_{36}. These species could then have high melting point cuticular lipids because of the n-alkane mixtures. In the case of *S.gregaria* the series of n-alkanes and the thick lipid layer on the wing may be necessary to withstand high desert temperatures.

Unlike the synthetic mixture, the natural mixture of methylalkanes, which contains the longest molecules among cuticular hydrocarbons, was partially effective in reducing water permeability, particularly when applied in large amounts. This observation agrees with those of Hadley (1977), Toolson (1982) and Toolson and Hadley

(1977, 1979) who relate the presence of high proportions of long-chain saturated methylalkanes to low cuticular permeability.

Some of the polar components investigated in the present work also have long chains and form efficient water barriers, for example, esters which are the longest molecules in the cuticular lipids of *L.migratoria* and *S.gregaria*. Alcohols also have long chains but their efficiency at reducing water permeability may also be related to their ability to spread over the cuticular surface.

Unsaturated components, such as the synthetic mixture of alkenes proved to be inefficient at reducing the water permeability of extracted wings. However, their good spreading features (Plate 10b) like those of methylalkanes, may well allow them to provide a matrix for waterproofing polar components with less spreading characters (Lockey, 1988). This could explain their presence in the cuticular lipids of many insects. In the two species of this study however, alkenes are absent and such a role is probably played by the methylalkanes (Plate 10a).

Unsaturation may also explain the difference in water permeability when the isolated natural free fatty acid mixtures were applied to the extracted wings of *L.migratoria* and *S.gregaria*. This was seen in the amount/unit area experiments. The higher water permeabilities of the *L.migratoria* mixtures when compared with those of *S.gregaria* may be explained by the higher proportion of unsaturation (15.7% in the former compared with 2.2% in the latter). On the other hand, the isolated natural triglyceride mixtures of the two species also show a difference in the amount of unsaturation. However, such a difference is not reflected in any difference in water permeability and the reason for this is not known.

The results of this study are similar to those of Grncarevic and Radler (1967) who reported that while hydrocarbons, and alcohols of

grape wax and paraffin wax were the most efficient components at reducing water permeability through artificial plastic membranes, fatty acids 22:0 and 26:0 did not reduce water permeability. They also emphasised that chain length is an important factor in reducing water evaporation as demonstrated in this study. The results obtained in this study also agree with those of Alexander *et al.* (1944) who reported that the water permeability of a layer of *n*-alkanes decreases as thickness increases. However, Alexander *et al.* (1944) showed that a 0.02μm thick layer of a polar lipid, such as beeswax, was effective at reducing the water permeability of a membrane, though increasing the thickness of the beeswax layer caused no further reduction in water permeability. Slightly different results were obtained with alcohols and esters in this study, where for both species and with both synthetic and natural mixtures, 0.13 mg/cm^2 caused a 43% to 50% reduction in percentage permeability and 0.38 mg/cm^2 and 0.64 mg/cm^2 caused a further 7% to 16% reduction in percentage permeability. Therefore the results of this study reveal that thickness may be an important factor with polar components such as alcohols and esters just as it is important with non-polar components.

Free fatty acids and triglycerides were found to be inefficient at reducing water permeability. This may be due to their poor spreading characteristics (Plates 13, 14b). However they may contribute in other ways to the water impermeability of cuticular lipid. For example, mixtures of free fatty acids and triglycerides could increase the hardness of a heterogenous cuticular lipid so that it could withstand abrasion by sand particles. This may explain the presence in the cuticular lipids of the two species of high proportions of triglycerides some of which is probable contamination from body lipid. But free fatty acids account for only low proportions

of the cuticular lipid of *L.migratoria* and *S.gregaria* and this could be regarded as a maladaption in the chemical composition of the lipids. Further, scanning electron micrographs showed that the cuticular lipids of *L.migratoria* and *S.gregaria* are of soft appearance rather than of hard crystalline appearance. This may be related to the fact that neither species burrows in sand but they seek shelters such as low bushes and rock crevices during unfavourable conditions (Uvanov, 1977). Such behaviour may also explain the low proportions of esters in both species. Several species with hard crystalline cuticular lipids have been reported. For example, the nymphs and adults of the sand cockroach, *Arenivaga investigata* (Jackson, 1983) have a high proportion of alkyl esters in their cuticular lipid which, as a result is hard and resistant to abrasion by sand particles. Free fatty acids, particularly saturated ones, may in another way contribute to the thermal stability of cuticular lipids. For example, the melting points of fatty acids, 16:0, 18:0 and 20:0 are 63, 70 and 75^{o}C respectively. Alternatively unsaturated fatty acids with their low melting points such as the commonly occurring fatty acids 18:2 (melting point -5^{o}C) and 18:3 (melting point -11^{o}C) (Bronk, 1973) could keep cuticular lipids fluid at low environmental temperatures. The higher unsaturation levels of free fatty acids in *L.migratoria* cuticular lipid may result in a lower melting point of the lipid.

The effect of lipid components in variable amounts on the water permeability of wings is shown by the lipid composition results. For example, high proportions of n-alkanes again proved to be efficient at reducing water permeability and the role of methylalkanes in increasing water permeability is clearly seen. With methylalkanes, a difference was observed between the wings of *L.migratoria* and *S.gregaria* in that the extracted wing of *S.gregaria* showed higher water permeability as the proportions of methylalkanes increased in

the mixture when compared to that of *L.migratoria*. This difference may be explained by the low ratio of methylalkanes to n-alkanes in *S.gregaria* wing lipid. At the same time, the presence in the cuticular lipid of *S.gregaria* of trimethylalkanes with their methyl side chains should increase water permeability (de Gier *et al.*, 1968).

Alkenes were not detected in the cuticular lipids of the two species. However, synthetic alkenes again proved to be inefficient at reducing water permeability, which is high even when alkenes are present in the lowest proportions in the ratio mixtures.

What is true for alkenes is also true for fatty acids and triglycerides. When unsaturation is high, so water permeability is high. With the natural mixtures of fatty acids and triglycerides, the wing of *L.migratoria* showed higher water permeability than that of *S.gregaria* when these components were present in mixtures and when they were in high proportions. For fatty acids, unsaturation accounts for 15.7% in *L.migratoria* and for 2.2% in *S.gregaria*. Further, the triglycerides of *L.migratoria* contain a significant proportion of unsaturated fatty acids (32.4%) (female) and 20.4% (male) while no unsaturated fatty acids were detected in the cuticular triglycerides of *S.gregaria*. These permeability results agree with those of Taylor *et al.* (1975) who showed that in artificial bilayers unsaturation increases pemeability.

Esters and alcohols seem to behave differently in that the water permeability of a mixture increases after their proportion exceeds 33%. For esters, this may be explained by their high crystallization and low spreading characteristics (Plate 14a). However, their presence may give the lipid more rigidity as has been shown in artificial bilayers (Ladbrooke *et al.*, 1968). Esters and alcohols occur in only low proportions of the cuticular lipids of the two species, so that

mixtures with high proportions of these components have a composition different from the naturally-occurring lipid.

The results of this study show that as the temperature increases, so there is an increase in the water permeability of the wing and tergal epicuticular lipids of *L.migratoria* and *S.gregaria*. Such an observation has been reported in other insect and arthropod species by many workers (Beament, 1945; Edney, 1977; Gunn, 1933; Ramsay, 1935a,b; Toolson *et al.*, 1979; Wigglesworth, 1945). The results also show a gradual increase in wing and tergal water permeability between 25°C to 45°C and a steep increase between 45°C and 65°C (Figures 12 & 13) confirming that water permeability increases over a range of temperature rather than at a precise temperature as proposed by Beament (1945). This rise occurs over a range of 40°C. This is true for the intact wings and terga of both species as well for the extracted wings with re-applied lipid. This range of 40°C agrees with the range found in *S.gregaria* nymphs by Wigglesworth (1986). Permeability increase over a range of temperature (22 to 35°C) is also reported by Toolson *et al.* (1979) in the scorpion *Centruroides sculpturatus*. A similar steep rise in the water permeability of *L.migratoria* was also found by Loveridge (1968b) between 46 to 48°C.

This study shows that mixtures of the isolated natural lipid components formed more impermeable layers than the synthetic mixtures. This observation agrees with those of Alexander *et al.* (1944) and Grncarevic and Radler (1967) and it may be that the efficiency of the natural lipid of *L.migratoria* and *S.gregaria* as a water barrier is due to the presence of complex mixtures of long chain molecules. The mixtures of synthetic lipids used in this study contained only short chain components.

Figure 8

Diagram of wing holder drawn to scale

Upper plate, 40mm diameter, 4mm thick

Lower disc, 40mm diameter, 14mm deep

Central well, 10mm diameter, 12mm deep

O-ring, 14mm diameter

Figure 8

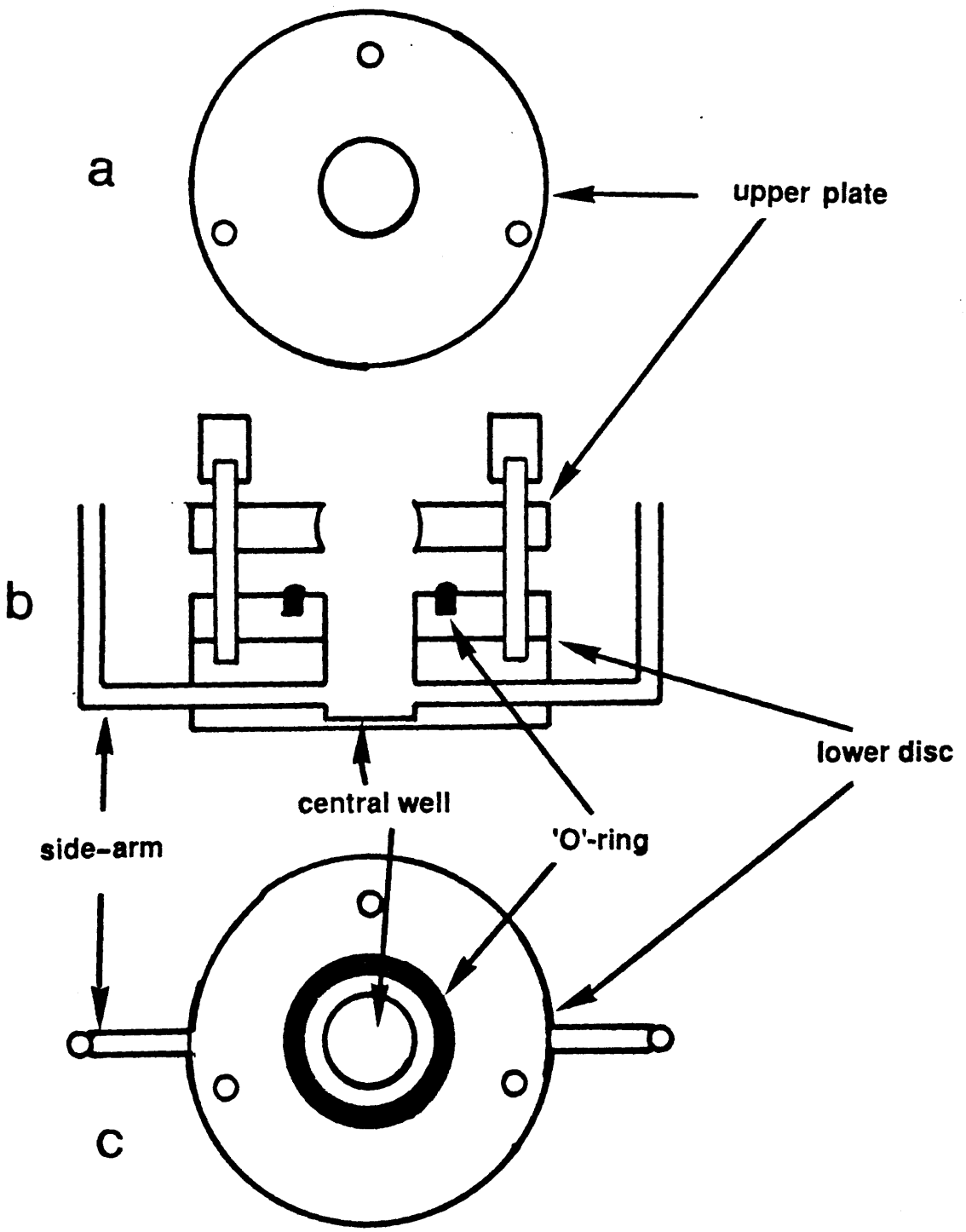


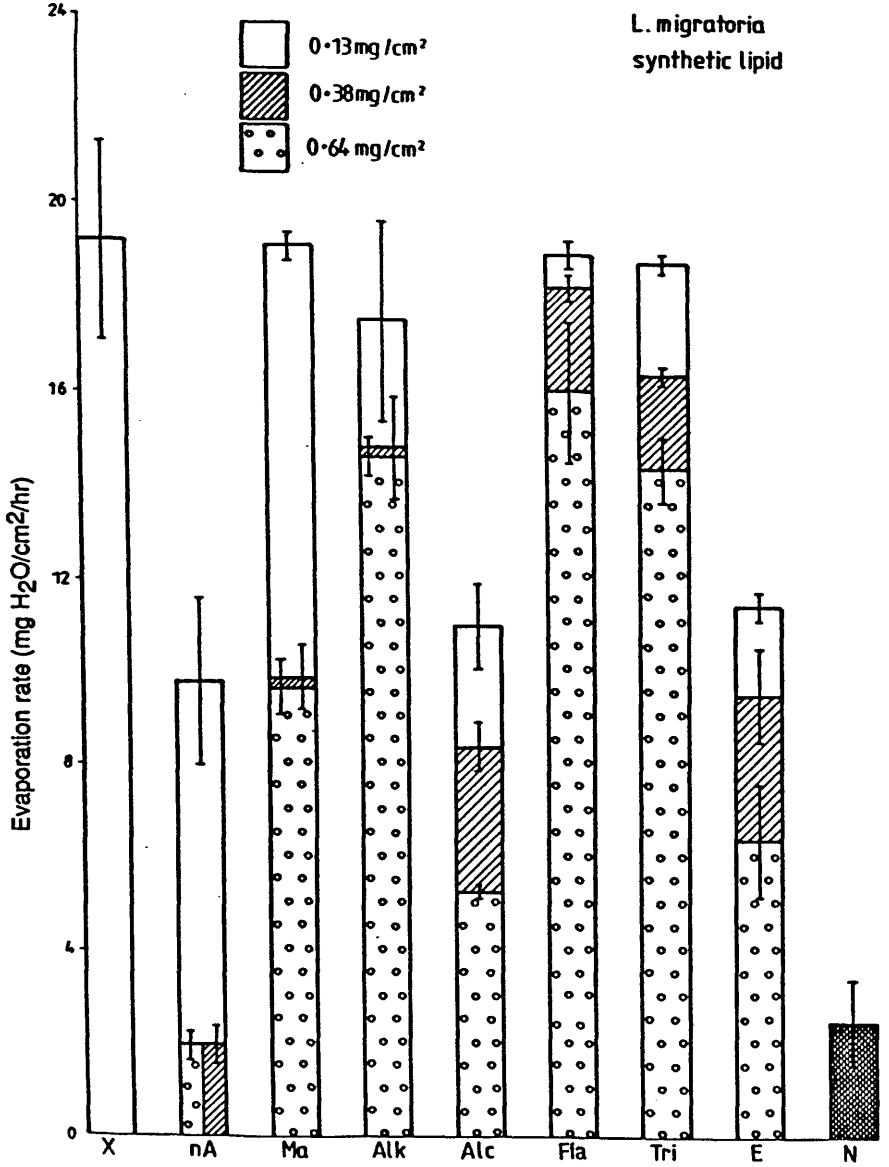
Figure 10a

Lipid amount (mg/cm^2) and water evaporation rates through extracted hind wings

Synthetic lipid spread on *L.migratoria* and *S.gregaria* wings at $0.13mg/cm^2$, $0.38mg/cm^2$ and $0.64mg/cm^2$

Lipids: Alc, alcohols; Alk, alkenes; E, esters; Ffa, free fatty acids; Ma, methylalkanes; nA, n-alkanes; Tri, triglycerides.

X, extracted wing; N, intact wing.


0·13mg/cm²
0·38mg/cm²
0·64 mg/cm²
L. migratoria
synthetic lipid
Evaporation rate (mg H2O/cm2/hr)
0
4
8
12
16
20
24
X
nA
Ma
Alk
Alc
Fla
Tri
E
N

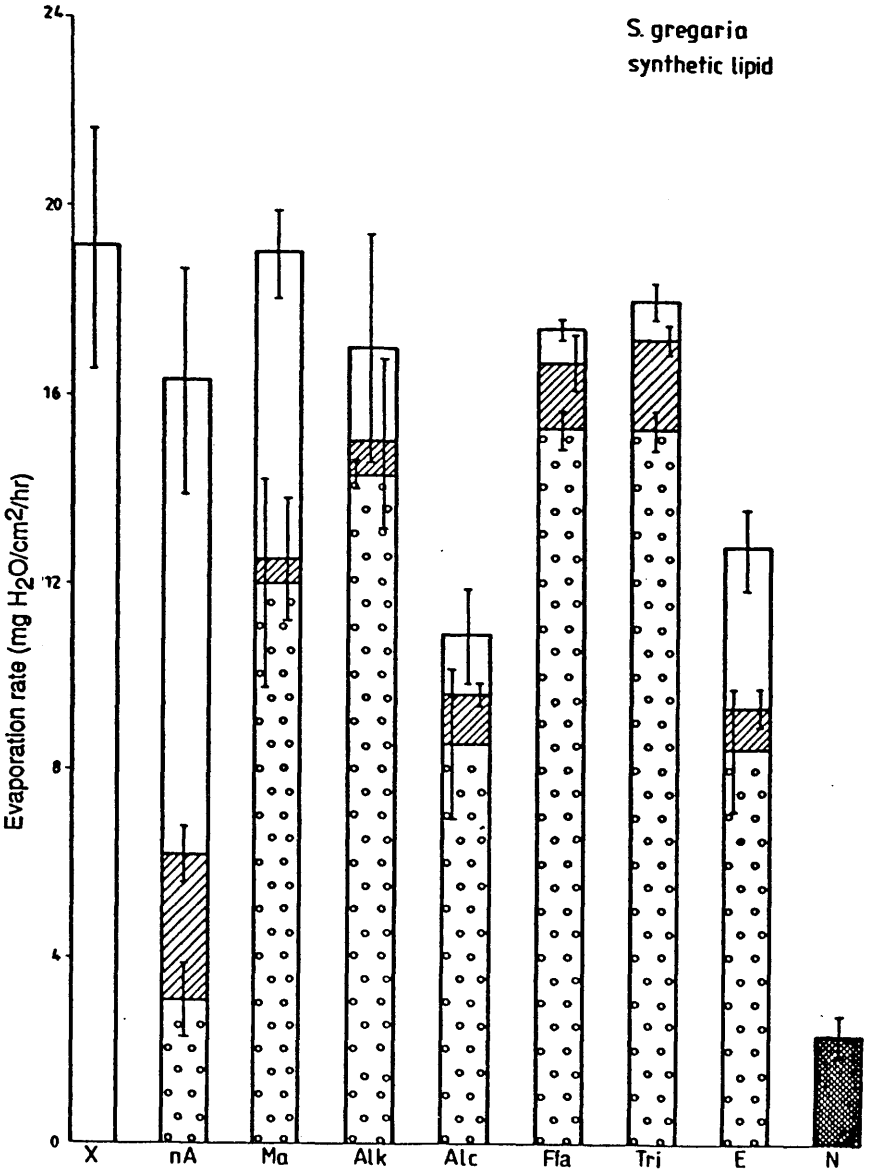

S. gregaria
synthetic lipid
Evaporation rate (mg H2O/cm2/hr)
0
4
8
12
16
20
24
X
nA
Ma
Alk
Alc
Fla
Tri
E
N

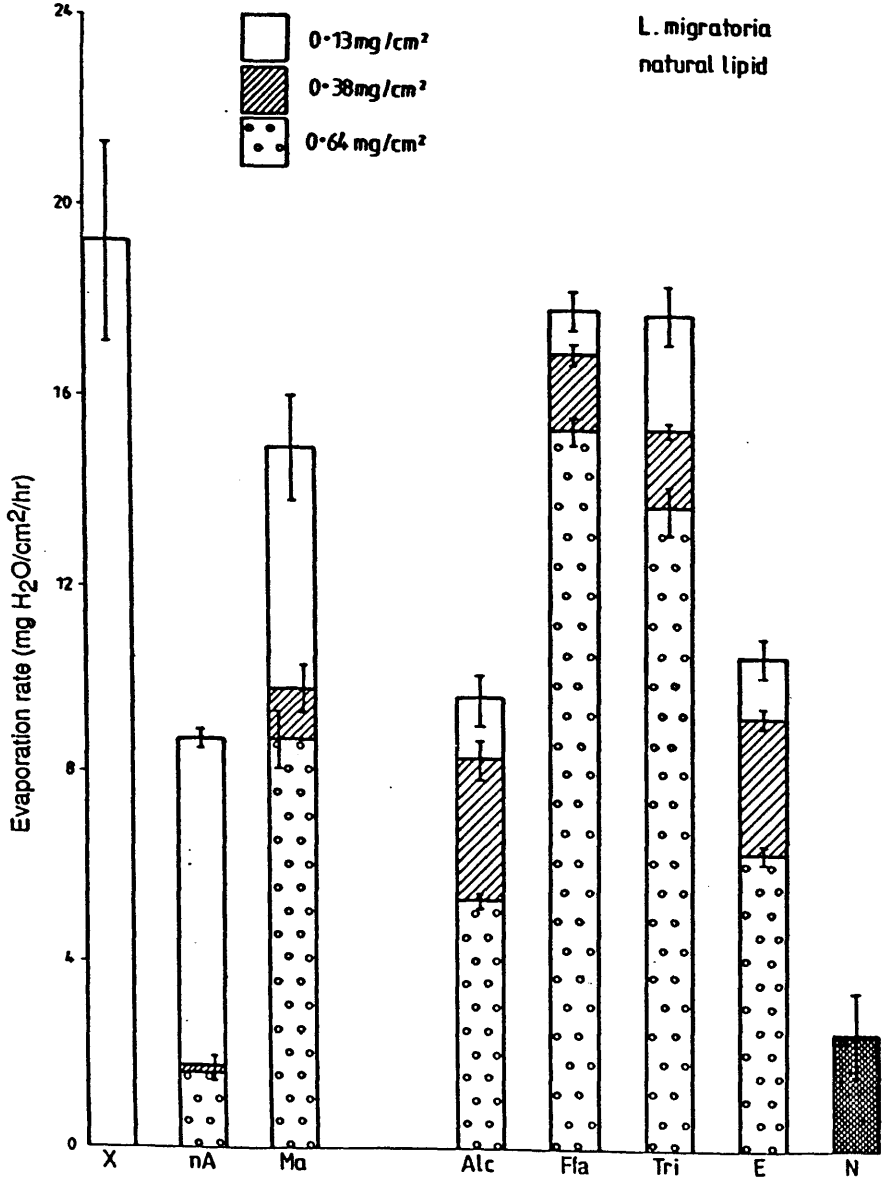
Figure 10b

Lipid amount (mg/cm^2) and water evaporation rates through extracted hind wings

Isolated lipid components of the natural lipid spread on *L.migratoria* and *S.gregaria* wings at $0.13mg/cm^2$, $0.38mg/cm^2$ and $0.64mg/cm^2$

Components: Alc, alcohols; E, esters; Ffa, free fatty acids; Ma, methylalkanes; nA, n-alkanes; Tri, triglycerides.

X, extracted wing; N, intact wing.


0·13mg/cm²
0·38mg/cm²
0·64 mg/cm²
L. migratoria
natural lipid
Evaporation rate (mg H2O/cm2/hr)
0
4
8
12
16
20
24
X
nA
Ma
Alc
Fla
Tri
E
N

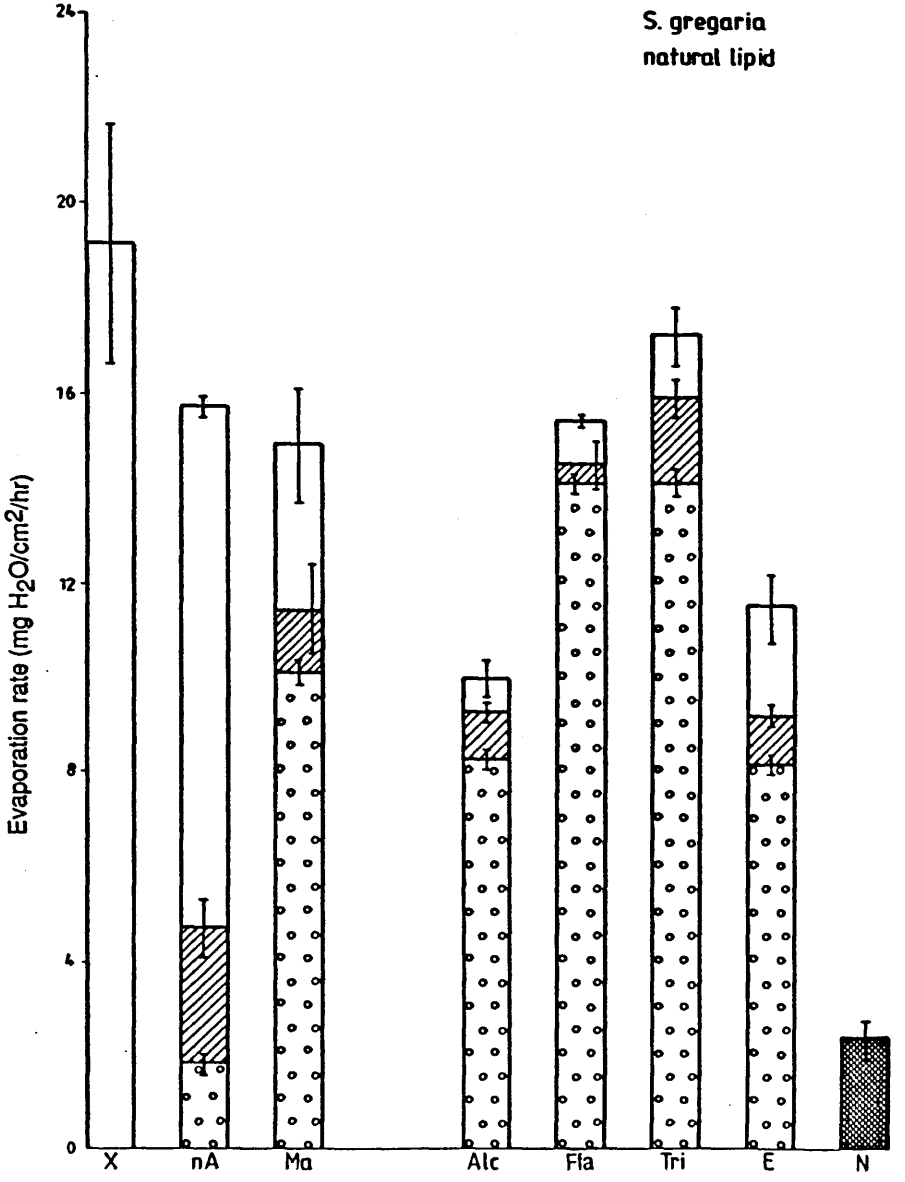

S. gregaria
natural lipid
Evaporation rate (mg H2O/cm2/hr)
0
4
8
12
16
20
24
X
nA
Ma
Alc
Fla
Tri
E
N

Figure 11a

Lipid composition and water evaporation rates through extracted hind wings. Synthetic lipids in three ratios (2:1, 1:1 & 1:2 v/v) spread on *L.migratoria* wing at 0.38mg/cm^2 and on *S.gregaria* wing at 0.76mg/cm^2.

Lipid mixtures: nA/Ma, n-alkanes + methylalkanes; nA/Alk, n-alkanes + alkenes; nA+Ma/Alk, n-alkanes + methylalkanes + alkenes (= Hc); Hc/Alc, preceding mixture + alcohols; Hc+Alc/FfA, preceding mixture + free fatty acids; Hc+Alc+FfA/Tri, preceding mixture + triglycerides; Hc+Alc+FfA+Tri/E, preceding mixture + esters.

X, extracted wing; N, intact wing.

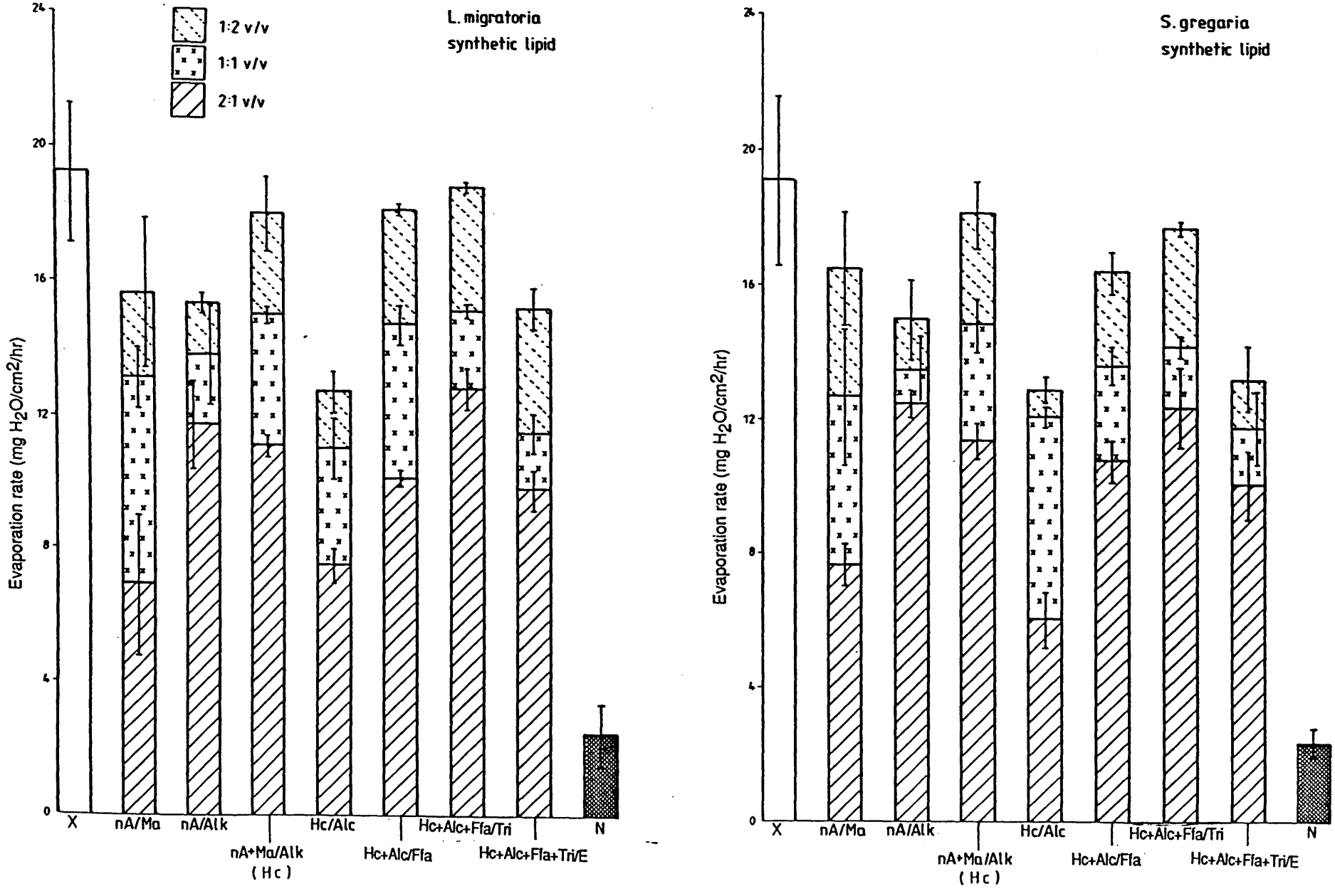
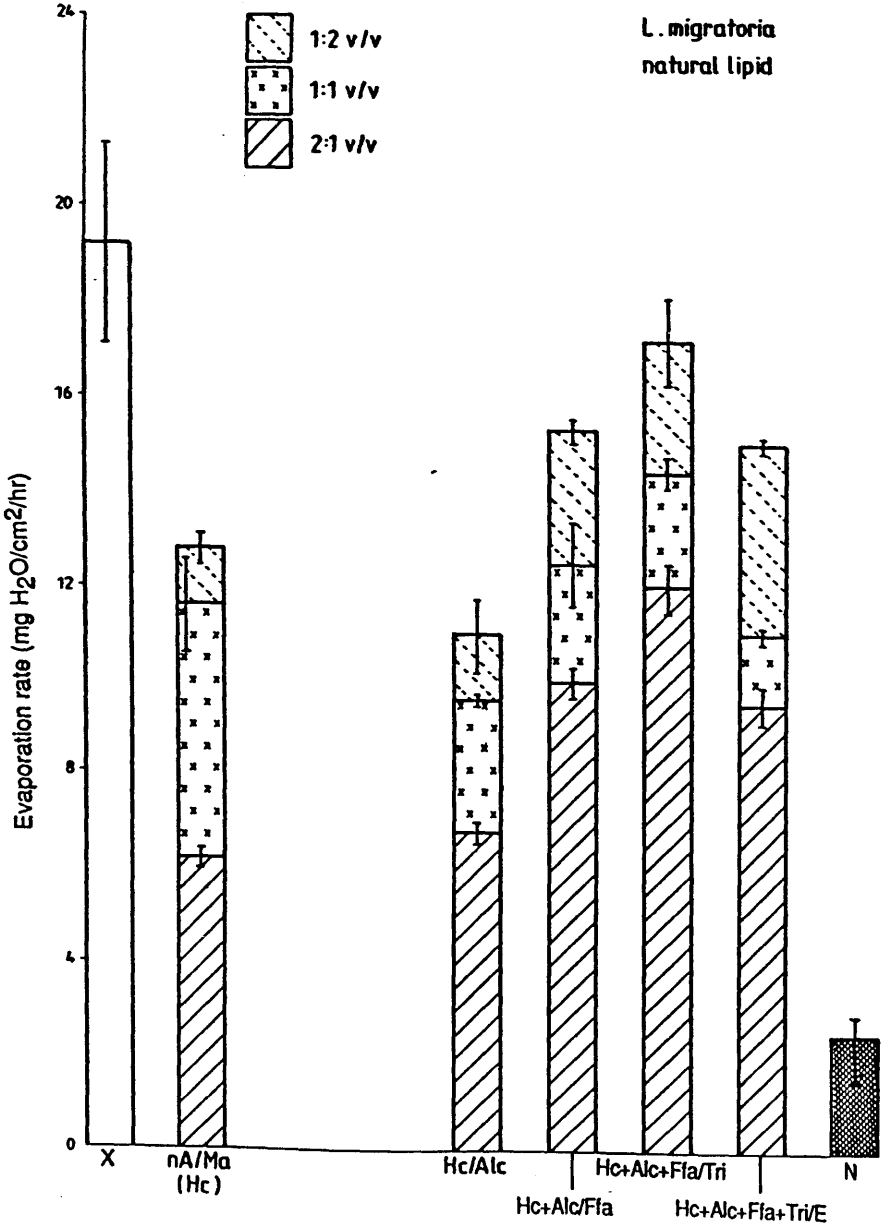

1:2 v/v
1:1 v/v
2:1 v/v
L. migratoria
synthetic lipid
S. gregaria
synthetic lipid
Evaporation rate (mg H2O/cm2/hr)
0
4
8
12
16
20
24
X
nA/Ma
nA/Alk
nA+Ma/Alk
(Hc)
Hc/Alc
Hc+Alc/Ffa
Hc+Alc+Ffa/Tri
Hc+Alc+Ffa+Tri/E
N

Figure 11b

Lipid composition and water evaporation rates through extracted hind wings. Isolated components of natural lipid in three ratios (2:1, 1:1 & 1:2 v/v) spread on *L.migratoria* wing at 0.38mg/cm^2 and on *S.gregaria* wing at 0.76mg/cm^2.

Component mixtures: nA/Ma, n-alkanes + methylalkanes (=Hc); Hc/Alc, preceding mixture + alcohols; Hc+Alc/Ffa, preceding mixture + free fatty acids; Hc+Alc+Ffa/Tri, preceding mixture + triglycerides; Hc+Alc+Ffa+Tri/E, preceding mixture + esters.

X, extracted wing; N, intact wing.


1:2 v/v
1:1 v/v
2:1 v/v
L. migratoria
natural lipid
Evaporation rate (mg H2O/cm2/hr)
0
4
8
12
16
20
24
X
nA/Ma
(Hc)
Hc/Alc
Hc+Alc/Ffa
Hc+Alc+Ffa/Tri
Hc+Alc+Ffa+Tri/E
N

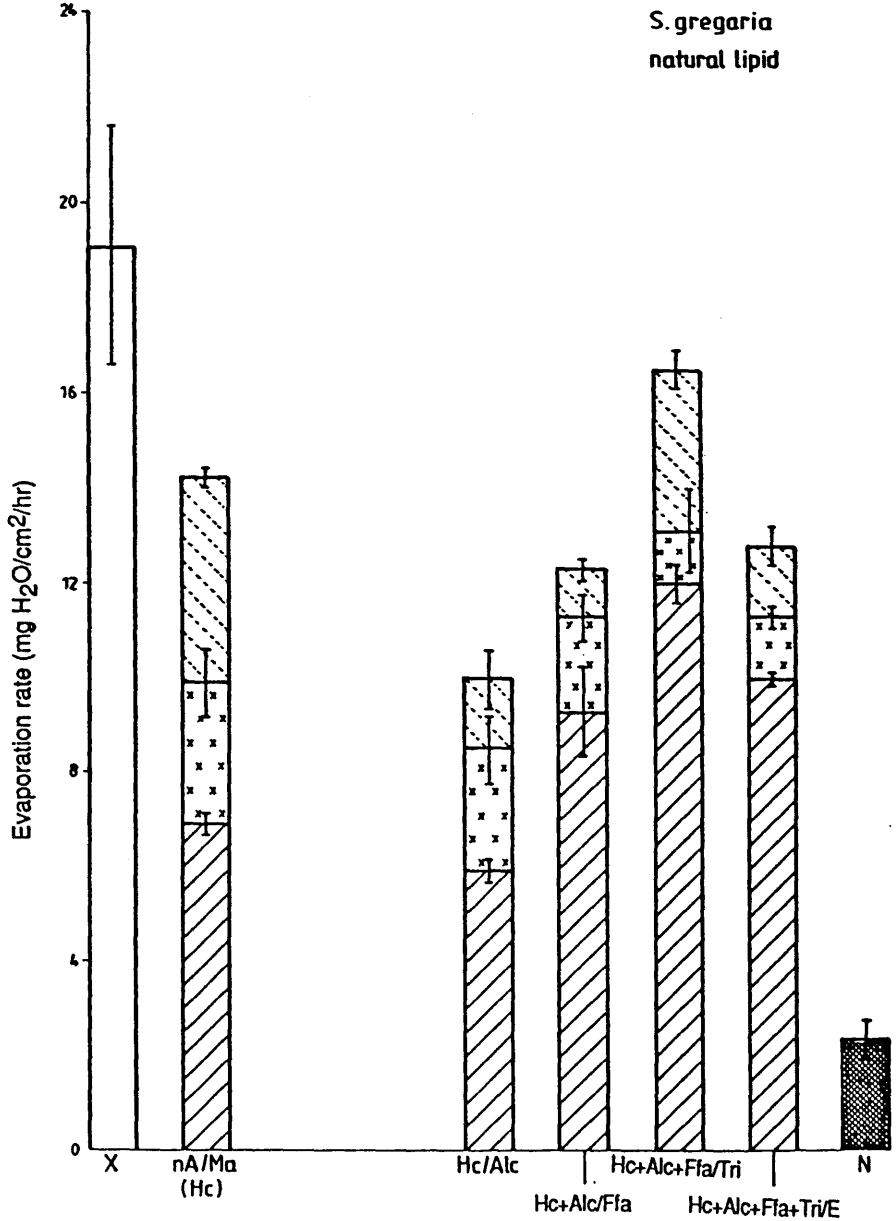

S. gregaria
natural lipid
Evaporation rate (mg H2O/cm2/hr)
0
4
8
12
16
20
24
X
nA/Ma
(Hc)
Hc/Alc
Hc+Alc/Ffa
Hc+Alc+Ffa/Tri
Hc+Alc+Ffa+Tri/E
N

Figure 12

The effect of temperature on water evaporation rates through the hind wings of *L.migratoria* and *S.gregaria*.

Circles, intact wings; squares, extracted wings with the isolated natural lipid re-applied at 0.38mg/cm^2 for *L.migratoria* and 0.76mg/cm^2 for *S.gregaria*.

Fig. 12

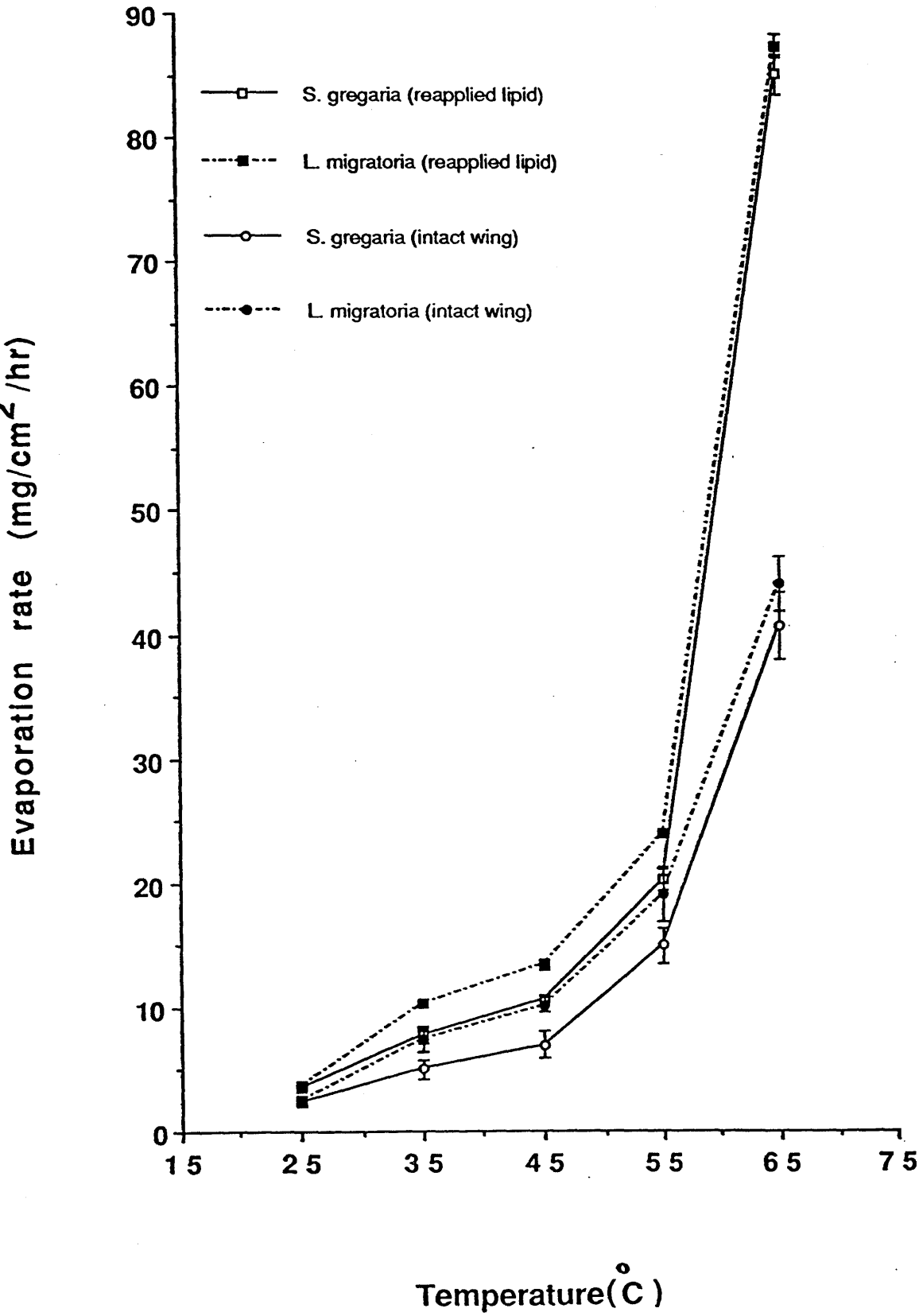

S. gregaria (reapplied lipid)
L. migratoria (reapplied lipid)
S. gregaria (intact wing)
L. migratoria (intact wing)
Evaporation rate (mg/cm2 /hr)
90
80
70
60
50
40
30
20
10
0
15
25
35
45
55
65
75
Temperature(°C)

Figure 13

The effect of temperature on water evaporation through the intact terga of *L.migratoria* and *S.gregaria*.

Fig. 13

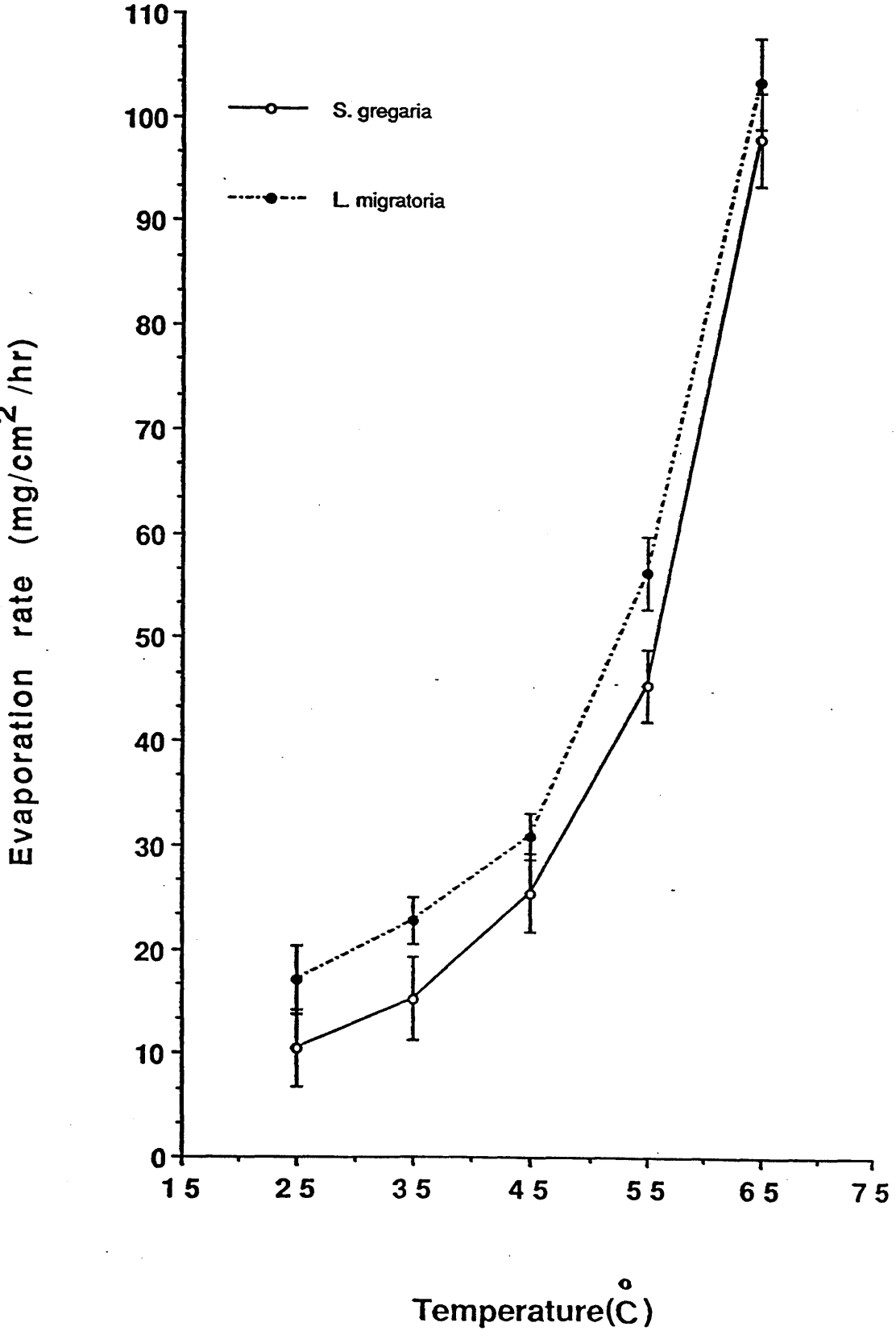

110
100
90
80
70
60
50
40
30
20
10
0
S. gregaria
L. migratoria
Evaporation rate (mg/cm2 /hr)
15
25
35
45
55
65
75
Temperature(°C)

Table 9: The effect of lipid amount/unit area on water permeability at 25°. Synthetic and natural lipid spread on extracted hind wings of L.migratoria and S.gregaria

Hind wing	Synthetic lipid	Amount mg/cm^2	Evaporation rate mg/cm^2/hr ± S.D.	% Permeability*	Natural lipid	Evaporation rate mg/cm^2/hr ± S.D.	% Permeability
L.migratoria	nA	0.13	9.8 ± 1.75	51	nA	8.7 ± 0.2	45
"	"	0.25	5.0 ± 1.0	26	"	4.7 ± 0.44	25
"	"	0.38	2.0 ± 0.36	10	"	1.8 ± 0.2	9
"	"	0.51	2.0 ± 0.21	10	"	1.8 ± 0.3	9
"	"	0.64	2.0 ± 0.3	10	"	1.7 ± 0.06	9
"	Ma	0.13	19.1 ± 0.3	100	Ma	14.9 ± 1.16	78
"	"	0.38	9.9 ± 0.72	52	"	9.8 ± 0.53	51
"	"	0.64	9.7 ± 0.55	51	"	8.7 ± 0.64	45
"	Alk	0.13	17.5 ± 2.10	91			
"	"	0.38	14.8 ± 1.07	77			
"	"	0.64	14.6 ± 0.40	76			
"	Alc	0.13	11.0 ± 0.90	57	Alc	9.6 ± 0.50	50
"	"	0.38	8.4 ± 0.46	44	"	8.3 ± 0.44	43
"	"	0.64	5.3 ± 0.1	28	"	5.3 ± 0.1	28
"	E	0.13	11.4 ± 0.32	59	E	10.5 ± 0.42	55
"	"	0.38	9.5 ± 1.03	50	"	9.2 ± 0.21	48
"	"	0.64	6.4 ± 1.16	33	"	6.3 ± 0.15	33
"	Ffa	0.13	18.9 ± 0.32	98	Ffa	17.8 ± 0.42	93
"	"	0.38	18.2 ± 0.26	95	"	16.9 ± 0.15	88
"	"	0.64	16.0 ± 1.50	83	"	15.3 ± 0.26	80
"	Tri	0.13	18.7 ± 0.15	97	Tri	17.7 ± 0.58	92
"	"	0.38	16.3 ± 0.15	85	"	15.3 ± 0.1	80
"	"	0.64	14.3 ± 0.68	75	"	13.6 ± 0.46	71
X			19.2 ± 1.2				
N			2.5 ± 0.4				
S.gregaria	nA	0.13	16.3 + 2.39	85	nA	15.7 + 0.21	82
"	"	0.25	9.8 ± 1.96	51	"	9.1 ± 0.67	48
"	"	0.38	6.2 ± 0.57	33	"	4.7 ± 0.55	25
"	"	0.51	4.0 ± 0.80	21	"	3.1 ± 0.15	16
"	"	0.64	3.1 ± 0.82	16	"	1.8 ± 0.15	9
"	"	0.76	2.0 ± 0.15	11	"	1.7 ± 0.1	9
"	"	0.89	2.0 ± 0.2	11	"	1.7 ± 0.1	9
"	Ma	0.13	19.0 ± 0.9	100	Ma	14.9 ± 1.21	78
"	"	0.38	12.5 ± 1.32	65	"	11.4 ± 0.91	60
"	"	0.64	12.0 ± 2.20	63	"	10.1 ± 0.15	53
"	Alk	0.13	17.0 ± 2.40	89			
"	"	0.38	15.0 ± 1.82	79			
"	"	0.64	14.3 ± 0.3	75			
"	Alc	0.13	10.9 ± 1.02	57	Alc	10.0 ± 0.35	52
"	"	0.38	9.6 ± 0.15	50	"	9.2 ± 0.15	48
"	"	0.64	8.6 ± 1.61	45	"	8.3 ± 0.15	44
"	E	0.13	12.8 ± 0.76	67	E	11.5 ± 0.7	60
"	"	0.38	9.4 ± 0.40	49	"	9.2 ± 0.15	48
"	"	0.64	8.5 ± 1.27	45	"	8.2 ± 0.2	43
"	Ffa	0.13	17.4 ± 0.21	91	Ffa	15.4 ± 0.1	81
"	"	0.38	16.7 ± 0.61	87	"	14.5 ± 0.45	76
"	"	0.64	15.3 ± 0.36	80	"	14.1 ± 0.15	74
"	Tri	0.13	18.0 ± 0.36	94	Tri	17.2 ± 0.56	90
"	"	0.38	17.2 ± 0.26	90	"	15.9 ± 0.35	83
"	"	0.64	15.3 ± 0.44	80	"	14.1 ± 0.15	74
X			19.1 ± 1.4				
N			2.4 ± 0.2				

Alc, alcohols; Alk, alkenes; E, esters; Ffa, free fatty acids; Ma, methylalkanes; nA, n-alkanes; Tri, triglycerides; N, intact wing; X, extracted wing.

$$\% \text{ permeability} = \frac{\text{Evaporation rate of extracted wing + lipid}}{\text{Evaporation rate of extracted wing alone}} \times 100$$

Table 10: The effect of lipid composition on water permeability. Mixtures of synthetic and natural lipids in three ratios (2:1, 1:1 & 1:2 v/v) spread on L.migratoria at 0.38 mg/cm^2 and on S.gregaria wing at 0.76 mg/cm^2

Hind wing	Synthetic lipid	Amount mg/cm^2	Ratio	Evaporation rate mg/cm^2/hr ± S.D.	% Permeability	Natural lipid	Evaporation rate mg/cm^2/hr ± S.D.	% Permeability
L.migratoria	nA/Ma	0.38	2:1	6.9 ± 2.06	36	nA/Ma (Hc)	6.2 ± 0.15	32
"	"	"	1:1	13.1 ± 0.93	68	"	11.6 ± 1.08	60
"	"	"	1:2	15.6 ± 2.21	81	"	12.8 ± 0.31	67
"	nA/Alk	"	2:1	11.7 ± 1.31	61			
"	"	"	1:1	13.8 ± 1.53	72			
"	"	"	1:2	15.3 ± 0.26	80			
"	nA+Ma/Alk (Hc)	"	2:1	11.1 ± 0.32	58			
"	"	"	1:1	15.0 ± 0.25	78			
"	"	"	1:2	18.0 ± 1.17	94			
"	Hc/Alc	"	2:1	7.5 ± 0.46	39	Hc/Alc	6.8 ± 0.20	35
"	"	"	1:1	11.0 ± 0.89	57	"	9.6 ± 0.1	50
"	"	"	1:2	12.7 ± 0.62	66	"	11.0 ± 0.75	57
"	Hc+Alc/Ffa	"	2:1	10.1 ± 0.2	53	Hc+Alc/Ffa	10.0 ± 0.32	52
"	"	"	1:1	14.7 ± 0.55	77	"	12.5 ± 0.85	65
"	"	"	1:2	18.1 ± 0.12	94	"	15.3 ± 0.25	80
"	Hc+Alc+Ffa/Tri	"	2:1	12.8 ± 0.61	67	Hc+Alc+Ffa/Tri	12.0 ± 0.46	63
"	"	"	1:1	15.1 ± 0.15	79	"	14.4 ± 0.31	75
"	"	"	1:2	18.8 ± 0.06	98	"	17.2 ± 0.85	90
"	Hc+Alc+Ffa+Tri/E	"	2:1	9.8 ± 0.62	51	Hc+Alc+Ffa+Tri/E	9.5 ± 0.44	50
"	"	"	1:1	11.5 ± 0.57	60	"	11.0 ± 0.1	57
"	"	"	1:2	15.2 ± 0.59	79	"	15.0 ± 0.1	78
X				19.2 ± 1.2				
N				2.5 ± 0.4				
S.gregaria	nA/Ma	0.76	2:1	7.7 ± 0.61	40	nA/Ma (Hc)	6.9 ± 0.21	36
"	"	"	1:1	12.7 ± 2.04	67	"	9.9 ± 0.7	52
"	"	"	1:2	16.5 ± 1.70	86	"	14.2 ± 0.2	74
"	nA/Alk	"	2:1	12.5 ± 0.35	65			
"	"	"	1:1	13.5 ± 0.95	71			
"	"	"	1:2	15.0 ± 1.23	79			
"	nA+Ma/Alk (Hc)	"	2:1	11.4 ± 0.52	60			
"	"	"	1:1	14.8 ± 0.79	78			
"	"	"	1:2	18.1 ± 0.95	95			
"	Hc/Alc	"	2:1	6.1 ± 0.76	32	Hc/Alc	5.9 ± 0.21	31
"	"	"	1:1	12.1 ± 0.25	63	"	8.5 ± 0.68	45
"	"	"	1:2	12.9 ± 0.42	68	"	10.0 ± 0.62	52
"	Hc+Alc/Ffa	"	2:1	10.8 ± 0.64	57	Hc+Alc/Ffa	9.3 ± 0.92	49
"	"	"	1:1	13.6 ± 0.53	71	"	11.3 ± 0.45	59
"	"	"	1:2	16.4 ± 0.61	86	"	12.3 ± 0.2	64
"	Hc+Alc+Ffa/Tri	"	2:1	12.4 ± 1.22	65	Hc+Alc+Ffa/Tri	12.0 ± 0.35	63
"	"	"	1:1	14.2 ± 0.25	74	"	13.1 ± 0.9	69
"	"	"	1:2	17.7 ± 0.20	93	"	16.5 ± 0.36	86
"	Hc+Alc+Ffa+Tri/E	"	2:1	10.1 ± 1.0	53	Hc+Alc+Ffa+Tri/E	10.0 ± 0.1	52
"	"	"	1:1	11.8 ± 1.06	62	"	11.3 ± 0.2	60
"	"	"	1:2	13.2 ± 0.9	69	"	12.8 ± 0.35	67
X				19.1 ± 1.4				
N				2.4 ± 0.2				

Alc, alcohols; Alk, alkenes; E, esters; Ffa, free fatty acids; Ma, methylalkanes; nA, n-alkanes; Tri, triglycerides, N, intact wing; X, extracted wing.

$$\% \text{ Permeability} = \frac{\text{Evoporation rate of extracted wing + lipid}}{\text{Evoporation rate of extracted wing alone}} \times 100$$

Plate 10

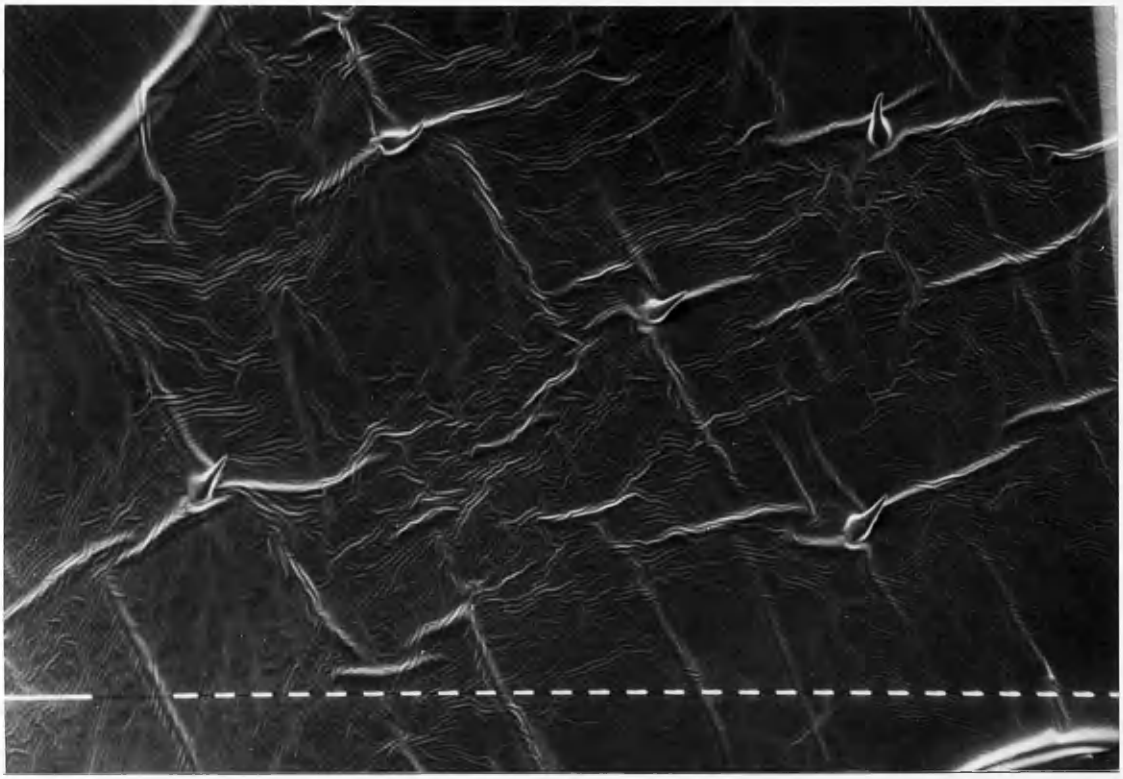
Spreading and physical characteristics of lipid.

a) Scanning electron micrograph of one drop of a solution of synthetic methylalkanes (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing uniform coverage.

b) Scanning electron micrograph of one drop of a solution of synthetic alkenes (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing uniform coverage.

(Bar = 10μ)

a



b

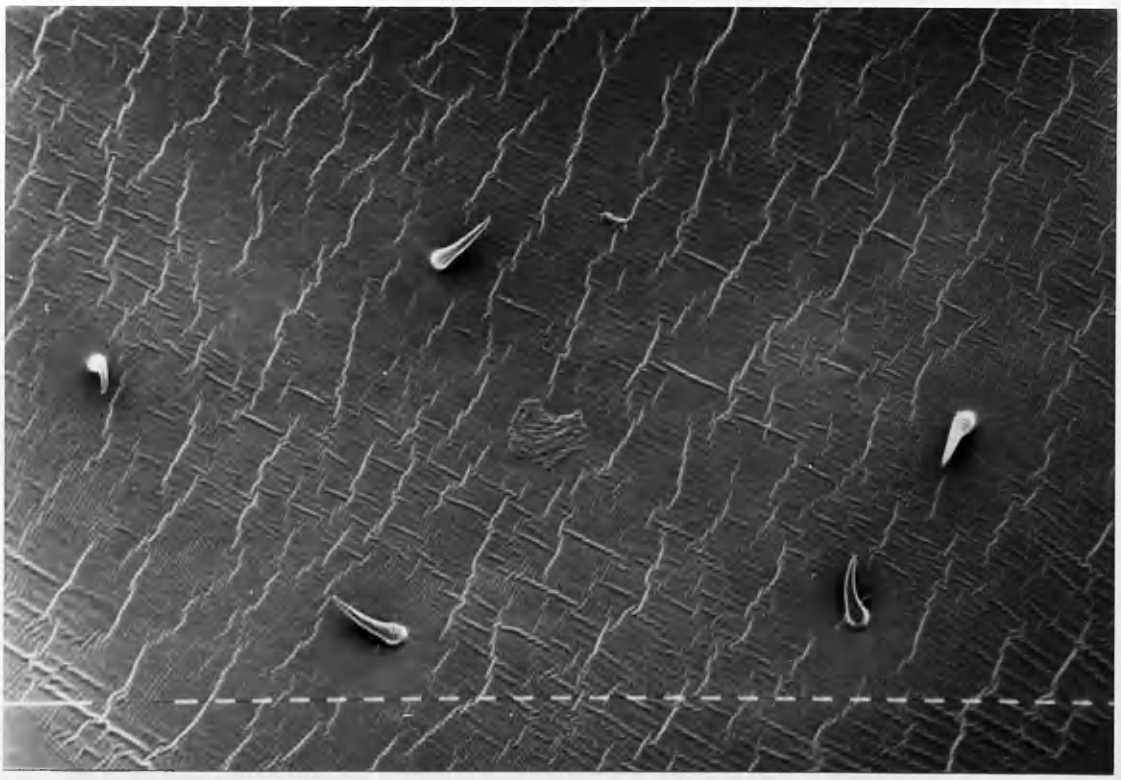


Plate 11

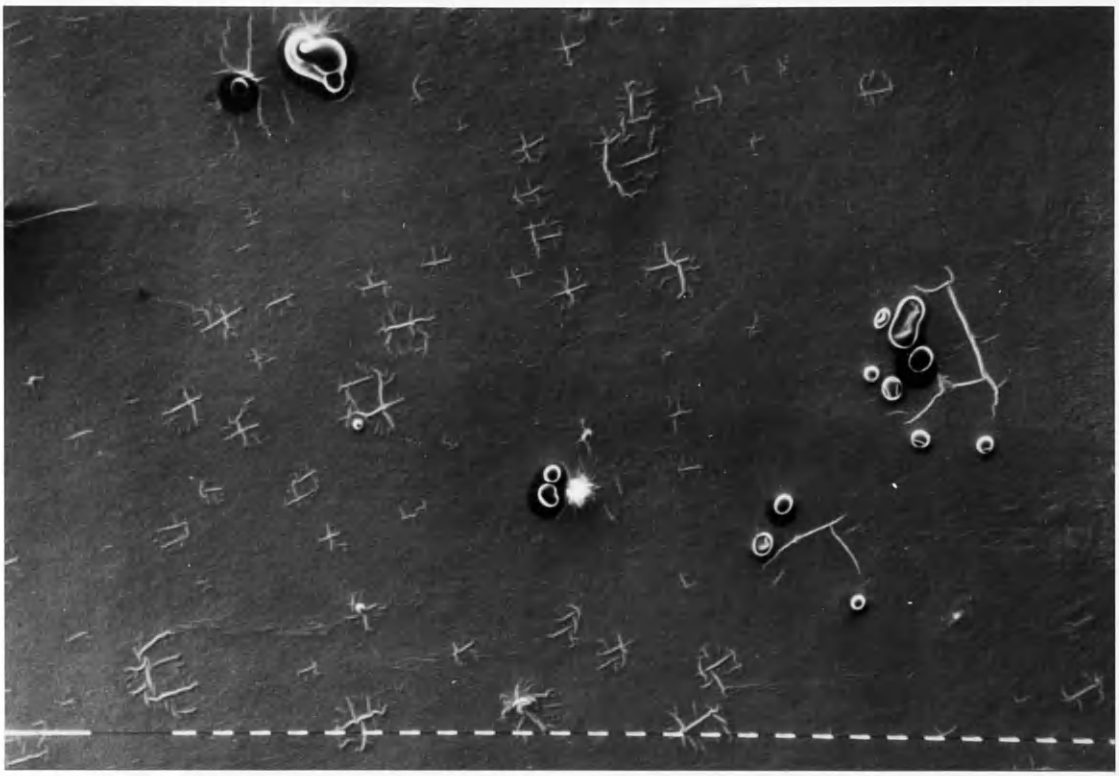
Spreading and physical characteristics of lipid.

a) Scanning electron micrograph of one drop of a solution of isolated natural methylalkanes (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing uniform coverage.

b) Scanning electron micrograph of one drop of a solution of isolated natural alcohols (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing uniform coverage.

(Bar = 10μ)

a



b

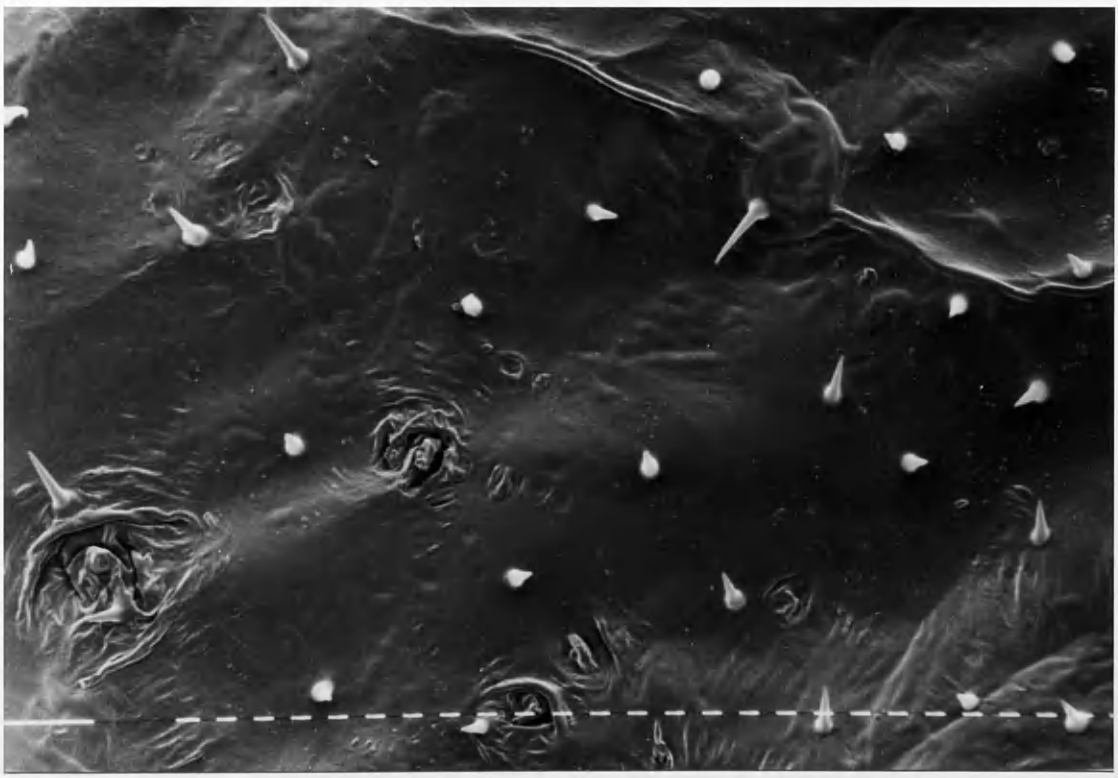


Plate 12

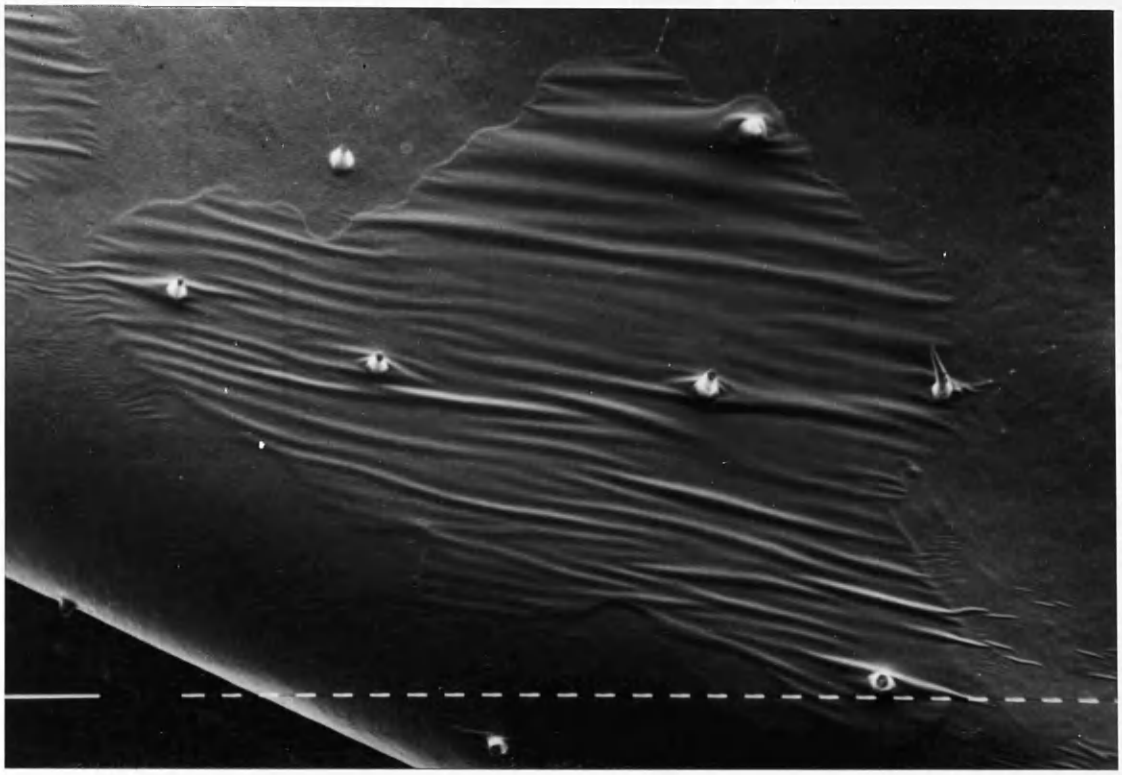
Spreading and physical characteristics of lipid.

a) Scanning electron micrograph of one drop of a solution of synthetic n-alkanes (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing incomplete coverage.

b) Scanning electron micrograph of one drop of a solution of isolated natural n-alkanes (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing the crystalline form assumed by n-alkanes.

(Bar = 10μ)

a



b

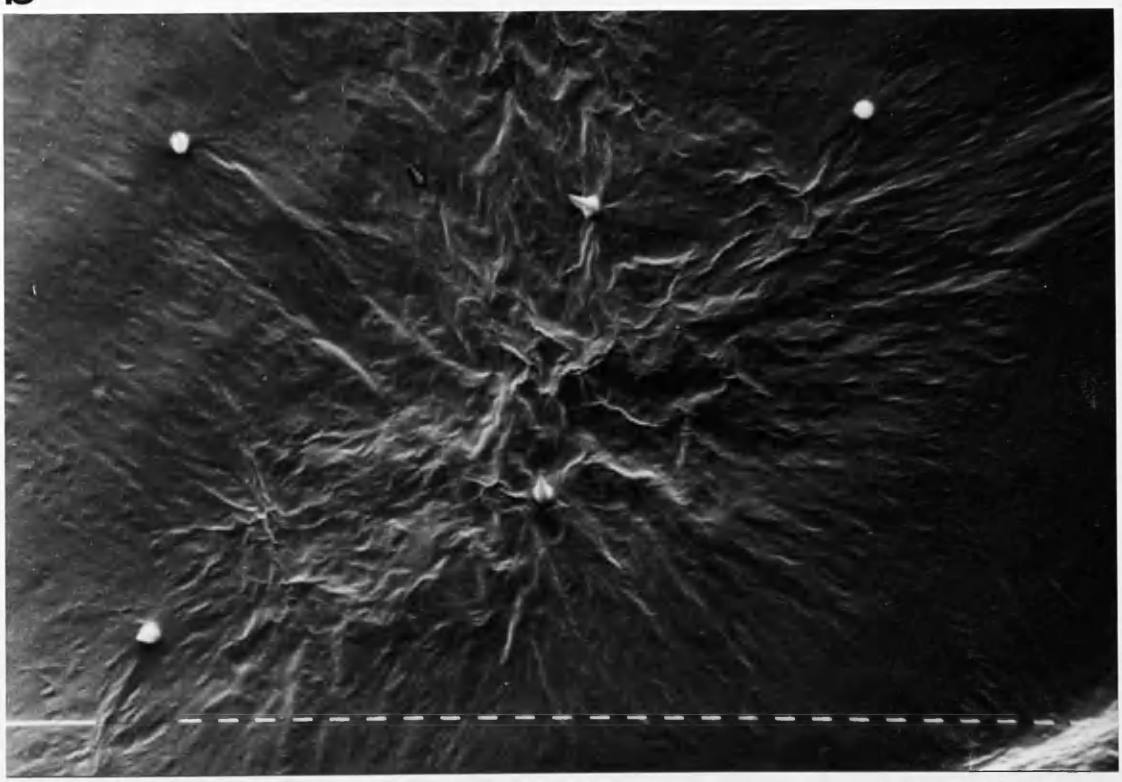


Plate 13

Spreading and physical characteristics of lipid.

Scanning electron micrograph of one drop of a solution of isolated natural free fatty acids (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing the limited coverage and the crystalline form assumed by the free fatty acids.

(Bar = 10μ)

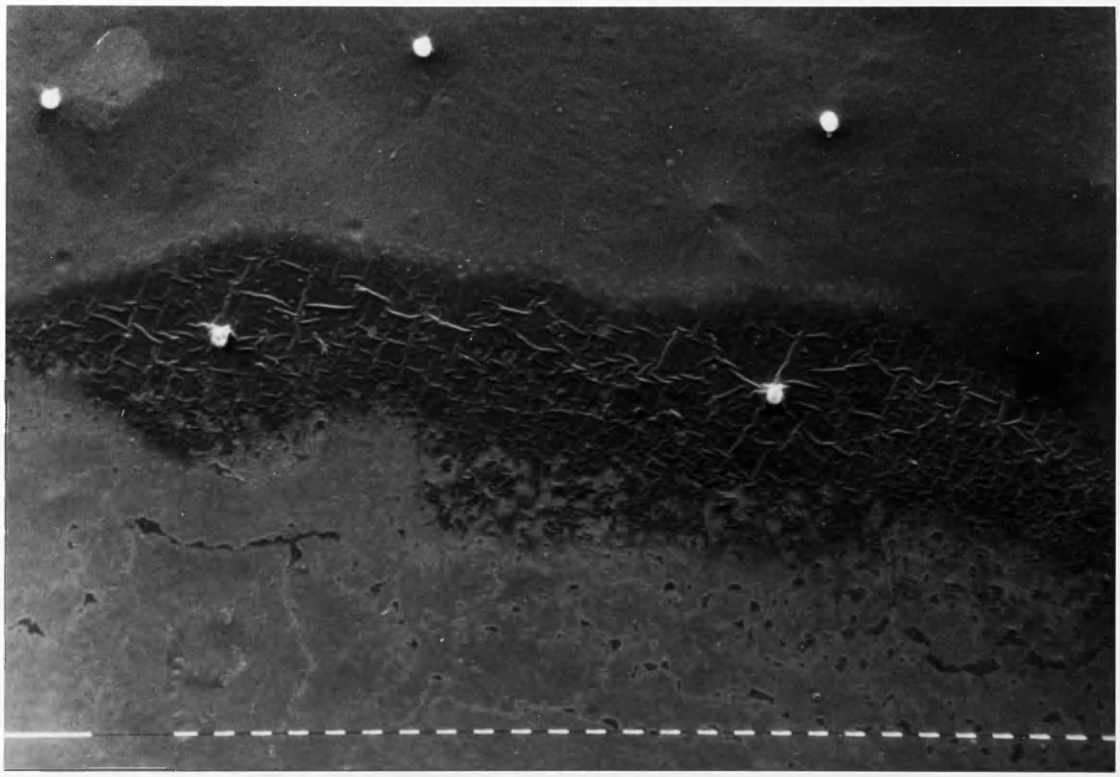


Plate 14

Spreading and physical characteristics of lipid.

a) Scanning electron micrograph of one drop of a solution of isolated natural esters (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing the limited coverage and the solid form assumed by the esters.

b) Scanning electron micrograph of one drop of a solution of isolated natural triglycerides (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria* showing limited coverage and the solid form assumed by the triglycerides.

(Bar = 10μ)

a

b

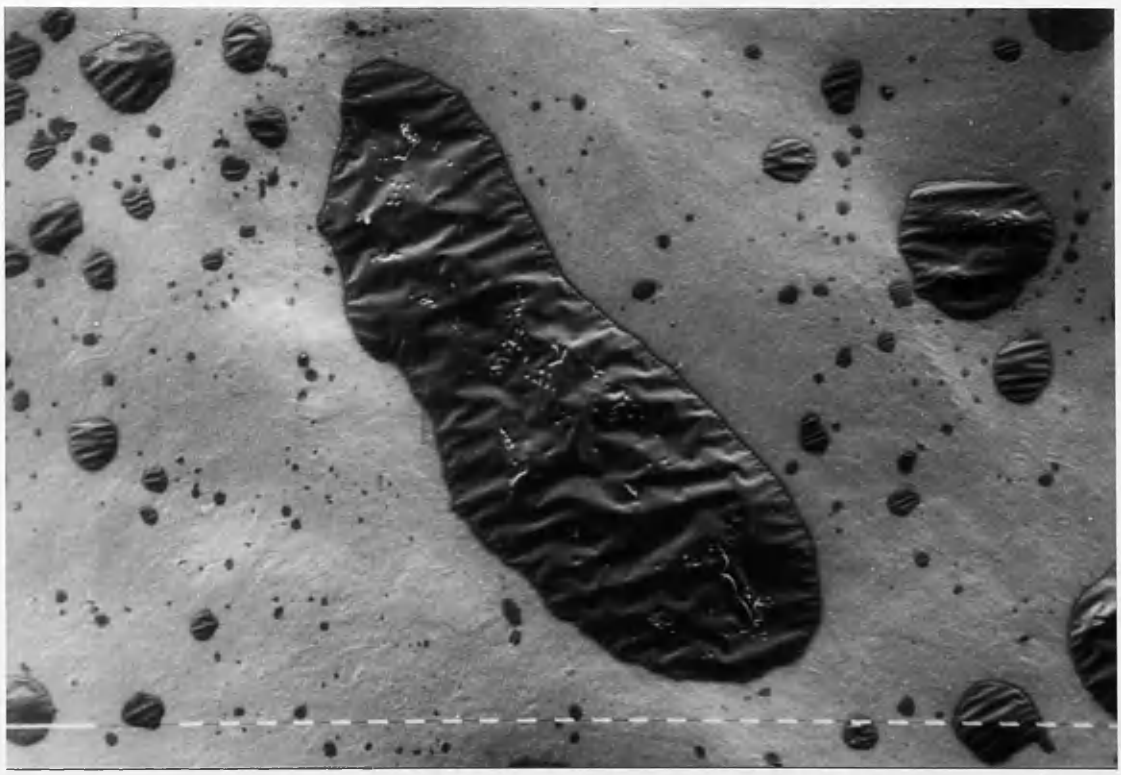


Plate 15

Spreading and physical characteristics of lipid.

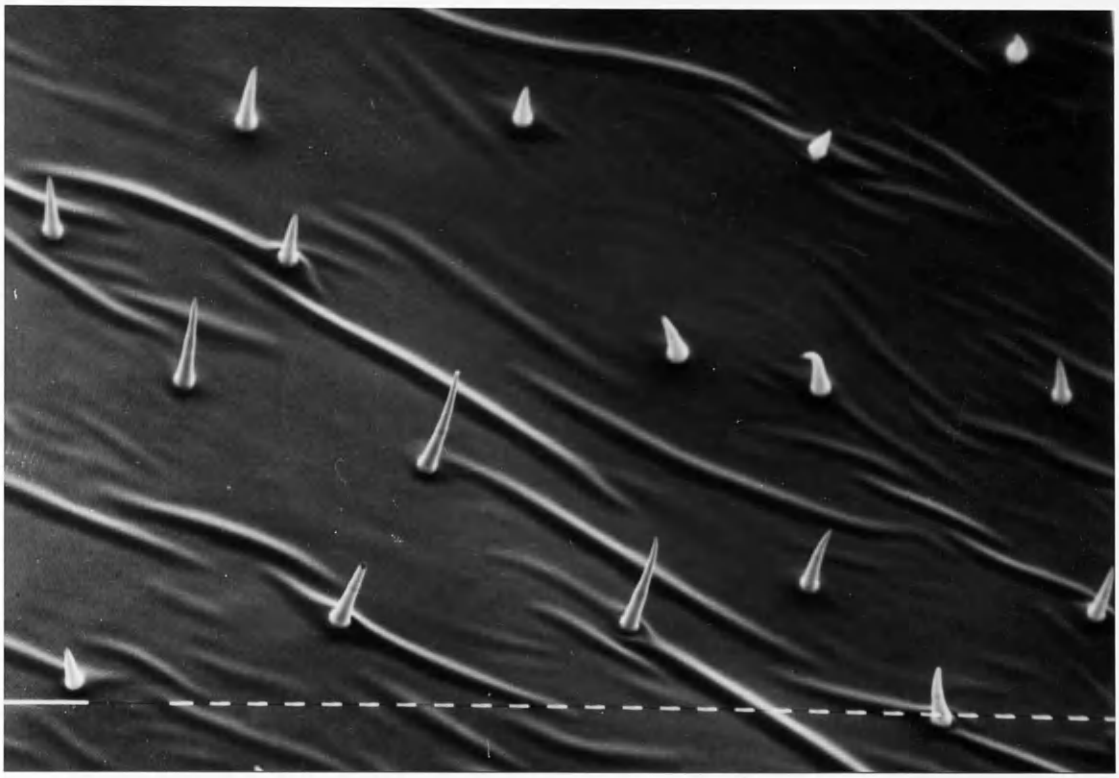
Scanning electron micrograph of one drop of a solution of a 1:1 (v/v) mixture of n-alkanes and methylalkanes (0.1 mg/ml) applied to the extracted hind wing of *S.gregaria*.

a) Synthetic mixture

b) Isolated natural mixture

(Bar = 10μ)

a



b

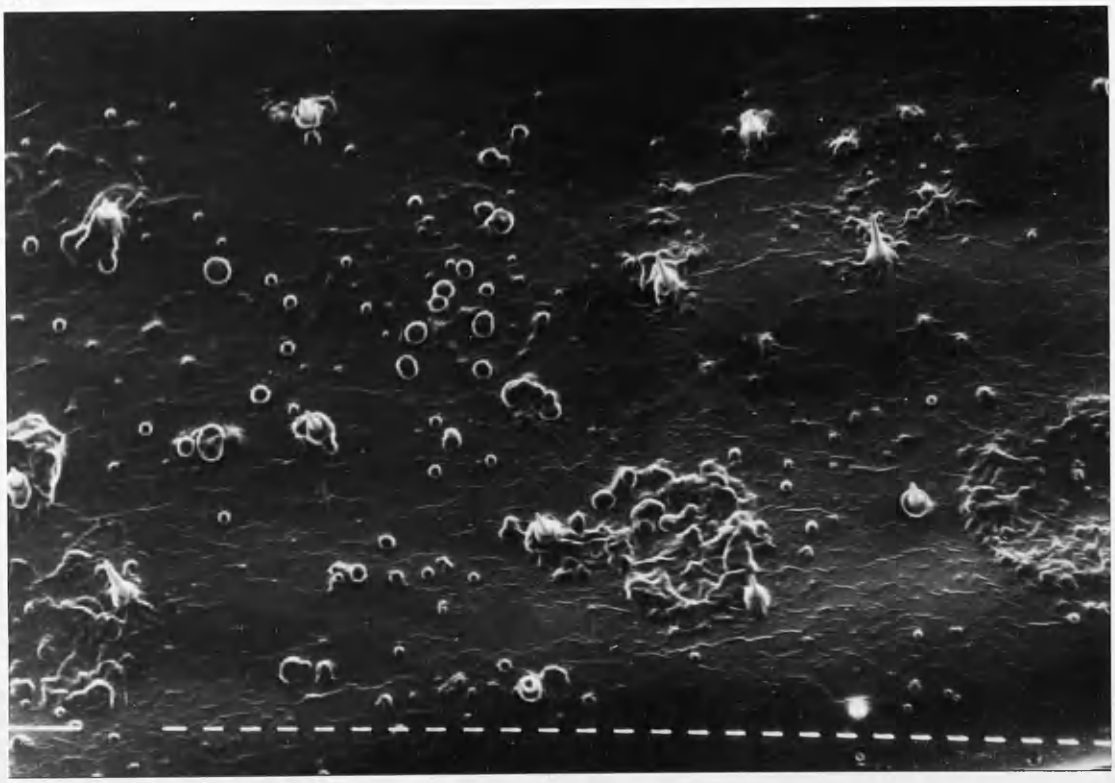


Plate 16

Spreading and physical characteristics of lipids

Scanning electron micrograph of one drop of a solution of a 1:1:1:1 (v/v) mixture of synthetic n-alkanes, methylalkanes, alkenes and esters (0.1 mg/ml). Note that the high percentage of esters inhibits hydrocarbon spreading and gives the layer a hard crystalline appearance.

(Bar = 10μ)

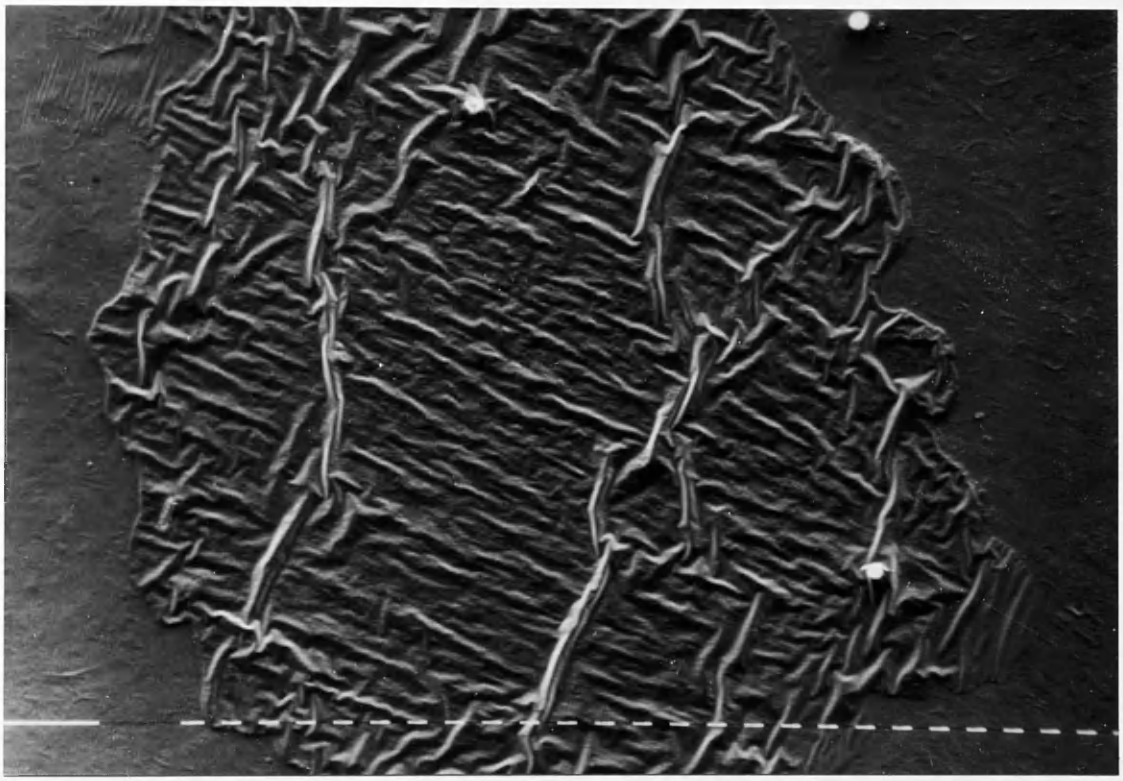


Plate 17a

Scanning electron micrograph of an intact hind wing of *S.gregaria*

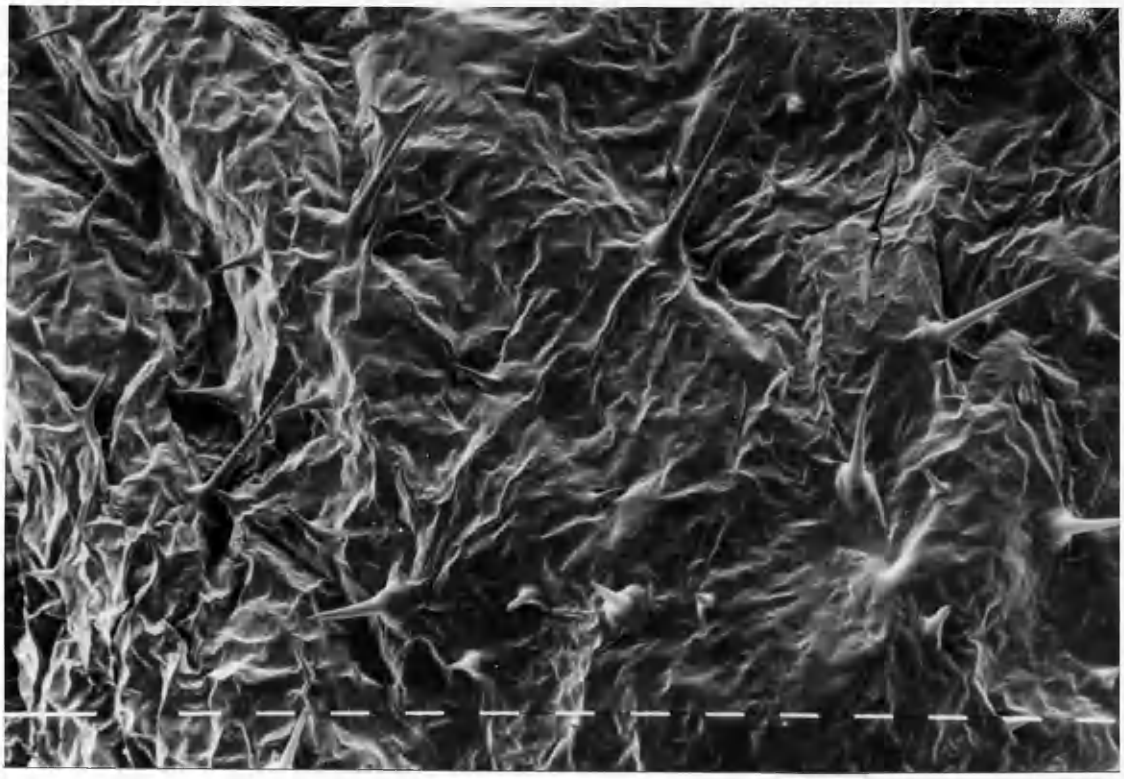
(Bar = 10μ)

Plate 17b

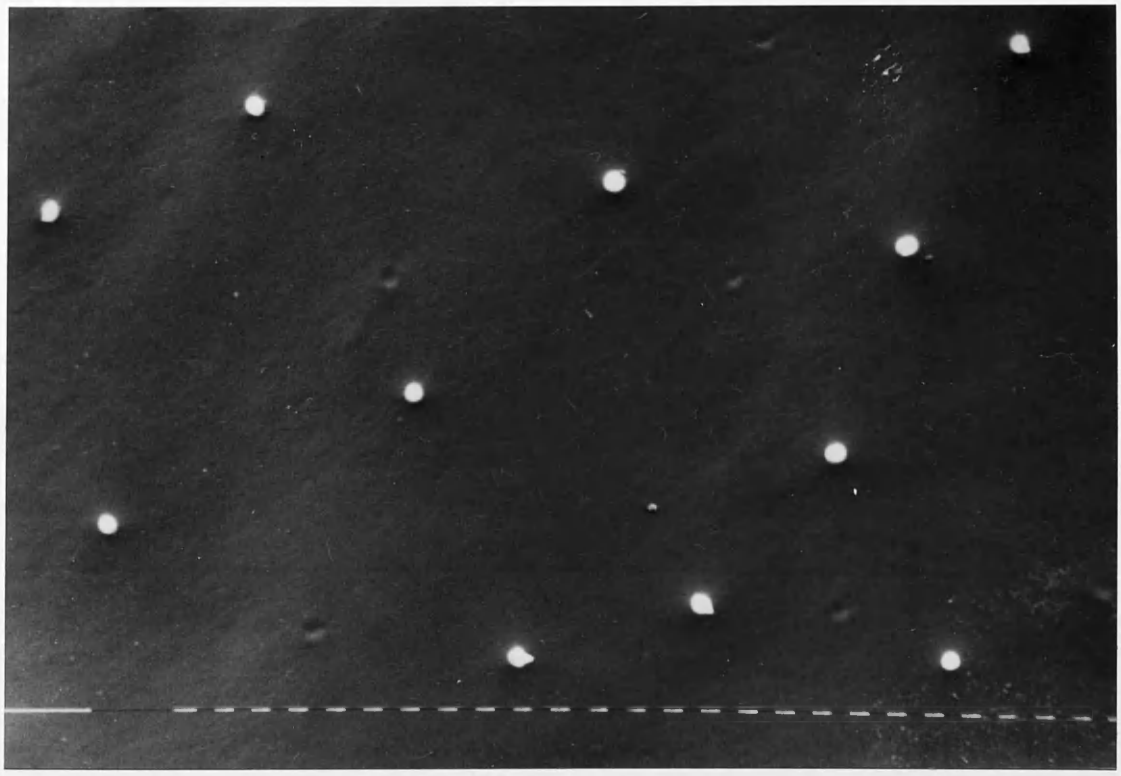
Scanning electron micrograph of an extracted hind wing of *S.gregaria*

(Bar = 10μ)

a



b



CHAPTER FIVE

GENERAL DISCUSSION

The environmental stresses encountered by the African migratory locust, *L.migratoria migratoriodes* and the desert locust, *Schistocerca gregaria* are different. This is due to the type of habitat they occupy. The vast plains of the middle reaches of the Niger river with their network of water-channels overgrown with tall grass and reeds are known to be the outbreak area of the African migratory locust, *L.migratoria migratoriodes*. This outbreak area is very similar to the reed-beds of the lower reaches of some Russian rivers which form the outbreak area of the Asian migratory locust, *L.migratoria migratoria* (Uvarov, 1977). These two similar habitats occupied by the African and the Asian subspecies are considered to be semi-arid. For *S.gregaria*, no outbreak area has been discovered and the species is only found in deserts (Baron, 1972), thus indicating a complete arid existence.

These semi-arid and arid environments determine the feeding and drinking habits of *L.migratoria migratoriodes* and *S.gregaria* in that there is a clear difference in feeding between *S.gregaria* which is omnivorous and feeds on a wide range of plants and *L.migratoria* which is graminivorous and feeds mainly on grasses. Although laboratory-reared species have been observed to drink water, there is some doubt as to whether this occurs in nature. Uvarov (1966) gives reports of marching hopper bands of the Asian migratory locust, *L.migratoria migratoria* stopping and drinking at pools of water. The same has been recorded for *Dociostaurus maroccanus* in Iraq (Uvarov, 1966). What is true for the Asian subspecies may also be true for the African subspecies, *L.m.migratoriodes* studied in this work, if its similar outbreak area is taken into consideration.

In *S.gregaria*, individuals in the desert do not drink and the source of water is food. Food affects both defaecation and the water

content of the body (Baron, 1972). When food is plentiful, the process from eating to the defaecation of small dark pellets normally takes from 30 minutes to a couple of hours. When food is in short supply, the rate of the process is slower and may take from 3 to 4 days during which *S.gregaria* is able to resist starvation. The water content of the body depends on the state of vegetation and it is exceptionally variable. Water content can drop by approximately half and still individuals are able to search for a new source of food. Uvarov (1966) gives details of the effect of food on the water content of the body of *S.gregaria*. At 28°C and at 40% relative humidity, water content falls from an original level of 71 to 76% to 53% with fresh food and to 43% with dry food. At 30°C and at 30% relative humidity, water content falls to 37.4% with dry food and to 36.5% (approximately half of the original) after 6 days of starvation. Such a wide tolerance to water content is due to the exceptional adaptation of *S.gregaria* to desert conditions.

Water regulation in *S.gregaria* includes the water content of faeces and the haemolymph. Norris (1961) observed that when adults of *S.gregaria* were fed on dry grass, the faecal pellets were very few and very dry, but that the pellets become very wet when fresh grass was eaten. Similar results were obtained by Walloff (personal communication - quoted by Uvarov, 1966) with *S.gregaria* swarms in Somalia. Here the pellets were very dry during the hot and dry season, but became full of moisture with the onset of the rainy season and the appearance of lush vegetation.

Water is mainly stored in the haemolymph and the variation in the water content of haemolymph is related to feeding. The variation is not only due to uptake or loss of water but also to the distribution of water between the haemolymph and body tissues (Uvarov, 1966). Under normal conditions, the water content of haemolymph is controlled by

secretion from the malpighian tubules and reabsorption by the rectum (Stobbart & Shaw, 1974). Albaghdadi (1987) describes the effect of prolonged dehydration on the volume and ionic balance of *S.gregaria* haemolymph. He found that after 7 days of dehydration, the ionic concentration (Na^+, K^+ & Cl^-) of the haemolymph showed only a slight change, but its volume decreased by 35 to 50%. 25% of the removed ions were excreted while 75% were distributed in the body. This process of ion removal from haemolymph to tissues maintains cell volume during dehydration.

Difference in habitat is reflected in morphological adaptations and this is seen in *L.migratoria* and *S.gregaria* when their cuticle structures are compared. Although no structural differences in wing cuticle were observed in the present work, the terga showed differences, mainly in the procuticle. In *L.migratoria*, the tergal procuticle is 3 times thicker than that of *S.gregaria* but in the latter species the tergal procuticle has well developed endocuticular lipid lamellae which are only poorly developed in *L.migratoria*.

The epicuticular lipid of wings and terga differ in *S.gregaria* and *L.migratoria*. Examination by SEM showed that the fore and hind wings of *S.gregaria* had a thicker epicuticular lipid layer than those of *L.migratoria*. Similarly, SEM examination showed that the tergal epicuticular lipid of *L.migratoria* formed a smooth layer while that of *S.gregaria* contained amorphous lipid masses.

The cuticular differences found in this work could account for the lower water permeability of *S.gregaria* cuticle when compared to that of *L.migratoria* and the lower permeability of *S.gregaria* cuticle can be related to the arid habitat the species occupies as opposed to the semi-arid habitat of *L.migratoria*.

The permeability results of this study are consisent with those

of Albrecht (1962) who compared the resistance of the hatchlings of *S.gregaria* and *L.migratoria* to moist and dry environments. He found that the hatchlings of *S.gregaria* survived better in a dry habitat (30% relative humidity) than those of *L.migratoria* which survived longer in a moist habitat (95% relative humidity). Albrecht (1962) explained this by suggesting that *L.migratoria*, which he called the marsh locust, is better adapted to moist conditions in the nymphal stages, whereas nymphs of the desert locust survive longer in an arid environment. Albrecht also noted that survival time in *S.gregaria* is longer than in *L.migratoria* which he explained by suggesting that the risks are spread over a longer period in *S.gregaria* than in *L.migratoria* and that *S.gregaria* is able to survive in a wider variety of situations.

The morphology of epicuticular lipids depends upon the chemical composition of the different lipid fractions comprising the lipid. The chemical composition is species-specific and in some species composition has been related to the habitat in which the species lives (Hadley, 1981). Such a relationship is seen in the present study. Although the same major fractions were detected in the epicuticular lipid of both species, the composition of the fractions was found to differ.

Concerning the hydrocarbons, both *S.gregaria* and *L.migratoria* have significant proportions of hydrocarbons in their epicuticular lipid. Both species possess the same classes of hydrocarbons, but *S.gregaria* has trimethylalkanes which *L.migratoria* lacks. In the wings of *S.gregaria*, hydrocarbons account for a higher proportion of the lipid than in *L.migratoria* and the higher proportion is accompanied by a higher proportion of n-alkanes compared with *L.migratoria*. The terga of *L.migratoria* however, have a higher proportion of hydrocarbons than those of *S.gregaria*. But n-alkanes occur in higher proportions in the

tergal lipid of *S.gregaria* than in that of *L.migratoria*. In the present study, n-alkanes proved to be the most efficient waterproofing component and this may account for the more impermeable cuticle of *S.gregaria*. These results agree with those of Armold *et al.* (1969), Armold and Regnier (1975), Bell *et al.* (1975), Coudron and Nelson (1981), Goodrich (1970), Hadley (1980b), Hegdekar (1979) and Lees (1955) who demonstrated a relationship in the species they examined, between the need for water conservation and lipid amount/unit area of cuticle and high proportions of n-alkanes.

Methylalkanes were detected in the epicuticular lipid of both *S.gregaria* and *L.migratoria* where they occur in higher proportions in the wings and terga of *L.migratoria* than in those of *S.gregaria*. Although methylalkanes can comprise some of the longest molecules in an insect hydrocarbon mixture, they were found to be only partially efficient at waterproofing in the permeability experiments (section 4.3.A). This agrees with the results of Hadley (1977), Toolson (1982) and Toolson and Hadley (1977, 1979). The methyl side chains of methylalkanes prevent close molecular packing, thus rendering the lipid layer in which they occur more permeable to water (Hadley, 1980a; Lockey, 1988). The presence of trimethylalkanes in the hydrocarbon mixture of *S.gregaria* could therefore cause the lipid layer to be more permeable to water, so decreasing the adaptation of this species to a desert habitat. However, the proportion of trimethylalkanes in the lipid is very low (1%). The function of trimethylalkanes in the cuticular lipid of *S.gregaria* is unknown, but their presence could be countered by the presence of a high proportion of n-alkanes in a comparatively thick lipid layer on the wings, and by well developed lipid lamellae in the terga. These features do not occur in *L.migratoria*.

Esters occur in only low proportion in both species with a slightly higher proportion in *L.migratoria*. Esters are the longest molecules in the cuticular lipid of the two species and both the synthetic and the natural mixtures proved to form efficient water barriers when spread on the wings of *S.gregaria* and *L.migratoria*. However, the esters of *L.migratoria* contain a higher proportion of unsaturated acids than those of *S.gregaria*. Unsaturation also occurs in the triglyceride fatty acids of *L.migratoria* and the free fatty acids of both species, so that 47% of the cuticular lipid of *L.migratoria* is unsaturated compared with 3% in *S.gregaria*. Unsaturation is known to increase the permeability of artificial bilayers (de Gier *et al.*, 1968; Ladbrooke *et al.*, 1968; Taylor *et al.*, 1975) and such high levels of unsaturation could account for the more permeable lipid layer of *L.migratoria* compared with that of *S.gregaria*. According to Toolson and Hadley (1977, 1979), unsaturation disrupts Van der Waals forces between the hydrocarbon chains of a lipid, rendering a layer of lipid more permeable to water.

The exceptional flying ability of *S.gregaria* allows the species to range over two continents, both in its solitary and gregarious phases, while *L.migratoria* flights are confined to one continent. Such long flights at high environmental temperatures combined with the greater wing and tergal surface areas of *S.gregaria* could result in lethal levels of cuticular transpiration without an efficient waterproofing lipid layer. Such a layer has been demonstrated in this study.

The results of this study also agree with those of Blackith (1961) who found that the cuticular transpiration of intact and abraded cuticle (the latter equivalent to extracted terga) of *S.gregaria* to be lower than those of *Nomadacris* and *L.migratoria*. From his work Blackith (1961) concluded that *S.gregaria* was better adapted

to the arid desert environment than either *Nomadacris septemfasciata* or *L.migratoria*.

Not all lipid components are equally efficient in reducing water permeability, as shown by the results of this study, and such an observation has been reported by Alexander *et al.* (1944), and Grncarevic and Radler (1967). If only a few lipid components form efficient water barriers, then the question arises as to why cuticular lipids are such complex mixtures. Part of the answer to this question is provided by the results from the spreading experiments of this study. Despite the efficiency of n-alkanes, alone they showed only limited spreading over the cuticular surface of the wing and this could explain the presence of methylalkanes in cuticular lipid. Methylalkanes spread well over the cuticular surface and therefore they could provide a matrix for n-alkanes and for the polar components of lipid (Lockey, 1988). The efficiency of alcohols in forming water impermeable layers may be due to their long alkyl chains and to their extensive spreading features. As polar components, they may also strengthen Van der Waals forces between components of a cuticular lipid layer. Esters however, have weak spreading features and they tended to crystallise over a small area of cuticle. Their presence however could give rigidity to the lipid layer as reported by Ladbrooke *et al.* (1968) who worked with artificial bilayers. Triglycerides formed only poor waterproofing barriers and they have the lowest spreading characteristics. Like esters, their presence could enhance the rigidity of the cuticular lipid layer and make the layer more crystalline. Fatty acids had limited spreading characteristics and they were inefficient at waterproofing. Their presence in cuticular lipid in the saturated form could increase the thermal stability of the lipid layer due to their high melting points

(Bronk, 1973).

Evaporation occurred over a range of 40°C in both species. This is similar to the range recorded by Wigglesworth (1986) for *S.gregaria* nymphs and it agrees with the view that there is a bulk change in the cuticular lipid (Lockey, 1988) rather than a sudden change in an orientated monolayer of lipid at the cuticular surface as proposed by Beament (1964). Though the water evaporation curves of both species have the same shape, the curve of *S.gregaria* is shifted to the right of that of *L.migratoria* which indicates that the lipid layer of *S.gregaria* is less permeable to water over a range of temperature. This is probably an adaptation to the desert temperatures which *S.gregaria* is likely to encounter.

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