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TEMPORAL PATTERNS OF SEX PHEROMONE TITERS AND RELEASE RATES IN *Holomelina lamae* (LEPIDOPTERA: ARCTIIDAE)

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Abstract—Six hydrocarbon components (2-methylhexadecane, *n*-heptadecane, 2-methylheptadecane, 2-methyloctadecane, *n*-nonadecane, and 2-methylnonadecane) were identified in sex-pheromone gland extracts and in airborne collections of *Holomelina lamae*. Low variability in the ratio of components among individuals indicates tight regulation of blend composition. Minor changes were evident in the quantity and ratio of the blend as a function of time of day. Based on gland extracts, the total quantity of the six components increased from day 1 ($\bar{X}$ = 6299 ng) to day 4 ($\bar{X}$ = 7498 ng) and subsequently decreased. No significant correlations were found between total gland contents and wet or dry weights of moths. Emission rates of *H. lamae* females were determined from pheromone adsorbed onto Porapak Q. Quantities released peaked shortly after the onset of calling and decreased rapidly as calling continued. Peak release rates ranged from 13 to 350 ng/10 min, and from 37 to 835 ng/60 min. Noncalling females did not emit detectable quantities of pheromone. Based on release rates and the rate of pulsation of the abdominal tip, the average amount released per pulse is not constant. The mean ratio of components released (0.78:7.45:84.80:2.84:2.59:1.53) was not very different from the ratio of components in gland extracts of 2-day-old females (0.70:4.19:90.12:1.65:1.91:1.42). We propose that the blend is atomized rather than volatilized from the gland, thus retaining nearly the same ratios in the female's effluvium as in her gland.

Key Words—Sex pheromone, pheromone release rates, pheromone titer, airborne pheromone collection, *Homomelius lamae*, Lepidoptera, Arctiidae, 2-methylheptadecane.

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INTRODUCTION

Most moths that employ pheromones for sexual recruitment employ blends of several pheromone components. A persistent problem in research on pheromones has been the correlation of surface washes or gland extracts with volatile components. Olsson et al. (1983) summarized the problem as follows: "Discussion of the relative amounts of compounds in a pheromone blend in terms of major and minor components are generally based on the relative amounts found in extracts of female glands. When the vapor pressures of the pheromone components are significantly different, the relative amounts in the vapor phase differ from those in the liquid phase." They showed that the pheromone components of the turnip moth, *Agrotis segetum*, decyl, (*Z*)-5-decenyl, (*Z*)-7-dodecenyl, and (*Z*)-9-tetradecenyl acetate, which were found in a ratio of 5.7:4.3:62.7:27.3 in abdominal tip extracts, were represented as 28.6:25.5:43.2:2.7, respectively, in the vapor phase. Few studies have combined airborne collections of pheromone components with gland extracts, and few behavioral evaluations of multicomponent pheromone blends have been based on the blend actually emitted by the female. Further, little work has been directed at determining how the ratios are modulated by factors such as age and time of day.

Roelofs and Cardé (1971) determined that 2-methylheptadecane was a major component of the sex pheromone of several sibling species in the *Holomelina aurantiaca* complex. Pulsing of the abdominal tip in this group yields a higher pheromone release rate than that obtained from artificially extruded glands (Schal and Cardé, 1985), and peak pheromone release occurs soon after the onset of calling even though calling in *H. lamae* persists for several hours (Schal and Cardé, 1986). Here we report on the composition of a multicomponent blend in *H. lamae* pheromone glands and airborne collections and constituent quantities and ratios as a function of time of day and age.

METHODS AND MATERIALS

Insects. The main *Holomelina lamae* colony (MI) originated from insects collected in Manistique, Michigan (R16W, T42N, sec 14) on August 10, 1978, and was maintained in the lab for ca. 60 generations. A second colony (ME) originated from 12 females collected at Seawall Bog, Acadia National Park, Maine, on August 12, 1984; second generation females were used. Larvae were reared individually on pinto-bean diet in 30-ml clear plastic cups at 27°C and 15:9 light–dark regime until the last instar, whereupon they were transferred to 24°C and 16:8 light–dark regime. Light intensity during photophase was 1400 lux. Insects were checked daily for pupation, and female pupae and adults were kept in clear plastic cups. For airborne volatile collections, females following ecdysis were kept in screen cages in constant 40 cm/sec wind.

Pheromone Extraction. Abdominal tips of females were excised with forceps and immersed for 1 hr in 150 μl of a 0.5 ng/μl solution of 2-methylpentadecane (2me-15c, as internal standard) in redistilled *n*-hexane. A subsequent extraction of the same gland for 24 hr liberated an additional 4.7% of 2-methylheptadecane (2me-17c), the major pheromone component, but also greatly increased the amount of debris which interfered with GLC analysis. In both extracts, all six components were liberated in similar proportions. Smaller volumes of solvent increased losses associated with removal of the extracted glands from the solvent; although quantitative analysis was unaffected because an internal standard was used, we attempted to minimize these losses in order to permit quantification of minor extractable components. Samples were stored in conical glass tubes made by drawing Pasteur pipets over a flame. They were placed in hexane-containing glass vials with Teflon-lined caps and stored at $-20°C$.

Airborne Collections. Two types of collection devices were used. For 10-min aeration of the abdominal tip, collection tubes were modified Pasteur pipets with one flared and one constricted end; they were silanized and filled with 200 mg of preconditioned Porapak Q between glass wool plugs (Schal and Cardé, 1985). Glass beads, glass wool, and charcoal adsorbants were unsatisfactory because the short-chain hydrocarbons were not retained as well as the long-chain compounds. Conditioning of Porapak Q was modified from Byrne et al. (1975). The adsorbant was washed with redistilled *n*-hexane, N_2 dried, heated at 180°C in a N_2 stream for 24 hr, packed into tubes, and washed again with hexane. Tubes thus treated were reused after extraction without further conditioning.

Females were placed on horizontal wooden dowels (0.5 cm diameter) where they assumed vertical calling positions with the abdomen hanging below the dowel. The collection tube was positioned such that the abdominal tip was ca. 3–5 mm below the outer rim of the flared end of the tube. Thus, tubes could be exchanged readily with no disturbance to the moth. Readsorption onto the female's body was minimized by turbulence-free unidirectional flow as determined with titanium tetrachloride "smoke."

For 60-min collections, the apparatus described by Schal and Cardé (1985) was employed. Individual females with clipped wings were placed on a screen perch within the upper portion of a chamber composed of two modified ground-glass joints (14/20) whose lower portion was packed with 150 mg of preconditioned Porapak Q. The inlet was packed with glass wool (prewashed with hexane and heated at 350°C) as an air filter.

For both 10- and 60-min collections, 210 $\pm$ 10 ml/min of room air was pulled through the collection tube with a vacuum pump. Room temperature was 23 $\pm$ 2°C. Pheromone was eluted with 800 μl of *n*-hexane in 100-μl aliquots, and 100 ng 2me-15c was immediately added as internal standard.

To determine the collection efficiencies for both devices, 5 μl of a hexane

solution, consisting of 1 μg of each of the six components [2-methylhexadecane (2me-16c), *n*-heptadecane (*n*-17c), 2-methylheptadecane (2me-17c), 2-methyloctadecane (2me-18c), *n*-nonadecane (*n*-19c), 2-methylnonadecane (2me-19c); all from K & K Laboratories] was applied to the glass-wool plug above the Porapak and aerated for 10 or 60 min. No breakthrough occurred in either set-up when two collection tubes were arrayed in series for 3 hr.

Quantitative Analysis. Extracts in hexane were reduced to 2 μl with a stream of prefiltered N_2. Absolute losses of the six components ranged from 9 to 30%, but their ratio relative to the internal standard remained the same. Gas–liquid chromatographic (FID) analysis was conducted on 2-m × 2-mm-ID and 1-m × 2-mm-ID glass columns packed with 3% SP-2100 (methyl silicone) on 100–120 mesh Supelcoport. Oven temperature was 155°C for 5 min and programmed to 210°C at 3°/min. To examine for possible trace amounts of other compounds, some runs were made on a polar 2-m × 2-mm-ID Silar 10CP column (10% Silar 10C on acid-washed 100–120 mesh Chromasorb W).

RESULTS

Gland Titers. In addition to 2me-17c, the major component reported in *Holomelina* (Roelofs and Cardé, 1971), five other hydrocarbons were extracted. Abdominal tips of newly ecdysed MI females ($N = 9$) averaged a total of 6299 ng of all six components combined (Figure 1, Table 1). Quantities and ratios of components extracted from pharate adults ($N = 5$) 24 hr before eclosion were not significantly different from freshly eclosed females. Two-day-old ME females (one generation in lab) had similar ratios of the six components (Figure 1, Table 1), but the titers were significantly lower than those of 2-day-old MI females (Table 1).

Relatively small diel changes in pheromone gland titers were evident over 13 days (Figure 1). The levels of all components decreased around 10 hr after lights on, coinciding with onset of calling (Schal and Cardé, 1986), but with the exception of 3-day-old females, these changes were not statistically significant ($P > 0.05$, SNK test). In 3-day-old females, the titer of all components rose to its highest level by 14 hr after lights on (Figure 1), although calling continued until lights off (Schal and Cardé, 1986).

The ratio of the six components in the gland did not vary on a diel basis ($P > 0.05$, SNK test; Figure 1). Small standard errors indicate tight regulation of the composition of glands in different females as do significant ($P < 0.01$) correlation coefficients (Pearson) among quantities of all six components.

Quantities of 2me-16c and *n*-17c increased with age up to 13 days, based on a linear model ($P < 0.01$) (Figure 1); 2me-19c decreased with age ($Y = -0.14X + 87.0$, $P = 0.03$), while the change in other components did not differ significantly from zero. For the first four days of calling, the titer of each

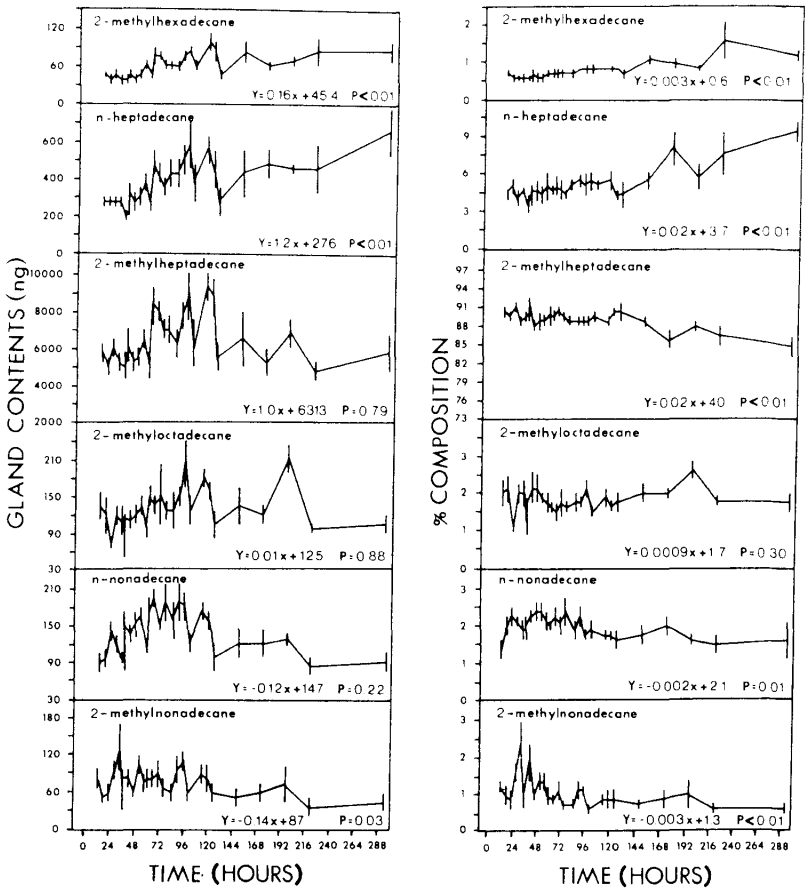

FIG. 1. Changes in quantities (left) and proportions (right) of the six components (±SEM) extracted from pheromone glands of 0- to 14-day old *H. lamae* females. An 8-hr scotophase occurred between 1600 and 2400 hrs daily. Samples sizes range from 8 to 14 for each time.

component except 2me-19c, and the titer of all components combined increased with age ($P < 0.05$) (Figure 1). On subsequent days, the quantities of all components did not change significantly until day 13.

Although only small changes were seen in the ratios of components as females aged (Figure 1), significant ($P < 0.01$) increases with age occurred in 2me-16c and *n*-17c, and significant decreases ($P < 0.01$) occurred in the ratios of 2me-17c, *n*-19c, and 2me-19c; the proportion of 2me-18c did not change with time ($P = 0.30$). In the first four days, the ratios of only 2me-16c and *n*-17c increased significantly; the proportions of the other components remained unchanged (Figure 1). On subsequent days (5–13), the proportion of *n*-

TABLE 1. QUANTITIES AND PROPORTIONS (±SD) OF SIX COMPONENTS IN PHEROMONE GLANDS OF MI AND ME *H. lamae* AT VARIOUS TIMES AND RELEASED BY 2-DAY-OLD MI FEMALES

	At emergence (N = 9)		Mean first 4 days (N = 164)	
Component	ng	%	ng	%
2-Methylhexadecane	45 ± 10	0.72 ± 0.05	55 ± 25	0.75 ± 0.11
n-Heptadecane	281 ± 103	4.36 ± 1.01	355 ± 186	4.90 ± 1.43
2-Methylheptadecane	5668 ± 1267	90.24 ± 2.33	6418 ± 2331	89.31 ± 2.19
2-Methyloctadecane	135 ± 77	2.06 ± 0.88	127 ± 76	1.79 ± 0.82
n-Nonadecane	90 ± 44	1.40 ± 0.54	147 ± 67	2.06 ± 0.67
2-Methylnonadecane	80 ± 40	1.22 ± 0.44	82 ± 50	1.18 ± 0.75

TABLE 2. PEARSON CORRELATION ANALYSIS OF CHANGE IN PERCENT COMPOSITION OF EACH EXTRACTED COMPOUND WITH SUM OF SIX COMPOUNDS[a]

	Day 2 (n = 19)		13 Days (N = 234)		Released (N = 39)	
	r_p	P	r_p	P	r_p	P
2-Methylhexadecane	0.31	0.19	−0.04	0.58	−0.17	0.31
n-Heptadecane	0.07	0.76	−0.01	0.99	−0.36	0.02
2-Methylheptadecane	0.11	0.64	0.10	0.13	0.08	0.65
2-Methyloctadecane	−0.24	0.33	−0.09	0.15	0.19	0.26
n-Nonadecane	0.03	0.92	−0.17	0.01	−0.01	0.99
2-Methylnonadecane	−0.18	0.47	−0.10	0.14	0.28	0.09

[a] r_p is the Pearson correlation coefficient, P is probability level.

17c increased ($P < 0.01$), that of 2me-17c decreased significantly ($P < 0.01$), and the others remained unchanged.

To determine whether gland composition changed with the total amount of extractable material, the proportion of each component was plotted against the total amount extracted for 2-day-old females and for all females over 13 days. With the exception of *n*-19c, which decreased in representation as the total blend increased, all correlations (Pearson) were insignificant (Table 2), indicating that the composition of the pheromone blend is independent of the total titer.

Correlation with Body Weight. Pupae and newly eclosed females were

Mean first 13 days (N = 234)		Day 2 (32–39 hr, N = 19)		ME females (day 2, N = 5)		Release ratio (N = 39)
ng	%	ng	%	ng	%	%
61 ± 31	0.84 ± 0.34	45 ± 16	0.70 ± 0.08	16 ± 5	0.73 ± 0.08	0.78 ± 0.22
391 ± 231	5.45 ± 2.24	262 ± 112	4.19 ± 1.04	125 ± 46	5.82 ± 1.85	7.45 ± 1.93
6461 ± 2644	88.87 ± 2.77	5726 ± 1751	90.12 ± 1.90	1861 ± 569	83.20 ± 3.52	84.80 ± 3.19
129 ± 72	1.80 ± 0.76	101 ± 39	1.65 ± 0.62	77 ± 40	3.91 ± 2.42	2.84 ± 1.40
140 ± 66	1.97 ± 0.70	123 ± 62	1.91 ± 0.65	123 ± 46	5.44 ± 0.67	2.59 ± 0.89
76 ± 49	1.06 ± 0.69	90 ± 65	1.42 ± 1.07	21 ± 10	0.90 ± 0.37	1.53 ± 0.85

weighed, their glands extracted, and the dry weights obtained after 24 hr in a drying oven (70°C). No significant relations were evident between total gland titer and either wet weight (r = 0.16, P = 0.50, N = 19) or dry weight (r = 0.61, P = 0.11, N = 8) for 2-day-old females or for 0- to 4-day-old females (r = 0.12, P = 0.15, N = 147; r = 0.11, P = 0.22, N = 136, respectively) (Figure 2A, B). Also, the quantities and proportions of all individual components did not vary significantly with body weight.

Release Rates. All components isolated from gland extracts were also collected from freely calling 2-day-old females (Table 1). Three collection tubes were changed successively every 10 min in the first 35 min of calling; subse-

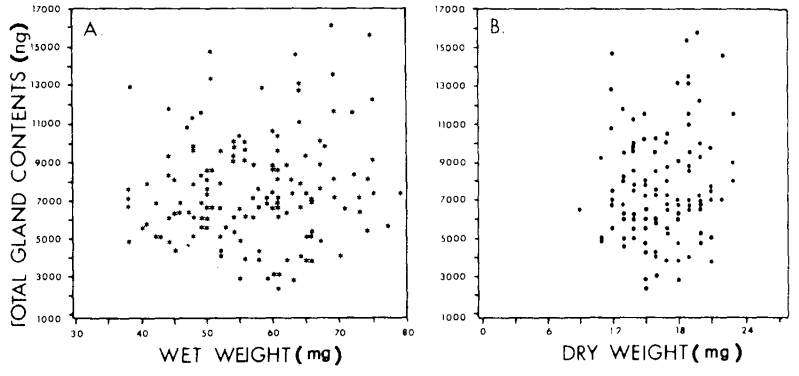

FIG. 2. Relation of total gland content of *H. lamae* with wet and dry weights.

quently, 10-min collections were made once every hour. An initial increase in the main component, 2me-17c, after the first 10 min of calling was followed by a rapid decrease, indicating that most of the material was released early in the calling period (Figure 3). This was borne out in successive 60-min collections from freely calling females where a rapid decline in the amount of pheromone released occurred after the first hour of calling (Figure 4).

The six components were represented in similar proportions in both air-

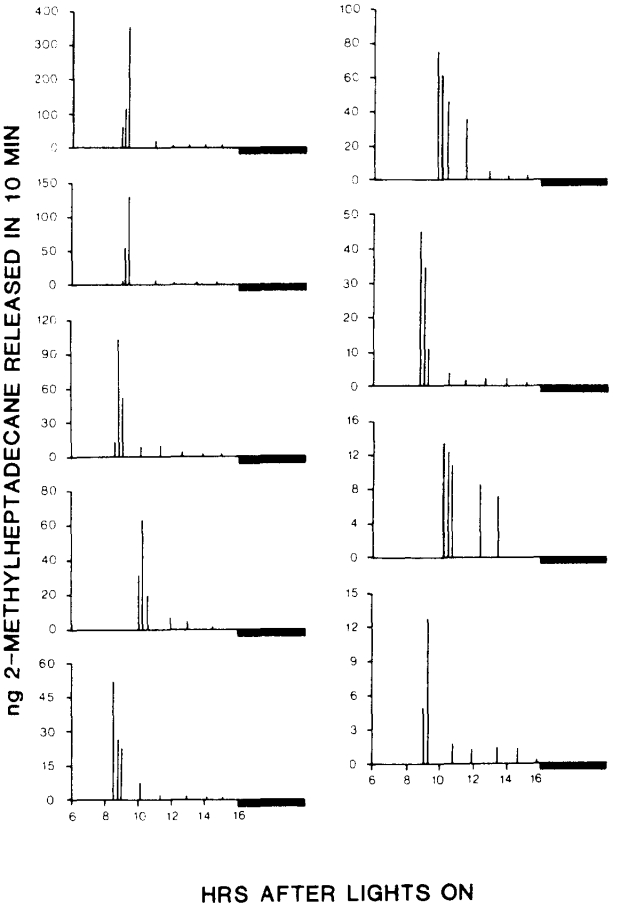

FIG. 3. Release rates per 10 min of nine *H. lamae* females throughout the scotophase (dark bars). Only 2-methylheptadecane is shown. Three collections of 10 min each were conducted in the first 35 min of calling; subsequently, 10-min collections were conducted once every hr.

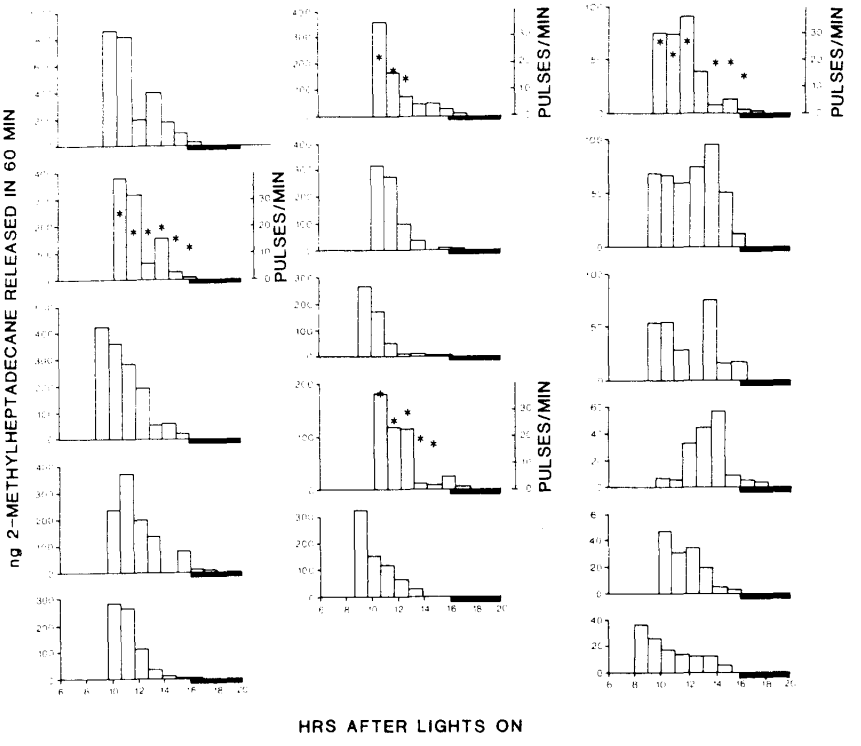

FIG. 4. Release rates per 60 min of 16 *H. lamae* females throughout the calling period. Only 2-methylheptadecane is shown. Successive 60-min collections were conducted from the onset of calling to its termination. For four females, the rates of extrusion of the terminal abdominal segments are also noted (asterisks).

borne collections and gland extracts (Table 1). Only the proportion of *n*-17c varied inversely with the total blend ($r = 0.36$, $P = 0.02$, $N = 39$; Table 2). The proportions of other components did not change as the total blend increased.

Pulsing Rate. H. lamae, like other arctiid moths, rhythmically extends and retracts the last two abdominal segments during calling. We determined the rate of pulsation of the abdominal tip for freely calling females in still air by counting extrusions per 1 min at 1-hr intervals throughout the calling period. At the onset of calling, pulsation frequency averaged 26/min (Figure 5) and duration of exposure of the terminal segments was long. The pulse rate rapidly peaked at 40/min where it remained for several hours. Toward the termination of calling, the pulse rate decreased gradually to a mean of 13/min (Figure 5).

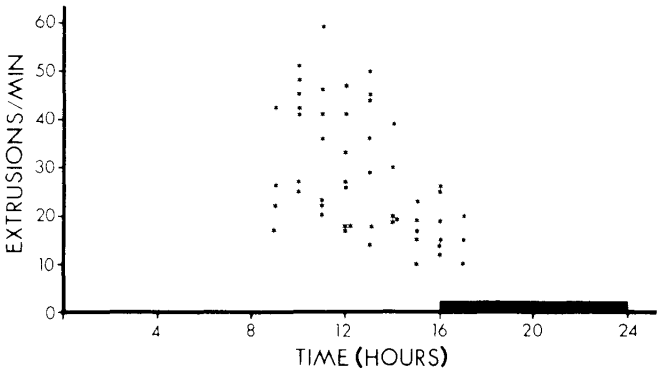

FIG. 5. Rate of extrusion and retraction of the terminal abdominal segments of *H. lamae* as a function of time of day.

DISCUSSION

Pheromone Profile. Roelofs and Cardé (1971) identified 2-methylheptadecane as the major component of the sex pheromone of a group of *Holomelina* sibling species. Males of several species were attracted to and trapped with baits of 2me-17c, but few *H. lamae* males were trapped, even though males oriented to within short distances of the traps. They suggested that missing components, greater release rates of the primary attractant, or both could be important in effecting close-range orientation (Roelofs and Cardé, 1971). We found five related components (2me-16c, *n*-17c, 2me-18c, *n*-19c, 2me-19c)[2] in gland extracts of females reared in the laboratory for ca. 60 generations and in first-generation offspring of feral females; the six components were also collected in air passing over calling females (Figure 1, Table 1). It is possible that other components are also volatilized, but at proportions below 1% of the total blend.

Among the hydrocarbon analogs of 2me-17c identified in *H. lamae* (Cardé et al., 1987), *n*-17c increases male trap catch two to threefold over 2me-17c alone, when released from separate rubber septa at charges of 1000 and 10 or 1000 and 1 μg, respectively. At charges of 100 μg with 1000 μg 2me-17c, *n*-17c diminishes trap catch twofold (Cardé et al., 1987). Thus, relative emission of *n*-17c within a range relative to 2me-17c is important to male attraction in *H. lamae*.

ME females had similar ratios of the six components in gland extracts (Table 1), but the quantity of pheromone extracted was significantly lower in ME compared to MI females ($P < 0.01$, t test). Minks (1971) reported a de-

[2] Prof. W. Roelofs and R. Cardé originally characterized these components by GLC and MS in 1970 at the New York Agricultural Experiment Station in Geneva.

crease in sex pheromone production in inbred strains of the summerfruit tortrix moth, *Adoxophyes orana*, and Miller and Roelofs (1980) showed that a lab colony of *Argyrotaenia velutinana* had significantly lower pheromone titers than field-collected moths. The differences observed in the two strains of *H. lamae* could be attributed to inherent differences in the two source populations or to inbreeding in the laboratory of the Michigan strain.

Diel and Age Effects on Gland Content. In the first four days, total gland content in *H. lamae* exhibited a diel periodicity only in 3-day-old females, decreasing abruptly at the onset of calling, but returning to its noncalling level within 2–4 hr while females still called (Figure 1). Means at different times in the diel were not significantly different from each other for 1-, 2-, and 4-day-old females. Thus, high emission rates at the onset of calling (Figures 3 and 4; Schal and Cardé, 1985) and lack of significant diel fluctuations in pheromone titer suggest rapid biosynthesis of pheromone during calling.

Similar results were found with *Trichoplusia ni* which does not have a daily rhythm in pheromone titer (Shorey and Gaston, 1965; Sower et al., 1972). However, Webster and Cardé (1982) related an abrupt drop in pheromone titer to the onset of calling in *Platynota stultana*; gland titer increased throughout the day and peaked at the initiation of calling. Similar relationships were obtained for surface washes of pheromone glands of *Plodia interpunctella* and *Ephestia cautella* (Coffelt et al., 1978), and *Heliothis zea* (Raina et al., 1986) in which peak calling coincided with maximal pheromone titer in the middle of the scotophase.

The pheromone content of virgin females changed little with age (Figure 1). However, the quantity of all components increased over the first four days, and subsequently decreased or remained unchanged. A similar pattern was observed by Lawrence and Bartell (1972) in *Epiphyas postvittana* based on male response to female extract. Miller and Roelofs (1977), Webster and Cardé (1982), and Raina et al. (1986) showed that pheromone titers in *A. velutinana*, *P. stultana*, and *H. zea* increased for the first two, four, and three days respectively, but decreased over the next few days.

The narrow coefficients of variation in the ratio of the pheromone components (Figure 1, Table 1) were independent of age (as in Miller and Roelofs, 1977) and indicate tight regulation of the blend. In *H. lamae*, although relative amounts of four of the six components changed with time, these changes are probably insignificant to behavioral response. For example, the change in 2me-16C was from ca. 0.7% on day 1 to 1.3% on day 13. Although statistically significant, it is doubtful that males detect such slight changes.

Correlations between total gland titers and proportion of individual components resulted in insignificant r values, indicating that blend composition is independent of blend quantity (Table 2).

Gland Content vs. Body Weight. Pheromone gland titer is unrelated to the wet or dry weights of pupae or adult females (Figure 2). Similar results were

obtained by Miller and Roelofs (1980) and by Charlton and Cardé (1982) for *A. velutinana*, and *Lymantria dispar*, respectively. Fitzpatrick et al. (1985) found that pupal weight did not correlate to male hairpencil contents (benzaldehyde) in *Pseudaletia unipuncta*. However, Shorey and Gaston (1965) reported that pheromone content in 4-day-old *T. ni* was correlated with the weight of females at emergence.

Release Rates and Ratios. Freely calling *H. lamae* females release all six components of the pheromone blend only during the calling period (Figure 1, Table 1); no detectable pheromone was collected from noncalling females either before or after calling. Although calling persisted for several hours in *H. lamae* (Schal and Cardé, 1986), the majority of the daily pheromone release occurred early in the calling period. A similar pattern is suggested in *Utetheisa*, in which females called for up to 200 min (Figure 6 in Conner et al., 1980), but males were attracted to calling females for an average of only 29 min, depending on the season. *T. ni* releases pheromone in calling bouts of ca. 20 min. Pheromone is released at a high rate early, but decreases asymptotically over the course of a calling bout (Bjostad et al., 1980). The strategy implicated is one of maximization of early pheromone release in some noctuids and arctiids, with concomitant rapid rates of pheromone biosynthesis as evidenced by the minor changes in gland content. Presumably, females tend to be located and mated early in the calling period or bout.

Although release rates later in the calling period may have little significance in the field because females may be mated early, they do have important implications for studies of pheromone release. Many such studies make the tacit assumption that release rates are constant throughout the calling interval, and therefore various periods of aeration of the gland can be corrected to yield a per minute or per hour release rate. The patterns exhibited by *H. lamae*, *H. armigera*, and *T. ni* clearly question such extrapolations. Similarly, other studies have documented large variability in the amount and temporal pattern of pheromone emission from individual females (e.g., Morse et al., 1982).

In situations where glands are forcibly extruded (chemically or mechanically) for 10 min, and it is known that a subsequent 30 min of aeration yields little additional material (e.g., Pope et al., 1984), volatilization from the gland surface declines exponentially. Release rates (ng/min) thus obtained will differ when averaged for all 10 min or for any interval shorter or longer than 10 min. It is not known whether actual release rates approximate the maximum rate obtained from forcibly extruded glands (probably the first minute), or an average over a certain interval. For instance, the single highest 10 min rate of release for *H. lamae* was 353 ng, which would extrapolate to 2118 ng/hr or double the single highest release rate obtained from 60-min collections.

H. lamae exhibits a relatively high rate of pheromone emission (Figures 3 and 4; Schal and Cardé, 1985). Why such high release rates? An obvious explanation is to increase the distance of communication. Furthermore, high re-

lease rates may function in reproductive isolation, but ratios of components, diel activity patterns, and geographic and microhabitat distributions (Roelofs and Cardé, 1971) are probably more important partitioning factors.

The rates of emission of a single pheromone component depend upon its vapor pressure, environmental factors (e.g., temperature, wind), and the size and form of the emitting surface. Steck et al. (1984) showed that 3% of Z9–14:Ac and 97% of Z11–16:Ac provided for sustained trapping of the noctuid *Mamestra configurata* over several weeks. But, because the Z9–14:Ac was released and thus depleted more rapidly than Z11–16:Ac, its proportion in the trap declined so that the lure then attracted *Enargia infumata*. Similar effects on ratios were obtained by Olsson et al. (1983) in a study of vapor pressures of sex pheromones. The differential release of various components from artificial sources and interactions among loaded compounds suggest some important cautionary notes in formulating pheromones. First, since different substrates release materials differentially, release rates and ratios must be determined for each substrate before field tests are conducted. Second, as compounds are added or omitted from rubber septa, as in studies of the roles of each component, release rates and ratios must be determined for each new combination of components to account for interaction among components. In fact, in a comparison of three substrates, Linn et al. (1984) found that Z7–14:Ac and Z9–14:Ac were released at a much lower rate relative to the loading when compared to the 12-carbon compounds, resulting in an inappropriate ratio and poor response by *T. ni*.

A central concern is how the blend of different components is regulated. Both emitted and extracted blends of *H. lamae* contained hydrocarbons ranging from 16 to 19 carbons in length which differ in vapor pressure. Yet, the ratio of volatilized and extracted components was nearly the same, and for both, blend quality did not change with blend quantity. The remarkable similarity between the profiles of the six-component blend in the female's gland and her effluvium indicates that evaporation of the blend from the gland surface is not responsible for the ratio of components emitted. Species in the *Holomelina aurantiaca* complex examined to date possess paired tubular pheromone glands (unpublished). We propose that upon each pulse of the abdominal tip, the blend is atomized in a fine mist, thus retaining the same ratio of components as found in the gland (Cardé et al., 1987). This mechanism could also account for the conspicuously high rate of emission. This novel interpretation of blend regulation allows the ratio of components biosynthesized and present in the gland to reflect the ratio released.

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