Melatonin in the drinking water also produced arrhythmia in sparrows (fig., table, B). The sparrows exhibited freerunning circadian perch-hopping rhythms while they were drinking tap water or tap water with ethanol. Concerning their responses, the 12 sparrows given melatonin and ethanol in their drinking water can be divided into three subgroups: a) four birds were arrhythmic, b) five birds showed a mixture of some arrhythmia and some rhythms, and c) three birds were unaffected. In all, 75% of the birds were affected by the melatonin. As with the capsules, some of the birds displayed responses to the melatonin or its removal within a cycle.

From water consumption tests we estimate the oral dose as less than 5 mg/day/bird. The response of arrhythmia requires a large dose. We note that we previously lowered sparrow body temperature and caused roosting behavior with intramuscular melatonin injections, but only with large doses (greater than 1.2 mg/bird[7]). We can conjecture about the requirement for large doses. First, sparrow melatonin synthesis is especially robust. Sparrow pineal glands have large amounts of activity of the enzymes that synthesize melatonin from serotonin – N-acetyltransferase activity increases 46-fold at night to reach levels of 3.2 nmoles/pineal/h, and sparrow pineal HIOMT is 0.7–0.8 nmoles/gland/h. Hemipineal glands from sparrows produced melatonin at rates as high as 8 ng/ml/h[8]. Possibly, large doses are needed experimentally because the response systems require high levels of melatonin. Second, when melatonin turnover was measured in other species, the rate was high (e.g. half-life of 17–23 min in rats[9]). Since we do not know the locus of the target, we do not know what effective level is required at what site.

Several further observations require comment. *First,* in both the capsule and oral experiments, melatonin may have altered the amount of activity. However, inspection of the birds' records showed reduced activity in some birds and increased activity in others. *Second,* there are apparent period changes (birds kept in the experiment longer increased period length 0.9 h, ethanol lenghened the period by 0.6 h, melatonin shortened the period 0.5–0.7 h). We advise caution in interpreting these small intraexperimental period changes because they are in the range of interexperimental variability for controls in our laboratory (24.1–24.9). *Third,* the response to melatonin may be dependent on individual sensitivity to the dose used (no response below threshold, period shortening at intermediate levels, arrhythmia at high levels). The sparrows were wild, trapped animals of varying sex, age, and history.

Oral melatonin administration has produced other effects beside arrhythmia in sparrows. The other effects are also related to pineal function – a) alteration of hamster testis size, b) advance of seasonally dependent events in white-tailed bucks, c) sedative effects in humans, and d) elevation of blood melatonin in sheep, goats and humans[10–15]. Large doses were required for most of the responses in which oral melatonin exhibited effects which correlate with pineal-related events such as reproduction, body temperature, seasonal cycles, and activity rhythms.

The similarity between melatonin administered continuously in capsules versus sporadically (rhythmically or nonrhythmically) in ad libitum oral doses may not be trivial since it implies a common final mode of action.

1 Support was provided to S. Binkley by NSF PCM 8314331.
2 Gaston, S., and Menaker, M., Science *160* (1968) 1125.
3 Binkley, S., Kluth, E., and Menaker, M., J. comp. Physiol. *83* (1972) 63.
4 Takahashi, J., in: Vertebrate Circadian Systems, p. 112. Eds J. Aschoff, S. Daan and G. Gross. Springer-Verlag, New York 1982.
5 Turek, F., McMillan, J., and Menaker, M., Science *194* (1976) 1441.
6 Hendel, R., and Turek, F., Physiol. Behav. *21* (1978) 275.
7 Binkley, S., Chronobiology, p. 472. Eds L. Scheving, F. Halberg and J. Pauly. Igaku Shoin, Ltd., Tokyo 1974.
8 Takahashi, J., Neural and endocrine regulation of avian circadian system, p. 169. Ph.D. thesis, U. Oregon 1981.
9 Gibbs, F., and Vriend, J., Endocrinology *109* (1981) 1796.
10 Pevet, P., and Haldar-Misra, C., Experientia *38* (1982) 1493.
11 Vriend, J., and Gibbs, F., Life Sci. *34* (1984) 617.
12 Vaughan, G., Pineal Res. Rev. *2* (1984) 141.
13 Kennaway, D., and Seamark, R., Aust. J. biol. Sci. *33* (1980) 349.
14 Bubenik, G., J. exp. Zool. *255* (1983) 155.
15 Waldhauser, F., Waldhauser, M., Lieberman, H., Deng, M., Lynch, H., and Wurtman, R., Neuroendocrinology *39* (1984) 307.

0014-4754/85/121615-03$1.50 + 0.20/0

Rhythmic extrusion of pheromone gland elevates pheromone release rate

C. Schal and R. T. Cardé

Department of Entomology, University of Massachusetts, Amherst (Massachusetts 01003, USA), 22 August 1984

Summary. In two arctiid species, *Holomelina lamae* and *H. aurantiaca,* which rhythmically extrude and retract their abdominal tips during pheromone emission, pheromone glands contain up to three orders of magnitude more of the major component than in most Lepidoptera examined to date. Using an effluent collection technique, relatively high rates of pheromone emission were obtained from freely calling females. In contrast, volatilization rates from forcibly extruded glands were about 25 times lower for both species, suggesting that pulsation of the gland functions to increase the release rate.
Key words. Sex pheromone; pheromone emission rate; calling behavior; pheromone gland content; Arctiidae.

Many organisms transmit pulsed olfactory signals, most notably moths in the family Arctiidae[3]. Cardé[4] summarized three adaptive roles of pulsing the ovipositor and pheromone gland: 1) Higher release rates may be accomplished by 'spreading the pheromone over a larger surface area of the intersegmental membrane' in a manner analogous to 'scent marking'[5,6]. 2) 'Pulsing could create a frequency modulation of the chemical message'[3,4]. Chemical cues provide weak temporal and spatial stimuli compared to light and sound cues[7]. Signal to noise ratios may be increased and sensory adaptation reduced by introducing intermittency to the signal, thus increasing the number of potential comparisons[8]. 3) 'Pulsed message might provide an equivalent distance of downwind communication but conserve pheromone'[4]. Intermittent signals may communicate species-specific codes, positional information within the plume, and proximity to the emitter[3]. Of course, these are not necessarily mutually exclusive functions.

In this paper we address the effects of rhythmic pulsation of the abdominal tip on release rate of pheromone in two arctiid moths, *Holomelina lamae* and *H. aurantiaca.* Both emit 2-methylheptadecane as a primary component of the sex pheromone[9]. Other components, their diel periodicities in the pheromone gland and in air-borne collections, and the effects of age, weight, and wind on calling and pheromone release will be discussed elsewhere. Here we concentrate on the release rate and gland content of the most abundant component.

Individual ovipositor tips were extracted for 60 min in a 150-µl solution of 0.5 ng/µl 2-methylpentadecane (as internal standard) in redistilled n-hexane. Controls to correct for experimental losses included: a) extraction efficiency determined by extracting ovipositor-sized glass bulbs with known loadings; b) extraction efficiency from serial extraction of the same gland – 24 h in hexane greatly increased the amount of debris extracted from the gland, but only an additional 4.7% of 2-methylheptadecane was liberated; and c) losses of the two compounds due to reduction of 150 µl in a N_2 stream were equal. GLC analyses were conducted with a glass column packed with 3% SP-2100 (methyl silicone) at 155 °C for 5 min and temperature programed at 3°/min to 210 °C.

Just before calling[10,11], hexane extracts of the terminal abdominal segments of 2-day-old *H. lamae* and *H. aurantiaca* averaged 6484 ng and 935 ng (N = 14 each) per female, respectively (table). These, particularly the former, are very large quantities of pheromones which are up to three orders of magnitude greater than in other Lepidoptera.

To determine how pulsing influenced the rate of pheromone release we adapted a technique first used by Sower et al.[12] and improved by Baker et al.[13]. Just prior to the onset of calling, each female was inserted into a 4-mm ID tube and pressure applied by a pipe cleaner used to forcibly extrude the abdominal tip through a hole at the base of the tube (fig.). Collection tubes were modified pasteur pipets with one flared and one constricted end; they were silanized and filled with 200 mg of preconditioned[14] Porapak Q between glass wool plugs. The gland was positioned 3–5 mm below the outer rim of the flared end of the collection tube. Identical tubes in the same configuration were used to collect from freely calling females (fig.). Thus, tubes could be exchanged readily without disturbing the moth.

For 60-min collections, individual females were placed on a screen perch within the upper portion of a chamber composed of two silanized ground glass joints whose lower portion was packed with 150 mg of Porapak Q (fig.). In all collections 210 ml/min of air filtered through glass wool passed through the collection tube.

Pheromone was eluted from the Porapak Q with 800 µl of n-hexane in 100-µl aliquots, and 100 ng 2-methylpentadecane was added as internal standard. As with gland extracts, collection and extraction efficiencies (95%) were determined and amounts corrected accordingly. No breakthrough occurred when two collection tubes were arrayed in series for 3 h.

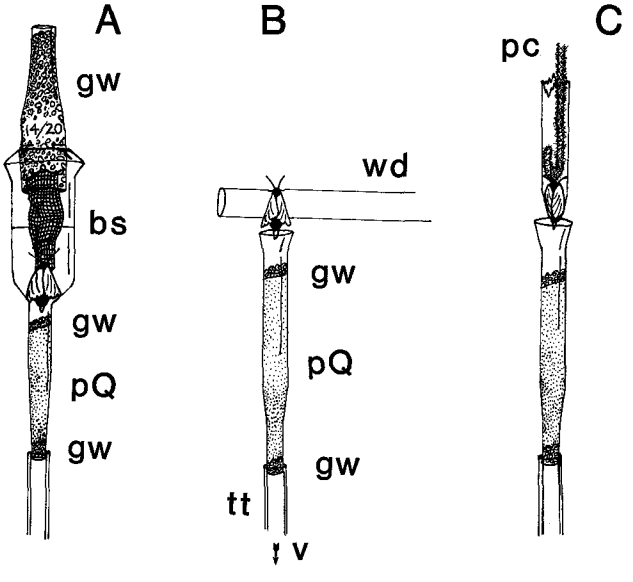
Collections of volatiles from freely calling females in the first 10 min of calling averaged 26.3 and 60.3 ng 2-methylheptadecane for *H. lamae* and *H. aurantiaca*, respectvely (table). Successive 10- and 60-min collections showed a rapid decrease in pheromone released. For both species, the quantities released in the first 60 min of calling were usually the maximum amounts released during the calling period, although calling persisted for 5–8 h in both species[10,11]. Presumably, females are located and mated early in the calling period, so that reduced release rates after the first h may have little significance in the field.

In contrast, 10-min collections from forcibly extruded glands resulted in significantly less pheromone, often indistinguishable from background levels (table). Pulsing is clearly required to effect emission of pheromone in *Holomelina*. Forcibly extruded abdominal tips release little pheromone, but pheromone may be extracted from these glands, and upon release, the female pulses her gland and pheromone is volatilized. These results clearly demonstrate that by modulating pulsation frequency, females may control the amount of pheromone they release.

The mechanism facilitating such control remains an enigma, but recent evidence (unpublished) indicates that specialized tubular glands, much like those documented for the arctiid *Utetheisa*[3] and the geometrid *Rheimaptera*[15], are involved. Bilaterally symmetrical tubes under tergites 7 and 8 are overlain by glandular epithelium, lined with spines, and end in two dorsal orifices between segments 8 and 9. The glands are lined with pheromone producing cells and spines which provide increased surface area and effect elastic reexpansion of the tubes. In *H. lamae*, extracted tubes contained ca. 95% of the total amount of 2-methylheptadecane extracted from the terminal segments. The mechanics of pheromone release in *Utetheisa* was suggested to involve rhythmic 'inhalation' of air into and 'exhalation' of pheromone-laden air out of the tubular glands. Without further histological evidence, we tentatively support this proposition but stress the importance of pulsing in increasing release rates, not in saving pheromone.

It appears that extrusion and retraction of the pheromone gland are important in species with internal, tubular glands[3,15]. However, significant discrepancies in release rates were found between forcibly extruded glands[12,13] and freely calling females[16] of the noctuid *Trichoplusia ni*, which does not pulse its abdominal tip and has a typical intersegmental pheromone gland. Moreover, surface washes after 10 min of vaporization from forcibly extruded glands resulted in no detectable pheromone[12], but up to 1200 ng of pheromone was recovered in whole gland extractions at the end of normal calling[16]. Hence, it appears that release rates may be elevated by either pulsation of specialized glands or other yet unknown transport mechanisms, involving intersegmental membranes. Caution must be exercised in the application of release rates obtained from forced mechanical[12,13,17] or chemical[18,19] extrusion of glands, as they may be significantly underestimated. This is particularly significant in view of the finding that in the noctuids *Heliothis zea* and *H. virescens*, increasing collection time from forcibly extruded glands from 10 to 30 min did not increase the amount of pheromone recovered[19]. Since males are attracted to freely calling females for several h, it is evident that transport and/or synthesis of pheromone are inhibited by artificial extrusion.

Specialized rhythmic behaviors for the dissemination of attractants are common in other animals. Male dung beetles rhythmically retract their hind legs against a sternal pheromone producing gland; extension of the legs expose the gathered pheromone to wind[20]. Females of the dermestid beetle, *Trogoderma glabrum*, extend their ovipositors and rhythmically pulsate their abdominal segments[21]. Oriental fruit moth males pulse their hairpencils 1–4 times during a 1.5-sec courtship display, possibly acti-



Apparatuses used to collect airborne pheromone emitted by individual moths. *A* The 60-min collection device for freely calling females. *B* The 10-min collection device for freely calling females. *C* The device for collection from forcibly extruded glands. bs, brass screen; gw, glass wool; pc, pipe cleaner; pQ, poprapak Q; tt, tygon tube; v, vacuum; wd, wooden dowel.

Mean ± SD (N) in ng of 2-methylheptadecane extracted from pheromone glands and collected as volatilized material from forcibly extruded glands and freely calling females

	Gland content	Forcibly extruded gland[a]	10-min collection Highest release period[b]	First release period[c]	60-min collection Highest release period[b]	First release period[c]
H. lamae	6484 ± 2143 (14)	3.6 ± 3.5 (9)	90.6 ± 105.5 (9)	26.3 ± 21.8 (9)	333.9 ± 303.5 (22)	322.3 ± 308.9 (22)
H. aurantiaca	935 ± 437 (14)	3.1 ± 2.7 (8)	84.9 ± 68.9 (7)	60.3 ± 51.8 (18)	370.8 ± 207.5 (10)	311.8 ± 184.9 (16)

[a] Terminal abdominal segments extruded for 10 min; [b] Mean of the highest release period for each female; [c] Mean of the initial calling period for each female.

vating a 'recharging' mechanism[22]. Some insects modulate release calling behavior (and probably release) throughout the calling period. The armyworm, *Pseudaletia unipuncta,* exhibits short calling bouts early (2 min) followed by considerably longer bouts[23]. The tortricid, *Choristoneura fumiferana,* pulsates the abdomen at the initiation of calling, but later the ovipositor remains extruded for up to 7 h[24]. If male arctiids utilize temporal information from pulses, they must modulate their responses according to pulsation frequency which, in addition to varying among individuals, also varies with time of day[3], temperature, and wind.

Wright[25] suggested that in odor plumes, flying insects modulate their internally generated zigzag paths by evaluating the frequency of 'on' and 'off' stimuli which they encounter by entering pheromone filaments and 'holes', respectively. Thus, as frequency and amplitude of pulses increase near the source, the insect narrows its lateral excursions. Conner et al.[3] liken pulsing of the gland to zigzag flight in 'continuous' plumes and argue that both may function to take the moth out of the plume periodically. However, the latter is unsupported, particularly by the findings that the zigzag legs narrow as pheromone concentration increases (e.g., Cardé[26]), opposite to what would be predicted by a disadaptation scheme.

Moreover, continuous emission was detected 2 m downwind of a source as bursts of about 250 msec duration occurring at a rate of twice per sec[27]. Since the separation between bursts must approach zero near the source, 'a rapid decrease in intermittency might be expected to be highly conspicuous to a flying insect'[28]. Given this potential mechanism for close-range orientation, how would a pulsing source increase its effectiveness? Indeed, pulsing pheromone to gypsy moth males in a wind tunnel did not alter the thresholds or latencies of responses, nor characteristics of upwind anemotactic flight when compared to a continuous source[28].

Pulsation of the gland may be used to increase the signal to noise ratio close to the source in insects with high release rates. With slow release it may not be necessary because peak concentrations are likely below the level required for sensory adaptation. However, *T. ni* and *Holomelina* have high release rates, but only the latter pulses. Clearly, information on male thresholds is requisite for elucidating this hypothesis.

Lastly, rhythmic pulsation may elevate the temperature of the gland, thus increasing the rate of pheromone emission[28]. We tested this hypothesis by remotely monitoring the temperatures of the abdominal tip and an adjacent abdominal segment with a Barnes Model RM-2A Infrared Microscope. During calling the temperature of the pheromone gland averaged 0.55 °C below abdominal surface temperature. Similar measurements for the gypsy moth, *Lymantria dispar* averaged 0.57 °C below abdominal temperature. Hence, pulsation did not elevate the emission rate of pheromone by increasing gland temperature. Indeed, the evaporative surface was slightly, but consistently cooler than the abdomen. Moreover, it seems that greater thermal influence upon release rates may be attained by selection of suitable microhabitats for calling, rather than by physiological heat production, especially in a day-active insect.

From the foregoing discussion we conclude that in arctiid females, pulsation of the abdominal tip elevates the rate of pheromone emission by effecting transport of pheromone from tubular glands to the surface. Lack of pulsing in many Lepidoptera, intermittency of continuously emitted signals, and analysis of flight tracks in a wind-tunnel suggest that frequency modulation of pheromone by pulsing of the gland probably does not provide males with additional information over non-pulsed sources.

1 We thank R. Charlton, R. Collins, J. Tang and R. Webster for valuable discussions, D. Smith for drawing of the figure, and Dr Robert W. Astheimer of Barnes Engineering for generously donating his time and an IR Microscope used in remote temperature measurements.

2 Present address: Department of Entomology and Economic Zoology, Cook College, P.O. Box 231, Rutgers, The State University of New Jersey. New Brunswick, New Jersey 08903, USA.

3 Conner, W.E., Eisner, T., Vander Meer, R.K., Guerrero, A., Ghiringelli, D., and Meinwald, J., Behav. Ecol. Sociobiol. *7* (1980) 55.
4 Cardé, R.T., in: Movement of Highly Mobile Insects: Concepts and Methodology in Research. Eds R.L. Rabb and G.G. Kennedy. North Carolina State University, 1979.
5 Colwell, A.E., Shorey, H.H., Baumer, P., and Vorhis Key, S.E. van, J. chem. Ecol. *4* (1978) 717.
6 Teal, P.E.A., McLaughlin, J.R., and Tumlinson, J.H., Ann. ent. Soc. Am. *74* (1981) 324.
7 Wilson, E.O., in: Chemical Ecology. Eds E. Sondheimer and J. Simeone. Academic Press, New York 1970.
8 Blaney, W.M., and Duckett, A.M., J. exp. Biol. *63* (1975) 701.
9 Roelofs, W.L., and Cardé, R.T., Science *171* (1971) 684.
10 Cardé, R.T., Can. Ent. *106* (1974) 933.
11 Schal, C., and Cardé, R.T., Physiol. Ent., submitted.
12 Sower, L.L., Shorey, H.H., and Gaston, L.K., Ann. ent. Soc. Am. *65* (1972) 954.
13 Baker, T.C., Gaston, L.K., Pope, M.M., Kuenen, L.P.S., and Vetter, R.S., J. chem. Ecol. *7* (1981) 961.
14 Byrne, K.J., Gore, W.E., Pearce, G.T., and Silverstein, R.M., J. chem. Ecol. *1* (1975) 1.
15 Werner, R.A., Ann. ent. Soc. Am. *70* (1977) 264.
16 Bjostad, L.B., Gaston, L.K., and Shorey, H.H., J. Insect Physiol. *26* (1980) 493.
17 Haynes, K.F., Gaston, L.K., Pope, M.M., and Baker, T.C., Envir. Ent. *12* (1983) 1597.
18 Pope, M.M., Gaston, L.K., and Baker, T.C., J. chem. Ecol. *8* (1982) 1043.
19 Pope, M.M., Gaston, L.K., and Baker, T.C., J. Insect Physiol. *30* (1984) 943.
20 Tribe, G.D., S. Afr. J. Sci. *171* (1975) 277.
21 Hammack, L., Ma, M., and Burkholder, W.E., J. Insect Physiol. *22* (1976) 555.
22 Baker, T.C., and Cardé, R.T., Ann. ent. Soc. Am. *72* (1979) 173.
23 Turgeon, J., and McNeil, J., Ent. exp. appl. *31* (1982) 402.
24 Sanders, C.J., and Lucuik, G.S., Can. Ent. *104* (1972) 1751.
25 Wright, R.H., Can. Ent. *80* (1958) 81.
26 Cardé, R.T., and Hagaman, T.E., Envir. Ent. *8* (1979) 475.
27 Murlis, J., and Jones, C.D., Physiol. Ent. *6* (1981) 71.
28 Cardé, R.T., Dindonis, L.L., Agar, B., and Foss, J., J. chem. Ecol. *10* (1984) 335.

0014-4754/85/121617-03$1.50 + 0.20/0