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Evolution of olfactory receptor in oriental fruit fly *Dacus dorsalis*

(olfaction/receptor/kairomone/secondary plant substances/coevolution)

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ABSTRACT **Male oriental fruit flies (*Dacus dorsalis*) from colonies in Taiwan and Hawaii were evaluated for limit of response to various analogues of methyl eugenol. The results are interpreted in terms of the geometry and allosteric requirements of the antennal receptor that triggers the characteristic methyl eugenol reflex. This receptor has evolved for complementarity to all portions of the methyl eugenol molecule and responds only to *ortho*-substituted benzenes with adjacent oxygen atoms or isoelectronic equivalents. Substantial differences in responses of Taiwan and Hawaiian *D. dorsalis* suggest that perceptible evolution of the receptor protein has occurred during the past 50 years. A plausible scheme for the coevolution of dacini flies with plants containing phenylpropionoid essential oils is outlined.**

When exposed to nanogram quantities of 3,4-dimethoxyallylbenzene (methyl eugenol), a widely distributed phenylpropionoid essential oil, the male oriental fruit fly *Dacus dorsalis* Hendel undergoes a characteristic sequential response of searching with pulsating mouthparts, arrest, and compulsive feeding (1). This sensitive behavioral response to a simple chemical provides a useful tool for investigations of the physicochemical interactions between kairomone and antennal receptor, of the neurological complexities of insect behavior, and of the coevolution of a large and rapidly evolving family of Diptera (Tephritidae) with various plant families. Previously (1) we concluded that the depolarization of the olfactory sensillum resulted from a conformational change in the lipoprotein receptor involving the unshared electron pairs of the *ortho*-dimethoxybenzene moiety. In the present investigation, we have extended the mapping of the active site of the methyl eugenol receptor to evaluation of the optimal size and shape of the lipoprotein patch that is complementary in structure to the methyl eugenol molecule. We have also begun the exploration of coevolutionary development of Dacinae flies with phenylpropionoid essential oils, comparing responses of *D. dorsalis* in Taiwan and Hawaii.

METHODS AND MATERIALS

Attractancy of methyl eugenol and related chemicals was measured as described (1). Aliquots of liquid attractants were applied by microliter disposable pipettes to the center of 9-cm Whatman no. 1 filter paper discs. Dilutions of crystalline compounds or amounts of liquids below the microliter range were prepared in reagent grade acetone and applied to the test paper by microliter pipette. The filter paper discs were placed on squares of aluminum foil with the corners folded over the filter paper to prevent the fruit flies from contacting the underside of the test paper. The treated filter paper discs were placed on the floor of 30-cm^3 (1 ft^3) aluminum-screen cages containing approximately 100 male laboratory-reared *D. dorsalis* age 12–20 days beyond eclosion. At 1, 2, 5, 10, and 20 min after introduction of the test paper, the number of male flies attracted to the treated area was recorded and observations were made of the typical methyl eugenol responsive sequence (1). When a positive response was obtained at ≈10 mg of test compound, progressive dilutions were made until the approximate threshold concentration producing a positive feeding response [limit of response (LR)] was determined. Measurements were made at 26–28°C. Two types of evaluation were made: (*i*) determination of olfactory threshold (LR) for each compound exposed on a single filter paper in the test cage, and (*ii*) determination of olfactory preference within a group of related compounds exposed simultaneously at equal dosages on several filter papers in the test cage. Three to five cages of flies were used in rotation to avoid fatiguing the olfactory receptor mechanism, although no evidence of this was noted.

The chemicals for evaluation were checked for purity by thin-layer chromatography on silica gel (Merck G-254 F). Aldrich and Eastman "white label" grades were purified by recrystallization or redistillation when necessary. The remaining compounds were synthesized by conventional methods and were purified to the properties indicated in the tables by vacuum distillation or by recrystallization. Methyl eugenol (bp 104–105°C at 1 mm Hg) (1) was used as a standard at 10 μg to evaluate the response of each cage of male flies before further investigations were made.

RESULTS

Time Sequence of Methyl Eugenol Response. The immediacy of the male *D. dorsalis* response was remarkably constant and was clearly a function of the amount of compound present. When various amounts of methyl eugenol were exposed together in a single cage, large numbers of flies were observed to feed at the treated spots (Table 1). The 2-min response represented an optimal exposure period because of the loss of methyl eugenol at the lower dosages, through feeding by the flies. When plotted as logarithm of dosage vs. number of flies responding, these data gave an excellent demonstration of the Weber–Fechner law (2): $P = K \log S$ in which P = sensation and S = stimulus.

The reproducibility of response is shown by the mean (±SD) number of male flies feeding on 10 μg of methyl eugenol at the indicated interval, from five replicate evaluations in cages of 100 male *D. dorsalis*: 1 min, 37.8 ± 11.4; 2 min, 55.0 ± 6.3; 5 min, 74.4 ± 15.5; 10 min, 77 ± 3.8.

Effects of Vapor Pressure. The response of male *D. dorsalis* in the cage tests described was not materially affected by the vapor pressure of the test compound. Methyl eugenol (bp 248°C; LR <0.01 μg) was at least 100 times more attractive than *o*-dimethoxybenzene (veratrole) (bp 207°C; LR 1–10). *o*-Dipropoxybenzene (bp 234°C; LR 100), *o*-diisopropoxybenzene (bp 220°C; LR 50), and *o*-dibutoxybenzene (bp 241°C;

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Abbreviation: LR, limit of response.

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Table 1. Number of male *D. dorsalis* responding to various concentrations of methyl eugenol

Time, min	No., by methyl eugenol concentration				
	0.1 μg	0.3 μg	1.0 μg	3.0 μg	10 μg
0.5	5	13	12	22	28
1	8	17	19	25	50
2	6	15	20	50	70
5	4	9	18	40	>70
10	3	12	20	40	>70
Mean ± SEM	5.2 ± 1.9	13.2 ± 3.0	17.8 ± 3.4	35.4 ± 11.6	57.6 ± 18.7

LR 3000) had similar boiling points but different LR. The independence of response and vapor pressure is demonstrated by the three isomeric dimethoxybenzenes: *ortho*, bp 207°C, LR 1–10; *meta*, bp 216°C, LR >10,000; and *para*, bp 213°C, LR 100.

Nature and Position of Ring Substituents. Of the 10 sets of *ortho-*, *meta-*, and *para*-substituted methoxybenzenes (Table 2), exposure to 6 sets produced the typical methyl eugenol reflex in male *D. dorsalis*. In all of these sets the *ortho*-substituted methoxybenzene was 10- to 100-fold more attractive than the *meta* or *para* isomer. The second substituent (and LR, in μg, for the active compounds in parentheses) were: CH_3O (1.0) < OH (10) < $OC(O)CH_3$ (100) = NH_2 (100) < $C(O)OCH_3$ (1000) = CH_2OH (1000). The only other active compounds investigated were *ortho*-substituted methoxybenzenes in which the second substituent was CH_3S (10), $N(CH_3)_2$ (1000), and $CH(CH_3)_2$ (10,000). The substituted methoxybenzenes in which the second substituent was *ortho, meta,* or *para* Cl, CH_3, NO_2, or COOH or *ortho* CH_3SO, CH_3SO_2, $N^+(CH_3)_3$, $C(O)CH_3$, or C_6H_5 were completely unattractive at a dosage 10^4 greater than the LR for *o*-dimethoxybenzene (Table 2).

These data conform to the previous hypothesis (1) that the attractants for male *D. dorsalis* trigger a conformational change in the receptor protein of the olfactory sensillum and this results in the depolarization of the sensory dendrite. The highly active attractants all contain a paired-electron-rich O atom together with an adjacent O atom, or its electronic equivalent S or N atom, containing other unshared electron pairs. The critical nature of the second unshared electron pair is demonstrated by the inactivity of *o*-methylsulfinylmethoxybenzene (CH_3SO) and *o*-methylsulfonylmethoxybenzene (CH_3SO_2) in which the electrons on the second substituent are coordinated with additional oxygen atoms and of the quaternary ammonium compound *o*-trimethylammoniummethoxybenzene [$(CH_3)_3N^+$] in which the electron pair of the *o*-dimethylaminomethoxybenzene is also shared.

The appreciable attractancy of *o*-methoxybenzyl alcohol (CH_2OH) and methyl *o*-methoxybenzoate [$C(O)OCH_3$] at first seemed anomalous. However, study of Fisher–Hirschfelder–Taylor molecular models of these *o*-CH_3O compounds showed that the O atoms one carbon atom removed from the benzene ring can assume configurations that place the two ether O atoms in almost identical spatial relationships to those of *o*-dimethoxybenzene (Fig. 1). The importance of two ether O atoms is shown by the complete inactivity of *o*-methoxyacetophenone [$C(O)CH_3$]. The inactivity of *o*-methoxybenzoic acid is also surprising and may be associated with the altered electronic character in the ionizable C(O)O group.

As shown by the inactivity of the methylenedioxybenzene (—OCH_2O—) compounds previously evaluated (1), the adjacent electron-rich O atoms must be freely rotatable in order to depolarize the olfactory sensillum. The methyl eugenol isosteres 3,4-dimethylallylbenzene and 3,4-dichloroallylbenzene previously found to be inactive were reevaluated against both Hawaiian and Taiwan flies and were completely inactive at 10,000 μg. These compounds at 10,000 μg had no effect on male fly response to 10 μg of methyl eugenol.

The consistently low LR associated with *ortho*-substituted methoxybenzenes is not difficult to explain because of the probable coevolutionary development of *D. dorsalis* with plant species containing the kairomone *o*-dimethoxyallylbenzene or methyl eugenol. Thus, the antennal receptor is completely complementary to the structure of methyl eugenol. In a previous investigation of the isomeric dimethoxyphenyl acetates (1), the lowest LR was found with the 3,4-dimethoxy isomer, whose configuration most closely approximates that of methyl eugenol. The 2,5-dimethoxy isomer was more attractive than the 2,6-dimethoxy isomer and the 3,5-dimethoxy isomer was inactive. From the data on the isomeric sets of substituted methoxybenzenes (Table 2) it can be generalized that the LR values for the *ortho* isomers are less than those for the *para* isomers and that the *meta* isomers are generally inactive. The exceptions are *m*-methoxyphenyl acetate and *m*-methoxyaniline, which seem to be definitely more attractive than the respective *para* isomers. This must result from configurational peculiarities of these two sets of compounds that are not yet obvious.

The presence of two adjacent ring substituents is essential for a substantial degree of attraction of the male flies. The following monosubstituted benzenes were completely unattractive at 10,000 μg: CH_3O, CH_3, Cl, NO_2, NH_2, OH, COOH, and $CH_3C(O)O$. Dimethylaniline [$(CH_3)_2N$] showed a feeble re-

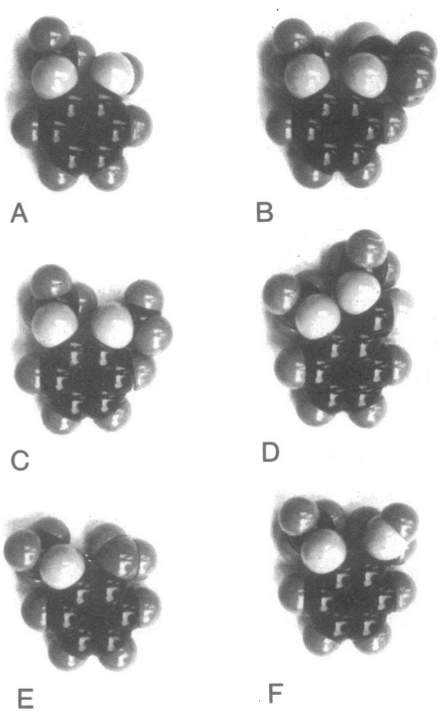

FIG. 1. Molecular models of active attractants showing appropriate juxtaposition of electron-rich O atoms (light color). (*A*) *o*-Methoxyphenol; (*B*) *o*-methoxyphenyl acetate; (*C*) *o*-dimethoxybenzene; (*D*) *o*-methoxymethylbenzoate; (*E*) *o*-methoxyaniline; (*F*) *o*-methoxybenzyl alcohol.

Table 2. Response of male *D. dorsalis* (Hawaiian) to substituted benzene derivatives

Compound	bp or mp, °C	LR, μg	Feeding response
$CH_3O—C_6H_4—OCH_3$			
I. *ortho*	bp, 207	1.0	+++
II. *meta*	bp, 216; mp, 55–60	>10,000	0
III. *para*	bp, 213; mp, 56–60	100	+
$CH_3O—C_6H_4—OH$			
IV. *ortho*	bp, 204–206	10	++
V. *meta*	bp, 113–115 at 5 mm Hg	>10,000	0
VI. *para*	bp, 243	1,000	+
$CH_3O—C_6H_4—OC(O)CH_3$			
VII. *ortho*	bp, 95–96 at 0.6 mm Hg	100	+++
VIII. *meta*	bp, 100–102 at 0.6 mm Hg	1,000–10,000	0
IX. *para*	mp, 32	>10,000	0
$CH_3O—C_6H_4—C(O)OCH_3$			
X. *ortho*	bp, 93–94 at 0.7 mm Hg	1,000	++
XI. *meta*	bp, 98 at 1.6 mm Hg	>10,000	0
XII. *para*	mp, 47–48	>10,000	+
$CH_3O—C_6H_4—CH_2OH$			
XIII. *ortho*	bp, 248–250	1,000	+
XIV. *meta*	bp, 255	>10,000	0
XV. *para*	bp, 258	>10,000	0
$CH_3O—C_6H_4$-NH_2			
XVI. *ortho*	bp, 225	100	+
XVII. *meta*	p, 251	1,000	+
XVIII. *para*	bp, 240–250	>10,000	0
$CH_3O—C_6H_4—COOH$			
XIX. *ortho*	mp, 98–100	>10,000	0
XX. *meta*	mp, 102–104	>10,000	0
XXI. *para*	mp, 182–185	>10,000	0
$CH_3O—C_6H_4—CH_3$			
XXII. *ortho*	bp, 170–172	>10,000	0
XXIII. *meta*	bp, 175–176	>10,000	0
XXIV. *para*	bp, 174	>10,000	0
$CH_3O—C_6H_4—Cl$			
XXV. *ortho*	bp, 195–196	>10,000	0
XXVI. *meta*	bp, 193	>10,000	0
XXVII. *para*	bp, 198–202	>10,000	0
$CH_3O—C_6H_4—NO_2$			
XXVIII. *ortho*	bp, 272	>10,000	0
XXIX. *meta*	mp, 35–37	>10,000	0
XXX. *para*	bp, 274; mp, 50–52	>10,000	0
$CH_3O—C_6H_4—SCH_3$			
XXXI. *ortho*	bp, 84–85 at 1.5 mm Hg	10	++
$CH_3O—C_6H_4—SOCH_3$			
XXXII. *ortho*	mp, 44	>10,000	0
$CH_3O—C_6H_4—SO_2CH_3$			
XXXIII. *ortho*	mp, 90–91	>10,000	0
$CH_3O—C_6H_4—N(CH_3)_2$			
XXXIV. *ortho*	bp, 74–76 at 1 mm Hg	1,000	++
$CH_3O—C_6H_4—N^+(CH_3)_3I$			
XXXV. *ortho*	dec.	>10,000	0
$CH_3S—C_6H_4—OH$			
XXXVI. *ortho*	bp, 103–104 at 21 mm Hg	10,000	+
XXXVII. *meta*	mp, 29	>10,000	0
XXXVIII. *para*	mp, 83–85	>10,000	0
$CH_3O—C_6H_4—C(O)CH_3$			
XXXIX. *ortho*	bp, 90–92 at 1 mm Hg	>10,000	0
$CH_3O—C_6H_4—CH(CH_3)_2$			
XL. *ortho*	bp, 62–65 at 1 mm Hg	10,000	+
$CH_3O—C_6H_4—C_6H_5$			
XLI. *ortho*	mp, 28–29	>10,000	0

sponse at 10,000 μg, and methyl benzoate [$CH_3OC(O)$] at 10,000 μg produced an evident feeding response in both sexes, indicating a different type of response.

Hydrophobic Interactions. The insect olfactory sensillum is generally considered to contain a lipoprotein patch (3). This suggests that lipophilic molecules should be more strongly adsorbed at the receptor site than hydrophilic molecules, provided that they possess the requisite stereochemical features. The series of substituted anisoles (Table 2) provides a range of octanol/water partition values (4, 5) suitable for evaluating this

Table 3. Response of male *D. dorsalis* (Hawaii) to *ortho*-dialkoxybenzenes

Benzene cmpd.	bp or mp, °C	LR, μg	Feeding response
o-Dimethoxy	bp, 225	1	+++
o-Diethoxy	mp, 43–45	5	+++
o-Dipropoxy	bp, 102–105 at 0.5 mm Hg	100	+++
o-Diisopropoxy	bp, 85–92 at 1 mm Hg	50–100	+++
o-Dibutoxy	bp, 125–128 at 0.7 mm Hg	10,000	+

hypothesis. The calculated Π values [logarithm (octanol/water partition)] for these compounds range from −3.85 for *o*-trimethylammoniumanisole to 4.07 for *o*-phenylanisole; neither of these was active in stimulating the methyl eugenol reflex (Table 2). The Π values for the eight active molecules ranged from 1.08 for *o*-$CH_3OC_6H_4CH_2OH$ to 2.72 for *o*-$CH_3OC_6H_4SCH_3$. There was no correlation of Π with LR. This lack of correlation is not unexpected because the requirement for allosteric interaction with the O atoms of the 3,4-dimethoxyphenyl moiety of methyl eugenol is of critical importance in determining activity.

Inasmuch as the aliphatic side chain of methyl eugenol ($CH_2CH{=}CH_2$) has a complementary receptor conformation, analysis of the role of Π in determining the activity of the 4-substituted veratrole molecules is more informative. Compounds evaluated ranged from Π = 1.06 (CH_2OH) to Π = 3.64 (C_3H_7) and the correlation between Π and LR for the 10 available compounds, $r = 0.62$, was statistically significant ($P = 0.05$). Thus, it appears that the methyl eugenol receptor contains a specifically localized lipophilic area that is structurally complementary to the allyl moiety of methyl eugenol.

Size of Alkoxy Groups. This was evaluated with the *o*-dialkoxybenzenes as shown in Table 3. In individual cage tests the LR increased progressively: methyl < ethyl < propyl = isopropyl ≪ butyl. This effect was clearly shown in the preference tests (Table 4). It appears that the methyl eugenol receptor is flexible enough to accommodate alkoxy groups of increasing Van der Waals' radii from methyl (2.0 Å) to propyl (6 Å) but the activity fell off markedly with butyl. This supports the hypothesis that interaction with the receptor lipoprotein patch occurs through the paired ether O atoms and that steric hindrance from larger aliphatic moieties decreases the efficiency of contact.

DISCUSSION

A few of the compounds evaluated here have been examined for attractancy by Beroza and Green (6) who evaluated the vapor attractancy as lures for trapping and by Lee and Chen (7) who used olfactometry from alcohol solution. These authors used attractancy indices (from 1 to 4) based on numbers of insects caught in traps and did not evaluate the male feeding response. Their results appear to be less precise than the olfactory thresholds (LR) used in the present investigation. However, the data are comparable to ours in that compounds **I-*o*** and **II-*o*** were highly attractive in both studies, **I-*p*** and **II-*p*** were moderately attractive, and **VIII-*o*, -*m*,** and **-*p*** were poorly attractive. Compounds **V-*o*** and **-*p*** were also rated as poorly attractive. The only serious discrepancy was in the anisic acids, **VII-*o*, -*m*,** and **-*p***, which in agreement with our study were found to be poorly attractive (6). However, Lee and Chen (7) reported **VII-*p*** as highly attractive, **VII-*m*** as moderately attractive, and **VII-*o*** as not attractive. This is a curious discrepancy in view of the total inactivity in our tests with flies from both Taiwan and Hawaii at high dosages.

These results suggest the value of assaying the olfactory threshold based on the total methyl eugenol reflex of attractivity, arrest, and feeding for precise evaluation.

Table 4. Preference tests of male *D. dorsalis* for methyl eugenol and various structural elements

Test	No. of flies attracted at various times			
	1 min	2 min	5 min	10 min
Taiwan, 1 μl				
Methyl eugenol	16	29	33	56
Methyl isoeugenol	4	5	10	12
3,4-Dimethoxypropylbenzene	1	3	7	5
Hawaii, 10 μl				
Methyl eugenol	>50	>50	>50	>50
Methyl isoeugenol	11	18	18	30
3,4-Dimethoxypropylbenzene	5	17	20	26
Dimethoxybenzenes				
Taiwan, 10 μl				
ortho	12	22	25	11
meta	0	0	0	0
para	0	0	0	0
Hawaii, 10 μl				
ortho	18	28	30	36
meta	0	0	0	0
para	0	1	0	0
o-Dialkoxybenzenes				
Taiwan, 1 μl				
CH_3O	8	13	28	22
C_2H_5O	4	2	5	5
C_3H_7O	0	0	0	6
iso-C_3H_7O	0	0	0	0
C_4H_9O	0	0	0	0
Hawaii, 10 μl				
CH_3O	75	72	70	54
C_2H_5O	9	17	24	7
C_3H_7O	12	16	19	6
iso-C_3H_7O	14	14	18	21
C_4H_9O	0	0	0	1

Evolution of Receptor. The available evidence indicates that the male *D. dorsalis* has evolved an antennal receptor specifically tailored to respond to methyl eugenol. Evaluation of >4000 organic compounds for attractivity to *D. dorsalis* showed none that were more attractive (6) or had a lower olfactory threshold (1) than methyl eugenol. Simultaneous exposure of the male flies to equal concentrations of compounds incorporating structural features or modifications of methyl eugenol (Table 4) showed a marked preferential response to all parts of the methyl eugenol molecule. Thus, methyl eugenol (LR, 0.01 μg) with the $-CH_2CH{=}CH_2$ side chain was strongly preferred over methyl isoeugenol (LR, 0.1) with $CH{=}CHCH_3$ and over 3,4-dimethoxypropylbenzene (LR, 0.01–0.1) with $-CH_2CH_2CH_3$. With the isomeric dimethoxybenzenes, the male flies showed exclusive preference for the *ortho* configuration as found in methyl eugenol (LR, 1 μg) over *meta* (>10,000) and *para* (100). The preference for the size of *ortho* alkoxy groups was clearly for methoxy (LR, 1 μg) > ethoxy (LR, 5 μg) > propoxy (LR, 100 μg) = isopropoxy (LR, 50–100 μg) ≫ butoxy (LR, 10,000 μg) (Table 3).

Despite this specific receptor design for methyl eugenol there were interesting differences in behavior between *D. dorsalis* from Taiwan where the fly was introduced in 1911 and from Hawaii where the fly was introduced in 1944. The Hawaiian flies were very responsive to *o*-methylthioanisole (LR, 10 μg) and fed avidly on this compound but Taiwan flies of the same age showed absolutely no response to as much as 10,000 μg of

this compound. Both populations of flies showed excellent response to methyl eugenol and to veratrol. Similarly, the Hawaiian flies responded to *o*-aminoanisole (LR, 100 μg) but the Taiwan flies did not respond to 10,000 μg. The Hawaiian flies responded well to *o*-dipropoxybenzene (LR, 100 μg) and to *o*-diisopropoxybenzene (LR, 50–100 μg) but the responses of the Taiwan flies were very slight. These differences are clearly shown in the preference tests of Table 4. In general, it appeared that the receptor of the Hawaiian flies was somewhat more elastic and responsive to a greater range of structural modifications of methyl eugenol than was that of the Taiwan flies. This suggests that the evolution of the methyl eugenol receptor is proceeding more rapidly in the Hawaiian strain than in the Taiwan strain.

Coevolution of Dacinae with Plants Producing Phenylpropionoids. Sexually mature males of the Dacinae (family Tephritidae) are strongly attracted to phenylpropionoid essential oils from a wide variety of plants. *D. dorsalis* males and closely related species areknown to be attracted to and feed at blossoms, fruits, and leaves of at least 14 species of plants in 10 families from which methyl eugenol has been isolated (1, 7–11). The melon fly *D. cucurbitae*, which does not respond to methyl eugenol, was found to be strongly attracted to a synthetic chemical anisyl acetone or *p*-methoxyphenyl-3-butanone (12); subsequently it was shown that *p*-acetoxyphenyl-3-butanone and its hydrolytic product *p*-hydroxyphenyl-3-butanone were superior as attractants (13). This latter compound has been isolated and identified as an important flavor constituent of the raspberry (14).

Drew (15) recently surveyed the Dacinae of the South Pacific for attraction to either methyl eugenol or *p*-hydroxyphenyl-3-butanone (Willison's lure) or its acetyl ester (cue-lure). Males of 79 species of Dacinae collected segregated into 23 species responding to methyl eugenol and 56 species responding to Willison's lure or cue-lure. No species responded to both types. The segregation of responses conformed substantially to morphologically determined taxonomic divisions within the Dacinae—i.e., *p*-hydroxyphenyl-3-butanone attracted species of both *Callantra* and *Dacus* and of subgenera *Dacus* and *Strumeta* whereas methyl eugenol attracted only species of the *Strumeta* subgenus. Thus, these olfactory responses clearly have evolutionary significance.

From considerations of plant evolution, the formation of both methyl eugenol and *p*-hydroxyphenyl-3-butanone can be traced back to a common precursor, the widely distributed *p*-hydroxycinnamic acid. Methyl eugenol can be formed from *p*-hydroxycinnamic acid by reduction of COOH, *ortho* hydroxylation, and subsequent O methylation (16). *p*-Hydroxyphenyl-3-butanone can be formed from *p*-hydroxycinnamic acid by conjugation of the SCoA derivative with malonyl-CoA to form 5-(*p*-hydroxyphenyl)-3-ketovaleric acid followed by decarboxylation to the 3-butanone as suggested for the analogous formation of zingerone or (3-methoxy-4-hydroxyphenyl)-3-butanone (17). It seems probable, therefore, that the primitive Dacinae coevolved with plants containing *p*-hydroxycinnamic acid and, as speciation progressed, two subgroups segregated, one responding to methyl eugenol and the other to *p*-hydroxyphenyl-3-butanone. Evolution was accompanied by definitive changes in the specificity of the antennal olfactory receptors of the two groups. Thus, *D. cucurbitae* (*Dacus* subgenus) is not attracted by 3,4-dimethoxyphenyl-3-butanone (12) which is highly attractive to *D. dorsalis* (*Strumeta* subgenus). Conversely, *D. dorsalis* responds only weakly to *p*-methoxybenzyl alcohol, but 3,4-dimethoxybenzyl alcohol was about 1000 times more attractive (1). It is pertinent to the above speculation that *p*-methoxycinnamic acid was reported to be moderately attractive to both *D. dorsalis* and *D. cucurbitae* (7).

Both methyl eugenol and *p*-hydroxyphenyl-3-butanone attract only male Dacinae, and scanning electron microscope studies of the scape of the antennae of *D. dorsalis* suggest that the male has specific sensilla for detection of methyl eugenol that are lacking in the female. The evolutionary significance of this difference has been subject to speculation. It has been suggested (11, 18) that the males respond to these kairomones because of fortuitous chemical resemblances to male-aggregating or female sex pheromones. However, this notion is improbable in view of the preceding discussion. It is much more plausible from the viewpoint of coevolution to suppose, as suggested by Bush (19), that host plant odors act as secondary sex attractants or rendezvous stimulants for the Tephritidae, to bring the sexes together in the environment of a suitable host plant.

The coevolution of the Dacinae with plants containing these widely distributed essential oils has led to the evolutionary development of specific olfactory sensillae complementary in structure to specific phenylpropionoids. The widespread distribution of such primitive secondary plant products may be a major factor in promoting speciation of the Dacinae and in their exploitation of very wide host ranges. The Tephritidae are believed to have originated during the Paleogene era, about 65 million years ago, as feeders on decaying fruits (20) and the family is still undergoing rapid evolution. The differences in olfactory perception found in our study of Taiwan and Hawaiian flies seem to indicate the importance of structural evolution of the olfactory mechanism in the development of the host-seeking pattern and range.

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