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DEVELOPMENTAL AND ENVIRONMENTAL SOURCES OF PHEROMONE VARIATION IN *Colias eurytheme* BUTTERFLIES[1]

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Abstract—Body size, age, ambient temperature, wing wear, and flight activity were investigated as possible sources of variation in the quantities and relative proportions of the three chemical components [*n*-heptacosane (C27), 13-methylheptacosane (13MH), and *n*-nonacosane (C29)] of the male courtship pheromone of *Colias eurytheme* butterflies. Size of the male has very little influence on the amount of any of the pheromone components present on the wings. Most of the deposition of all three components onto the surface of the hindwing occurs between 3 and 9 hr after emergence from the pupa. 13MH is deposited more rapidly than C27 and C29, and C27 more rapidly than C29. After the first 12 hr posteclosion, the pheromone phenotype of an individual male remains relatively constant through at least 96 hr of age. Experiments showed that none of the three chemicals volatilize to any appreciable extent at temperatures likely to be experienced in the field. The pheromones of actively searching and courting males did not differ from those of less-active feeding and resting males, suggesting that volatilization induced by flight activity is not an important source of pheromone variation. Loss of scales with age does seem to affect pheromone phenotype, but not in a readily interpretable way. Although the quantity of 13MH was lower in worn males than in fresh, C27 was higher.

Key Words—*Colias*, Lepidoptera, Pieridae, courtship pheromone, pheromone variation, pheromone production, cuticular hydrocarbons, *n*-heptacosane, 13-methylheptacosane, *n*-nonacosane.

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INTRODUCTION

Female *Colias eurytheme* (Pieridae) are able to recognize conspecific males using both ultraviolet reflectance patterns and the male courtship pheromone as cues (Silberglied and Taylor, 1973, 1978; Taylor, 1973). The pheromone is located on the dorsal surface of the hindwing (Silberglied and Taylor, 1978; Rutowski, 1980) and consists of *n*-heptacosane (C27), 13-methylheptacosane (13MH), and possibly *n*-nonacosane (C29) (Grula et al., 1980). These hydrocarbons are found commonly in the epicuticular wax layer of many different insects (Sappington, 1989). Surveys of pheromone composition of males from several populations of *C. eurytheme* have shown that both the quantities and relative proportions (blends) of the three components vary greatly among individuals (Sappington, 1989; Sappington and Taylor, 1990a). Disruptive sexual selection on the courtship pheromone imposed by the two female morphs (orange and alba) (Sappington, 1989; Sappington and Taylor, 1990a) is probably responsible for maintaining some of the observed variation, but part of it derives from developmental and/or environmental sources (Sappington and Taylor, 1990a,b).

Many developmental sources of variation in pheromones and hydrocarbons have been identified in various insects. Age is frequently cited. In the rice stem borer, *Chilo suppressalis* (Pyralidae), quantities of the female pheromone decrease daily over the first three days posteclosion (Usui et al., 1988). The amount of pheromonally active (*Z*,*Z*)-9,19-alkadienes in female *Pikonema alaskensis* (Hymenoptera) increased from 10.2 μg at eclosion to 25.0 μg at 5 days old, while the amount of (*Z*,*Z*)-9,19-dienes remained constant with age (Bartelt et al., 1984). Young male rove beetles (*Aleochara curtula*) have hydrocarbon profiles similar to those of females and are therefore unattractive to them (Peschke, 1987). Antony and Jallon (1982) found that the level of surface hydrocarbons remain constant in virgin females from 3 to 5 days old in *Drosophila melanogaster*. In *D. virilis*, the quantity of hydrocarbons increases over the first four days of adulthood in both sexes and continues to increase to 8 days of age (Jackson and Bartelt, 1986). In addition, the average chain length of hydrocarbons decreases as the flies age, and two hydrocarbons change differentially depending on sex after 4 days of age. Other *Drosophila* species (Bartelt et al., 1986), the fleshfly *Sarcophaga bullata* (Jackson et al., 1974), and the milkweed bug *Oncopeltus fasciatus* (Jackson, 1983) also show age-related changes in hydrocarbon profiles. Kuwahara (1979) presented evidence that the ratio of the two major components of the courtship pheromone of *Pieris melete* varies with age of the individual.

Body size is not correlated with pheromone component quantities in *Chilo suppressalis* (Usui et al., 1988) or in *Aleochara curtula* (Coleoptera) (Peschke, 1987). However, Anderbrant et al. (1985) suggested that differing degrees of larval competition in *Ips typographus* (Coleoptera) may contribute to intraspe-

cific variation in the quality and quantity of the aggregation pheromone through its effects on the weight and fat content of the emerging adults.

It is possible for temperature to differentially affect the rate of volatilization of various components of a surface pheromone. For example, female rove beetles (*Aleochara curtula*) maintained at 27°C for 14 days show a greater loss of cuticular alkenes than of saturated hydrocarbons (Peschke, 1987).

In this study, we examined the effects of age, body size, ambient temperature, wing wear, and flight activity on the pheromone phenotypes of male *C. eurytheme*.

METHODS AND MATERIALS

Pheromones of individual males were extracted with hexane and were analyzed using gas chromatography. Procedural details may be found in Sappington and Taylor (1990a). The pheromone characters quantified for each male included micrograms of C27, 13MH, and C29; C27 and C29 combined (C27 + C29); total pheromone (C27 + 13MH + C29); and the ratios 13MH:C27, 13MH:C29, 13MH:(C27 + C29), and C27:C29, which were $\log_{10}$-transformed for the reasons presented in Sappington and Taylor (1990a).

Size of a male may influence the quantity of pheromone on the wings. Forewing length in *Colias* is correlated with dry weight (Gray 1967a, b) and was used as an index of body size. Regressions of pheromone characters on size and calculation of coefficients of determination (r^2) were performed for males collected randomly or in mated pairs from several populations (dates and locations of modern samples are described in Sappington and Taylor, 1990a) and from a laboratory colony (Sappington and Taylor, 1990a). Populations from Orchard Lake, Minnesota, Crystal Lake, Minnesota, and Tempe, Arizona, were sampled in 1966 and 1967 (see Taylor 1972 for specific dates and sampling methods). These samples have been stored in plastic bags in large cardboard drums during the intervening years, and little volatilization or degradation of the pheromones has occurred (Sappington, 1989). This is not too surprising given the high molecular weight of the chemicals involved and their low volatilities (Grula et al., 1980; data herein), nor is such long-term stability unprecendented (Carlson and Brenner, 1988; Huyton et al., 1980).

Two different experiments were performed to determine if pheromone quantity or quality change with age of the male. Laboratory-reared males were kept in small cylindrical (80-mm-diam. × 100-mm-tall) eclosion cages made of plastic screening, where flight activity was restricted, and allowed to age unfed for 1, 8, 12, 24, 48, 72, or 96 hr. Pheromones then were extracted and analyzed. In the second test, males were allowed to age a certain number of hours, after which a single hindwing was removed with scissors and the pheromone immediately extracted. The male was then returned to its cage to age a specified number of hours further before the other wing was removed and

extracted. The paired age groups were 1–3, 3–6, 6–9, 9–12, 12–16, 16–24, 24–48, 24–72, 48–96, and 144–192 hr. Amputation caused no obvious harm except possibly in 1-hr-old butterflies in which drops of hemolymph occasionally exuded from cut wing veins.

Males collected in Lawrence, Kansas, in 1986 and 1987 during unrelated experiments were classified by wing wear as either fresh (little or no scale loss) or worn (moderate to heavy scale loss). Pheromone component quantities and ratios were compared between the groups to determine if loss of scales causes a loss of pheromone as well.

We determined if ambient temperature causes volatilization of the pheromone components, and if so, whether they volatilize at different rates. The *n*-alkanes C27 and C29 are solids at room temperature, while 13MH is a liquid (Grula et al., 1980), but this does not necessarily mean that 13MH is more volatile than the solids (R. Carlson, University of Kansas, personal communication). Two tests were conducted in which one wing from each experimental male (lab-reared) was removed and the pheromone extracted, while the other wing was exposed to a heat-producing light bulb. In the first test, the wings were exposed for 3 hr at 38°C, while in the second test they were exposed for 24 hr at 54°C. There was no air movement over the wings during this time.

It is known that males spend most of their time seeking females by flying low over the vegetation and inspecting orange, yellow, or white objects (Taylor, 1972; Graham et al., 1980). It is also known, however, that not all males are as active as others, due in part to their PGI genotypes (Watt et al., 1985). We hypothesized that if pheromones, or parts of the pheromone, volatilize more readily during periods of flight activity (e.g., because of increased air flow), then males that are out searching for and courting females should have different pheromone compositions than males that are resting or feeding. On three consecutive days in August 1987 in a weedy alfalfa field in Lawrence, Kansas, 8–12 dead females were pinned to dowel sticks painted green (Silberglied and Taylor, 1978). They were placed in a natural resting position with wings closed and were exposed just above the alfalfa canopy where they were clearly visible to searching males. Males that approached and courted one or more of the dead females were captured and the pheromones compared to those of males captured while resting or feeding at flowers during the same time of day (1200–1500 hr CST). Ambient temperatures during the periods of collection ranged between 32 and 38°C.

RESULTS

Size of a male does not seem to be an important factor in determining its pheromone phenotype. Regressions of pheromone component quantities on forewing length (Table 1) show that only occasionally is a character signifi-

TABLE 1. REGRESSION COEFFICIENTS (b) AND COEFFICIENTS OF DETERMINATION (r^2) FOR PHEROMONE COMPONENT QUANTITIES REGRESSED ON FOREWING LENGTHS

			μgC27		μg13 MH		μgC29		μg(C27 + C29)		μg Total	
Population	Year	N	b	r^2	b	r^2	b	r^2	b	r^2	b	r^2
Orchard Lake, Minnesota	1966	78	0.02	0.00	0.29[a]	0.08	0.12	0.02	0.14	0.01	0.43	0.04
Crystal Lake, Minnesota	1966	146	0.03	0.01	0.02	0.01	0.20	0.01	0.22	0.01	0.10	0.02
Tempe, Arizona	1967	65	0.05	0.01	0.06	0.01	0.16	0.01	0.21	0.01	0.27	0.01
Antelope, Arizona	1988	97	0.01	0.00	0.19	0.21	0.27	0.01	0.28	0.02	0.48	0.02
Antelope, Arizona (lab-reared sons)	1988	110	0.02	0.01	0.14	0.03	0.12	0.01	0.14	0.01	0.28	0.02
Lawrence, Kansas	1987	80	0.03	0.00	0.62[b]	0.20	0.32[b]	0.17	0.36[a]	0.09	0.98[b]	0.02
Lawrence, Kansas	1988	65	0.03	0.01	0.43[a]	0.06	0.21	0.02	0.24	0.02	0.67	0.04

[a] Significant at 0.05 level.
[b] Significant at 0.01 level.

cantly related to size and even then the amount of variation accounted for by wing length is slight (r^2s < 0.25). Ratios of component quantities were only rarely related to wing length and never explained more than 7% of the variance.

It is not known whether *Colias* males secrete pheromone components throughout their life or if production is shut down soon after eclosion. In a preliminary test, pheromones from males of seven age classes were analyzed and compared (Table 2). A Kruskal-Wallis test across all age classes indicated that at least one age class differed significantly from the others for all pheromone characters except $\log_{10}$ (13MH : C29). Removal of the 1 hr age class from the test resulted in no significant differences, suggesting that an individual's pheromone phenotype stabilizes after the first 12 hr posteclosion.

The second age test involved comparing the pheromone on one wing removed from an individual at one age with that on the other wing removed at a different age. The means for each of 12 age classes ranging from 1 hr to 192 hr are presented in Table 3. As in the first test, pheromone phenotypes seem to stabilize by 9–12 hr posteclosion. The mean quantities of the three components seem much greater in the 144-hr and 192-hr age classes, but sample sizes are very small for these groups and the standard errors are quite large. The paired-comparison t tests (Table 4) indicate that at the level of individual butterflies, most of the differences between age classes in butterflies greater than 9 hr of age (Table 3) are not real, although again the sample size for the oldest age classes is very small. One exception is the significant increase in micrograms of C29 between 24 and 48 hr of age. Phenotypic correlations among component quanitities also vary with age (Table 5), peaking at age 9 hr and decreasing thereafter, suggesting differential environmental effects on pheromone components that accumulate with age.

Although age per se does not greatly affect pheromone phenotypes after the first 9–12 hr posteclosion, older males in natural populations may have different pheromone configurations than young males because of loss of scales with age. Rutowski (1985) found that an increase in wing wear causes a decrease in ultraviolet reflectance, and loss of pheromone-bearing scales from the dorsal surface of the hindwing also can be presumed. If pheromone components are not replenished, then one would expect a decrease in component quantities with an increase in wing wear. Males collected in Lawrence, Kansas, in 1986 and 1987 were classified as fresh or worn and pheromone characters were compared with a Mann-Whitney U test for both years (Table 6). As expected, worn males have less 13MH than fresh males, but, surprisingly, worn males have significantly more C27 than young males, and there is no difference in the quantity of C29 present. Thus, these age-related differences must be associated with some factor other than simple scale loss. These differential changes are reflected in the general breakdown of phenotypic correlations among component quantities as males become worn (Table 7), although this also could be related to male age per se (Table 5).

TABLE 2. MEANS ± SE FOR PHEROMONE QUANTITIES AND LOG_{10} RATIOS FROM FIRST AGE TEST USING MALES FROM LAB COLONY (G GENERATION)

Age (hr)	N	Amount (μg)			13MH/C27	13MH/C29	13MH/(C27 + C29)	C27/C29	C27 + C29 (μg)	Total (μg)
		C27	13MH	C29						
1	12	0.70 ± 0.13	0.27 ± 0.07	1.00 ± 0.27	−0.43 ± 0.08	−0.53 ± 0.11	−0.81 ± 0.09	−0.10 ± 0.10	1.71 ± 0.31	1.98 ± 0.33
8	2	1.45 ± 0.25	0.97 ± 0.23	2.47 ± 0.49	−0.18 ± 0.3	−0.04 ± 0.02	−0.61 ± 0.02	−0.23 ± 0.01	3.92 ± 0.74	4.89 ± 0.97
12	8	2.13 ± 0.37	3.51 ± 1.09	3.19 ± 0.67	0.07 ± 0.13	−0.07 ± 0.11	−0.31 ± 0.12	−0.14 ± 0.03	5.32 ± 1.04	8.83 ± 2.04
24	14	2.06 ± 0.29	2.53 ± 0.47	3.21 ± 0.40	0.05 ± 0.08	−0.16 ± 0.09	−0.37 ± 0.09	−0.20 ± 0.02	5.26 ± 0.68	7.79 ± 0.94
48	14	2.11 ± 0.35	2.86 ± 0.63	2.95 ± 0.53	0.03 ± 0.09	−0.08 ± 0.94	−0.33 ± 0.09	−0.12 ± 0.03	5.05 ± 0.88	7.91 ± 1.38
72	12	2.79 ± 0.41	2.93 ± 0.74	3.87 ± 0.82	−0.09 ± 0.10	−0.20 ± 0.08	−0.46 ± 0.09	−0.11 ± 0.04	6.66 ± 1.20	9.59 ± 1.70
96	4	2.73 ± 0.50	3.73 ± 1.87	2.89 ± 1.12	−0.08 ± 0.21	−0.20 ± 0.16	−0.45 ± 0.18	−0.12 ± 0.05	6.62 ± 1.61	10.4 ± 3.45

TABLE 3. MEANS ± SE FOR PHEROMONE QUANTITIES AND LOG_{10} RATIOS FROM SECOND AGE TEST USING MALE OFFSPRING OF WILD FEMALES

Age (hr)	N	Amount (μg) C27	13MH	C29	13MH/C27	13MH/C29	13MH/(C27 + C29)	C27/C29	C27 + C29 (μg)	Total (μg)
1	12	0.52 ± 0.09	0.29 ± 0.12	0.30 ± 0.06	−0.44 ± 0.23	0.28 ± 0.22	−0.76 ± 0.18	0.16 ± 0.19	0.82 ± 0.14	1.11 ± 0.22
3	22	0.46 ± 0.03	0.31 ± 0.03	0.42 ± 0.07	−0.19 ± 0.05	−0.03 ± 0.11	−0.46 ± 0.06	0.16 ± 0.09	0.88 ± 0.08	1.20 ± 0.90
6	19	0.70 ± 0.09	0.60 ± 0.09	0.76 ± 0.07	−0.07 ± 0.05	−0.13 ± 0.05	−0.42 ± 0.04	−0.07 ± 0.06	1.46 ± 0.13	2.06 ± 0.20
9	20	1.62 ± 0.56	2.01 ± 1.0	1.49 ± 0.51	−0.03 ± 0.07	−0.02 ± 0.07	−0.33 ± 0.07	0.00 ± 0.05	3.11 ± 1.1	5.12 ± 2.1
12	26	0.84 ± 0.12	0.91 ± 0.14	0.91 ± 0.10	−0.03 ± 0.10	−0.10 ± 0.09	−0.39 ± 0.09	−0.08 ± 0.06	1.75 ± 0.18	2.67 ± 0.28
16	26	1.31 ± 0.28	1.03 ± 0.15	1.47 ± 0.22	−0.06 ± 0.08	−0.17 ± 0.08	−0.43 ± 0.07	−0.12 ± 0.06	2.81 ± 0.44	3.87 ± 0.55
24	29	1.54 ± 0.15	2.07 ± 0.20	2.08 ± 0.17	0.14 ± 0.09	−0.03 ± 0.06	−0.28 ± 0.07	−0.17 ± 0.06	3.62 ± 0.30	5.69 ± 0.34
48	12	1.48 ± 0.12	2.48 ± 0.32	1.90 ± 0.14	0.17 ± 0.10	0.06 ± 0.09	−0.19 ± 0.09	0.11 ± 0.03	3.38 ± 0.23	5.87 ± 0.42
72	10	1.11 ± 0.12	2.09 ± 0.34	2.03 ± 0.23	0.21 ± 0.13	−0.05 ± 0.12	−0.25 ± 0.12	−0.27 ± 0.07	3.14 ± 0.29	5.22 ± 0.50
96	5	1.33 ± 0.25	2.69 ± 0.40	1.64 ± 0.26	0.32 ± 0.06	0.22 ± 0.11	−0.05 ± 0.08	−0.10 ± 0.09	2.97 ± 0.43	5.66 ± 0.66
144	3	2.20 ± 5.7	2.87 ± 0.12	2.31 ± 0.30	0.14 ± 0.09	0.10 ± 0.04	−0.18 ± 0.06	−0.04 ± 0.08	4.51 ± 0.82	7.38 ± 0.92
192	3	3.87 ± 1.0	3.84 ± 0.32	2.48 ± 0.54	0.02 ± 0.08	0.21 ± 0.09	−0.21 ± 0.03	0.18 ± 0.16	6.35 ± 0.94	10.2 ± 1.3

TABLE 4. MEAN DIFFERENCES IN PHEROMONE QUANTITIES AND LOG_{10} RATIOS FROM PAIRED COMPARISONS AGE TEST

Age 2–Age 1 (hr)	N	Amount (μg)			Ratio				C27 + C29 (μg)	Total (μg)
		C27	13MH	C29	13MH/C27	13MH/C29	13MH/(C27 + C29)	C27/C29		
3–1	12	0.00	0.05	−0.13	0.22	0.39	0.31	0.17	0.09	0.14
6–3	36	0.19	0.38[a]	0.48[b]	0.18[a]	0.02	0.09	−0.15[a]	0.62[b]	1.00[b]
9–6	9	1.09	2.44	1.07	0.27[a]	0.13	0.21[a]	−0.14	2.35	4.79
12–9	11	−0.18	0.09	−0.33	0.02	−0.01	0.01	−0.03	−0.28	−0.19
16–12	15	−0.04	−0.08	0.12	−0.02	0.00	0.01	0.02	−0.17	−0.25
24–16	12	−0.38	0.10	0.31	0.08	0.08	0.11	−0.01	−0.38	−0.07
48–24	7	0.38	0.78	0.83[a]	−0.01	0.01	0.00	0.02	0.90	1.67
72–24	10	−0.30	−0.53	0.61[a]	−0.17	−0.16	−0.14	0.02	−0.46	−0.98
96–48	5	−0.19	−0.06	0.13	0.07	0.07	0.06	0.00	−0.43	−0.49
192–144	3	1.67	0.97	0.28	−0.12[c]	0.10	−0.03	0.22	1.84	2.81

[a] Significant at 0.05 level.
[b] Significant at 0.01 level.

TABLE 5. PHENOTYPIC CORRELATIONS BETWEEN PHEROMONE COMPONENT QUANTITIES AT DIFFERENT AGES

Age (hr)	*N*	C27 vs. 13MH	C29 vs. 13MH	C27 vs. C29
1	12	0.43	0.21	0.56
3	22	0.04	0.04	0.11
6	19	0.49[a]	0.73[b]	0.26
9	20	0.98[c]	0.99[c]	0.97[c]
12	26	0.39[a]	0.48[a]	0.50[b]
16	26	0.61[d]	0.57[b]	0.47[a]
24	29	−0.28	0.13	0.64[d]
48	12	0.32	0.01	0.54
72	10	0.19	0.23	0.27
96	5	0.68	−0.18	0.41
144	3	0.77	0.99[a]	0.73

[a] Significant correlation at 0.05 level.
[b] Significant correlation at 0.01 level.
[c] Significant correlation at 0.0001 level.
[d] Significant correlation at 0.001 level.

TABLE 6. COMPARISON OF PHEROMONES AMONG FRESH (F) AND WORN (W) MALES, LAWRENCE, KANSAS (MEANS)

		1986		1987	
		F(36)	W(9)	F(25)	W(30)
μg:	C27	1.05	2.36[a]	1.14	2.00[a]
	13MH	6.35	4.30[b]	4.52	2.87[a]
	C29	2.48	2.56	2.72	2.80
	(C27 + C29)	3.53	4.92[c]	3.86	4.80[b]
	Total	9.88	9.22	8.38	7.67
Log_{10}:	$\frac{13MH}{C27}$	0.77	0.28[a]	0.55	0.14[a]
	$\frac{13MH}{C29}$	0.40	0.24[b]	0.17	−0.04[c]
	$\frac{13MH}{(C27 + C29)}$	0.24	0.07[a]	0.01	−0.29[a]
	$\frac{C27}{C29}$	−0.37	−0.04[c]	−0.38	−0.18[a]

[a] Significant difference at 0.001 level.
[b] Significant difference at 0.05 level.
[c] Significant difference at 0.01 level.

TABLE 7. PHENOTYPIC CORRELATIONS AMONG PHEROMONE COMPONENT QUANTITIES FOR FRESH AND WORN MALES, LAWRENCE, KANSAS

Year	Class	C27 vs. 13MH	C29 vs. 13MH	C27 vs. C29
1986	Fresh	0.30	0.41[a]	0.52[b]
	Worn	−0.27	0.00	0.09
1987	Fresh	0.47[a]	0.35	0.56[b]
	Worn	−0.13	−0.01	0.58[b]

[a] Significant correlation at 0.05 level.
[b] Significant correlation at 0.01 level.

TABLE 8. MEAN DIFFERENCES IN PHEROMONE QUANTITIES AND LOG_{10} RATIOS FROM PAIRED-COMPARISONS VOLATILIZATION EXPERIMENTS (FRESH–EXPOSED)

		3 hr @ 38°C (N = 18)	24 hr @ 54°C (N = 16)
μg:	C27	0.013	0.299
	C29	0.063	0.246
	13MH	0.097	0.032
	(C27 + C29)	0.202	0.838[a]
	Total	0.299	0.870[b]
Log_{10}:	$\frac{13MH}{C27}$	0.029	0.004
	$\frac{13MH}{C29}$	−0.032	−0.092
	$\frac{13MH}{(C27 + C29)}$	−0.004	−0.043
	$\frac{C27}{C29}$	−0.061	−0.096

[a] Significant difference at 0.01 level.
[b] Significant difference at 0.05 level.

DISCUSSION

Male pierids typically disseminate courtship pheromones from specialized scent scales on the wings (Bergstrom and Lundgren, 1973; Rutowski, 1977, 1980). Little is known about the process of secretion onto the wing scales in most

TABLE 9. COMPARISON OF PHEROMONES AMONG COURTING AND FEEDING MALES, LAWRENCE, KANSAS 1987 (MEANS ± SE)

		Courting (N = 41)	Feeding (N = 39)
μg:	C27	1.40 ± 0.14	1.48 ± 0.19
	13MH	4.80 ± 0.39	4.69 ± 0.39
	C29	3.30 ± 0.22	3.29 ± 0.22
	(C27 + C29)	4.70 ± 0.30	4.77 ± 0.37
	Total	9.50 ± 0.55	9.47 ± 0.57
Log_{10}:	$\frac{13MH}{C27}$	0.56 ± 0.05	0.53 ± 0.05
	$\frac{13MH}{C29}$	0.16 ± 0.03	0.11 ± 0.05
	$\frac{13MH}{(C27 + C29)}$	0.00 ± 0.04	−0.05 ± 0.05
	$\frac{C27}{C29}$	−0.41 ± 0.04	−0.42 ± 0.05

species, including *C. eurytheme*. The scent-producing scales of the closely related species *Colias philodice* are associated with individual cells in the integument (Rutowski, 1980), which are presumably secretory in nature, and the same situation may exist in *C. eurytheme*. Unlike the esters that make up the pheromone in the former species (Grula et al., 1980), however, the three hydrocarbons making up the *C. eurytheme* pheromone are typical cuticular hydrocarbons found in many insects. In fact, C27 and C29 are found on the wings of female *C. eurytheme* in quantities comparable to that of males (Grula et al., 1980; Sappington, 1989; Sappington and Taylor, 1990a). In the whitemarked tussock moth (*Orgyia lencostigma*), several odd-numbered *n*-alkanes on the scales, including C27, release male copulatory behavior after it has been stimulated with the female's long-range pheromone (Grant et al., 1987). The authors proposed that these common hydrocarbons act as sign stimuli to elicit copulation behavior after the male has made contact with the pheromone source. Evidence from the house fly, *Musca domestica* (Howard and Blomquist, 1982) suggests that it does not produce its cuticular pheromone in unique cells or glands, but rather in the same (perhaps modified) cells as other cuticular hydrocarbons, and this might be true for *C. eurytheme* also. Cuticular hydrocarbons in general are synthesized in cells associated with the epidermis or the peripheral fat body (Howard and Blomquist, 1982). Transport to the surface is prob-

ably through pore canals that penetrate the endocuticle and epicuticle (Jackson and Baker, 1970).

In the fly *Sarcophaga bullata*, there is a period of moderate hydrocarbon accumulation (5–8 μg/day) during the four days preceding ecdysis, and synthesis is controlled by ecdysterone (Arnold and Regnier, 1975). There is evidence that production of cuticular pheromones in the house fly *Musca domestica* (Howard and Blomquist, 1982) and the bruchid beetle *Zabrotes subfasciatus* (Pimbert and Pouzat, 1988) is induced by factors released at the onset of vitellogenesis. In *Pieris melete*, the total quantity of four pheromone components increases for the first two days and remains relatively constant thereafter through 100 hr/of age. During this period each of the components increases in quantity at different rates, causing changes in blend composition (Kuwahara, 1979). The author also concluded that the total quantity changed with time of day (1200 hr vs. 1800 hr), but this is based on a sample of only four and three individuals per group, respectively, whose ranges overlap, certainly making this an unwarranted inference. Lundgren and Bergstrom (1975) reported that pheromone production and deposition onto the scent scales of the lycaenid butterfly, *Lycaeides argyrognomon*, started during the first day posteclosion in lab-reared stock, but no details were given.

In *Colias eurytheme* the greatest accumulation of all three pheromone components occurs between 3 and 9 hr posteclosion (Table 4). It also is apparent from the ratio data (Table 4) that during the first 12 hr, 13MH is deposited more rapidly than C27 and C29, and C27 more rapidly than C29. Blends remain remarkably constant throughout life after the first 12 hr, even though quantities of the components fluctuate a bit, suggesting that any small age-related changes after this period affect all components equally.

In *Colias philodice*, the ability of male wings to induce an acceptance response in females was reduced significantly by exposure of detached wings to room temperature for 24 hr, suggesting significant volatilization of one or more pheromone components (Rutowski, 1980). There is no evidence that any of the components in the *C. eurytheme* pheromone volatilize to any appreciable degree at temperatures that are experienced in the field (Table 8) or even elevated temperatures above those reached while basking or overheating (Watt, 1968, Watt et al., 1983; Kingsolver and Watt, 1983; Kingsolver, 1983a, b). Nor does the greater flight activity of actively searching males cause a detectable loss of pheromone (Table 8). Watt et al. (1985) have shown that males with PGI genotypes that permit more flight activity under prevailing climatic conditions are more successful at obtaining copulations than those with less kinetically favorable genotypes. They propose that this differential success is related to differences in male persistence during courtship. It is not known if PGI genotype has pleiotropic effects on pheromone composition, but the lack of differences in the pheromones of courting vs. resting males (Table 9) indi-

cates that PGI genotype is not correlated with pheromone phenotype through the effects of differential volatilization associated with differential flight activity.

The low volatility of the components (Table 8) ensures that depletion will occur very slowly over the life of the male. Low volatility also requires that this pheromone be detected by females either via contact or aerially at very close range. In bioassays in which detached wings were presented to females, contact of the wings with the antennae was necessary to elicit a response in *Eurema lisa* (Rutowski, 1977), *Colias philodice* (Grula et al., 1980; Rutowski, 1980), and *C. eurytheme* (Grula et al., 1980; Sappington, personal observation). In all of these species, courting males flutter vigorously around the perched female and buffet her with their wings (Rutowski, 1980; Grula et al., 1980).

Pheromone composition in *C. eurytheme* does seem to change with increased wing wear in wild populations, although not in a way that is readily interpretable (Table 6). The quantity of 13MH is less in worn males than in young ones, which is to be expected if 13MH is carried on scales that are lost as the butterflies age. However, the quantity of C27 increases with wing wear, suggesting that it continues to be secreted. The data from the age tests (Table 2–4), however, indicate C27 does not increase with age, at least between the ages of 12 hr and 96 hr. The situation after 96 hr posteclosion is less clear. The noctuid moth, *Trichoplusia ni*, emerges from the pupa with the wax layer consisting primarily of methylalkanes that are produced only in the larval stage, but *n*-alkanes, produced exclusively by adults, increase in amount to day 5 (De Renobales and Blomquist, 1983). It is possible that C27 is produced by older *C. eurytheme* males to replace the molecules lost during courtship or through wing wear, while 13MH is not. Elucidation of this problem awaits further study.

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