Biosynthesis of 2-ketones in Drosophila buzzatii
by Paul Joseph Skiba

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry
Montana State University
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Abstract:
The analysis of pheromone biology in Drosophila is in its infancy, but promises to provide important information about many aspects of the signalling system which appear to have evolved during speciation. While there have been numerous studies on the identification and characterization of Drosophila pheromones, little was known about the biosynthesis of these important compounds.

Using radiolabeled acetate, the ejaculatory bulbs from mature male D. buzzatii were found to be the only tissues capable of biosynthesizing the 2-ketones in vitro. Although radiolabel was incorporated into lipids at all ages tested, label was not detected in pheromone components until the insects were four days post eclosion. The testes and the accessory glands, which are both closely associated with the ejaculatory bulb, did not incorporate label into the 2-ketones.

All of the subcellular fractions from the ejaculatory bulbs from mature males incorporated label from [1-14C]-acetate into the 2- ketones to some extent. However, the microsomes incorporated the most radiolabel into the 2-ketones, with about 7% of the recovered label present in 2-tridecanone and (Z)-10-heptadecen-2-one.

The use of specific heavy atom labeled fatty acyl-CoA esters allowed the biosynthetic pathways for the production of 2-ketones to be determined. The incorporation of [2H23]-lauroyl-CoA into 2-tridecanone and [l,2-13C]-palmitoleoyl-CoA into (Z)-10-heptadecen-2-one, indicated that D. buzzatii produce their 2-ketones via a type of chain elongation.

Furthermore, the incorporation of the fatty acyl-CoA esters in the presence and absence of the acetyl-CoA carboxylase inhibitor avidin indicated that the 2-ketones were biosynthesized by a microsomal chain elongation system and not by a microsomal fatty acid synthetase. The in vitro production of 2-tridecanone and (Z)-10-heptadecen-2-one by the microsomal fraction from ejaculatory bulbs required malonyl-CoA and lauroyl-CoA and palmitoleoyl-CoA, respectively.

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ABSTRACT

The analysis of pheromone biology in Drosophila is in its infancy, but promises to provide important information about many aspects of the signalling system which appear to have evolved during speciation. While there have been numerous studies on the identification and characterization of Drosophila pheromones, little was known about the biosynthesis of these important compounds.

Using radiolabeled acetate, the ejaculatory bulbs from mature male D. buzzatii were found to be the only tissues capable of biosynthesizing the 2-ketones in vitro. Although radiolabel was incorporated into lipids at all ages tested, label was not detected in pheromone components until the insects were four days post eclosion. The testes and the accessory glands, which are both closely associated with the ejaculatory bulb, did not incorporate label into the 2-ketones.

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The use of specific heavy atom labeled fatty acyl-CoA esters allowed the biosynthetic pathways for the production of 2-ketones to be determined. The incorporation of [$^{2}H_{23}$]-lauroyl-CoA into 2-tridecanone and [1,2-^{13}C]-palmitoleoyl-CoA into (Z)-10-heptadecen-2-one, indicated that D. buzzatii produce their 2-ketones via a type of chain elongation.

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INTRODUCTION

General

Chemical communication is an information channel that arthropods have nearly perfected. Chemicals participate in recruitment, defense, caste determination, sexual attraction, aggregation, and other behaviors. Insects also use chemicals for interacting with other arthropods, their plant and animal hosts, and their general environment. These chemicals, which convey information between organisms are called semiochemicals.

If a semiochemical conveys intraspecific information (i.e. aggregation or sexual attraction), it is called a pheromone. Therefore, pheromones are substances which are secreted to the outside by an individual and received by a second individual of the same species in which they elicit a specific reaction.

Pheromones are excellent transmitters of messages because they can pass around obstacles and are potentially capable of being detected at very low dilutions and so convey messages over long distances. They are also useful over extremely short distances, even to the level of contact chemoreception. In addition, their ability to be effective in very small quantities makes them energetically efficient, as does their ability to endure and to continue to convey their signal for extended periods, as in scent trails.

Bossert and Wilson (1963) reasoned that because of the nature of chemical communication, only a limited range of molecules would be suitable. They should have between 5 and 20 carbons and a molecular weight between 80 and 300. This was because, at low molecular weights, only a small number of molecules are feasible signals, while above a certain weight, the diversity of alternatives is more

than is required and the cost of synthesizing these molecules becomes progressively high. On the functional side, large molecules have lower volatility, which makes them unsuitable for certain roles.

The first pheromone identified was the sex pheromone of Bombyx mori, the silkworm moth (Butenandt et al., 1959). The elucidation of bombykol [(E,Z)-10,12-hexadecadien-1-ol] required twenty years and 500,000 female abdomens. Thirty years after the discovery of bombykol, hundreds of insect pheromones have been characterized and purified. Biological studies of their behavioral function and specificity have frequently revealed that a group of compounds, a pheromone blend, rather than a unique molecule are involved. These mixed compounds are usually structurally related.

Although all insect orders use pheromones for communication, the highly social Hymenoptera and Isoptera have developed the most complex and sophisticated pheromone systems. Among subsocial insects, pheromones have been shown to play major roles in the initiation of gregarious behavior during group oviposition among certain mosquitoes (Hudson and Mclintock, 1967) and the desert locust (Norris, 1963); mate attraction among species that maintain a solitary lifestyle(Inscoe, 1977); dispersal behavior among generally gregarious species during predator attack (Nault and Phelan, 1984); the synchronization of gamete maturity among species exhibiting aggregative behaviors (Blum, 1981, 1983); and the formation of aggregations at food sites, particularly among scolytid beetles (Birch, 1984) and Drosophila species (Bartelt and Jackson, 1984). An aggregation pheromone elicits behavior in conspecifics leading to an increase in their density in the vicinity of the pheromone source. Aggregation can benefit the individual insect in a number of ways including defense against predators, overcoming the resistance of a host, location of food, and mate selection.

Aggregation pheromones are widespread in insects, being found in cockroaches, social Hymenoptera (bees and wasps), and many beetles in addition to Drosophila.

Pheromones play a role in many important behavioral and physiological processes of Drosophila (Bartelt et al., 1985; Jallon, 1984; Brieger and Butterworth, 1970; Manning, 1967). These pheromones and the responses they mediate are adaptions that increase reproductive success or survival. All of the Drosophila species studied to date use intraspecific chemical communication to locate food, habitats, and mates. These pheromones are believed to play a role in the reproductive isolation of individual Drosophila species (Brieger and Butterworth, 1970).

Pheromone Biosynthesis

Initial studies on the biosynthesis of pheromones were begun in the 1970's when Kasang et al. (1974) induced female gypsy moths to synthesize tritiated dispalure and Jones and Berger (1978) succeeded in inducing female cabbage looper moths to produce ^{14}C-labeled (Z)-7-dodecenyl acetate by injecting [1-^{14}C]-acetate. Although many insect pheromones have been isolated and their structures determined, until recently relatively little was known about their biosynthesis (Bjostad and Roelors, 1983, 1984; Chu and Blomquist, 1980).

Lipids have assumed considerable functional significance during the evolutionary history of the class Insecta. They are essential structural components of the cell membrane and cuticle, they provide a rich source of metabolic energy for periods of sustained energy demand, they facilitate water conservation both by the formation of an impermeable cuticular barrier and by yielding metabolic water upon oxidation, and they include important hormones and pheromones.

Most insect pheromones are lipids and are believed to be derived from fatty acids. The chemicals used as pheromones exhibit considerable diversity, reflecting a variety of unique biochemical processes that occur in pheromone-producing tissues. The biosynthetic pathways for the pheromones from a number of species have been determined, and work is progressing toward description of the enzymes involved. From the results of studies to date, it appears that these specific and unique chemicals are produced by the addition of one or two ancillary enzymes to alter the products of "normal" metabolism, rather than the elaboration of an entire set of unique enzymes in the pheromone producing glands.

Over the past few years, a substantial amount of information of fatty acid derived pheromone biosynthesis in a number of Dipteran and Lepidopteran species has become available. The method used by most insects seems to be that of de novo synthesis, although food components may be used by some species (Blomquist and Jackson, 1979).

By characterizing which types of reactions are involved and in what order, a number of different biosynthetic pathways have been shown to exist Pennanec'h et al., 1992 for a review). There are a variety of common reactions, such as fatty acid synthesis, elongation, chain shortening, and desaturation. A few steps are specific, depending on the functional group involved.

Insects produce a vast number of compounds which are basically straight-chain, and can be formally derived from fatty acids. Most of these compounds probably originate in the classic acetate-malonate pathway.

Acetyl-CoA and malonyl-CoA are the basic building blocks used for the de novo biosynthesis of fatty acids in living organisms (Volpe and Vagelos, 1973, 1976). Acetyl-CoA normally arises in the cell as a product of the glycolytic cycle or by the ß-oxidation of fatty acids in the mitochondria. In addition, acetyl-CoA

can also be formed by direct esterification of acetate in the cytoplasm by acetyl-CoA synthetases. The synthesis of malonyl-CoA is catalyzed by acetyl-CoA carboxylases which condense carbon dioxide with acetyl-CoA in an ATP-dependent reaction. Fatty acids are then synthesized by condensation of two-carbon units from malonyl-CoA with a growing fatty acyl chain covalently linked to fatty acid synthase in eukaryotic systems; the release of carbon dioxide provides the driving force for the condensation reaction. The ß-ketoacyl derivative is reduced, dehydrated, and reduced again to form the fatty acyl derivative two carbons longer. The cycle is then repeated with condensation of another malonyl-CoA molecule with the elongated fatty acyl chain. Seven different activities are involved in the reaction catalyzed by fatty acid synthetase.

De novo biosynthesis of medium and long chain pheromones and of their fatty acyl precursors, especially palmitate and stearate from radiolabeled acetate have been documented in many insect species (Bade, 1964; Bjostad and Roelofs, 1984; de Renobales and Blomquist, 1984). These studies have shown the ability of insects to condense small carbon units such as acetyl- and malonyl-CoA to form medium size fatty acids, as it occurs in other eucaryotic organisms.

Insect fatty acid synthetases (FAS) have been isolated from the pea aphid, Acyrthosiphon pisum (Ryan, et al., 1982), and from two different fruit flies: Ceratitis capitata (Municio et al., 1979), and Drosophila melanogaster (deRenobales and Blomquist, 1984). In all three cases, the FAS activity was found in the 105,000 g particle-free supernatant. Acetyl-CoA and malonyl-CoA were substrates for the enzyme which in all cases required NADPH. Fatty acid products were all released in the free form. All of these insect FAS showed marked similarities with the few vertebrate enzymes which have been purified: complex structure, similar molecular weight, and a preference for NADPH.

The major proportion of the fatty acid complement of insect is represented by eight fatty acids. These are the saturated fatty acids: myristic acid, palmitic acid, and stearic acid; the monounsaturated fatty acids: myristoleic acid, palmitoleic acid, and oleic acid, and the polyunsaturated fatty acids, linoleic acid and linolenic acid. Examination of the fatty acids into which the label from [1-^{14}C]-acetate becomes incorporated indicates that the primary products of de novo fatty acid synthesis are palmitic, palmitoleic, stearic, and oleic acids Keith, 1967; Zebe and McShan, 1959).

Acetyl-CoA carboxylase contains covalently bound biotin as a prosthetic group, and catalyzes the carboxylation of acetyl-CoA to the three carbon compound malonyl-CoA. Malonyl-CoA then condenses with acetyl-CoA in a series of reactions catalyzed by the fatty acid synthetase complex to yield a four carbon butyryl intermediate. There follows a successive sequence of similar condensations with the growing acyl intermediate until an acyl chain of appropriate length has been achieved. It is evident that this pathway results in the production of fatty acids containing an even number of carbon atoms.

Following the synthesis of a long chain saturated fatty acid, the monounsaturated homologue may be formed by direct dehydrogenation (Bade, 1964; Lambremont et al., 1965;, 1966; Takaya and Miura, 1968; Stephen and Gilbert, 1969) and have demonstrated that, in locust this capacity resides within the microsomal fraction. Similar to other desaturases, the locust desaturase shows an absolute dependence on oxygen and requires reduced pyridine nucleotide (NADH being more active than NADPH), a finding that has been confirmed in Ceratitis capitata (Gonzalez-Buitrago et al., 1979).

Two insect orders have been the subject of intensive studies revealing two pheromone types which have different properties: volatile pheromones common

among Lepidoptera and contact pheromones common in Diptera. Most have aliphatic chains with a limited number of double bonds in a variety of positions and usually of the cis (Z) configuration. Volatile pheromones show chain lengths which are usually less than eighteen carbons and bear a functional group, very often an alcohol, acetate, aldehyde, or ketone. For contact pheromones, the chains are usually longer than twenty-one carbons and often very long, with an odd number of carbons and no functional group; most are linear hydrocarbons. These pheromones have been proposed to be biosynthesized by either a chain elongation pathway or a chain shortening pathway.

A large number of examples of derivatives of long chain fatty acids are found in other insect groups, particularly the Hymenoptera (Blum et al., 1983; Williams, et al., 1980; Graham, et al., 1979). The Dufour's gland and some other abdominal glands of ants often contain long chain fatty acid derivatives. Hydrocarbons and saturated acetate esters predominate, but unsaturated acetates are occasionally detected. The other group of compounds found commonly is ketones, particularly methyl ketones but including some ethyl ketones. The methyl ketones usually have odd-carbon chains and range from 2-tridecanone to 2-nonadecanone.

Biosynthetic studies of the Dufour's gland have not demonstrated conclusively the incorporation of acetate units into either hydrocarbons or the main chain of the oxygenated compounds, although rapid incorporation into the acetate portion of the esters is evident (Graham et al., 1979).

The exocrine secretions of many species of bee contain very similar compounds as found in ants. Alcohols are particularly common, including a number of methyl carbinols and the related methyl ketones.

Several of the compounds produced in the defensive secretions of termites,

particularly from the family Rhinotermitidae, are also similar. Among these are several compounds with unusual structures, including a nitroalkene, a series of conjugated enones, and enolic ß-ketoaldehydes (Spanton and Prestwich, 1981).

Apart from these examples, derivatives of long-chain fatty acids have been found in a wide variety of insects from different orders. In some cases the same compound can have very different functions in different insects; for example, hexadecyl acetate is a pheromone in Lepidoptera, but is found, in larger amounts, in the defensive secretions of beetles, ants and bees.

Chain Shortening Pathway

Many Lepidopteran sex pheromones share the following features: a long nonbranched carbon chain with an oxygen function (alcohol, aldehyde, ester) on one terminal carbon, and one or more double bonds, usually far removed from the oxygenated carbon. Species specificity is achieved by varying these characteristics (chain length, oxidation state, double bond site) and the mixing of two or more components in specific ratios. The structures of these sex pheromones suggested that their synthesis may be linked to or similar to that of fatty acids Roelofs and Wolf, 1988).

Although the structural characteristics of typical lepidopteran sex pheromones suggest an origin in either the fatty acid synthesis or fatty acid degradation patyhways, studies to confirm this and elucidate the details of the insertion of unsaturation, and modification of the functional group, have only been performed recently (reviewed in Roelofs and Brown, 1982).

Total lipid analysis of the sex pheromone gland of the redbanded leafroller moth, Argyrotaenia velutinana, shows that a wide range of saturated and unsaturated fatty acids is present, but the use of radio-labeled precursors has

demonstrated that the primary route for biosynthesis of the sex pheromones is via a chain-shortening pathway (Roelofs and Wolf, 1988). Essentially, hexadecanoic acid can be thought of as the initial precursor, which is first shortened, possibly by hexadecanoyl-CoA oxidase, to tetradecanoic acid. The double bond is then introduced by two desaturase enzymes, one producing the trans (E)-isomer and one the (Z)-isomer. The resulting acids are then reduced and acetylated to give two of the pheromone components, Z11-14:Ac and E11-14:Ac (Figure 1). The acid moieties are normally bound to glycerol, usually as triacylglycerols. The E/Z ratio of the tetradecenoyl moieties was found to be 61:39 in triacylglycerols, and approximately 31:69 in the phosphatides, contrasting strongly with the observed 9:91 ratio in the acetates emitted in the pheromone. No evidence was found for an isomerase which could correct the ratio of precursors, and the fate of the excess (E)-component is at the present time unknown, as is the way in which the final ratio of acetates is controlled.

A central problem of lepidopteran pheromone biochemistry, the control of component proportions within a given blend, is still a mystery. The specificity and relative quantities of the various enzymes of a biosynthetic sequence could play a major role in determining the proportions of homologous compounds in a mixture.

The stereospecificity of individual enzymes for certain substrates and products might explain the fixed proportions of geometric isomers found in species like Argyrotaenia velutinana. Most of the enzymes involved in biosynthesis have not been purified and protein chemistry studies are thus very limited.

Similar processes can be postulated to produce very many of the common constituents of Noctuid and Totricid sex pheromones although evidence for most of these steps is sparse. Many of the known double bond positions can be accounted for by the involvement of either the 11-desaturase detected in A.

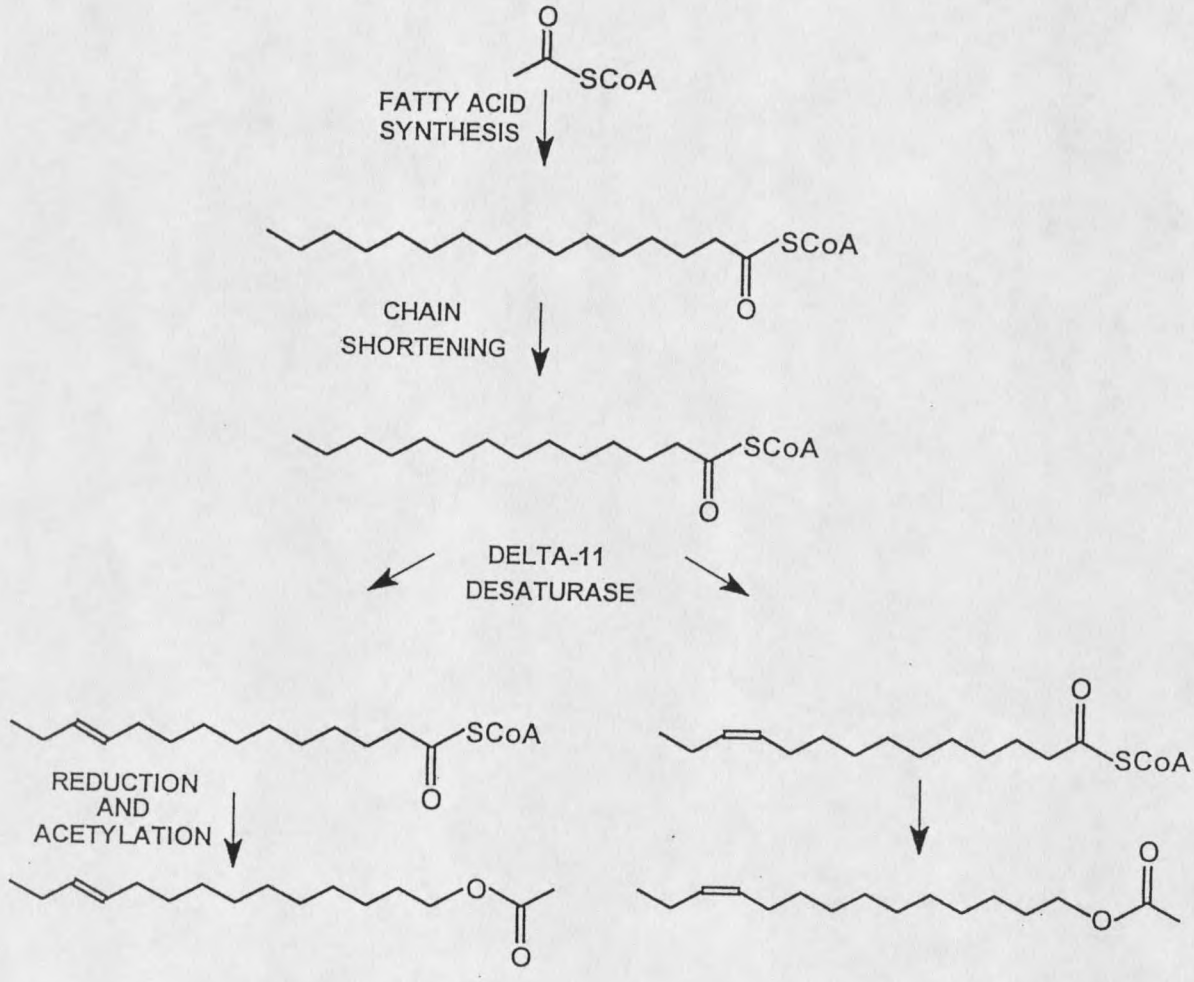

Figure 1. Biosynthesis of Z11-tetradecenyl acetate and E11-tetradecenyl acetate, the sex pheromone components of Argyrotaenia velutinana Bjostad and Roelofs, 1981).

velutinana (Bjostad and Roelofs, 1981), apparently unique to insects, or a 9-desaturase, examples of which are widespread, and which give rise, for example, to common fatty acids, oleic and palmitoleic. It is likely that, as more species are investigated, more diverse routes of biosynthesis will become apparent.

Chain shortening consists of the removal of one or more two-carbon units. Keith (1967) showed that D. melanogaster larvae reared on radioactive palmitic and stearic acids were able to shorten stearic acid to myristic acid but unable to shorten palmitic acid. The first in vivo evidence for chain shortening was found in Argyrotaenia citrana and Argyrotaenia velutinana where the pheromone gland was shown to be capable of shortening hexadecanoic acid to tetradecanoic acid (Bjostad and Roelofs, 1981). Further studies with cell free preparations from A. citrana showed that [U-^{14}C]-palmitate was converted to [^{14}C]-tetradecanoate while [1-^{14}C]-palmitate was not converted to labeled tetradecanoate (Wolf and Roelofs, 1983). In addition, in vivo studies on Trichoplusia ni (Bjostad and Rolofs, 1983) (Z)-11-hexadecenoate was chain-shortened to (Z)-9-tetradecenoate, which in turn was shortened to (Z)-7-dodecenoate, the immediate fatty acyl precursor to the pheromone, (Z)-7-dodecenyl acetate (Figure 2).

There is much evidence to suggest that the metabolic pathway for fatty acid oxidation in insects is the same as in other eucaryotes. The initial activation of fatty acid is catalyzed by the microsomal enzyme fatty acyl-CoA synthetase which has been demonstrated in several insect species (Hoskins, et al., 1956; Zebe and McShan, 1959; Domroese and Gilbert, 1964; Beenakkers and Henderson, 1967; Stevenson, 1972). In addition, ß-hydroxyacyl-CoA dehydrogenase and ß-ketoacyl-CoA thiolase have been demonstrated in mitochondrial preparations from several

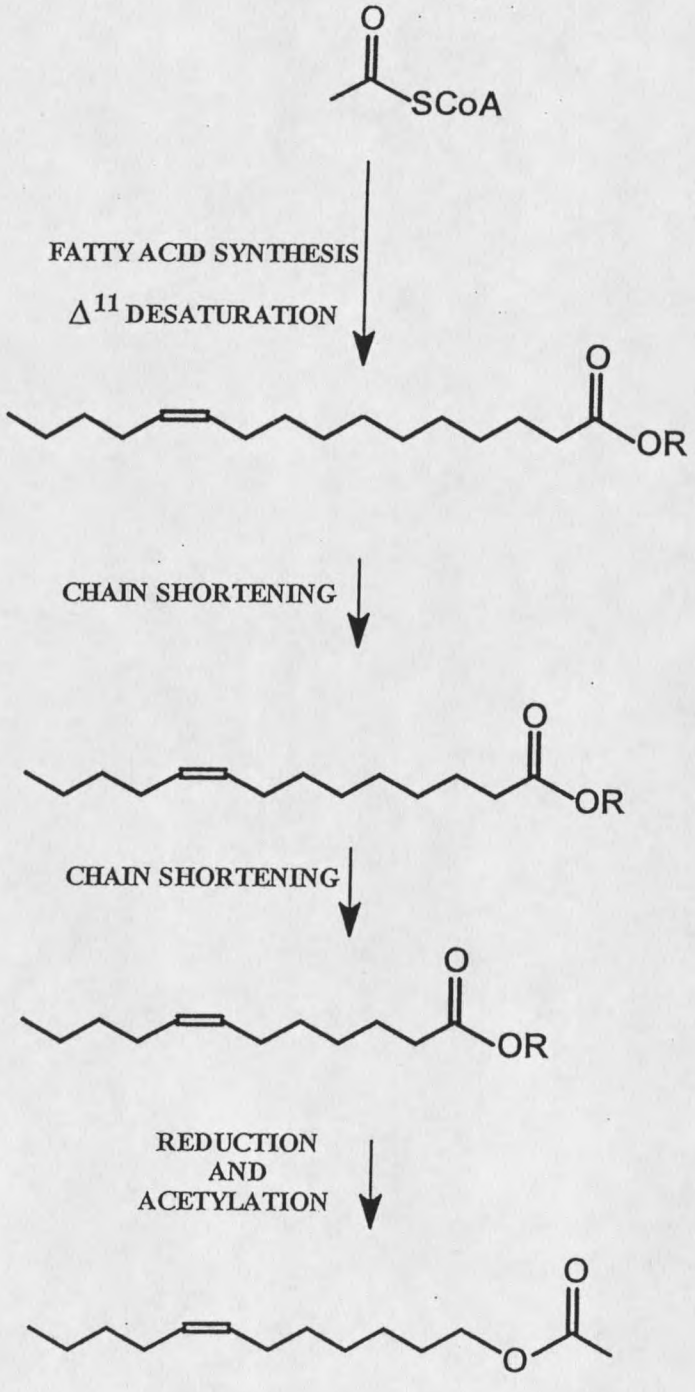

Figure 2. Biosynthesis of Z7-dodecenyl acetate, the sex pheromone of Trichoplusia ni (Bjostad and Roelofs, 1983).

species (Zebe and McShan, 1959; Beenakkers and Henderson, 1967; Crabtree and Newsholme, 1972), indicating that the complete complement of ß-oxidation enzymes are present in insects. Although the enzymatic mechanism of chain shortening in insects has not been studied, it appears to be similar to the ß-oxidation steps which occur in peroxysomes.

Chain Elongation Pathway

Fatty acyl elongation consists of successive additions of two carbon units to the medium size fatty acyl moieties which result from fatty acid synthesis. In some insects, the involvement of chain elongation has been shown to be involved in the biosynthesis of very long chain fatty acids which are precursors to hydrocarbons and acetates.

Only a few in vitro studies of the elongation reaction have been carried out. Gonzales-Buitrago et al. (1979) studied the microsomal elongation system of Ceratitis capitata, which utilized malonyl-CoA as a substrate.

Based on the structural similarity between cuticular hydrocarbons and the 2-ketones, it was suggested that the 27 and 29 carbon 2-ketones of the German cockroach may be formed from oxidation at the 2 position of pre-formed hydrocarbons, or alternatively from failure to reduce the carbonyl group during an elongation reaction by which the hydrocarbons would otherwise be synthesized (Jurenka et al., 1989).

Microsomes from Musca domestica were able to quickly transform stearoyl and oleoyl-CoA to fatty acids containing between 20 and 28 carbons (Vaz, et al., 1988a). The cofactors necessary for the elongation reaction are malonyl-CoA, and NADPH or NADH. Since fatty acids longer than 18 carbons are not usually observed in vivo, it appears that elongation and decarboxylation are closely

coupled.

To produce (Z)-9-tricosene and other cuticular hydrocarbons, long chain fatty acids have to be decarboxylated. The final decarboxylation of tetracosenoic acid to tricosene has not been directly tested, but a similar process has been demonstrated in termite and cockroach microsomes (Chu and Blomquist, 1980; Major and Blomquist, 1978). Very little is known about the decarboxylation reaction. It is suggested that a reductive decarboxylation through an alpha-hydroxy-intermediate takes place, by analogy with plants (Kolattukudy, 1976; Tchoy-Pek et al., 1986). All studies have found a condensation-reduction pathway similar to that found in bacteria, and have also described a strong link between elongation and decarboxylation (Jurenka et al., 1989; Chase et al., 1990; Juarez, et al., 1992).

3,11-Dimethyl nonacosanone, the sex pheromone of the German cockroach has been shown to be produced by a chain elongation reaction (Chase et al., 1990, Juarez, et al., 1992). This methyl branched ketone is formed by the insertion of methylmalonyl-CoA units in the microsomal chain elongation reaction (Figure 3).

As in other insects, much more is known about the biology and chemistry of dipteran pheromones than about their biosynthesis. Extensive biosynthetic studies on pheromones have been done only with the housefly, Musca domestica, and the pathways in this insect are now well understood (Figure 4). The sex pheromones of the housefly are closely related to the hydrocarbon components of the epicuticular wax layer. The biosynthetic pathways for cuticular hydrocarbons have been studied in a number of non-dipteran species and apply directly to dipteran sex pheromone biosynthesis (Blomquist and Dillwith, 1985).

The model systems for pheromone biosynthesis show several common steps involving similar enzymes: fatty acid synthesis, chain shortening, elongation,

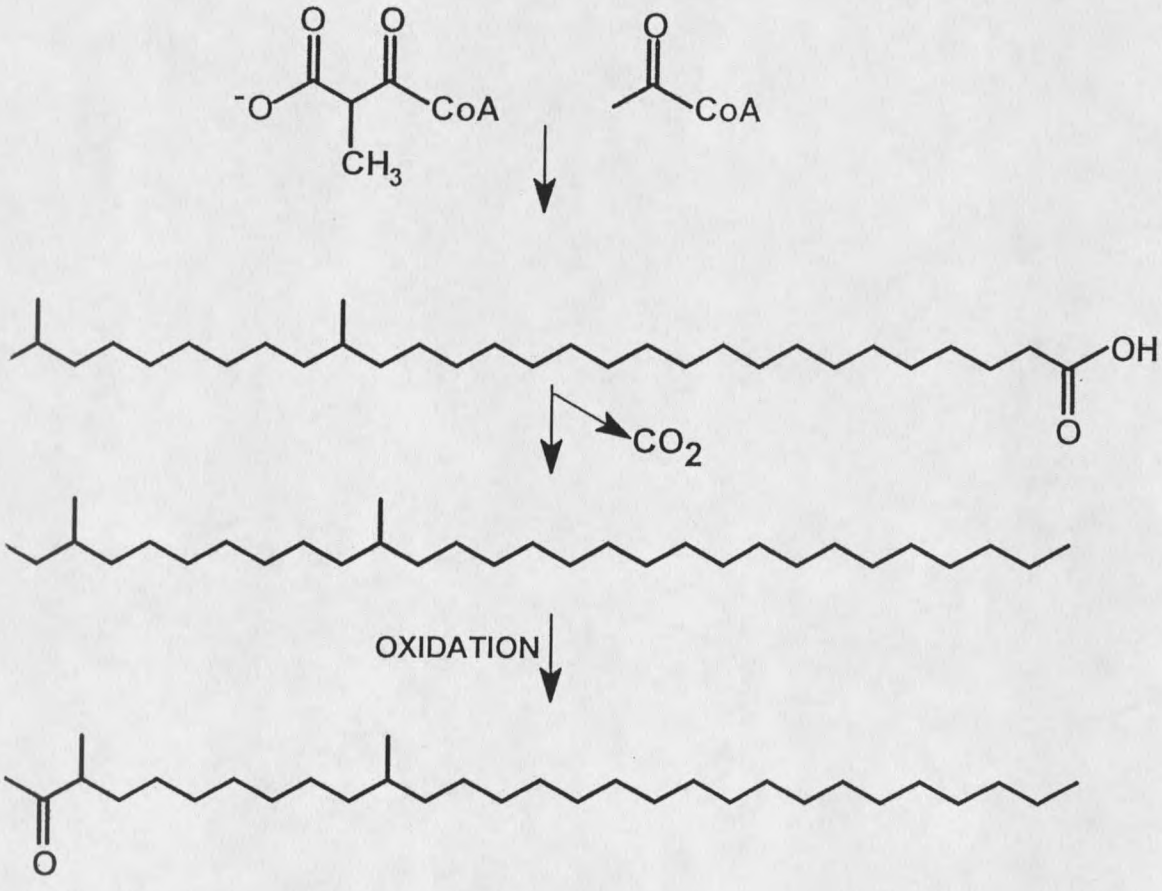

Figure 3. Biosynthesis of 3,11-dimethyl-2-nonacosanone, the sex pheromone of the German cockroach (Juarez, et al ., 1992).

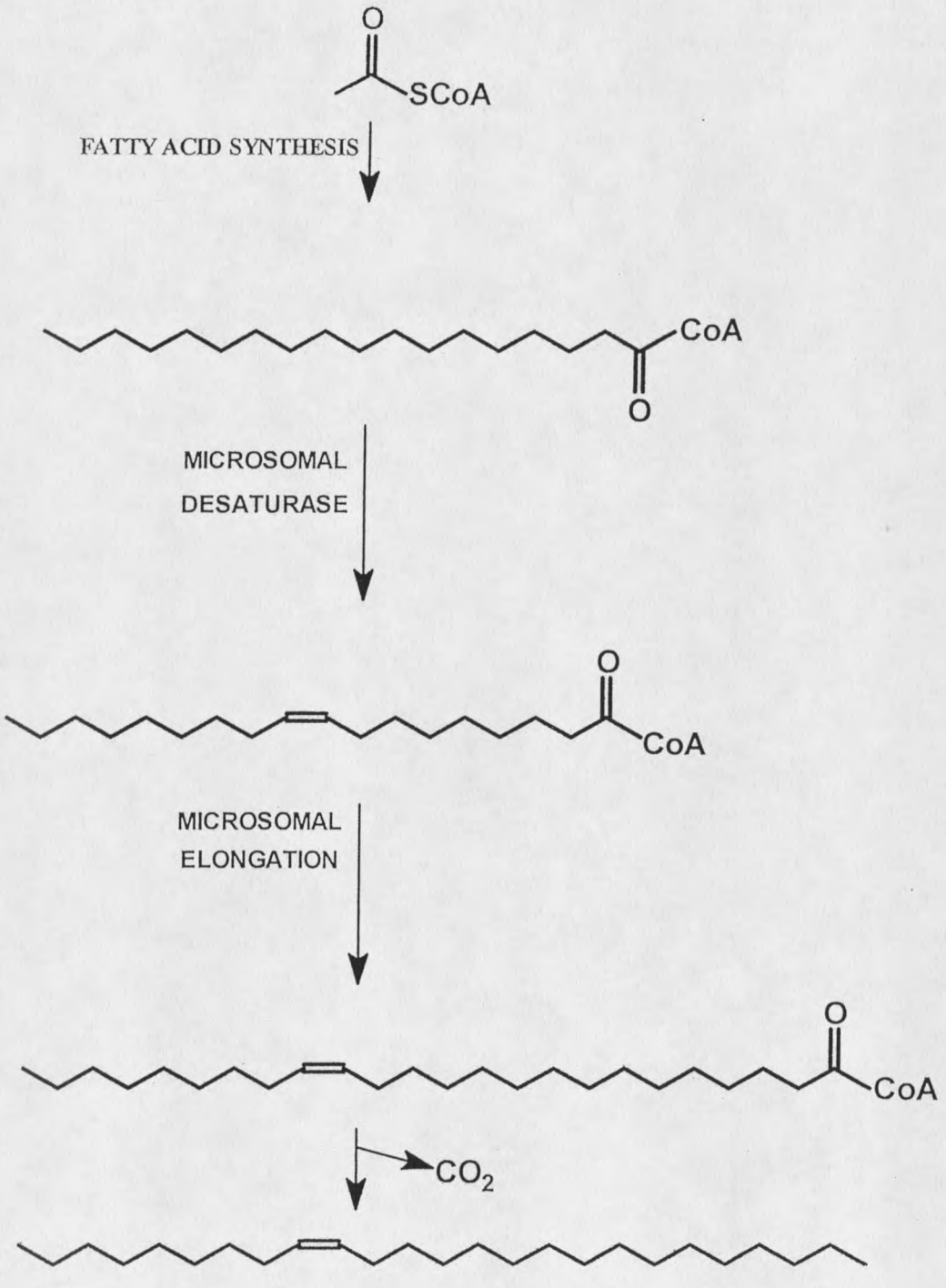

Figure 4. Biosynthesis of Z9-tricosene, the sex pheromone of the housefly (Vaz et al., 1988).

desaturation, and a few specific steps: decarboxylation, esterification, hydrolysis, oxidation, and reduction.

Drosophila Pheromones

Although a number of investigators have studied the involvement of pheromones in close range courtship behavior (Antony and Jallon, 1987; Antony et al., 1985; Jallon, 1984), less is known about how the flies initially come together in the field so that close range courtship stimuli can operate. The contribution of pheromones to this process had probably been overlooked because in most Drosophila species, feeding, mating, and oviposition occur at the same site and attraction to food odors could account for aggregation. However, in certain Hawaiian species, mating occurs in male mating territories away from the feeding site (Ringo, 1976). In these species, the males appear to emit pheromones which may be long range attractants. Further work (Bartelt, et al., 1985a, 1985b, 1986, 1988; Schaner, et al., 1987, 1989a, 1989b, 1989c, 1989d, 1989e) has shown that many of the Drosophila species which do not use separate feeding and mating sites, nevertheless, emit pheromones which contribute to the aggregation of individuals.

Just eight years ago, Bartelt ans Jackson (1984) demonstrated that aggregation behavior in Drosophila was regulated in part by chemicals produced by the flies. Since that first paper, the aggregation pheromones of over thirty species of Drosophila have been characterized and identified. The results are an exciting addition to the understanding of the chemical ecology, evolution, and molecular systematics of the species studied. The composition of the aggregation pheromones vary from single compounds that are common to a number of species to mixtures of a variety of components that are species-specific.

The use of aggregation pheromones to locate feeding sites and mating partners is probably a general phenomenon in the Drosophila . The presence of male-produced aggregation pheromones has been demonstrated in seven species of the D. virilis species group, in eight species of the D. melanogaster species group, and in six members of the D. repleta species group. In all cases, the pheromone attracts nearly equal numbers of both sexes and synergizes food odors.

Pheromones play a role in many important behavioral and physiological processes of Drosophila. These pheromones and the responses they mediate are adaptions that increase reproductive success or survival. All of the Drosophila species studied to date use intraspecific chemical communication to locate food, habitats, and mates. These pheromones are believed to play a role in the reproductive isolation of Drosophila species (Ewing, 1983) .

The pheromones produced by Drosophila species represent several different classes of compounds. Pheromone components can be hydrocarbons, both monounsaturated and diunsaturated, usually longer than twenty carbons. The functional groups identified in Drosophila thus far include tiglic esters (Bartelt et al., 1985), ketones (Moats et al., 1987), and acetate esters (Schaner et a:., 1989a, 1989e). The diversity of pheromone structure has been suggested to be responsible for the separation of species. At the chemical level, the biosynthesis of these pheromones has become so finely tuned that components are biosynthesized not only to a high degree of chemical purity, but geometrical and optical isomerism is also precisely controlled.

Mature males of the thirty Drosophila species studied to date produce relatively nonpolar aggregation pheromones which attract both males and females of any age (Table 1). The male-produced aggregation pheromones are synergistic with volatile compounds related to food odors and/or oviposition sites. These

chemical cues are not present in newly eclosed males or in virgin females of any age. However, over a two to three day period during sexual maturation there is a dramatic increase in the amount of pheromone stored in the male's ejaculatory bulb. In many Drosophila species, the pheromone components are stored in the male's ejaculatory bulb and transferred to the female during mating. The female later releases the pheromone onto the food and/or oviposition site (Schaner et al., 1989a, 1989b, 1989c).

Most of the species of the melanogaster group use the single component (Z)-11-octadecenyl acetate (ZVA) as their aggregation pheromone; however, in the evolution of the bipectanata complex there appears to be a mutation to production of (Z)-11-eicosenyl acetate which is two carbons longer. In addition, the pheromone perception by species studied in the bipectanata complex is not as specific as those in the melanogaster complex, since they will respond to both (Z)-11-eicosenyl acetate and (Z)-11-octadecenyl acetate (Schaner et al., 1989a; Schaner et al., 1989b).

Several Drosophila species produce two chemically different classes of chemicals in their aggregation pheromone blend. The aggregation activity of components of the two classes is usually synergistic. All species of the virilis group make esters of tiglic acid (mostly ethyl tiglate) as a low molecular weight component of their aggregation pheromone blend (Bartelt et al., 1985; Bartelt et al., 1986b). Most of the virilis subgroup also make a high molecular weight component, (Z)-9-heneicosene, as a coattractant (Bartelt et al., 1986b); however, it appears that when D. virilis split off, they developed the ability to make and respond to (Z)-10-heneicosene (Bartelt and Jackson, 1984). D. lummei and all of the montana subgroup lost the ability to produce the hydrocarbon component, but

Table 1. Phylogeny of the the melanogaster, virilis, and repleta groups and their aggregation pheromone components.

Species	Pheromone
melanogaster group	
suzuki subgroup	
rajasekarii	Z-11-18:Ac
melanogaster subgroup	
yakuba	Z11-18:Ac
simulans	Z11-18:Ac
mauritiana	Z11-18:Ac
melanogaster	Z11-18:Ac
ananassae subgroup	
malerkotliana	Z11-20:Ac
bipectinata	Z11-18:Ac
ananassae	Z11-18:Ac and Z11-20:Ac
virilis group	
virilis subgroup	
virilis	Z10-21 and ET
lummei	ET
novamexicana	Z9-21 and ET
americana	Z9-21 and ET
texana	Z9-21 and ET
montana subgroup	
borealis	ET
littoralis	ET
repleta group	
hydei subgroup	
hydei	2-tridecanone and ET
repleta subgroup	
repleta	2-tridecanone and ET
mulleri subgroup	
mulleri	(S)-2-tridecyl acetate
serido	(Z)-10-heptadecen-2-one
borborema	(Z)-10-heptadecen-2-one
buzzatii	(Z)-10-heptadecen-2-one

Z-11-18:ac= (Z)-11-octadecenyl acetate; Z-11-20:Ac= (Z)-11-eicosenyl acetate;

Z10-21= Z10-heneicosen; Z9-21= Z9-heneicosene; ET= ethyl tiglate

lummei and some species of the montana subgroup retained the ability to respond to both (Z)-9- and (Z)-10-heneicosenes which they do not make (Bartelt et al., 1988). These data suggest that production and perception of (Z)-9-heneicosene and ethyl tiglate are primitive traits for the virilis group. In speciation to virilis, there were mutations to production and perception of (Z)-10-heneicosene. However, in speciation to lummei and the montana subgroup, there was a loss of (Z)-9-heneicosene production, but not a loss of (Z)-9-heneicosene perception.

The hydrocarbon and ester components of the aggregation pheromone in all members of the virilis group are transferred directly to the food source by mature males (Bartelt, et al., 1985).The pheromone and food odors then act in concert to attract both males and females of any age. Virilis is the only group studied to date in which the pheromones are transferred to the food source directly by the male.

Although the production of tiglate esters in the pheromone blend extends into the repleta group (Moats et al., 1987), hydrocarbons have not been observed as pheromone components in this group. Instead, one or more 2-ketones and acetate esters of 2-alcohols are the major components of the aggregation pheromone in most species of the repleta group studied thus far (Bartelt et al., 1989; Schaner and Jackson.,1992).

In members of the mulleri subgroup of the repleta group, the ejaculatory bulbs contain saturated and unsaturated 13, 15, and 17 carbon 2-ketones and in some species (S)-2-tridecanyl acetate. The most abundant and most active component present in D. mulleri was (S)-2-tridecanyl acetate (Bartelt et al., 1989). This compound was unique as the first pheromone component with a chiral carbon observed in Drosophila. D. wheeleri and D. aldrichi also contain large quantities of (S)-2-tridecanyl acetate. Much smaller amounts of 2-pentadecanone,

(Z)-10-pentadecen-2-one, 2-heptadecanone, and (Z)-10-heptadecen-2-one were also observed in the ejaculatory bulbs of wheeleri and mulleri. Only the seventeen carbon ketones of mulleri were active in the pheromone blend.

In the martensis complex, D. martensis had nearly equal amounts of 2-tridecanone and (Z)-10-heptadecen-2-one in their ejaculatory bulbs. The (Z)-10-heptadecen-2-one had aggregation pheromone activity, while 2-tridecanone was not active (Schaner et al.,1992). D. buzzatii likewise had large quantities of 2-tridecanone and (Z)-10-heptadecen-2-one in their ejaculatory bulbs; however in this case, 2-tridecanone inhibited the aggregation activity promoted by (Z)-10-heptadecen-2-one (Schaner et al., 1992). This was the first observation of an ejaculatory bulb component from Drosophila inhibiting aggregation activity. The other two closely related species in the buzzatii cluster, D. serido and D. borborema, did not have 2-tridecanone in their ejaculatory bulbs, but only (Z)-10-heptadecen-2-one, which was their aggregation pheromone. 2-Tridecanone was present in most species of the mulleri cluster, but was not active as an inhibitor or as a component of the pheromone except in buzzatii.

The mulleri complex of the repleta group consists of 23 described species from six clusters. The buzzatii cluster consists of three South American species, D. buzzatii, D. serido, and D. borborema. Of the members of the mulleri complex, Drosophila buzzatii males store the largest quantities of 2-tridecanone (230 ng/male) and (Z)-10-heptadecene-2-one (690 ng/male) in their ejaculatory bulbs (Schaner and Jackson, 1992). From the study of insect pheromones to date, it appears that these specific and unique lipid molecules are biosynthesized via either the chain shortening or chain elongation pathways. While the biosynthesis of Drosophila aggregation pheromones has not been investigated, the 2-ketones made by males of the mulleri group could be produced by either one of these pathways.