ASPECTS OF FATTY ACID METABOLISM
IN ICHNEUMONID PARASITOIDS AND THEIR HOSTS

by

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ABSTRACT

In contrast to many other insects whose characteristic fatty acid compositions are influenced to a limited extent by dietary fats, the fatty acid composition of the total lipid of hymenopterous parasitoids of the family Ichneumonidae is very similar to that of their diet, that is, the hosts on which they are reared. In cases where such parasites were reared on two different species of host with very different fatty acid patterns, the parasitoids, *Exeristes comstockii* and *Itoplectis conquisitor* retained no characteristic fatty acid composition of their own. In contrast, the hosts *Lucilia sericata* and *Galleria mellonella*, are influenced very little by dietary fats. This lack of control over pool size of fatty acids was characteristic of, but not exclusive to, all the ichneumonids examined in these studies. Isolated cases were found in other families analyzed, which included the Aphidiidae, Brachonidae, Pteromalidae and Eulophidae.

More detailed analyses demonstrated that although the parasitoid *I. conquisitor* appears to have little qualitative or quantitative control over the fatty acid composition of its neutral lipids, it does exert some quantitative control over that in its phospholipids. Although the fatty acid composition of the phospholipids varied considerably, and adopted many of the characteristics of the composition of the phospholipids of the host, consistent ratios of the fatty acid classes were maintained, thus the physical nature of the phospholipids, would be maintained.

Radioisotope studies demonstrated that the fatty acid pattern of the parasitoid, *E. comstockii* is maintained by active metabolic processes, and that the fractional turnover rates of the individual fatty acids are

much greater than those in either of the hosts, *L. sericata* and *G. mellon-ella*. The pattern was also shown to be maintained partially by direct incorporation of host fats.

PREFACE

A great deal of data are now compiled concerning physiology and metabolism in insects (Gilmour 1961, Rockstein 1965). Most of this has accumulated from the study of phytophagous or free living insects. As the importance of parasitic insects in the control of other insect populations is recognized, it is essential that the nutrition and biochemistry of these groups be studied and the interactions which take place between the parasite and its host be investigated. In reviewing the present state of knowledge concerning the physiology and biochemistry of endoparasitic Hymenoptera, Fisher (1971) has pointed out, "Proposals to use parasitic insects more widely in the biological control of pests will have to take account of the full physiology of the host-parasitoid relationship, the cellular defence reactions of insects towards new or alien parasitoids as well as the physiological ecology of both species and the integration of life cycles".

The nutritional requirements of insect parasitoids, as well as other insects associated with animal parasitism, have been reviewed by House (1958, 1965). Our present understanding is largely a result of work carried out on the endoparasitic viviparous sarcophagid, *Agria housei* (also referred to in the literature as *Pseudosarcophaga affinis* and *Agria affinis*) (House 1954, 1959), which was used in biological control of the spruce bud worm, *Choristoneura fumiferana*. In general it has the same requirements as phytophagous forms, although it is not intended by this to underemphasize the difference between it and free living insects, or between phytophagous forms. "In general, all insects seem to have similar

qualitative nutritional requirements regardless of feeding habits, or taxonomic positions; not withstanding the fact that requirements vary in certain species due to specific synthetic abilities or because of the involvement of symbiotic organisms" (House 1965). Success has been achieved at rearing A. housei axenically on a chemically defined meridic diet. More recently this same diet has been used to rear the ichneumonid parasite Itoplectis conquisitor (Yazgan and House 1970). Previously, another ichneumonid parasite, Pimpla turionellae had been reared on an artificial diet of pork liver (Bronskill and House 1957).

The axenic culture of insects in general has been described in a series of papers published by the New York Academy of Sciences (Dougherty 1959) and compilations of artificial diets for insects are available (House 1967, House, Singh and Batsch 1971).

It is now quite apparent that nutrition involves more than mere nutritional requirements; that is, more than the basic chemical factors essential to the adequacy of the diet. Other chemical and physical factors are important to the acceptance of the diet and normal feeding behaviour. Fraenkel (1953) stated, "Green leaves are excellent sources of all the food materials which insects seem to require", however, insects often grow poorly or not at all on feed that is presumably nutritionally adequate. Chemical and physical factors responsible for acceptance have been discussed by Beck (1956) and are well documented for species such as the commercial silkworm, Bombyx mori (Hamamura 1959, Hamamura and Naito 1961, Hamamura, Hayashiya and Naito 1961). In the case of insect parasitoids such factors are of a more complex and dynamic nature, since the diet is

another living insect.

Fisher (1971) stated, "Although much is known about the nutritional requirements of some parasitoids, information about their precise relationship with their hosts is still largely lacking. The relationship is not simply that of carnivore and prey ... A hormonal integration synchronizes the development of the parasite with that of its host, so that both species are regulated by external environmental control. Physiological means of interaction between competing larvae which are of widespread occurrence in parasitic species also point to a dependence on particular conditions of the host insect". The resistance of insect parasitoids to the defence mechanisms of their hosts have been reviewed by Salt (1970). Fisher (1971) has reviewed the chemosensory mechanism of perception and the criteria for the selection and acceptability of the host. Many parasitoids have been shown to discriminate between normal and parasitized hosts, but until recently nothing was known about the biochemical mechanism which elicits such selective responses. Intimate biochemical relationships have now been established between the parasitoid and its host.

An abnormal electrophoretic pattern in the hemolymph of *Pristiphora erichsonii*, the larch sawfly, when parasitized by the ichneumonid, *Mesoleius tenthredinis* has been described by Barlow (1962). This was one of the early reported biochemical changes associated with parasitism, and established the presence of an intimate biochemical relationship between a parasite and its host. Fisher and Ganesalingam (1970) have since reported changes in the amino acid composition of hemolymph in *Agnasta kuehnilla* after parasitism by the ichneumonid *Nemeritis canescens*. Such

biochemical changes may be involved in the mechanism by which parasitoids distinguish between healthy and already parasitized hosts. Arthur, Hegedekar and Rollins (1969) recently isolated a hemolymph protein component in Galleria mellonella, the greater wax moth, which induces oviposition by I. conquisitor. Perhaps such oviposition inducing protein components are absent after parasitism.

During attempts to determine the factors that restricted the ichneumonid Exeristes comstockii to lepidopterous hosts, Bracken and Barlow (1967) demonstrated that this parasitoid has no regulation over pool size of fatty acids, and duplicates the fatty acid pattern of its host. Again, a distinct biochemical interaction between parasitoid and host was shown.

Since I was interested in lipid biochemistry and in the physiological interactions between parasite and host, I undertook to carry on the studies of Dr. Barlow, under his direction, concerning the composition and metabolism of fatty acids in ichneumonid parasitoids and their hosts.

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To all the other members of the Department of Biological Sciences who assisted me during this period, I am indeed grateful.

GENERAL INTRODUCTION

A great deal of qualitative and quantitative data have accumulated on lipid metabolism in insects. Several excellent reviews have appeared including those of Gilbert (1967) and Fast (1970). Insect lipids are of endogenous or biosynthetic and exogenous or dietary origin. The relative contribution from each of these sources determines the composition of lipids which are present in the insect's tissues. The work described here concerns the comparative study of fatty acid composition and synthesis in insects and the effects of nutritional variation in fatty acids on lipid composition. The significance of these relationships to biological control or more specifically, to integrated control, is discussed.

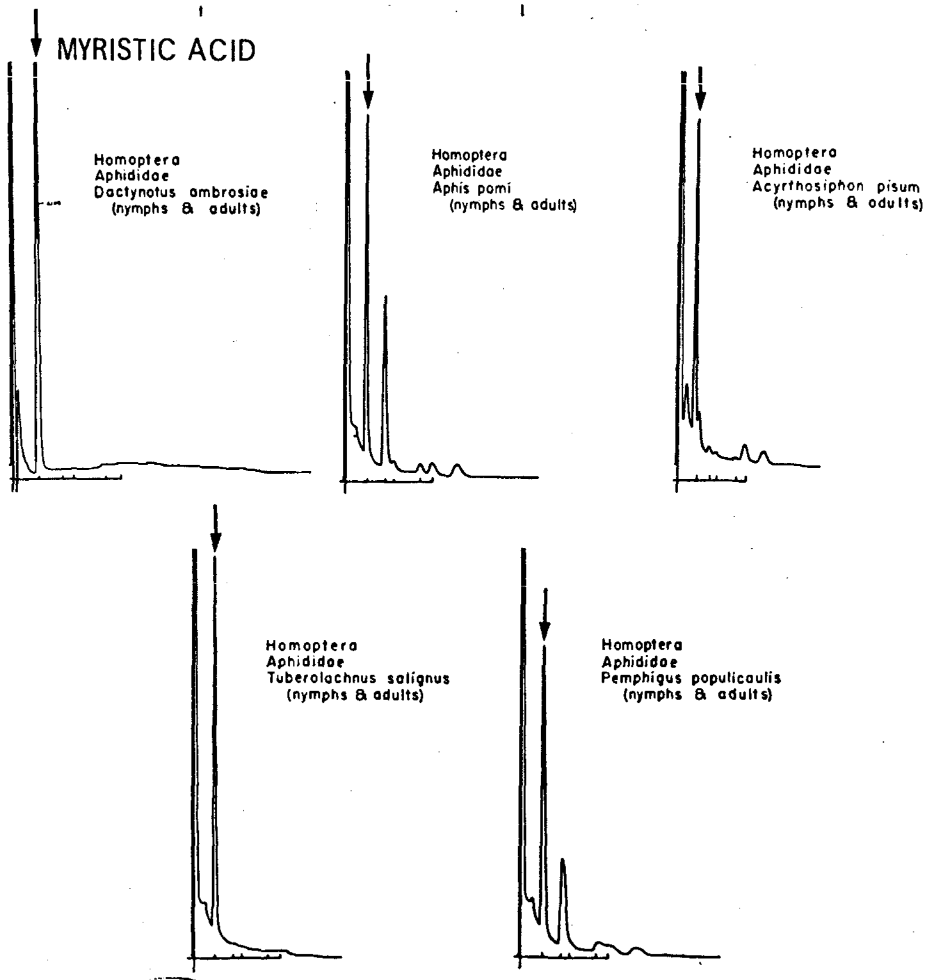
It has been conclusively established that insects synthesize saturated and *cis* 9-monounsaturated fatty acids (see reviews above). All the major synthetic pathways present in more highly evolved animals appear to be present in insects (for a review of these see Thompson 1970); that is, *de novo* synthesis, elongation synthesis and aerobic desaturation. Previous studies from our laboratory and some of the work described here have been concerned with the characterization and synthesis of fatty acids in phytophagous insects which we use as hosts for ichneumonid parasitoids. These include *Galleria mellonella*, the greater wax moth (Thompson 1970, Thompson and Barlow 1971, 1972) and the blowfly, *Lucilia sericata* (Lindsay and Barlow 1970, 1971). Recently, I have isolated and purified the fatty acid synthetase enzyme complex in *L. sericata* (unpublished). Insects cannot synthesize polyunsaturated fatty acids even if dietary linoleic or

linolenic acids are present, and in this way differ significantly from more highly evolved animals which are able to synthesize an extremely wide spectrum of ω 3, 6 and 9 polyunsaturates if either of these precursors is present. There is no doubt, however, that synthesis contributes to the saturated and monounsaturated fatty acid composition of insect tissues.

Studies with fatty acid free diets would reveal the extent to which synthesis contributes to the pool size of individual fatty acids. Few attempts have been made at rearing insects on such diets, and many insects have been shown to have specific fatty acid requirements (Fast 1970). However, three dipterous insects have been reared successfully on fatty acid free diets and this work will be described later.

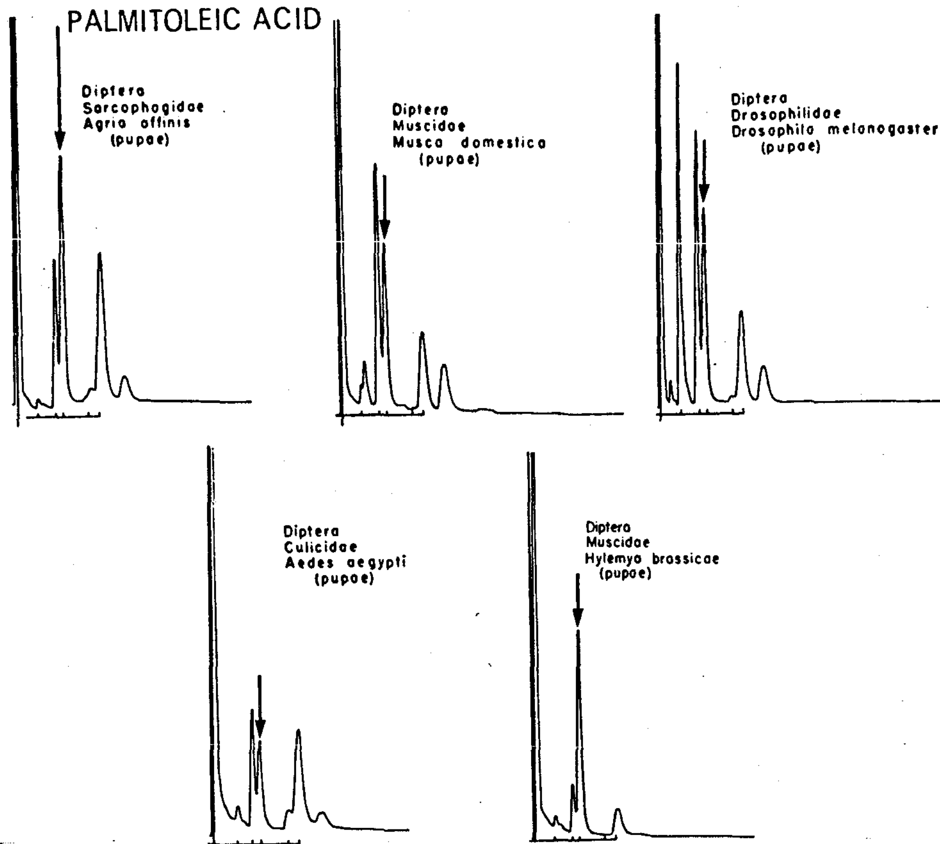
The influence of dietary fatty acids on the fatty acid composition in insect tissues is in question. There has been much disagreement in the past over the degree of correlation which exists between the lipid composition of the diet and the insect. Prior to about 1960 there was though to exist a direct correlation between the two, particularly with regard to the polyunsaturates. However, it was soon realized that since insects cannot synthesize polyunsaturates, but in many cases require them, they must be incorporated into the insect's fat directly from the diet, and this appears to be the case. Some correlation, therefore, does exist between the quality of fatty acids found in the diet and those in the insect. However, it is now quite evident that quantitatively little correlation exists (see Chapter 1 discussion). The relative levels of fatty acids in the diet have a limited influence on the levels within many insects.

Indeed, Barlow (1963, 1964) demonstrated that certain characteristics of the fatty acid composition of some insects are related to accepted taxonomic groupings. For example, insects of the family Aphidiidae have relatively high concentrations of myristic acid in their fats:



Myristic acid is normally found in trace or undetectable amounts in other insects and other animals in general. After this finding, Strong (1963) demonstrated this characteristic in 21 other species of this family. Similarly, Barlow (1964) demonstrated that the order Diptera is character-

ized by having relatively high palmitoleic acid levels:

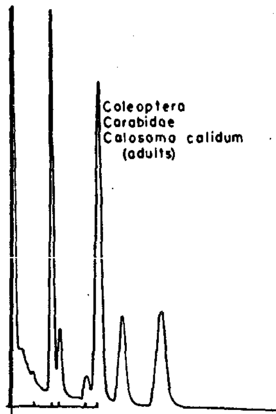


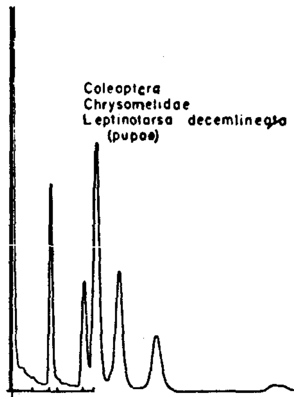
Palmitoleic acid, like myristic acid is normally present in only minor amounts in other taxa. Subsequently, additional species of this order have been demonstrated to display this characteristic (Fast 1966). Fast found that certain primitive Diptera of the family Cecidomyiidae do not have the characteristic, however. Another noteworthy exception exists. A small group of lepidopterous insects characterized by overwintering as larvae show similar high palmitoleic acid levels (Bracken and Harris 1969). These two striking examples appear to represent taxonomic characteristics,

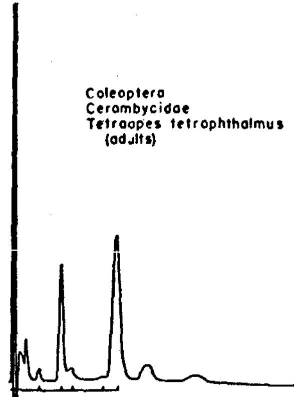
and these are retained regardless of dietary fatty acid composition. This was demonstrated by Strong (1963) in the aphids, and it is evident that the dipterous species described by Barlow (1964) and illustrated above differ considerably in their natural diet.

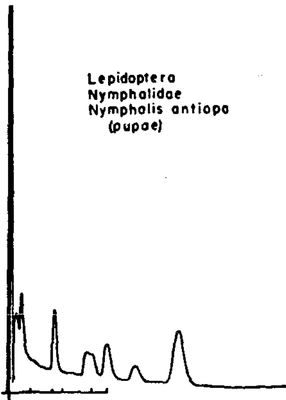
After these initial studies Barlow demonstrated conclusively with the use of chemically defined diets that the fatty acid composition of three dipterous species, *Agria affinis* (1965), *Musca domestica* (1966a) and *L. sericata* (1966b), were influenced to a very limited extent by the fatty acid composition of the diet. The high palmitoleic acid level was retained in all three species. The major fatty acids of these insects when reared on fatty acid free diets were approximately 30% palmitic acid, 40% palmitoleic acid, 5% stearic acid and 25% oleic acid, demonstrating that the high level of palmitoleic acid is due to active synthesis and not dietary incorporation alone. In general, this pattern is typical of this group regardless of the diet, indicating the limited degree of dietary influence. Polyunsaturates were, of course, present only when present in the diet.

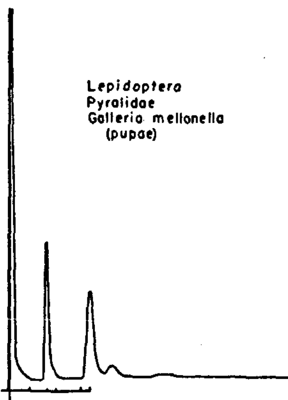
In contrast to the groups of insects just described, coleopterous, lepidopterous and free living hymenopterous insects display a "typical" pattern of the type found in most animals, in which palmitic and oleic acids comprise about 80 to 90% of the total fatty acid compliment:

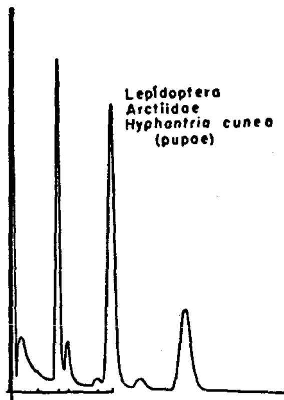

Coleoptera
Carabidae
Calosoma calidum
(adults)

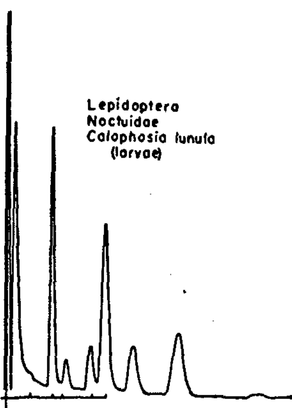

Coleoptera
Chrysomelidae
Leptinotarsa decemlineata
(pupae)

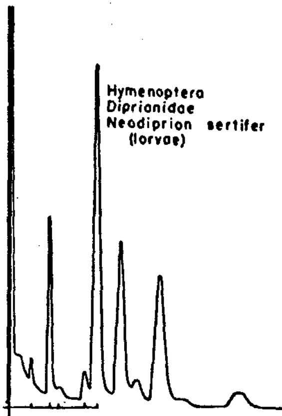

Coleoptera
Cerambycidae
Tetraopes tetrophthalmus
(adults)

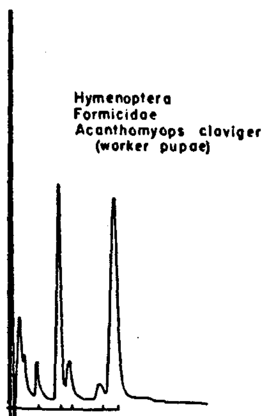

Lepidoptera
Nymphalidae
Nymphalis antiopa
(pupae)


Lepidoptera
Pyralidae
Galleria mellonella
(pupae)


Lepidoptera
Arctiidae
Hyphantria cunea
(pupae)


Lepidoptera
Noctuidae
Calophasia lunula
(larvae)


Hymenoptera
Diprionidae
Neodiprion sertifer
(larvae)


Hymenoptera
Formicidae
Acanthomyops claviger
(worker pupae)

The recognition of distinct fatty acid compositions as characteristic of certain taxa led to the proposition that antimetabolites or inhibitors of these apparently taxonomically specific fatty acids could be developed as insecticides directed against these taxa. This is discussed at some length in the general discussion.

In contrast to most insects which retain certain definite characteristics of their fatty acid patterns regardless of diet, Bracken and Barlow (1967) demonstrated that the ichneumonid parasitoid, *Exeristes comstockii* has no characteristic fatty acid pattern which could be considered its own when reared on different hosts with widely varying fatty acid compositions. In those studies *E. comstockii* usually found as an external parasite of *Rhyacionia buoliana*, the pine shoot moth, was reared in the laboratory on three hosts, *G. mellonella*, *L. sericata* and *Neodiprion sertifer*, a sawfly. All three hosts have distinctly different fatty acid patterns. In each case the fatty acid composition of the parasitoid was very near the same as the host on which it was reared, and the pattern remained the same throughout pupation and eclosion.

Since previous studies had been carried out to determine the influence of dietary fatty acids on those in the tissues of *L. sericata*, one of the hosts used to rear *E. comstockii*, studies described in chapter 1 were carried out to determine similar relations in *G. mellonella*, the second host used to rear the ichneumonid. These two studies together with those described previously from our laboratory, which characterized the synthetic activity and lipid composition in these two host insects result-

ed in a significant body of information on lipid composition and metabolism, which I could relate and compare to similar studies on ichneumonid parasitoids carried out subsequently and described in the remaining chapters. These studies were directed at determining the generality of the duplication phenonmenon in parasitic Hymenoptera, to further characterize its nature, and to determine the origin of fatty acids and mechanism of dupliication.

GENERAL METHODS AND MATERIALS

REARING TECHNIQUES

During these studies, the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), the European corn borer, *Ostrinia nubilalis* Hubn. (Lepidoptera: Pyralidae) and the blowfly *Lucilia sericata* L. (Diptera: Calliphoridae) were used as host species for the two parasitoids *Exeristes comstockii* Cress. (Hymenoptera: Ichneumonidae) and *Itoplectis conquisitor* Say (Hymenoptera: Ichneumonidae). All of these insect cultures were originally obtained from the Canada Department of Agriculture laboratory in Belleville, Ontario.

G. mellonella - The greater wax moth was reared on a cereal based artificial diet described by Dutky, Thompson and Cantwell (1962). The rearing conditions were 35% relative humidity (R.H.) and 30 to 35 C. Brands of mixed cereals obtained locally were used. The vitamin mixture described by Dutky *et al*. was replaced with Ostaco® vitamins (Charles E. Frost and Co., Montreal, Quebec). The diet was mixed as described; slow and careful

addition of the premixed liquid portion of the diet to the cereal was necessary in order to obtain the proper consistency.

One batch of diet, approximately 1000 g was placed in a 28 ounce cookie jar with a nylon screen cover. Approximately 1000 eggs were placed on the diet and development proceeded until pupation. The larval stage of development was generally four weeks, the pupal stage two weeks. After adult emergence, folded (accordian style) wax paper strips held with a paper clip were placed in the jars and oviposition occurred on these. The eggs were then scraped carefully onto a new diet.

O. nubilalis - The European corn borer was reared axenically on the chemically defined diet of Beck and Stauffer (1950), under similar conditions as G. mellonella. Egg masses were sterilized with 1% sodium hypochlorite solution and placed in small snap cap vials. After hatching, several dozen larvae were placed on six ounce batches of diet in one quart plastic food containers with nylon screen tops. Strips of corregated cardboard were placed on top of the diet and after development larvae pupated between the paper layers. Pupae were removed and placed in snap cap vials. After emergence, adult pairs were placed individually in 12 inch wax paper tetrahedrons supplied with water soaked cotton rolls through a hole in one of the sides. Oviposition occurred on the sides and egg masses were removed with wax paper intact, and placed in hatching vials.

O. nubilalis was reared with some difficulty, since diapause occurred in the last instar larval stage and could not be broken. After some time, the colony was depleted and was abondoned. The corn borers used in the

phospholipid studies were obtained from Dr. R. McClanahan at the Canada Department of Agriculture laboratory in Harrow, Ontario.

L. sericata - The blowfly was reared by the method of W.F. Dean (Simon Fraser University) as outlined by Williams and Smith (1970). Four hundred to 500 eggs were placed onto blocks of fresh beef liver three to four inches on a side, which were in turn placed over a sawdust layer in one quart food containers with screen tops. The eggs hatched within 24 hours and larvae pupated within the sawdust layer in 5 to 7 days. Pupae were removed and placed in rearing cages of the type described by Nicholls (1970). After emergence, adults were fed sugar cubes and water in soaked cotton rolls. After one week, small slices of beef liver in petri plates were added to the cage and eggs were collected from these in about three days. Rearing was carried out at 25 C and 40% relative humidity.

E. comstockii - Stock colonies of *E. comstockii*, an external parasitoid of the pine shoot moth, *Rhyacionia buoliana*, were maintained successfully in the laboratory on *G. mellonella*. Eggs were placed singly in punctured gelatin capsules and placed in a desiccator with a saturated solution of sodium carbonate. After hatching, which occurred 24 to 48 hours later, the capsules were dried and opened, and coddled last instar *G. mellonella* larvae (immobilized by placing in water at 54 C for approximately 45 seconds) were placed inside with the parasite larvae. The host was replaced after it turned black due to bacterial infection. After the parasite pupated the host was removed, and when the adults emerged, the males and females were isolated in separate cages. A dozen males were placed in a

small cage (4 x 4 x 2.5 inches) with a plastic front. Females anesthetized with carbon dioxide were added individually to the male cage and were removed after mating. Adult emergence and mating took place on the same day. Mated females were put singly or in pairs in small cages, and coddled host larvae in oviposition blocks were added immediately. Oviposition blocks were small rectangular wooden blocks, 2.5 x 1 x 1 cm, with a three mm hole bored lengthwise, and a slot connecting the bore to the outside of one side of the block. Coddled host larvae were drawn into the block by the end of the abdomen with forceps, and 4 mm of the abdomen were left exposed for the adult to feed on. After three to 5 days, females began to deposit eggs on the coddled host larvae. All rearing procedures were carried out at 25 C, and the adult parasitoids were supplied with sugar cubes, raisins, and water from small bent glass tubes.

During the course of these studies, *E. comstockii* was reared on alternate hosts including *L. sericata* and *O. nubilalis*. The parasitoid was reared on *L. sericata* with great difficulty. The larvae deteriorated quickly even when the blowfly was reared axenically on a chemically defined diet which was being used in our laboratory at that time (Barlow and Kollberg 1971). Experiments involving the addition of streptomycin to the usual liver diet did not alter this situation, and *L. sericata* reared on liver alone were totally unsuitable as hosts. The effects of endemic and other bacteria present in host species on the success of parasitism have been described by Bucher and Williams (1967) and Bracken and Bucher (1967).

The search for a suitable host with a fatty acid composition which differed considerably from that of G. mellonella was ended when Bracken and Harris (1969) reported the presence of high levels of palmitoleic acid in a small group of lepidopterous insects including O. nubilalis. Whenever possible this insect was used rather than L. sericata as an alternate host for E. comstockii and I. conquisitor.

I. conquisitor - I. conquisitor, an endoparasitoid of the pine shoot moth, was reared very easily on the alternate laboratory host, G. mellonella. Eggs were deposited by mated females directly into newly formed wax moth pupae. Development proceeded completely within the host puparium. After emergence, the adults were placed in a rearing cage similar to that used for rearing L. sericata, and mating occurred in a few minutes. Mated females were removed and placed individually in small petri plates with single host pupae, and after oviposition were returned to the mating cage. Parasitized G. mellonella were placed in an empty cage until the adult parasitoids emerged. The adults were fed sugar cubes and water in soaked cotton rolls. During the course of these studies, I. conquisitor was also reared on the alternate hosts L. sericata and O. nubilalis.

BIOCHEMICAL TECHNIQUES

The common biochemical techniques used in these studies are outlined. Techniques used in specific aspects of the work will be described in the appropriate sections.

Lipid extraction - Total lipid extracts were prepared by the method of

Bligh and Dyer (1959), modified to be used with microquantities. The monophasic extraction mixture contained chloroform, methanol and water, 1:2:0.8 by volume; the water came from the tissue to be extracted. Extraction was carried out in tissue grinders in three ml of chloroform:methanol, 1:2 by volume, and if the tissue to be extracted contained less than 0.8 ml of water (assuming 80% wet weight as water) distilled water was added to make up the deficit. After homogenizing for two to three minutes, one ml chloroform and one ml water were added to the initial homogenate with mixing after each addition. The resulting biphasic mixture was then poured into a centrifuge tube and centrifuged for 15 minutes at 3000 rpm. After centrifugation, the chlorofrom layer was removed and the solvent evaporated. The lipid was redissolved in petroleum ether (B.P. 30-60 C) and washed with an equal volume of distilled water in a separatory funnel. After standing for 12 or more hours, the petroleum ether was removed by evaporation leaving the extracted lipid.

Separation of lipid classes - Individual lipid classes were isolated from the total lipid extract by elution chromatography on silicic acid products. The first technique used 7% hydrated Florisil® (Floridin Co.) (activated magnesium silicate) as described by Carroll (1961). Hydrocarbons, sterol esters, triglycerides, sterols, diglycerides, monoglycerides, free fatty acids and phospholipids were eluted in a stepwise manner with 60 ml hexane, 100 ml 4% diethyl ether in hexane, 180 ml 15% diethyl ether in hexane, 180 ml 25% diethyl ether in hexane, 180 ml 50% diethyl ether in hexane, 150 ml 2% methanol in diethyl ether, 150 ml 4% acetic acid in diethyl ether and

200 ml methanol respectively from 36 g columns (20 mg sample/g packing).

The second and more commonly used technique was that described by Borgstrom (1962) for the separation of phospholipids from neutral lipids and free fatty acids. The column packing was 10 g of Unisil® (Clarkson Chemical Company) (silicic acid). The lipid extract was adsorbed (20 mg sample/g packing) and neutral lipids and phospholipids were eluted in a stepwise manner with 200 ml chloroform and 200 ml of methanol respectively. All column techniques described were monitored colorimetrically as described by Amenta (1964). Small samples of elutant were isolated, the solvent evaporated and a few drops of 7% potassium dichromate in sulphuric acid added. After heating in a boiling water bath for two to three minutes the characteristic colour reaction of reduced dichromate appears if organic matter is still present in the eluant. This technique can be quantitated spectrophotometrically. After elution of neutral and phospholipids as described, the solvents were evaporated and the lipid fractions redissolved in petroleum ether and washed with water to remove any traces of Unisil.

Saponification and isolation of free fatty acids - Saponification of lipid extracts or isolated lipid classes was carried out by the method of the Association of Official Agricultural Chemists (Lepper 1950). The lipid was dissolved in three ml of hot ethanol and 0.1 ml of 60% potassium hydroxide solution was added. This mixture was then refluxed for 30 minutes. After saponification, 5 ml of water were added and the ethanol-water phase was washed with petroleum ether (B.P. 30-60 C) to remove non-saponifiable

lipids. After removal of the petroleum ether, the ethanol phase was acidified with 0.5 ml of concentrated hydrochloric acid and washed again with petroleum ether. Free fatty acids were obtained after evaporation of the petroleum ether.

Preparation of fatty acid methyl esters for gas-liquid chromatography (GLC) - Esterification of fatty acids, a preparative step for GLC, was carried out by two procedures. Free fatty acids, isolated after saponification, were esterified by the method of Schlenk and Gellerman (1960), with diazomethane. The reaction apparatus consisted of a three chambered system with all chambers connected gas phase to liquid phase with tubes. The first chamber was filled with diethyl ether, the second contained the diazomethane generator and the third the free fatty acid mixture in diethyl ether containing 10% methanol. Nitrogen gas saturated with ether after passing through the first chamber carried the diazomethane gas from the second chamber into the third chamber containing the free fatty acid-ether solution. The reaction was monitored colorimetrically, being complete when the ether solution in the third chamber turned yellow from excess dissolved diazomethane. After the diethyl ether was evaporated from the esterified fatty acid mixture, the fatty acid methyl esters were taken up in petroleum ether (B.P. 30-60 C), and the excess methanol was removed from the sample by washing with water.

The second method of esterification; transesterification, was carried out with boron-trifluoride by the method of Morrison and Smith (1964) on isolated lipid classes or free fatty acids.

The lipid sample was placed in a small centrifuge tube with pressure tight teflon lined screw top caps. After the solvent was evaporated,14% boron trifluoride in methanol was added to the sample (1 ml/10 mg lipid). The pressure tight cap was then screwed on and the tube was placed in a boiling water bath. The reaction time varied with the lipid class: 15 minutes for neutral lipids, 10 minutes for phospholipids and two to 5 minutes for free fatty acids. After cooling, the reaction vessel was opened and the reaction mixture transferred to a clean centrifuge tube. Pentane was added to the mixture (four volumes/one volume reaction mixture) and the tube was centrifuged at 3000 rpm for 15 minutes. The fatty acid methyl esters were then recovered from the pentane layer.

Class isolation and purification of fatty acid methyl esters - Purification and separation of saturated, monounsaturated and polyunsaturated fatty acid classes was achieved by modifications of the method of De Vries (1963) with the use of silicic acid impregnated with silver nitrate. Silicic acid-silver nitrate column packing was prepared as described. The fatty acid methyl ester sample was added to the prepared column (10 mg lipid/1 g packing), and the saturates eluted with 20 ml of 10% benzene in petroleum ether (B.P. 30-45 C), the monounsaturates with 10 ml of 7% diethyl ether in petroleum ether (B.P. 30-45 C) and the polyunsaturates with diethyl ether. The isolated fatty acid methyl esters were redissolved in toluene for gas-liquid chromatography (GLC) after evaporation of the eluting solvents.

<u>Gas-liquid chromatography of fatty acid methyl esters</u> - Methylated fatty acids were analyzed using a Carlo Erba Fractovap gas-liquid chromatograph. The carrier gas was helium and the column packing most commonly used was 15% diethylene glycol succinate on Chromosorb W (AW), mesh 60/80. During initial studies 15% Apeizon L packing was used alternatively for verification of identifications. Carrier gas pressures from 0.5 to 1.0 lb/sq. in. and column temperatures from 190 to 200°C were used throughout the course of these studies. Methylated fatty acid standards, including myristic (C14:0), myristoleic (C14:1), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (18:1), linoleic (C18:2) and linolenic (C18:3) acids were run with each sample for identification and quantitative determinations.

GLC data were treated in two ways. For directly comparative studies, that is, in studies involving direct comparison of fatty acid patterns, the areas under the peaks were determined using height x width at half height calculations and the percent composition of the fatty acids calculated as the percent of the area of an individual fatty acid of the total of the areas of all the fatty acids. However, although the quantitative GLC response to each fatty acid is almost linear, the response varies with the different fatty acids. Therefore, for more accurate percent composition calculations and specific activity determinations during the radioisotope studies, the actual amounts of fatty acids present were calculated by comparing the peak area of each individual fatty acid to the peak area of the corresponding fatty acid in the standard of known weight/unit area. The percent composition was determined by the actual weight of each in-

dividual fatty acid as a percent of the total weight of all the fatty acids.

CHAPTER 1

The consistency of the fatty acid pattern of *Galleria mellonella*, reared on fatty acid supplemented diets

INTRODUCTION

Previous studies have demonstrated to a limited extent that the fatty acid composition of the diet has some influence or is reflected in the pattern of the insect which is reared on it (Yuill and Craig 1937, Kiyoku 1953, House, Riordan and Barlow 1958, Cherry 1959, Lambremont, Blum and Schrader 1964, Schaefer 1968, Vanderzant 1968). Still other workers have ascribed unusual fatty acid patterns to dietary influences (Grindley 1952). However, there are some studies which demonstrated that regardless of the fatty acid composition of the diet, the pattern of the insect remains unchanged (Barlow 1964, 1966a,b, Van Handel and Lum 1961, Fast and Brown 1962). Many others have shown that the relative composition of fatty acids in the insect are distinctly different than in the diet.

The purpose of these experiments was to document the influence of dietary fatty acids on their relative composition in the wax moth.

METHODS AND MATERIALS

Wax moth larvae were reared on Pablum based diets as described in the General Methods and Materials section. To these diets were added supplements of free stearic, palmitoleic and linoleic acids (3 g/100 g diet). Stearic and palmitoleic acid were used as the saturated and monounsaturated supplements, since these are present in very low quantities and addition of relatively small amounts significantly increased the percentage in the diet. Linoleic acid was used as a polyunsaturate supplement rather than linolenic acid, which is present in much smaller amounts, because linolenic

acid would have a greater tendency to oxidize in the diet. Preliminary experiments with ^{14}C (U) oleate supplements demonstrated that free fatty acid dietary supplements are digested and incorporated into the insect's fat.

After 24 hours starvation to allow elimination of gut contents, last instar larvae were homogenized and the free fatty acids extracted and analyzed by GLC as described previously. The unsaturated fatty acid fractions were not degraded to determine the specific isomers present.

RESULTS

It is apparent that even after significant increases in the concentrations of stearic (Table 1-1), palmitoleic (Table 1-2), and linoleic acids (Table 1-3), only minor changes occur in the pattern of the larvae. The larval content of both C16:1 and C18:2 fatty acids increase somewhat when palmitoleic and linoleic acids respectively were added to the diet. Frass analysis shows that the acids in excess of the relative concentration within the larvae are excreted (Table 1-1, 1-2, 1-3). Similar results were obtained with diets supplemented with palmitic and oleic acids, and also in experiments using triglycerides.

DISCUSSION

Many workers have conclusively established that insects synthesize fatty acids (Zebe and McShan 1959, Tietz 1961, Bade 1964, Lambremont,

Table 1-1. The fatty acid composition of Galleria mellonella and its frass when reared on stearic acid supplemented diets.

Fatty Acid*	Per Cent Composition					
	Control Diet			Stearic Acid Supplemented Diet		
	Diet	Larvae	Frass	Diet	Larvae	Frass
C14:0	0.2	0.5	0.7	0.3	Tr	0.7
C16:0	15.0	36.1	15.7	12.7	34.4	15.3
C16:1	Tr	2.7	2.4	Tr	3.2	2.3
C18:0	1.9	3.5	1.6	34.1	2.2	28.8
C18:1	37.7	47.9	51.1	25.6	50.9	39.6
C18:2	44.0	9.3	28.4	26.5	9.4	13.4
C18:3	1.3	-	-	0.7	-	-
C20:1	Tr	-	-	Tr	-	-

*The first number represents the carbon chain length, the second, the number of double bonds. Stearic acid is C18:0.

Table 1-2. The fatty acid composition of _Galleria_ _mellonella_ and its frass when reared on palmitoleic acid supplemented diets.

Fatty Acid*	Per Cent Composition: Control Diet			Per Cent Composition: Palmitoleic Acid Supplemented Diet		
	Diet	Larvae	Frass	Diet	Larvae	Frass
C14:0	0.2	0.5	0.7	0.3	Tr	0.2
C16:0	15.0	36.1	15.7	13.4	36.4	14.3
C16:1	Tr	2.7	2.4	23.6	6.4	21.4
C18:0	1.9	3.5	1.6	2.2	2.1	1.0
C18:1	37.7	47.9	51.1	29.1	45.8	41.7
C18:2	44.0	9.3	28.4	30.6	9.4	21.4
C18:3	1.3	-	-	0.8	-	-
C20:1	Tr	-	-	Tr	-	-

*The first number represents the carbon chain length, the second, the number of double bonds. Palmitoleic acid is an isomer of the C16:1 group.

Table 1-3. The fatty acid composition of Gallerina mellonella and its frass when reared on linoleic acid supplemented diets.

Fatty Acid*	Per Cent Composition: Control Diet			Linoleic Acid Supplemented Diet		
	Diet	Larvae	Frass	Diet	Larvae	Frass
C14:0	0.2	0.5	0.7	0.2	Tr	0.3
C16:0	15.0	36.1	15.7	12.3	35.3	14.9
C16:1	Tr	2.7	2.4	0.4	1.1	1.6
C18:0	1.9	3.5	1.6	1.1	1.7	1.1
C18:1	37.7	47.9	51.1	26.8	44.2	42.2
C18:2	44.0	9.3	28.4	58.5	13.6	40.0
C18:3	1.3	-	-	0.7	-	-
C20:1	Tr	-	-	-	4.2	-

*The first number represents the carbon chain length, the second, the number of double bonds. Linoleic acid is an isomer of the C18:2 group.

Stein and Bennett 1965, Stephen and Gilbert 1969, Thompson and Barlow 1971). There seems to be little doubt, therefore, that fat synthesis contributes to the fat deposited in the insect. Studies with fatty acid free diets would determine to what extent synthesis effects pool size of individual fatty acids. However, only a very few attempts at rearing insects on such diets have been made (Barlow 1965, 1966a,b). In many cases their use would be limited, since some insects have been shown to have specific fat requirements (Fast 1970). For example, the wax moth requires polyunsaturates for successful emergence (Dadd 1964).

The influence of dietary fatty acids on tissue pools however, is in question. Many earlier studies concluded that there is a direct correlation between the two (Yuill and Craig 1937, *Lucilia sericata*; Melampy and Maynard 1937, *Blattella germanica*; Kiyoku 1953, *Callosobruchus chinesis*; House *et al*. 1958, *Pseudosarcophaga affinis*; Cherry 1959, *Phormia terraenovae*). Many of these studies involved the addition of polyunsaturates to the diet. Fast (1964) has pointed out, "Since we are now certain that many insects cannot synthesize these acids (polyunsaturates), but do need them for certain metabolic purposes, they must be readily taken up from the diet. If the insects are incapable of metabolizing or excreting the excessive quantities of feed, these acids would accumulate ...".

Some more recent studies have also demonstrated this correlation. Lambremont *et al*. (1964) demonstrated in *Anthonomus grandis* that the relative amounts of fatty acids in the insect vary with diet. Similar results were obtained by Schaefer (1968), Vanderzant (1968) and Barnett and

Berger (1970) in Heliothis zea, and by Keith (1967) in Drosophila melanogaster. Certain ichneumonid parasites have recently been shown to duplicate the fatty acid patterns of their hosts (Bracken and Barlow 1967, Exeristes comstockii; Thompson and Barlow 1970, Itoplectis conquisitor).

Contrary to all these previous studies, Van Handel and Lum (1961) have demonstrated in Aedes sollicitans that palmitoleic acid is present in relatively high concentrations independently of the diet. Similar results were obtained by Fast and Brown (1962) in Aedes aegypti.

Barlow (1964) has demonstrated that certain characteristics of the composition of fatty acids may be taxonomic. For example, most dipterous insects have relatively high concentrations of palmitoleic acid in their fats. This appears to be characteristic of many Diptera thus far studied, regardless of their varying diets. Barlow also demonstrated in L. sericata (1966b), Agris affinis (1965) and Musca domestica (1966a), that only minor changes in the percent composition of fatty acids occurred as the relative concentrations in the diet varied, except the polyunsaturate linoleic acid, which did vary with the diet, particularly in M. domestica. Aside from the lack of polyunsaturates the patterns were very near the same when these insects were reared on fatty acid free diets.

Other workers have noted that the relative amounts of fatty acids in the insect are distinctively different than the diet (Lambremont and Blum 1963, Lambremont, Stein and Bennett 1965, in A. grandis; Nakasone and Ito 1967, Bombyx mori; Nelson and Sukkestad 1968, Trichoplusia ni; Moore and Taft 1970, H. zea; Yendol 1970, G. mellonella).

In the present experiments, G. mellonella appears to retain a characteristic fatty acid pattern regardless of the relative composition in the diet, and the excess fatty acid supplements are excreted (Table 1-1, 1-2, 1-3). Yendol (1970) has shown that hemolymph of the wax moth has a fatty acid composition closer to that of the insect's fat than the diet, and it therefore appears that some control over the fatty acid composition occurs at the gut level. Two possible mechanisms which may be operative in retaining the pattern are: 1) selective absorption by the gut wall, 2) absorption of fatty acids into the gut tissue in the same proportions as in the diet followed by increased fractional transport rates and selective excretion of the supplemented acids out of the gut tissue. Further experiments will have to be carried out to determine if either mechanism is operative.

At the present time it appears that every degree of dietary influence on the relative levels of tissue fatty acids is exhibited in the insect world. However, most insects appear to be influenced only to a limited extent by the relative composition of fatty acids in the diet.

CHAPTER 2

A survey concerning the influence of host fatty acid composition on that of ichneumonoid and chalcidoid parasitoids

INTRODUCTION

A great deal of data are now compiled concerning the fatty acid composition of insects (Fast 1970, Gilbert 1967). Certain characteristics of these compositions are taxonomic in nature (Barlow 1964) and persist regardless of dietary fatty acid composition (Barlow 1966b).

Most free-living Hymenoptera have a "typical" fatty acid pattern characteristic of a great many insects and vertebrate tissue in which palmitate and oleate comprise about 85% of the fatty acids. In contrast, the ichneumonid parasites, Itoplectis conquisitor and Exeristes comstockii, reared on hosts with widely different patterns have patterns very similar to their hosts and retain no characteristics that can be considered their own (Bracken and Barlow 1967, Thompson and Barlow 1970).

The definitive study of the duplication phenomenon by rearing parasites on alternate hosts is very difficult and perhaps impossible in the case of the highly advanced chalcidoids. However, a comparison of the fatty acid composition of a parasite and its host suggests if the phenomenon is present. The present study surveys parasitic wasps representative of a number of familites to determine the apparent generality of this phenomenon.

METHODS AND MATERIALS

Parasites and their corresponding hosts were obtained from wild populations and laboratory reared colonies from various Canada Department of Agriculture laboratories across Canada.

The lipids were extracted with chloroform-methanol, separated into neutral and phospholipid fractions, transesterified, and the fatty acid methyl esters analyzed by GLC, all as previously described in the General Methods and Materials section (Only one pooled sample of 50 to 100 insects was obtained from each species due to lack of material).

Two statistical indices were calculated in order to compare the overall similarity of the patterns of a parasite and its host. The first index is the variance of the quantitative differences of the individual fatty acids or the mean square distance (MSD) (Boyce 1969). The second index is the variance of the differences from unity of the ratios of the individual fatty acids (highest percentage/lowest percentage), which we have termed the mean square distance of the ratios (MSDR). This latter index is dependent upon proportionality rather than absolute differences. It reduces the effect of a difference which is proportionally small in abundant components, and increases the effect of a similar difference which is proportionally large in less abundant components. Fatty acids which were less than 5% of the total in both the parasite and the host were not included in the calculations.

RESULTS AND DISCUSSION

The fatty acid composition of the ichneumonoid and chalcidoid hymenopterous parasites studied resembles to varying degrees that of their hosts (Table 2-1, 2-2; Fig. 2-1). Qualitatively, the pattern of each parasite is identical to that of its host, but quantitatively they differ

between and within families.

In general, considering the percent composition as well as both indices, members studied from the family Ichneumonidae appear to be most influenced by their hosts (Fig. 2-1). This is particularly evident in the cases of *E. comstockii* and *I. conquisitor*, which were both reared on more than one host with widely varying patterns (Fig. 2-2). The pteromalid, *Spalangia cameroni* and the eulophid, *Dahlbominus fuscipennis*, also appear to duplicate the patterns of their host to much the same degree as the ichneumonids, but other members of the families Pteromalidae, Eulophidae, Brachonidae and Aphidiidae do so to a lesser degree or not at all (Fig. 2-1).

The close correlation of the fatty acid composition of the parasites and their hosts appears to be a general phenomenon in the Ichneumonidae, and may occur in some species of other families as well, but is not general in all parasitic Hymenoptera.

Table 2-1. The fatty acid composition of ichneumonoid wasps and their corresponding hosts.

Parasite and Host	% Composition									
	12*	14:0	16:0	16:1	18:0	18:1	18:2	18:3	MSD	MSDR
Family Aphidiidae										
1. *Aphidius smithi*	1	10.4	14.5	3.5	8.7	23.8	32.2	5.8	384.58	4.29
Acyrthosiphon pisum (Hom.)	2.4	51.4	4.1	2.8	6.4	10	14.4	8.2		
Family Brachonidae										
1. *Aphaereta pallipes*	-	3.8	15.8	16.5	5.7	24.0	34.1	-	135.72	0.71
Agria affinis (Dip.)	-	2	19	29	4	32	13	-		
2. *Microplitis croceipes*	Tr	0.9	12.0	4.3	9.2	28.5	28.6	16.5	102.96	0.75
Heliothis zea (Lep.)	-	0.4	15.5	0.6	6.9	23.4	47.6	6.0		
3. *Macrocentrus ancylivorus*	-	1.0	27.6	5.6	15.4	33.6	7.8	9.1	39.14	2.60
Gnorimoschema operculella (Lep.)	-	0.4	32.1	13.8	3.3	34.0	8.2	8.3		
Family Ichneumonidae										
1. *Itoplectis conquisitor*	-	0.6	20.6	42.0	1.0	25.6	10.1	-	3.86	0.01
Ostrinia nubilalis (Lep.)	-	0.6	22.5	43.6	1.0	22.6	9.6	-		
2. *Itoplectis conquisitor*	0.1	Tr	38.4	1.4	1.2	44.2	10.9	3.7	45.25	0.06
Galleria mellonella (Lep.)	0.2	Tr	29.7	2.5	1.2	51.5	13.5	1.3		

Table 2-1 Cont'd

Parasite and Host	% Composition									
	12*	14:0	16:0	16:1	18:0	18:1	18:2	18:3	MSD	MSDR
Family Ichneumonidae (cont'd)										
3. Exeristes comstockii	-	0.5	15.8	1.3	7.8	36.6	38.0	-	7.8	0.02
Galleria mellonella (Lep.)	-	0.3	19.2	2.0	7.8	32.2	38.4	-		
4. Exeristes comstockii	-	0.8	23.9	17.2	2.4	46.5	9.1	-	20.38	0.04
Lucilia sericata (Dip.)	-	1.5	28.7	20.2	2.6	39.7	7.3	-		
5. Exeristes comstockii	-	1.1	11.9	1.9	4.4	48.2	11.5	20.9	0.76	0
Neodiprion sertifter (Hymenop.)	-	1.5	11.5	1.7	3.2	49.8	11.9	20.5		
6. Pimpla turionella	Tr	Tr	37.0	1.4	1.0	48.8	11.7	-	21.27	0.03
Galleria mellonella (Lep.)	0.2	Tr	29.7	2.5	1.2	51.5	13.5	-		
7. Pleolophus basizonus	Tr	0.6	13.2	2.8	6.5	46.0	10.4	20.5	1.45	0.01
Neodiprion sertifter (Hymenop.)	Tr	1.2	11.9	1.2	5.5	46.9	12.3	20.9		

*The first number represents the carbon chain length, the second, the number of double bonds.

Table 2-2. The fatty acid composition of chalcidoid wasps and their corresponding hosts.

Parasite and Host	% Composition								MSD	MSDR
	12*	14:0	16:0	16:1	18:0	18:1	18:2	18:3		
Family Pteromalidae										
1. *Spalangia cameroni*	-	1.2	28.0	12.7	4.0	28.8	20.4	4.9	10.47	0.04
Musca domestica (Dip.)	-	2.1	29.5	16.8	3.0	24.3	22.0	2.4		
2. *Nasonia vitripennis*	-	0.4	15.7	10.3	6.3	25.3	29.2	12.8	67.93	3.54
Musca domestica (Dip.)	-	2.1	29.5	16.8	3.0	24.3	22.0	2.4		
Family Eulophidae										
1. *Encarsia formosa*	-	0.7	12.0	1.9	10.2	33.6	22.6	19.1	117.81	1.01
Trialeurodes vaporariorum (Hom.)	-	0.5	21.9	0.6	6.6	49.5	14.6	6.4		
2. *Dahlbominus fuscipennis*	0.2	1.3	13.0	3.7	2.7	37.1	12.6	29.5	24.55	0.14
Neodiprion lecontei (Hymenop.)	0.5	2.5	19.6	1.6	3.9	39.8	8.6	23.9		

*The first number represents the carbon chain length, the second, the number of double bonds.

Fig. 2-1. The MSD and MSDR of the fatty acid compositions of parasitic Hymenoptera and their hosts.

*Host names are given in cases where the parasite was reared on more than one host.

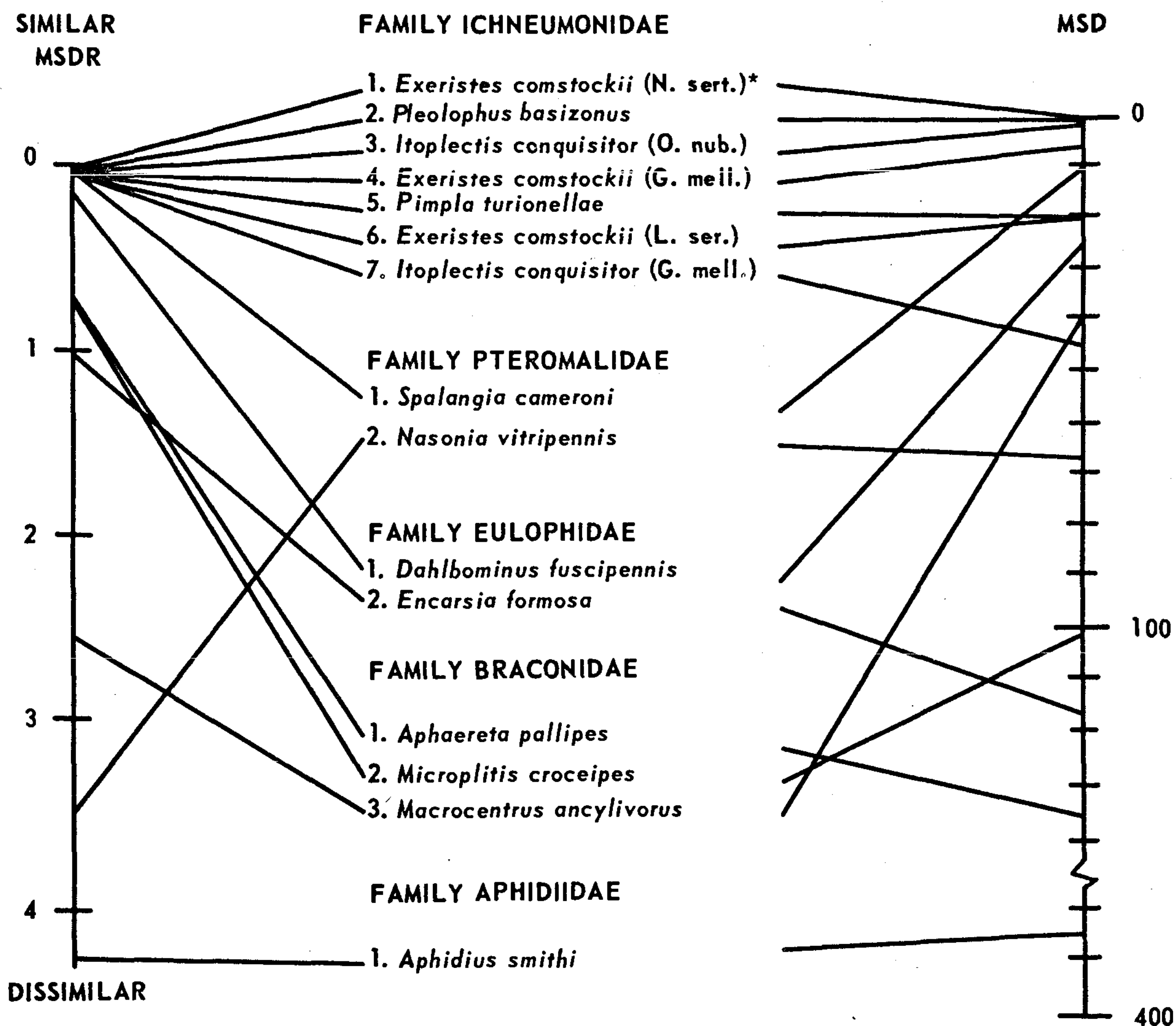
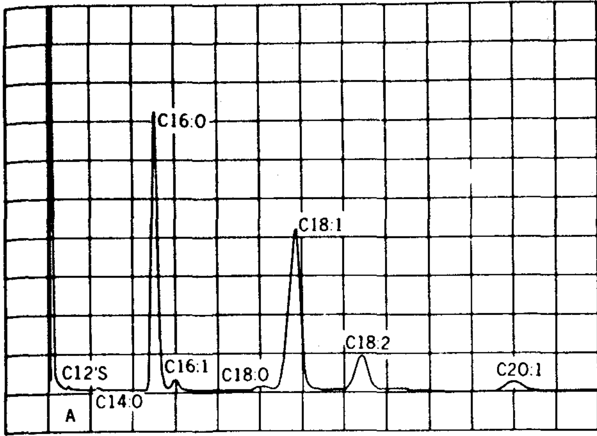

SIMILAR
MSDR
FAMILY ICHNEUMONIDAE
MSD
1. Exeristes comstockii (N. sert.)*
2. Pleolophus basizonus
3. Itoplectis conquisitor (O. nub.)
4. Exeristes comstockii (G. mell.)
5. Pimpla turionellae
6. Exeristes comstockii (L. ser.)
7. Itoplectis conquisitor (G. mell.)
FAMILY PTEROMALIDAE
1. Spalangia cameroni
2. Nasonia vitripennis
FAMILY EULOPHIDAE
1. Dahlbominus fuscipennis
2. Encarsia formosa
FAMILY BRACONIDAE
1. Aphaereta pallipes
2. Microplitis croceipes
3. Macrocentrus ancylivorus
FAMILY APHIDIIDAE
1. Aphidius smithi
0
1
2
3
4
DISSIMILAR
0
100
400

Fig. 2-2. The fatty acid patterns of *Itoplectis conquisitor* and *Exeristes comstockii* and their corresponding hosts. A. *Galleria mellonella*, B. *Itoplectis conquisitor* reared on *Galleria mellonella*, C. *Ostrinia nubilalis*, D. *Itoplectis conquisitor* reared on *Ostrinia nubilalis*, E. *Lucilia sericata*, F. *Exeristes comstockii* reared on *Lucilia sericata*, G. *Neodiprion sertifer*, H. *Exeristes comstockii* reared on *Neodiprion sertifer*.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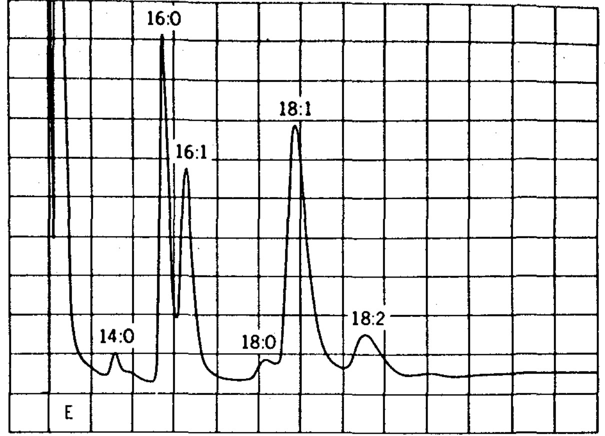
C18:1
C18:2
C12'S
C14:0
C16:1
C18:0
C20:1
A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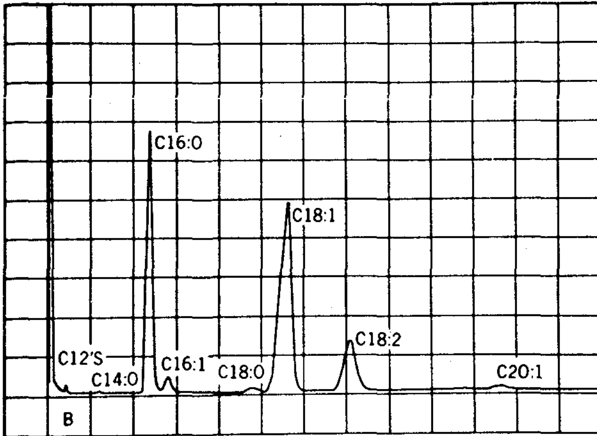
E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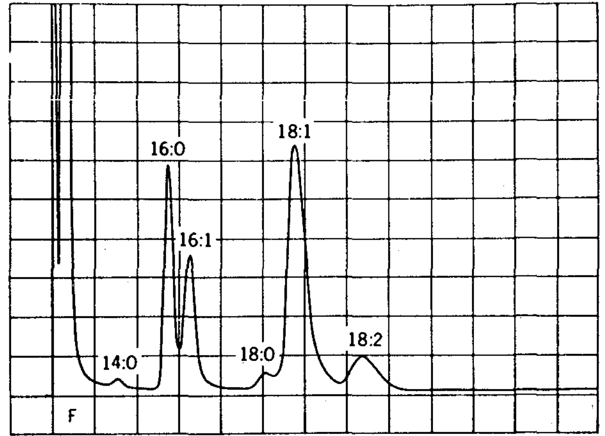
C18:1
C18:2
C12'S
C14:0
C16:1
C18:0
C20:1
B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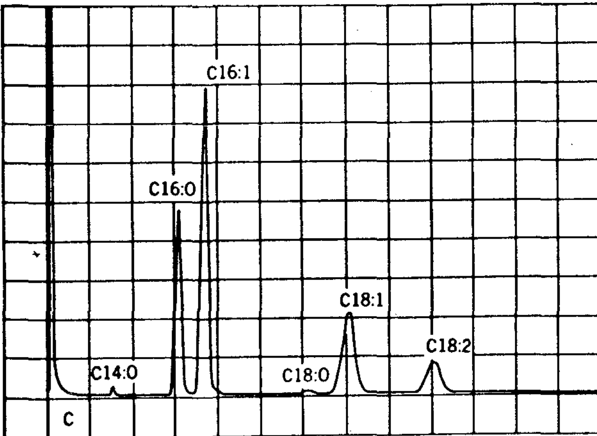
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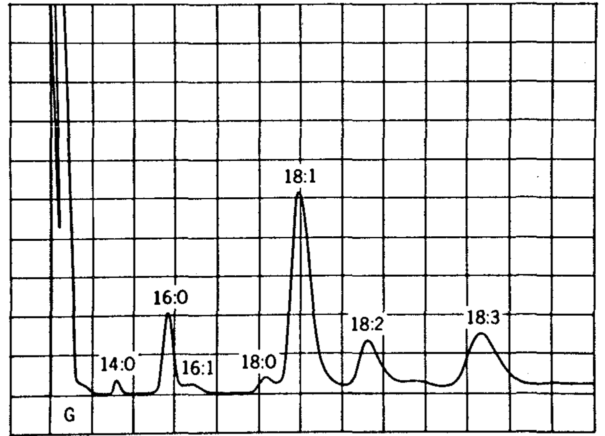
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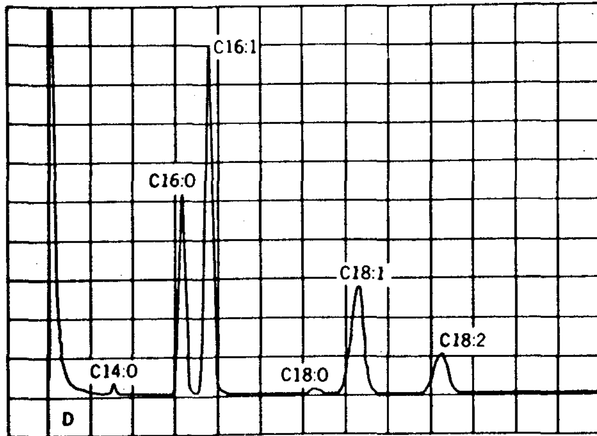
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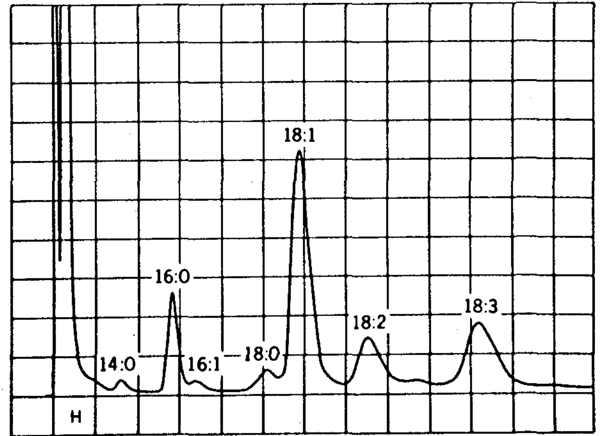
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CHAPTER 3

The inconsistent phospholipid fatty acid composition in the insect parasitoid, *Itoplectis conquisitor*

INTRODUCTION

The fatty acid compositions of the total lipids of hymenopterous parasites of the family Ichneumonidae, are qualitatively and quantitatively very similar to those of the hosts on which they are reared. In cases where species were reared on two different species of host with very different fatty acid patterns, the parasites, *Exeristes comstockii*, and *Itoplectis conquisitor*, retained no characteristic fatty acid composition of their own in contrast to *Lucilia sericata* and *Galleria mellonella*, the hosts, which are influenced very little by dietary fats. Indeed, most other insects retain certain characteristics of their fatty acid patterns, and these are influenced to a limited extent by dietary fats (Barlow 1964, Barlow 1966b, Fast 1964).

The characterization and significance of the fatty acid composition of phospholipids has been well documented (Demel, Van Deenan and Pethica 1967), and the question arose as to whether these ichneumonid parasites have a consistent fatty acid composition in their phospholipids as other organisms do. The purpose of the present study was to determine the effects of nutritional variation in fatty acid composition on that in the phospholipids of the ichneumonid parasite, *I. conquisitor*.

METHODS AND MATERIALS

I. conquisitor was reared on the two hosts, *G. mellonella* and *Ostrinia nubilalis* as described in the General Methods and Materials section. Lipid purification and fatty acid analyses were also carried out as previously

described.

RESULTS AND DISCUSSION

The fatty acid compositions of the separated neutral and phospholipid fractions (Table 3-1) of I. conquisitor reared on the two hosts, show the same differences as that of the total lipids. The similarity between the fatty acid composition of the neutral lipids and total lipids is due to the fact that neutral lipids make up 90 percent of the total lipids in these insects. The relatively high level of unsaturates in the phospholipids as compared to the neutral lipids is a characteristic of phospholipids and has been described in numerous other insects (Fast 1966).

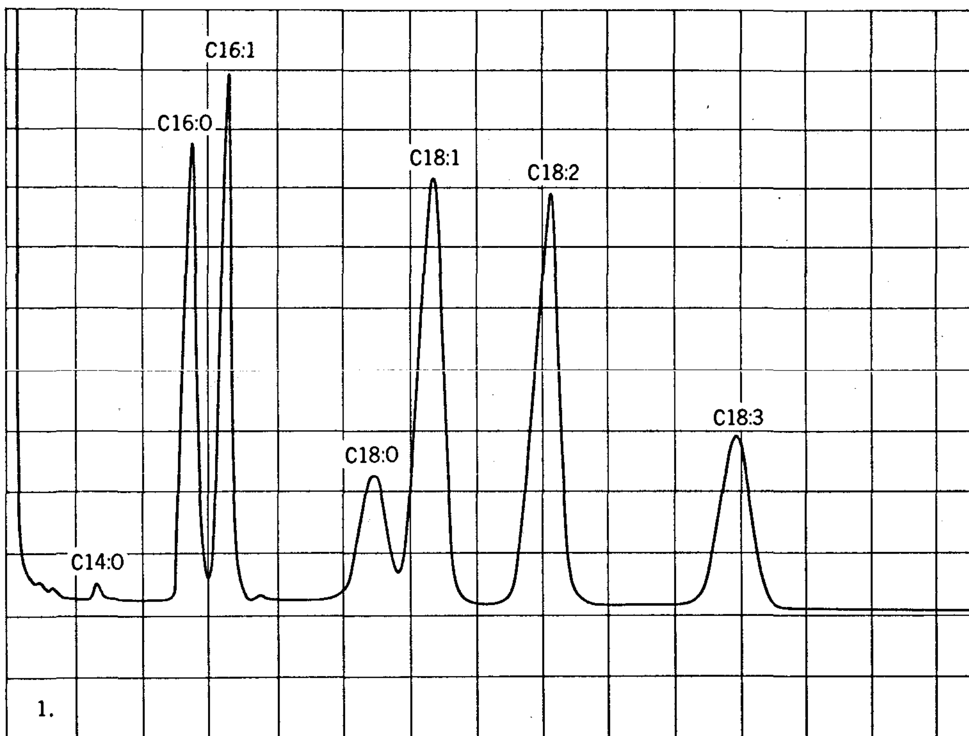
Although the fatty acid composition of the phospholipids of the parasite varies considerably when reared on different hosts (Fig. 3-1), and adopts many of the characteristics of the composition of the phospholipids in the host, namely the high C16:1 content, the composition is not influenced to the extent of that of the neutral lipids. In the case of the phospholipids, consistent ratios of the fatty acid classes are maintained, and the physical nature of the phospholipids appears to be unaltered (Table 3-2). This, however, is not the case with the neutral lipids, in which the ratios are altered considerably when the parasite is reared on different hosts (Table 3-2). The parasite, therefore, appears to exert more control over the fatty acid composition of the phospholipids than it does over that in the neutral lipids. However, this control is not as stringent as that exerted by most other insects over their fatty

Table 3-1. The fatty acid composition of the phospholipids and neutral lipids of *Itoplectis conquisitor* reared on two hosts, *Galleria mellonella* and *Ostrinia nubilalis*.

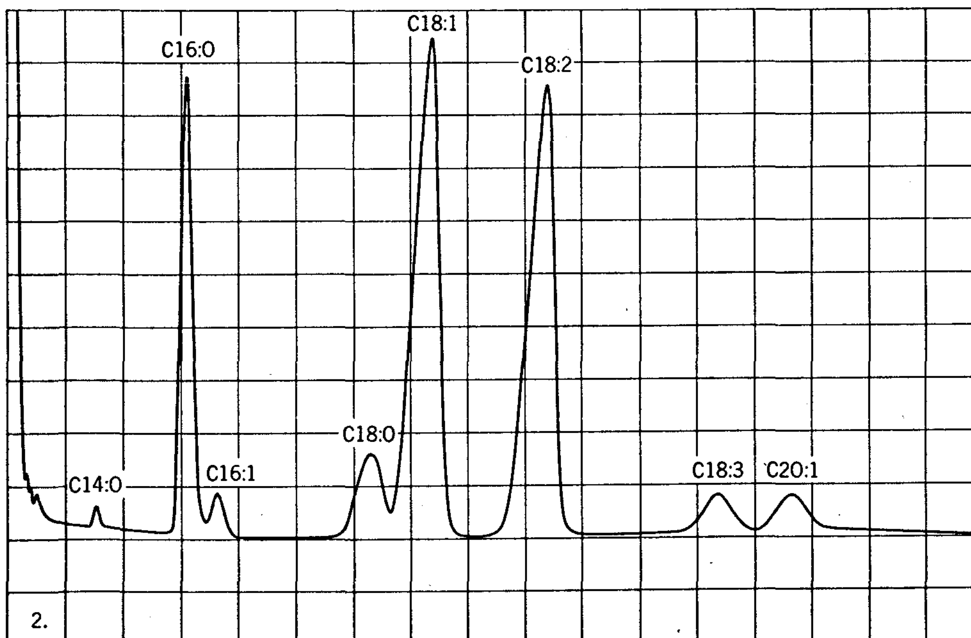
Source				% Composition				
	C14:0*	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:1
I. conquisitor reared on *G. mellonella*								
Phospholipids	Tr	14.1	1.6	6.5	36.1	34.9	3.3	3.4
Neutral lipids	Tr	31:0	1.7	1.5	43.4	9.9	Tr	12.4
I. conquisitor reared on *O. nubilalis*								
Phospholipids	Tr	13.6	15.4	8.2	24.8	25.6	12.2	--
Neutral lipids	Tr	25.9	35	0.9	26.9	6.5	4.8	--

*The first number represents the carbon chain length, the second, the number of double bonds.

Fig. 3-1. GLC chromatograms of the fatty acid patterns of the phospholipids of *Itoplectis conquisitor*. 1. *I. conquisitor* reared on *Ostrinia nubilalis*, 2. *I. conquisitor* reared on *Galleria mellonella*.



C16:1
C16:0
C18:1
C18:2
C18:3
C18:0
C14:0
1.



C18:1
C16:0
C18:2
C18:0
C14:0
C16:1
C18:3
C20:1
2.

Table 3-2. The ratio of fatty acid classes of the phospholipids and neutral lipids of *Itoplectis conquisitor* reared on two hosts, *Galleria mellonella* and *Ostrinia nubilalis*.

	Saturates / Unsaturates	Saturates / Monounsaturates	Saturates / Polyunsaturates	Monounsaturates / Polyunsaturates
Phospholipid Source				
I. conquisitor reared on *G. mellonella*	0.26	0.50	0.54	1.08
I. conquisitor reared on *O. nubilalis*	0.28	0.54	0.58	1.06
Neutral lipid Source				
I. conquisitor reared on *G. mellonella*	0.48	0.57	3.28	5.81
I. conquisitor reared on *O. nubilalis*	0.37	0.43	2.37	5.48

acid compositions.

The overall importance of the phospholipids in the structure and function of cells is now well-known (Korn 1966). However, the significance of the variation in individual components of the phospholipid molecule is still in question. The significance of the fatty acid composition in the phospholipids appears to be the requirement for phospholipids with certain physical properties to fulfill specific structural and/or functional requirements; the degree of saturation is, therefore, of great importance (Demel *et al*. 1967). In microorganisms, nutritional alternations in the fatty acid composition of the phospholipids are compensated for by substitutions which result in the maintenance of the physical character of the phospholipids. For example, lack of unsaturated fatty acids, can be compensated for by substitution of shorter chain saturated acids, cyclopropane fatty acids and methyl-branched chain acids, but in each case the physical properties of the phospholipids are maintained (Meyer and Bloch 1963, Law, Zalkin and Keneshire 1963, MacFarlane 1961). Such compensations have not previously been reported in more highly evolved organisms, and these organisms generally maintain a relatively specific fatty acid composition.

In the present experiments, although the fatty acid composition of the phospholipids varies considerably due to nutritional variation, the degree of saturation remains the same, and the parasite, therefore, compensates, such that the physical properties of the phospholipids are maintained. The parasite appears to have no qualitative or quantitative con-

trol over the fatty acid composition of its neutral lipids, but does have some quantitative control over that in its phospholipids.

CHAPTER 4

Synthesis of fatty acids by the parasitoid *Exeristes comstockii* and two hosts, *Galleria mellonella* and *Lucilia sericata*

INTRODUCTION

The effect of diet on tissue fat is quite variable within the insect world. In general, most insects are influenced to a limited extent by the composition of fatty acids in the diet and certain characteristics of the fatty acid composition are taxonomic in nature (see Chapter 1). In contrast, it has been shown that the ichneumonid parasite, *Exeristes comstockii* duplicates the fatty acid pattern of its host, and is unable to control pool size of fatty acids (Bracken and Barlow 1967). More recently another ichneumonid parasite of Lepidoptera, *Itoplectis conquisitor*, was found to exhibit a similar relationship to its host (Thompson and Barlow 1970).

The purpose of the present experiments was to investigate the rates and pathways of synthesis of fatty acids in *E. comstockii* and two hosts, *Galleria mellonella* and *Lucilia sericata*, on which the parasite was reared.

METHODS AND MATERIALS

G. mellonella larvae were obtained from a colony reared as described in the General Methods and Materials section. Last instar larvae were injected with a solution of ^{14}C-1-acetate (sodium salt, 57 mCi/mM) in distilled water (1 μCi/μl), through the anus into the hemocoel, with a Gilson micrometer syringe (1 μCi/10 mg wet weight). Weight-based injections were made to allow direct comparison and analysis of specific activities in all groups. Four groups of 5 insects each were injected. Last instar *L. sericata* larvae, obtained from a colony reared on calves liver, were injected similarly with ^{14}C-1-acetate into the dorsal aorta. Four groups of 20

to 30 insects were injected. Mortality was approximately 30%. Last instar E. comstockii larvae reared on G. mellonella were removed from the host, and injected with ^{14}C-1-acetate in the same manner through the 3rd or 4th body segment, anterior to the hind gut. Two groups of 20 to 30 insects were injected. Mortality was approximately 50%. Prior to injection, G. mellonella and L. sericata larvae were starved for 24 hours in order to eliminate the possibility of food in the gut biasing percent composition and specific activity (S.A.) determinations, and also to minimize bleeding. Any insect which bled to any appreciable extent or appeared badly wounded or dead was not included in the analysis and was added to the mortality calculations.

After 24 hours exposure, the insects were homogenized in a tissue grinder, the total lipid extracted saponified and esterified with diazomethane as described previously. The fatty acids were purified and fractionated, according to degree of satuation, on silicic acid-silver nitrate columns, and fatty acid analyses were carried out by GLC.

The individual fatty acid fractions were collected from the column effluent in side arm collection tubes immersed in a dry ice-acetone bath. Recovery was 90 to 100%. Radioactivity was determined in a Beckman LS-250 liquid scintillation spectrometer with the use of a toluene base cocktail containing 5% 2,5 diphenyloxazole. Quench corrections were applied and the activity was determined as disintegrations per minute (dpm).

The fatty acids were decarboxylated by the method of Phares (1951) as modified by Brady, Bradley and Trams (1960). The reaction was carried out in 25 ml Warburg flasks with center wells and side arm tubes. Amounts

of fatty acid up to one μmole were placed in the flask with 50 mg of sodium amide and a miniature magnetic stirring bar. After placing a 6 x 35 mm test tube in the center well, the flask was sealed with an upside down serum stopper and hose clamp. One half ml of sulphuric acid (3 parts concentrated:1 part fuming) was added to the side arm and the side arm was sealed with a glass stopper. The reaction was initiated by pouring the sulphuric acid into the main compartment of the flask and the reaction vessel was then placed in a 70 C water bath for one hour. After this reaction period the flask was removed from the water bath and placed in ice water for 5 minutes. One half ml of Hyamine® hydroxide (Rohm and Haas Co.) (1 molar solution) was then injected with a hypodermic syringe into the test tube in the center well through the serum stopper. After removal of the hypodermic needle another 6 x 35 mm test tube was put into the bottom of the serum stopper to prevent any leakage. The flask was then removed from the ice water and placed in a 37°C water bath for one hour. The vessel was removed every 15 minutes and the reaction mixture stirred with a magnetic stirrer to break up the carbon dioxide bubbles in the sulphuric acid. The flask was then opened and the test tube in the center well removed and added directly to a cocktail vial and radioactivity determined. Recovery was 95% on 9 trials with a ^{14}C-1-stearic acid standard. For the decarboxylation procedure all the insect replicates were pooled and three samples decarboxylated.

Oxidative cleavage of the monounsaturated fatty acids was carried out by the method of Tinoco and Miljanich (1965). Individual fatty acids

(0.1-10 mg) were added to ground glass tissue homogenizers containing 0.1 ml of glacial acetic acid and mixed for 30 seconds. Eight mg of potassium permanganate/mg of fatty acid were then added and mixed for an additional minute. After the addition of 0.1 ml of water, potassium metabisulphite was added until the mixture turned colourless. Two drops of 9N sulphuric acid and three ml petroleum ether (B.P. 30-60 C) were then added and the mono- and dicarboxylic acid products were isolated in the petroleum ether. The monocarboxylic (derived from the methyl end of the unsaturated fatty acid) and the dicarboxylic (derived from the carboxyl end of the unsaturated fatty acid) acids were analyzed by GLC (temperature program 50-190 C). Standard mixtures of mono- and dicarboxylic acids were run before and after each sample for identification purposes.

RESULTS AND DISCUSSION

The fatty acid patterns of *E. comstockii* and its two hosts, *G. mellonella* and *L. sericata* are shown in Fig. 4-1. Qualitatively and quantitatively, the fatty acid composition of the parasite is uniquely similar to the host on which it is reared. The major differences between the two hosts are the different levels of palmitolic acid (C16:1), high in *L. sericata* and low in *G. mellonella*, and the presence in *G. mellonella* of eicosenoic acid (C20:1) which is absent from *L. sericata*. These differences are equally evident in *E. comstockii* when it is reared on these two hosts. Previous studies have shown that the pattern of the parasite is retained through pupation and eclosion (Bracken and Barlow 1967). In

contrast, the fatty acid composition of many other insects including *L. sericata* (Barlow 1966b) and *G. mellonella* (see Chapter 1) is influenced very little by dietary fats.

The percent distribution of activity in the fatty acids of *G. mellonella* and *L. sericata* (Table 4-1) is consistent with that found in other lepidopterous and dipterous insects (Gilbert 1967). *E. comstockii* has an activity distribution more similar to *G. mellonella* than *L. sericata*. The major synthetic product in all cases is palmitic acid (Table 4-1).

The high percent activity incorporated and specific activity in the saturated fatty acids particularly palmitic and stearic acids in all three insects is consistent with the *de novo* and elongation systems of fatty acid synthesis (Thompson and Barlow 1971).

Decarboxylation of the individual fatty acid fractions of *G. mellonella* and *E. comstockii* further substantiated the presence of these two pathways (Table 4-2). *De novo* synthesis from ^{14}C-1-acetate would result in a molecule with every odd numbered carbon atom labelled, and each of these labelled atoms would contain 12.5% of the total activity of the acid in the case of palmitic acid and 14.3% of the activity in the case of myristic acid. The percent activity found in C_1 of myristic and palmitic acids is somewhat consistent with this distribution. The *de novo* synthesis of myristic acid has not been previously reported, probably because of exceedingly small pool size. The specific activity of the acid, indicative of the fractional turnover rate (the fraction of the pool turned over per unit of time), is very high (Table 4-1), but the total activity

Table 4-1. Incorporation of ^{14}C-1-acetate into fatty acids of Galleria mellonella, Lucilia sericata and Exeristes comstockii reared on G. mellonella, after 24 hours exposure (± S.E.).

	Exeristes comstockii reared on Galleria mellonella			Galleria mellonella			Lucilia sericata		
	% composition	% total activity	Specific activity (dpm/g)	% composition	% total activity	Specific activity (dpm/g)	% composition	% total activity	Specific activity (dpm/g)
C14:0*	0.5	6.68 ± 0.19	794.68 ± 22.37	0.5	9.99 ± 0.01	159.89 ± 15.42	1.5	5.14 ± 0.53	19.45 ± 1.79
C16:0	37.8	67.51 ± 2.76	106.13 ± 4.33	44.8	50.06 ± 7.99	8.95 ± 1.43	28.7	51.42 ± 5.85	10.10 ± 1.16
C16:1	4.1	5.33 ± 0.45	77.34 ± 6.56	3.7	2.5 ± 0.25	5.47 ± 0.49	20.2	19.15 ± 0.53	5.36 ± 0.15
C18:0	1.5	12.30 ± 1.13	487.17 ± 44.79	1.5	26.22 ± 2.25	140.11 ± 11.82	2.6	5.14 ± 0.18	10.99 ± 0.55
C18:1	43.3	8.18 ± 0.22	11.23 ± 0.29	37.6	7.99 ± 0.25	1.7 ± 0.064	39.7	19.15 ± 0.71	2.71 ± 0.1
C18:2	7.1	0	0	5.4	0	0	7.3	0	0
C20:1	5.7	0	0	6.5	3.25 ± 0.75	3.94 ± 0.89	-	-	-

*The first number represents the carbon chain length, the second, the number of double bonds.

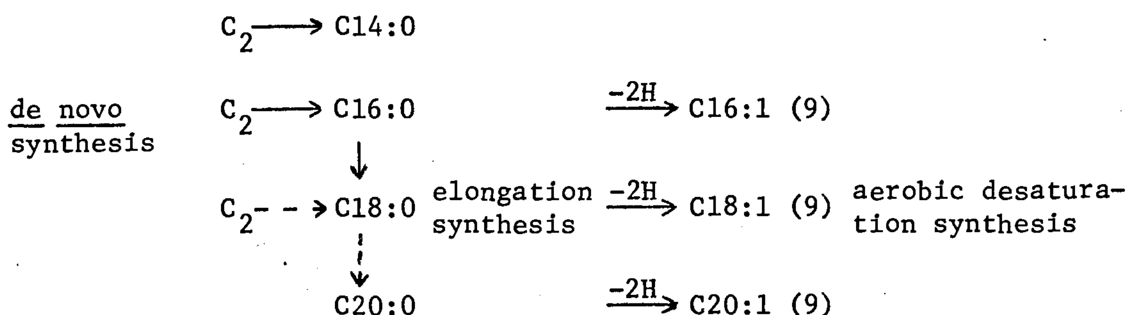
present, indicative of the turnover rate (weight turned over per unit time) is very small (Table 4-1), and would be undetectable if the pool size were small enough. Although myristic acid has the highest fractional turnover rate of all the fatty acids in all three insects, palmitic acid is the major *de novo* synthetic product, since more total activity was found in this acid (Table 4-1). Decarboxylation of stearic acid indicates its synthesis by elongation of plamitic acid. The relatively high activity in C_1 is most likely due to the rapidity of the elongation reaction as compared to the *de novo* synthesis of the substrate, palmitic acid. This difference in rates has been described previously (Thompson and Barlow 1971).

In general, both monounsaturated fractions have considerably less activity and lower specific activities than their corresponding saturates in all species (Table 4-1). However, oleic acid (C18:1) has more activity than stearic acid (C18:0) in *L. sericata*. Oxidative cleavage demonstrated that all of the monounsaturated acids are Δ 9 isomers. This is consistent with previous work demonstrating that insects synthesize monounsaturates by direct desaturation of the corresponding saturates (Bade 1964, Sridhara and Bhat 1965), and that direct or "aerobic" desaturations result in Δ 9 isomers (Bloomfield and Bloch 1960). However, decarboxylation of the monounsaturates results, in general, in a greater percent radioactivity in C_1 than in the corresponding saturates, and also greater variance. The high acitivty in C_1 may indicate that insects can synthesize monounsaturates by another pathway besides the direct desaturation described. The variance may be due to varying degrees of cleavage during the procedure

with subsequent decarboxylation of the $^{14}C9$ of the dicarboxylic acid product. *In vitro* work, in progress, may further determine the significance of these activity distributions.

The C20:1 monounsaturated fraction synthesized in *G. mellonella* (Table 4-1, Table 4-2), was absent from *L. sericata* and, although present in *E. comstockii* reared on *G. mellonella*, was not radioactive. In previous studies with various insects, no detectable amounts of fatty acids greater than 18 units were reported synthesized. Decarboxylation of this acid indicates synthesis by elongation of stearate and desaturation. Since eicosanoic acid was undetected, it is probably present in exceedingly small amounts and has a very high turnover rate. It is also possible that the eicosanoic acid pool remains attached to the enzyme system, and would, therefore, be undetectable using our techniques.

In summary, it would appear that these insects synthesize fatty acids by the following generalized scheme:



(The dotted line represent inconclusively established reactions; C 20 reactions apply only to *G. mellonella*.)

Table 4-2. Per cent radioactivity in C_1 of the individual fatty acids of Galleria mellonella and Exeristes comstockii reared on G. mellonella.

Fatty Acid	G. mellonella	E. comstockii reared on G. mellonella
C14:0*	15.09 ± 0.73†	18.40 ± 0.26
C16:0	11.11 ± 0.94	11.65 ± 0.13
C16:1	23.14 ± 3.09	26.11 ± 9.07
C18:0	47.94 ± 1.16	32.02 ± 0.47
C18:1	64.82 ± 5.11	28.23 ± 6.56
C20:1	64.19 ± 6.61	--

*The first number represents the carbon chain length, the second, the number of double bonds.

†These statistics apply to technical variance only.

Although E. comstockii duplicates the fatty acid pattern of its host, it does appear to have a distinct fatty acid metabolism of its own, with respect to synthesis and turnover. The parasite appears to lack the mechanism of pool size regulation normally present. Recent studies have shown that dietary fatty acids act to control the synthesis of fatty acids in insects, in a feedback inhibition manner similar to that described in vertebrates (Horie and Nakasone 1971). In this manner, dietary fats influence the levels of fatty acids in the insect. Perhaps the parasite, E. comstockii, lacks to a great extent such control mechanisms.

The fatty acids in E. comstockii originate partially from direct incorporation of host fat as well as synthesis. This is indicated by the presence of C20:1 which was synthesized by G. mellonella, but not by E. comstockii.

The activities of the fatty acids in E. comstockii are consistently higher than those of either G. mellonella or L. sericata (Table 4-1), and it would appear that E. comstockii has relatively high rates of fat turnover. Injections were made on a weight basis (i.e. 1 μCi/10 mg) in order to give equal dosages to each insect of all three species, and thereby compare specific activites directly. However, since E. comstockii larvae contain stored waste products, the dosage administered per mg weight of metabolically active tissue might be greater than that given the other species.

If fatty acid metabolism in the three species were quantitatively the same, the ratio of the S.A. of a fatty acid prominent in each species

(C18:1) to any other fatty acid in that species would be the same among the species, e.g.

$$\frac{\text{S.A. C16:0 } (\underline{G.}\ \underline{\text{mellonella}})}{\text{S.A. C18:1 } (\underline{G.}\ \underline{\text{mellonella}})} = \frac{\text{S.A. C16:0 } (\underline{E.}\ \underline{\text{comstockii}})}{\text{S.A. C18:1 } (\underline{E.}\ \underline{\text{comstockii}})}$$

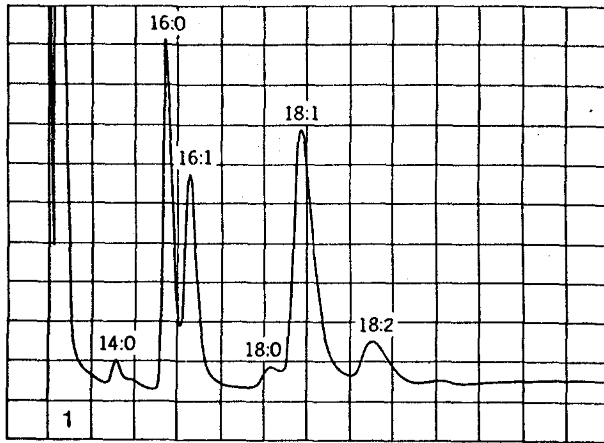
However, this was not the case (Table 4-3), and *E. comstockii*, therefore, appears to have rates of synthesis which are greater and distinctly different than either host.

Table 4-3. The ratio of the specific activities of the individual fatty acids of *Lucilia sericata*, *Galleria mellonella* and *Exeristes comstockii* to the specific activity of their respective C18:1 fatty acid fractions.

Specific Activity Ratios	C14:0*/C18:1	C16:0/C18:1	C16:1/C18:1	C18:0/C18:1	C20:0/C18:1
G. mellonella	94.05	5.27	3.22	82.42	2.32
L. sericata	7.18	3.73	1.98	4.06	--
E. comstockii reared on *G. mellonella*	70.76	9.45	6.89	43.38	--

*The first number represents the carbon chain length, the second, the number of double bonds.

Fig. 4-1. The fatty acid patterns of 1. *Lucilia sericata*, 2. *Exeristes comstockii* reared on *L. sericata*, 3. *Galleria mellonella*, 4. *E. comstockii* reared on *G. mellonella*.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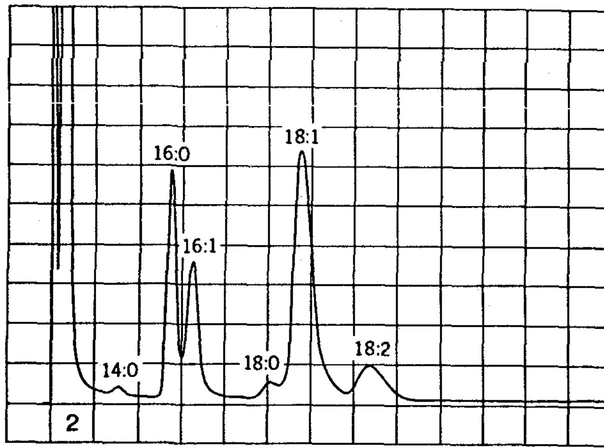
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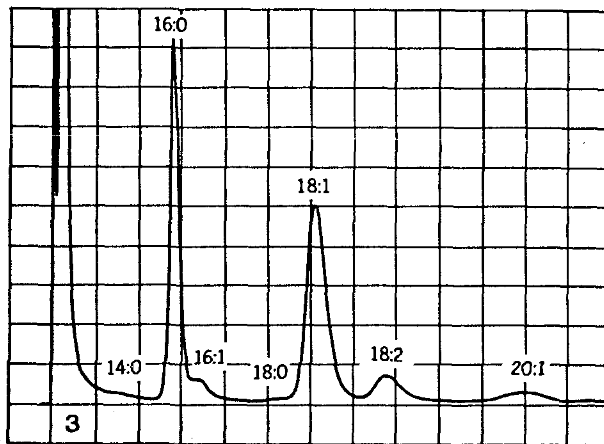
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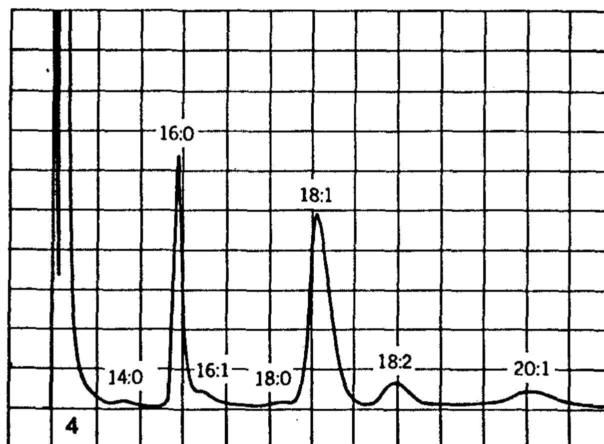
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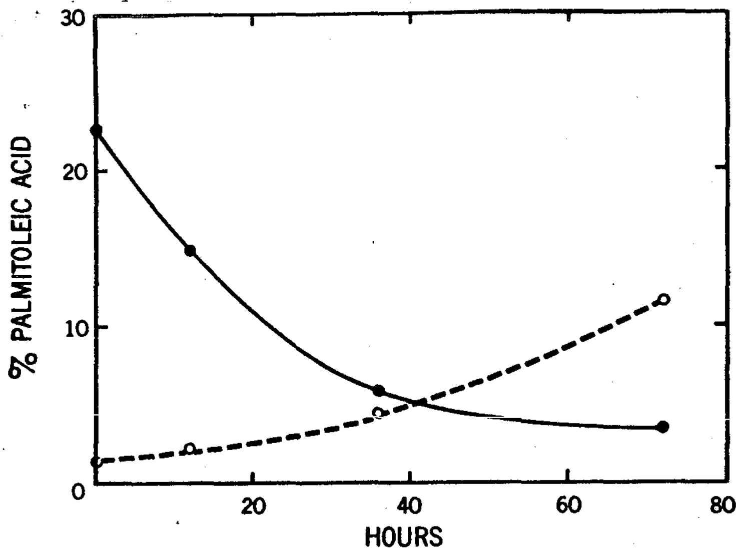
GENERAL DISCUSSION

It has been demonstrated in these studies that lipid metabolism in ichneumonid parasitoids differs from that in other insects. Specifically, these parasites do not have a characteristic fatty acid composition. Qualitatively, the composition is identical to that of the host, and quantitatively, the levels of fatty acids are very similar to and appear to be controlled by the concentration in the host. Nevertheless, the parasites do exert a limited degree of quantitative control of the fatty acid composition of their phospholipids. The levels of saturated and monounsaturated fatty acids are maintained by active metabolic processes and the parasites synthesize fatty acids by *de novo*, aerobic desaturation and elongation syntheses.

Studies carried out at the same time as those described here demonstrated that *Exeristes comstockii* is capable of changing its fatty acid composition rapidly when the developing larvae are transferred to different hosts (Barlow and Bracken 1971). Second instar parasite larvae were transferred from *Galleria mellonella* to *Lucilia sericata* and vice versa, and fatty acid analyses were carried out 12, 36 and 72 hours after transfer. The changes observed in palmitoleic acid were as follows:



The palmitoleic acid concentration is reduced rapidly when the parasite is transferred from L. sericata to G. mellonella (solid line), 50% in about 20 hours. Synthesis is probably reduced immediately and oxidation continues at a decreasing rate until equilibrium is reached at the lower level. When the parasite is transferred from G. mellonella to L. sericata (dotted line), the increase in palmitoleic acid in its fats occurs at a considerably slower rate than the reduction observed in the former case. Fifty percent of its final concentration was not reached until after 60 hours. The rapid rates of turnover of fatty acids in E. comstockii described in the present studies partially explains these rapid metabolic alterations in pool size.

The ichneumonid parasites described in these studies resemble the parasitic platyhelminth worms, which also have fatty acid compositions very similar to their hosts. However, the platyhelminths do not synthesize saturated or monounsaturated fatty acids. They lack de novo and aerobic

desaturation synthesis pathways (Meyer, Meyer and Bueding 1970). Some direct incorporation of host fatty acids occurs in addition to synthesis in the parasites. This is shown by the presence of polyunsaturated fatty acids, which are not synthesized by the host or the parasite, and by the presence of C20:1 which is synthesized by G. mellonella, but not by E. comstockii.

It is apparent that such ichneumonid parasites as E. comstockii and Itoplectis conquisitor could be resistant to any antimetabolites of fatty acid metabolism, which could possibly be developed for other taxa. The fatty acid compositions of these parasites can be varied to a great extent, without any apparent effects on the parasite.

The proposal that antimetabolites of fatty acid metabolism would prove effective as insecticides is based on the assumption that the characteristic fatty acid compsoition in insects is of physiologcal importance and that upsetting the balance of this composition would result in adverse effects. This was supported by studies which demonstrated that feeding cottonseed oil to the face fly and the housefly resulted in reduced fecundity and low order chemosterilization (Beroza and La Brecque 1967, Lang and Treece 1971). For years it has been known that discoloration occurs in eggs laid by hens fed cottonseed oil, extracted from plants of the order Malvales (Sherwood 1928), and that feeding cottonseed oil to animals causes hardening of their fats (Deuel 1955). Sterculic 8 (2 octyl-1-cyclopropenyl) octanoate, the cyclopropene derivative of oleic acid and malvalic 7 (2 octyl-1-cyclopropenyl) heptanoate acids

were identified as the constituents of cottonseed oil responsible (Masson, Varich, Heywong and Kemmerer 1957). I have recently demonstrated that cottonseed oil reduces the synthesis of palmitoleic acid and oleic acid in *L. sericata*. The proportion of the saturates, palmitic and stearic acids, increased and their corresponding monounsaturates decreased. This work has led to *in vitro* studies on the enzymes which synthesize fatty acids in an attempt to determine the metabolic origin of the fatty acid compositions characteristic of lepidopterous and dipterous insects.

Classical studies have shown that palmitoleic and oleic acids are synthesized by a single desaturating oxidase (Bloomfield and Bloch 1960), and generally an approximate ratio of 1:10 palmitoleic acid to oleic acid ratio results. This ratio is approximately the same in lepidopterous insects and the desaturase enzyme may be the same as that described in mammalian systems. Dipterous insects, however, generally have a 1:2 approximate ratio of palmitoleic to oleic acids, and the origin of these fatty acids may be different in this case. Three possible explanations for the different ratios are: a single enzyme system which has a different specificity than that of lepidopterous insects, two enzyme systems each responsible for the synthesis of one of the monounsaturates, and differing specificities in transacylase enzyme systems, which are responsible for incorporating free fatty acids into glycerides. Future studies should be carried out in attempts to further determine the physiological significance of the fatty acid composition, and the metabolic relationship responsible for the compositions in various insects.

SUMMARY

Dietary supplements of stearic, palmitoleic and linoleic acids, as representative of saturated, monounsaturated and polyunsaturated fatty acids respectively, did not appreciably affect the composition of fatty acids in wax moth larvae. Frass analysis demonstrated that most of the fatty acids in excess of their relative composition in the insect are excreted and speculations are made as to the possible mechanism involved in the retention of the characteristic pattern.

The fatty acid composition of parasitic wasps from various families of the Ichneumonoidea and Chalcidoidea appears to be influenced to varying degrees by the composition of their hosts, or diets. Qualitatively, each parasite is identical to its host. Quantitatively, the representatives studied from the family Ichneumonidae appear to be influenced to the greatest extent. The latter duplicate the fatty acid patterns of their hosts, and retain no characteristics that could be considered their own.

The insect parasitoid, *Itoplectis conquisitor* has no requirement for a specific fatty acid composition in its phospholipids. This composition varies markedly when the parasite is reared on different hosts. However, the parasite does exert a limited degree of quantitative control over this composition, and maintains the ratio of saturated/unsaturated fatty acids, such that the physical character of the phospholipids is maintained.

Radioisotope studies demonstrated that *Exeristes comstockii*, *Galleria mellonella* and *Lucilia sericata* incorporate ^{14}C from ^{14}C-1-acetate into fatty acids with radioactivity distributions consistent with *de novo*,

elongation, and desaturation synthetic mechanisms.

E. comstockii was found to incorporate ^{14}C-1-acetate into fatty acids at higher rates than either host. It appears, therefore, that the parasite has a fatty acid metabolism of its own, with respect to synthesis and turnover, although the fatty acids are qualitatively and quantitatively very similar to those in the host. The origin of the fatty acids of *E. comstockii* is partially explained by synthesis of fatty acids at different rates than its host, and direct incorporation of host fat, but the parasite appears to lack to a great extent the control over the pool size normally present in other insects.

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