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Pheromone component patterns of moth evolution revealed by computer analysis of the *Pherolist*

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Summary

1. The Pherolist internet site listing moth sex pheromone components reported in the literature was downloaded and processed by a BASIC program into a database with 2931 combinations of 377 unique chemical names of sex pheromone attractants used by 1572 moth species in 619 genera and 49 families. Names of pheromone compounds were analysed for aliphatic chain length, unsaturation position, geometric configuration, functional group (aldehyde, alcohol, acetate, epoxide, methyl-branched and hydrocarbon) and number of instances such combinations are used by species and families.

2. The analyses revealed pheromone blends of species ranged from one to eight components (45% species with one component, 36% two, 12% three, 5% four, 1% five, ≤ 0·5% for ≥ six). The numbers of different components of various chain lengths and functional groups, the numbers of instances such compounds are used by species and the numbers of species using such compounds are presented.

3. The average number of pheromone components per species increased as the number of species in a family increased based on linear regression of components in the 10 largest families, with species numbers ranging from 19 to 461. Pooling the four largest families gave a mean of 1·96 components per species that was significantly greater than the mean of the next 14 smaller families (1·63). Because related species in a large family would need more communication channels, this suggests that these species on average evolved to produce and detect more components in their pheromone blends to achieve a unique communication channel than was needed by species in smaller families.

4. Speciation in moths would entail evolutionary changes in both pheromone biosynthetic and sensory systems that avoided competition for communication channels of existing species. Regression analysis indicated that the more species in a family the more unique pheromone components, but the increase diminishes progressively. This suggests that, as the number of components increases with species number in a family, new species are more likely to evolve a unique blend comprising a communication channel from among existing components of the family.

Key-words: biosynthesis, computer program software, database, Lepidoptera, semiochemicals.

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Introduction

Pheromones are intraspecific communication signals that have been identified in animals ranging from bacteria and nematodes to mammals (Dunny & Leonard 1997; Lewis, Barbarosa & Gaugler 2002; Dulac & Torello 2003; Johnston 2003). More than 20 000 studies involve pheromones with the majority on insects (about 71%) and of these about 46% concern Lepidoptera, primarily moths (BIOSIS Previews® 1970–2004). Thus, more than 6000 research studies concern the chemical ecology of moths, due primarily to their importance in agriculture and forestry. On the internet, the *Pherolist* reports published knowledge of moth pheromone components and analogues used by more than 1683 species in 50 families (as of June 2002). The list was published first as a book (Arn, Toth & Priesner 1992)

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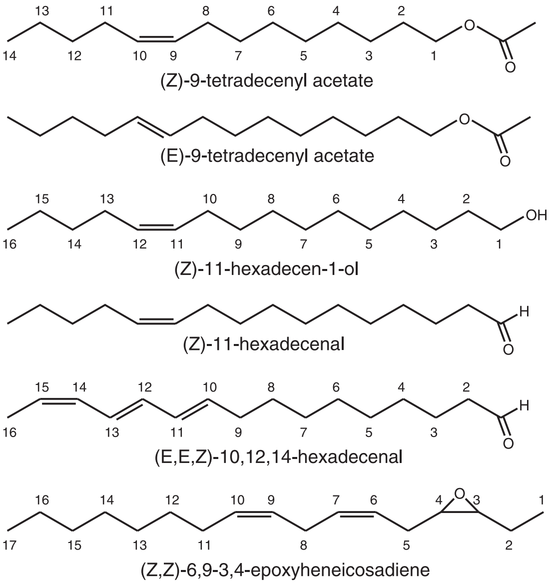

Fig. 1. Examples of moth pheromone components: (*Z*)-9-tetradecenyl acetate or Z9-14:Ac in commonly used abbreviated form (the most common component used by 199 species) (*E*)-9-tetradecenyl acetate or E9-14:Ac (component of 17 species) (*Z*)-11-hexadecen-1-ol or Z11-16:OH (most common alcohol, used by 50 species) (*Z*)-11-hexadecenal or Z11-16:Al (most common aldehyde, used by 119 species) (*E*,*E*,*Z*)-10,12,14-hexadecatrienal or E10E12Z14-16:Al (used by tobacco hornworm, *Manduca sexta*) and (Z,Z)-6,9–3,4-epoxyheneicosadiene or Z6Z9-3,4epo-21:Hy (epoxide hydrocarbon used by 14 species) (data compiled from *Pherolist*: Arn *et al.* 1992; Arn 2001).

and then assembled subsequently into a large website of html (hypertext markup language) files, with 693 such files on genera and species as well as 544 files on attractive components of species (Arn 2001). The *Pherolist* is organized into indexes of chemicals, families, genera, species and common names. In this study, only the chemicals that are characterized as being attractive to males were considered as sex pheromone components.

The majority of these pheromone components are related structurally, being unsaturated olefinic chains from 10 to 22 carbons with an end functional group (either alcohol, aldehyde or acetate). The positions of unsaturated, carbon–carbon double bonds can be in either the *E*- (*trans*-) or *Z*- (*cis*-) configuration, except at the end opposite the functional group (Fig. 1). Usually there are one, two and sometimes three unsaturations in the components of moths investigated to date (Byers 2002, 2005). Less commonly, moths have evolved olefinic epoxide pheromones (Millar 2000) by epoxidizing double bonds in the *cis*-configuration to yield (*R*,*S*) and (*S*,*R*) chiral forms (Fig. 1). The number of isomers of these olefinic chains is limited, so the number of communication channels comprised of single molecules that are available to moths may constrain their speciation (Byers 2005). However, blends of two or more components are found typically in moth species and these combinations are practically unlimited, although phylogenetic diversification might constrain biosynthetic systems and limit the evolution of new pheromone components during speciation (Byers 2005). Thus, the question arises of whether species in a taxon compete for use of the limited number of components that may comprise communication channels (Cardé & Baker 1984). An effect of such competition, based on combinatorial mathematics, could be that taxons with larger numbers of species have species that, on average, use more components per blend than smaller taxons. In addition, larger taxons may have relatively fewer different kinds of components on a per-species basis compared to smaller taxons.

Numerical summaries of pheromone component use by moths were not carried out previously because of the tedious and time-consuming nature of counting, for example, how many species use 14-carbon chain lengths (611 species, as described subsequently) or how many times 14-carbon chains are used by species (1006 instances). The relative counts summarizing patterns of use of conjugated or isolated unsaturations in all chain lengths or counts of *E*- and *Z*-configurations of monounsaturated components according to functional group is also difficult and confusing without the aid of computerized data-mining.

Thus, my objective was to develop a computer program that could search the *Pherolist* web site and automatically compile a separate database of linkages between chemicals, families and species. This database could in turn be searched methodically and summarized by computer algorithms to quantify patterns of pheromone component structure and use in moths. Some of the resulting patterns may show relationships between components and species per family that play a role in pheromone communication and the speciation process in moths (Linn & Roelofs 1995; Byers 2005). The emergent patterns may also provide insights into the ecological, evolutionary and physiological processes involved in moth pheromone communication.

Methods

The entire *Pherolist* website was downloaded (June 2002) to a personal computer for purposes of extracting data to be used in a separate database for off-line analyses. A program coded in QuickBASIC was made to search each of the 544 html files on chemical components for pertinent data. For example, the name of the compound was extracted from the title tag, (E,E)-8,10-dodecadien-1-ol of the html page (in short form, 8E10E-12:OH). Similarly, unique text strings were extracted that specified the family and genus species reported to be attracted to the component. A random access file of 2931 records was made containing record fields linking each chemical used by each species in a family/subfamily/tribe. Sex pheromone components of insects are either attractive alone to the responding sex, or must be presented together with at least one other component in a synergistic mixture, resulting in a greater attraction than the sum of the individual activities (Baker 1989; Byers 1992). Thus, it was assumed that the male-attractive compounds listed in the *Pherolist* are moth sex pheromone components characterized correctly by authors of peer-reviewed research articles.

A second BASIC program (available from the author) was made to view and search the random access file for various combinations of text strings, and excluded text strings that together indicate the chain length, functional group, chirality and the unsaturation configuration and position of chemicals, e.g. (Z,Z)-3,6-(9R,10S)-9,10-epoxyoctadecadiene. These data, in combination with species and family records, were counted and summarized by the program. Chain lengths from 5 to 10 carbons were determined if the compound name had any of the strings: 'pent', 'hex', 'hept', 'oct', 'non' or 'dec' but not 'pentadec', 'hexadec', 'heptadec', 'octadec' or 'nonadec', respectively. In addition, a chain length of 10 would contain 'dec' but not have any of the latter five strings just mentioned, nor 'undec', 'dodec', 'tridec' or 'tetradec'. Chain lengths 11–23 would contain each of the strings from 'undec' to 'nonadec' or 'eicos', 'heneicos', 'docos' or 'tricos'. Functional groups, or their lack, were determined by 'epox', 'methyl', '-one', 'oate' anywhere in the compound name and by 'nal', '-1-ol', 'acetate', 'ane' and 'ene' at the end of the name. Positions of unsaturations were recognized by strings of numbers (e.g. '-2,4,6-') enclosed with dashes, while configuration was determined by finding the first set of parentheses and looking for 'E' or 'Z'. Other determinations were whether the numbers represented conjugated or isolated double bonds and if they were odd or even numbers. Chirality was determined if 'R' or 'S' was found within parentheses. Alphabetical sorting of species and family lists, as well as ranking of counts, was performed with the *combsort* method of Lacey & Box (1991).

Searches of the database were conducted to count how many species use one, two, three or more components in the attractive sex pheromone. Similarly, the database was summarized by family. Counts were made of the numbers of different components of various functional groups and chain lengths reported in all species or for the families. Similarly, counts were made of the instances that various functional groups and chain lengths are used by moth species or families. Also, the numbers of species using various functional groups and chain lengths were counted. The numbers of instances were counted of all species using components with two unsaturations (conjugated or isolated) or three unsaturations (all conjugated, combinations of isolated/conjugated or all isolated). Similarly, the number of instances of all species were counted that use (a) monounsaturated components with configurations of *E* or *Z* in even or odd positions, (b) diunsaturated component instances of either (*E*,*E*) (*Z*,*Z*) (*E*,*Z*), or (*Z*,*E*) and (c) triunsaturated instances of either (*E*,*E*,*E*) (*Z*,*Z*,*Z*) or mixed *E* and *Z*. Other searches counted all instances or species using components with one, two or three unsaturations for various functional groups. Still other searches counted and ranked the most common components used by species as well as the number of families, genera and species per family. Some data sets were fitted to various equations using least-squares linear and curvilinear regression (Sokal & Rohlf 1995).

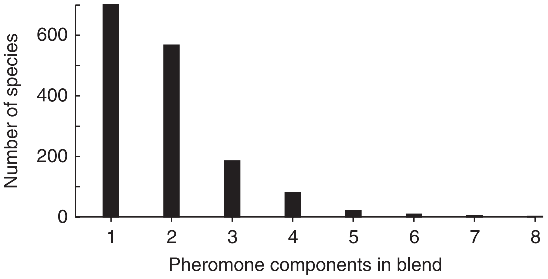

Fig. 2. Number of species with various numbers of reported components in pheromone blends used for long-range attraction.

Results

In the database there are 702 moth species reported to use at least one component in their sex pheromone, while 567, 185, 80 and 21 species have at least two, three, four and five component sex pheromones, respectively (Fig. 2). The average number of components reported per species is 1·86 ± 0·05 [± 95% confidence interval (CI)]. The species with the most reported components in their sex pheromone is *Heliothis virescens* (F) with 10 components, followed by *Adoxophyes* sp. and *Manduca sexta* (L.) with eight components, *Eucosma sonomana* Kearfott, *Euxoa messoria* Harris, *Mamestra brassicae* L., *Agrotis segetum* (Schiff.) and *Plutella xylostella* L. with seven components and *Cucullia umbratica* L., *Diaphania hyalinata* L., *Cydia pomonella* (L.), *Ostrinia nubilalis* (Hübner), *Eupoecilia ambiguella* Hübner and *Trichoplusia ni* (Hübner), with six components each.

There are 18 families reported to have at least 10 species each that range from 1·32 to 2·72 components on average per species of a family. The families in descending order are: Yponomeutidae (49 components/18 species = 2·72), Noctuidae (869/424 = 2·05), Pyralidae (210/105 = 2), Zygaenidae (33/17 = 1·94), Tortricidae (871/461 = 1·89), Geometridae (189/102 = 1·85), Cosmopterigidae (18/10 = 1·8), Gracillariidae (53/30 = 1·77), Sessidae (160/94 = 1·7), Sphingidae (22/13 = 1·69), Oecophoridae (27/16 = 1·69), Lymantriidae (30/19 = 1·58), Coleophoridae (55/36 = 1·53), Arctiidae (25/17 = 1·47), Tineidae (25/17 = 1·47), Argyresthiidae (23/16 = 1·44), Gelechiidae (87/66 = 1·32) and Pterophoridae (29/22 = 1·32).

In the 10 largest families with reported pheromones (19–461 species each), the average number of pheromone components per species in a family increases as a function of the number of species in the family (Fig. 3). The four largest families with more than 100 species each (total 1092) together have 2139 components that give an average of 1·96 components per species. This is

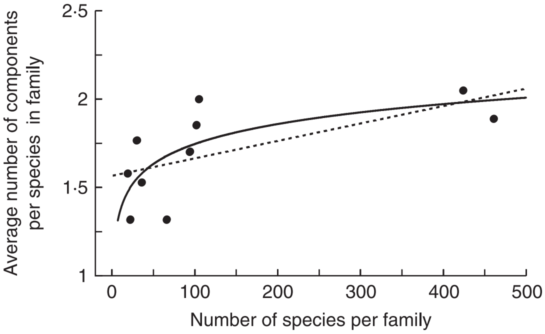

Fig. 3. Average number of components per species related to the number of species per family (10 largest families analysed with from 19 to 461 species each). The linear regression, Y = 1·57 + 0·000988X with R^2 = 0·39, P = 0·05. Logarithmic regression, Y = 0·997 +0·163ln X with an R^2 = 0·49; P = 0·02 gave a slightly better fit.

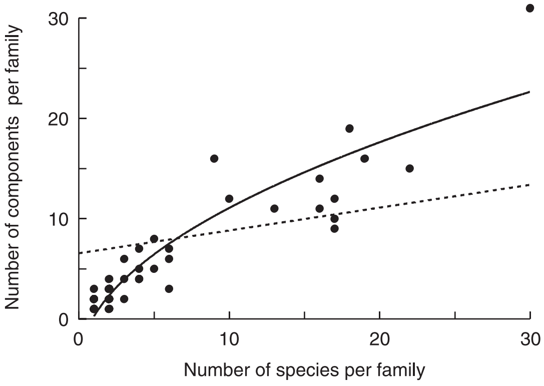

Fig. 4. Number of components per family related to the number of species per family (49 families analysed, with seven not shown above 30 species each). The curvilinear regression, Y = $5X^{-0·5}$ – 4·75 with R^2 = 0·86, P < 0·001, fitted the data better than a linear regression, Y = 0·2274X + 6·56 with R^2 = 0·76.

more than the 14 families with less reported species ranging between 10 and 100 species each (total 391) with 636 components and an average of 1·63 components per species. A comparison of the frequencies of the components and species for the four larger families (2139/1092) to the 14 families with less species (636/391) showed them to be significantly different (χ^2, P = 0·04).

The number of different components used reportedly by different families increases as a square root function of species number per family (Fig. 4). The function can be used to predict approximately the number of different components attractive to species of a particular family given the number of its species. As the number of components (X) in a family increases, the number of two-component combination blends (Y) that are available for communication channels can be calculated as X!/[2(X-2)!] or for three-component blends as X!/[6(X-3)!], which increase as a quadratic (Y = X2/2–X/2) or cubic (Y = X3/6 – X2/2 + X/3), respectively.

The numbers of different components reported in all moth species ranging in chain lengths from five to 23 carbons show that even-length chains from 12 to 18 carbons are the most prevalent (Fig. 5a). Chain lengths of 12 and 14 carbons are approximately equally abundant. The numbers of methyl-branched components are greatest at chains of length 17, and range from five to 19 carbons. The hydrocarbon components (no functional group) also are greatest at 17 carbon length chains, while epoxides peak at 19 carbons, with both types ranging from 17 to 21 carbons (Fig. 5b). The aldehyde, alcohol and acetate components have chain lengths that range from 10 to 18 carbons, with even chain lengths being most abundant (Fig. 5c). Aldehydes, alcohols and acetate are most abundant at 16, 12 and 14 carbons, respectively.

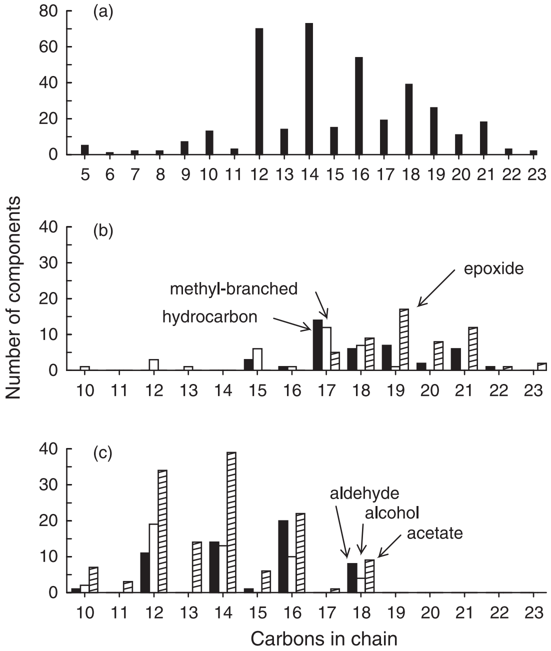

Fig. 5. (a) Number of unique pheromone components used by moths with various chain lengths from five to 23 carbons. (b) Number of components classified as hydrocarbon, methyl-branched or epoxides. (c) Number of components classified as aldehydes, primary alcohols or acetates.

The number of instances species have evolved to use components with chain lengths of five to 23 carbons is reported more often for even-length chains than for odd, with a peak at 14 carbons (Fig. 6a). Hydrocarbon chains occur about equally at 17, 19 and 21 carbon lengths while methyl-branched chains of 17 carbons are used most often. Epoxides are found in chains from 17 to 21 carbons, with 19 carbon chains used most frequently, followed closely by 21 carbon lengths (Fig. 6b). Aldehyde, alcohol and acetate components range from 10 to 18 carbons, predominately in even chain lengths, with peaks at 16, 12/14 and 16 for each functional group, respectively (Fig. 6c). The patterns in the usage of aldehyde, alcohol and acetate components by moths (Figs 5 and 6) are due mainly to four large families, the Tortricidae, Noctuidae, Pyralidae and Sesiidae, which together account for 78% of the instances for all 49 families.

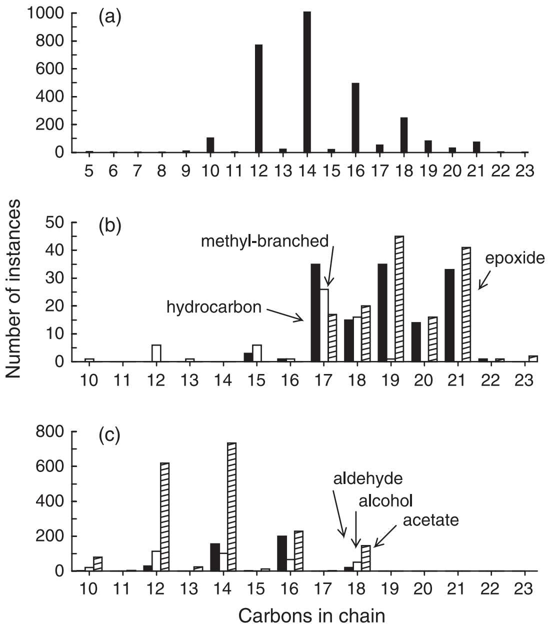

Fig. 6. (a) Number of instances of pheromone components with chain lengths from five to 23 carbons used by moth species. An instance is defined as a component-species use combination. (b) Number of instances of hydrocarbon, methyl-branched or epoxide pheromone components with chain lengths of 10–23 carbons used by moth species. (c) Number of instances of aldehyde, primary alcohol or acetate pheromone components with chain lengths of 10–23 carbons used by moth species.

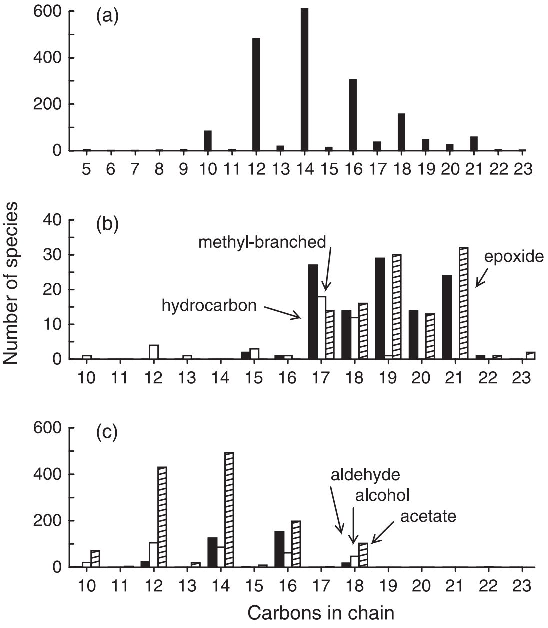

Fig. 7. (a) Number of species using pheromone components with chain lengths from five to 23 carbons. (b) Number of species using hydrocarbon, methyl-branched or epoxide pheromone components with chain lengths of 10–23 carbons. (c) Number of species using aldehyde, primary alcohol or acetate pheromone components with chain lengths of 10–23 carbons.

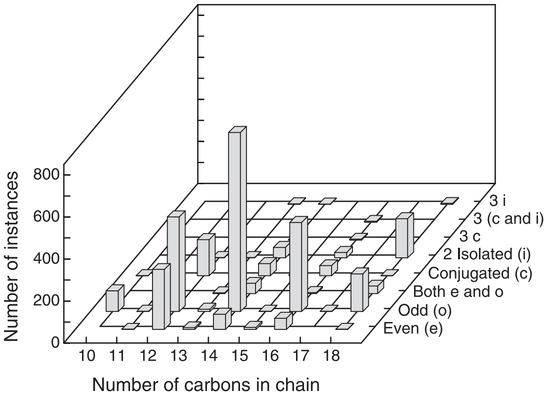

Fig. 8. Number of instances of moths using aldehyde, alcohol or acetate components with one or more unsaturations at even, odd or both even- and odd-numbered positions in carbon chains from 10 to 18 carbons. Similarly, the number of instances of moths using components with two unsaturations that are conjugated (c) or separated by a single bond, isolated (i) with more than a single bond separation, or with three or more unsaturations with mixtures of either conjugated or isolated positions.

Any particular component may be used by several species. For example, the most commonly used component is an acetate, Z9-14:Ac (199 species), followed by Z11-14:Ac (172), Z11-16:Ac (159), Z7-12:Ac (136), E11-14:Ac (126), an aldehyde Z11-16:Al (119), Z9-12:Ac (69), Z3Z13-18:Ac (64), Z8-12:Ac (60) and E8E10-12:Ac (54). Not surprisingly, the patterns for number of species that use components with chain lengths from five to 23 carbons (Fig. 7a) or hydrocarbons, methyl-branched chains and epoxides (Fig. 7b) or aldehydes, alcohols and acetates (Fig. 7c) are similar to the patterns for number of instances (Fig. 6). The numbers of instances reported are always greater than the numbers of species as a species could use Z7-14:Ac and Z9-14:Ac, counting as two instances of a 14-carbon chain length and as one species using such a chain length.

Pheromone components with unsaturation (double bonds) and an aldehyde, alcohol or acetate functional group range in chain length from 10 to 18 carbons (Fig. 8). The number of instances that moths use these components with a double bond in only even positions (430 instances) occurs most frequently in 12-carbon length chains (285 instances). The instances of component use with an unsaturation only in odd positions (2021) occurs most frequently for 14-carbon length chains (852), and is in all cases more numerous than instances of components having only even positions (Fig. 8; χ^2, $P < 0{\cdot}001$). The instances of conjugated double bonds (separated by a single bond) are most frequent in 12-carbon lengths (172), while isolated double bonds (separated by more than one single bond) are most usual in 18-carbon chains (187). In the same components with the functional groups above, the double bonds within the chain can take either of two configurations and be in either even or odd positions (Fig. 9).

The *Z*-configuration (*cis*) with 1553 instances is significantly more frequent than the *E* (*trans*), with 437 instances ($P < 0{\cdot}001$). The patterns for the higher frequency of odd positions compared to even positions

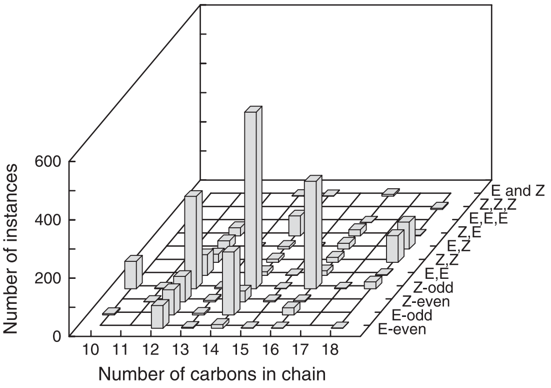

Fig. 9. Number of instances of species using aldehyde, alcohol or acetate components with one unsaturation at even- or odd-numbered positions with *E*- or *Z*-configurations or two unsaturations being (*E*,*E*), (*Z*,*Z*), (*E*,*Z*) or (*Z*,*E*) or with three unsaturations being all *E* or all *Z* or a mixture in carbon chains from 10 to 18 carbons. In components with two unsaturations, the lower position is given first.

(Fig. 8) are not altered by *E* and *Z* designations (Fig. 9). However, the *E*-even and *Z*-even instances are similar, and most frequent in 12-carbon length chains, while *E*-odd occurs less often (339) than the corresponding *Z*-odd (1415), but both are most usual in 14-carbon length chains (Fig. 9). Diunsaturated components are reported less frequently than monounsaturated ones. (*E*,*E*) is most common in 12-carbon chains (*Z*,*Z*) and (*E*,*Z*) in 18-carbon chains and (*Z*,*E*) in 14-carbon chains. However, the total instances for all chains of the four possible diunsaturated components are similar (105, 147, 154, 119, respectively, above), in contrast to the larger difference in monounsaturated *E* and *Z* frequencies (Fig. 9).

The 12 families with the most reported instances of aldehyde (Al), alcohol (OH) or acetate (Ac) components are shown according to the number of carbons in the chain length (Fig. 10). The Sesiidae utilize the most 18-carbon chain components (zero Al, 39 OH and 121 Ac), while the Tortricidae use the most 12-carbon (six Al, 71 OH and 373 Ac) and 14-carbon (70 Al, 53 OH and 274 Ac) length components. The Noctuidae most often use 16-carbon (101 Al, 43 OH and 140 Ac) length chains (Fig. 10). Only four of the 12 families have chains, with between 17 and 22 carbons with an epoxide group and various unsaturations (142 instances, Fig. 11). Arctiidae utilize only 21-carbon chains with an epoxide (eight instances), Geometridae use mostly 19-carbon chains (39 or 46%), Lymantriidae mostly 18-carbon chains (nine or 82%) and in Noctuidae 21-carbon chains are most common (18 or 47%). One other family, Amatidae, produces one epoxide of an 18-carbon chain. Moths use epoxides of the *cis*-configuration that are (*R*,*S*) or (*S*,*R*) and although *trans*-stereoisomers are possible as (*S*,*S*) or (*R*,*R*), they have not been found in moths (Millar 2000; *Pherolist*). About half the epoxides (all *cis*-) of moths have not been described with regard to chirality (*R*,*S*) or (*S*,*R*). It is striking that all unsaturated epoxide isomers are the *Z*-configuration.

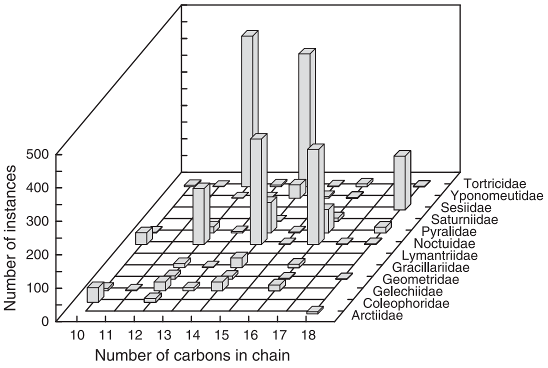

Fig. 10. Number of instances of species in the 12 moth families using the most aldehyde, alcohol or acetate components in 10–18 carbon chains.

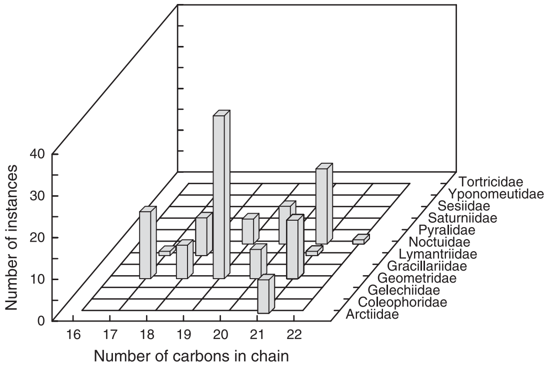

Fig. 11. Number of instances of species in 12 moth families (as in Fig. 9) using epoxide components in 17–22 carbon chains.

Discussion

There are an estimated 185 000 moth species (Holloway, Bradley & Carter 1987), from which about 377 pheromone components have been identified in 1572 species of 619 genera as of 2001 (Arn 2001; Byers 2005). These moth pheromone components are related structurally, the majority being acetates, alcohols or aldehydes of olefinic chains from seven to 22 carbons (mainly 12, 14 or 16 carbons). Only two species use chain lengths of 23 carbons (two epoxides by two species of Noctuidae), and one species of Lymantriidae uses tetracosane (24:Hy) and pentacosane (25:Hy) as pheromone components (*Pherolist*). It is surprising that a few species use these large compounds because of their relatively low volatility. Using the table and formula of Schlessinger (1971), saturated hydrocarbons of 24 and 25 carbons have vapour pressures at 15 °C that range from 3·2E-6 to 8·9E-7 mmHg, which is from 1275 to 4580 times less volatile than 14-carbon tetradecanal (used by three species). The compound Z9-14:Ac is used by 199 species and is between 39 and 142 times more volatile (Olsson *et al.* 1983) than 24:Hy and 25:Hy at 15 °C, respectively. Given that females of many moth species produce pheromone amounts in nanograms (Baker 1989), the males of lymantriid species would need to be more sensitive to minute quantities of 24:Hy and 25:Hy, or be attracted over shorter distances, than most other species using more volatile pheromone components.

The observed patterns of sex pheromone component occurrence in moths are probably related to underlying systems of biosynthetic enzymes with common phylogenetic origins as influenced by competition for limited communication channels. Pheromone components are biosynthesized in females from fatty acid precursors of 12–18 carbons converted to fatty acyl CoA esters. These are unsaturated by Δdesaturase enzymes of various numbered types (5, 9, 10, 11, 12, 13 and 14) corresponding to the position in the chain that is unsaturated (Bjostad, Wolf & Roelofs 1981; Foster & Roelofs 1988, 1996; Roelofs & Wolf 1988; Jurenka & Roelofs 1989; Zhao, Löfstedt & Wang 1990; Roelofs & Jurenka 1996; Jurenka 1997; Tillman *et al*. 1999). The unsaturated fatty acyl CoA esters then are chain shortened by *B*-oxidation in 2-carbon pieces from the CoA end (Jurenka *et al*. 1994). Thus, incremental chain shortening results in reducing the unsaturation position number by two, but maintains the odd or even attribute. The unsaturated fatty acyl CoAs can be reduced by a reductase to alcohols, at which time the *E* and *Z* ratios of components in some moths are configured, or when the alcohols are converted to acetates by acetyl transferase (Jurenka & Roelofs 1989; Tillman *et al*. 1999). The alcohols can be converted to aldehydes that in turn can be made into hydrocarbons and then to epoxides (Roelofs 1995; Tillman *et al*. 1999; Millar 2000).

Almost all the moth pheromone components discovered range from 10 to 21 carbons in chain length. The vast majority of these have chains of 12 (19%), 14 (19%), 16 (14%) or 18 (10%) carbons, but odd-numbered length chains still account for 29% of the components (epoxides of 17, 19 and 21 carbons make up 57% of the odd-length chains). These patterns of component use by chain length become altered when viewed in terms of the instances evolved in species. In this case, the proportion of species with odd-numbered chain lengths nearly disappears. Acetate functional groups are used relatively more than aldehydes and alcohols. Instances of moth usage of aldehyde, alcohol and acetate components with single unsaturations in odd numbered positions are much more numerous (82%) than instances of even ones (18%), and show a different pattern across the chain lengths. Instances of using components with two conjugated unsaturations are more common in 12-carbon chains, while two isolated double bonds are found usually in 18-carbon chains. Uses of components with a single unsaturation of *Z*-configuration are more common (78%) than those with an *E* (22%). However, when two unsaturations are found in the component there are no significant differences in the frequencies of *Z*,*Z* and *E*,*E* except for 18-carbon chains. The predominance of *Z*-configurations in moth components is not surprising, as fatty acid precursors with unsaturations are more common and more stable than *E*-forms in organisms (Lehninger 1970). The prevalence of unsaturations in odd-numbered positions is due to *Z*9-oleic acid as a precursor as well as the odd-numbered (5, 9, 11 and 13) Δdesaturases.

During speciation, not only would mutations in the genes coding the biosynthetic enzyme system of females need to occur but also in the sensory/behavioural system of males as constrained by phylogeny, interspecific competition for communication channels and limited numbers of possible chemical isomers (Byers 2005). As a family increased in number of species during evolution, there should be an increase in selection pressures on these related species to use more components in their pheromone blends in order to reduce mating mistakes, especially with sibling species. This hypothesis is supported by the increase in average number of components per species with an increase in species abundance per family based on a relatively wide range of species numbers (19–461) in 10 families. A linear relationship with a *y*-axis intercept above one component could mean that as families radiate, their species retain components of pheromone systems evolved earlier (all species have a pheromone with at least one component by definition). A linear function has the theoretical drawback, that as family size increases the mean components per species increases proportionally, which is unlikely as component blend combinations available for communication channels increase as a quadratic (two-component blends) or cubic (three-component blends) with an increase in total components. In this regard, a power function would seem more appropriate than a linear one, but might predict too low a number of components for small families.

When looking at all 49 families in terms of numbers of unique chemical components per family, the more species-rich a family is the more different kinds of components, but the increase is not proportional (decreasing curvilinear slope with increasing number of species). It appears that as the number of evolved components and species in a taxonomic group increases, it is more likely that a new species can evolve a unique blend from among existing components in the family by mutations affecting biosynthetic/sensory systems. The first ancestral species in a taxon must evolve at least one component, the second species to evolve is then constrained to produce a new component to avoid reproductive interference, while the third species can either evolve a third component or use a blend of the other two components. Furthermore, assuming that species of a small family can evolve easily to make any of 10 components and use them in two-component blends, then there are 45 unique blends possible, while species of a larger family with 50 components could make 1225 different two-component blends (Byers 2005). Thus, a species of the larger family undergoing speciation would have a higher probability of a successful biosynthetic/sensory mutation allowing evolution of new components in a free communication channel than would a species in the smaller family. In addition, species at low densities would have higher extinction rates when in competition with higher-density species using the same sex pheromone components.

The patterns and hypotheses presented here are based on pheromone components reported in 49 families, or about 79% of known moth families, and all the 16 superfamilies (Borror & DeLong 1971). Thus, the database coverage on moth pheromones encompasses much of the taxonomic breadth of moths, generally agreeing quantitatively with perceptions of common and rare families listed in Borror & DeLong (1971). The pheromone database undoubtedly contains errors in research reports due to false positives and incomplete analyses. For example, some of the 702 species reported to have a single component may have one or more additional ones that aid in attraction but simply have not yet been discovered. In spite of these errors and gaps in pheromone identifications of moths, the patterns of pheromone usage based on research over more than 30 years appear generally representative.

The 41 hydrocarbon, 54 epoxide, 55 aldehyde, 48 alcohol and 135 acetate components of moth pheromones as well as several million possible analogues (Byers 2005) can be drawn and viewed by software on the internet (Byers 2002). Since finishing this study, the *Pherolist* has changed management (Witzgall *et al.* 2005), while another database, the *Pherobase* (El-Sayed 2005), covers pheromones of all insect orders. The data-mining software as applied to the *Pherolist* serves as a model for revealing patterns of pheromone component use in moths among taxonomic groups. Further interpretation of the patterns in pheromone databases may aid in understanding the evolution of communication channels in moths and other insects.

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