STUDIES OF THE EMERGENCE RHYTHM OF THE EYE GNAT *HIPPELATES PUSIO* LOEW (DIPTERA: CHLOROPIDAE)

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Periodic phenomena are common in organisms, from plants of certain species which show them by diurnal or nocturnal attitudes, up to insects and other animals, where they appear as daily rhythms of activity. Bouvier (1922) reviewed some of the early literature of rhymicity, and Brown (1944) has brought the review more up-to-date. Biological clocks, circadian rhythms, exogenous and endogenous mechanisms controlling animal activity are reviewed and discussed at length in the Cold Spring Harbor Symposium on Quantitative Biology (1960). Harker (1961) gives an up-to-date, thorough review on phase setting.

The purpose of my investigation was to determine if the emergence of eye gnats (*Hippelates pusio* Loew) follows a circadian rhythm and if there is any difference in the emergence pattern of the sexes.

The first of three experiments was conducted under field conditions. The second test was conducted in constant temperature and humidity to determine the effect of continuous light, continuous dark, and simulated field photoperiod. The third test was conducted in constant temperature and humidity to determine the effect of rearing eye gnats with the light-dark cycle 12 hours out of phase with the normal day-night cycle in July at Gainesville, Florida.

The insects used in these experiments were from a colony of *Hippelates pusio* Loew in the F_{66} through F_{69} generations. For general rearing methods and handling techniques see Jay (1961) and Scherer (1963).

EMERGENCE UNDER FIELD CONDITIONS

Materials and Methods. Approximately 6,000 one-day old eye gnat eggs were used to seed each of two bains-marie two-thirds filled with rearing medium.

The tops of the egg-charged bains-marie were covered with sail cloth and taken into a fenced area on 21 May 1963 and buried so that the surface of the sand on the medium was approximately even with the surrounding soil surface. The site afforded intermittent sun and shade. The containers and the surrounding soil were covered by a 30-inch by 36-inch piece of ¼-in plate glass one foot above the ground to keep rain from filling the test containers while allowing light to enter freely. Ten days after egg setting the sail cloth covers were removed. Two emergence chambers were constructed by cutting 8-inch diameter holes into the bottoms of two cans 10 inches in diameter and 14 inches high. A circular piece of 30-mesh screen was soldered to each of these openings to admit air and light. The outer ring of a mason jar was soldered into a hole cut on the side very near the bottom of each can. These collection chambers

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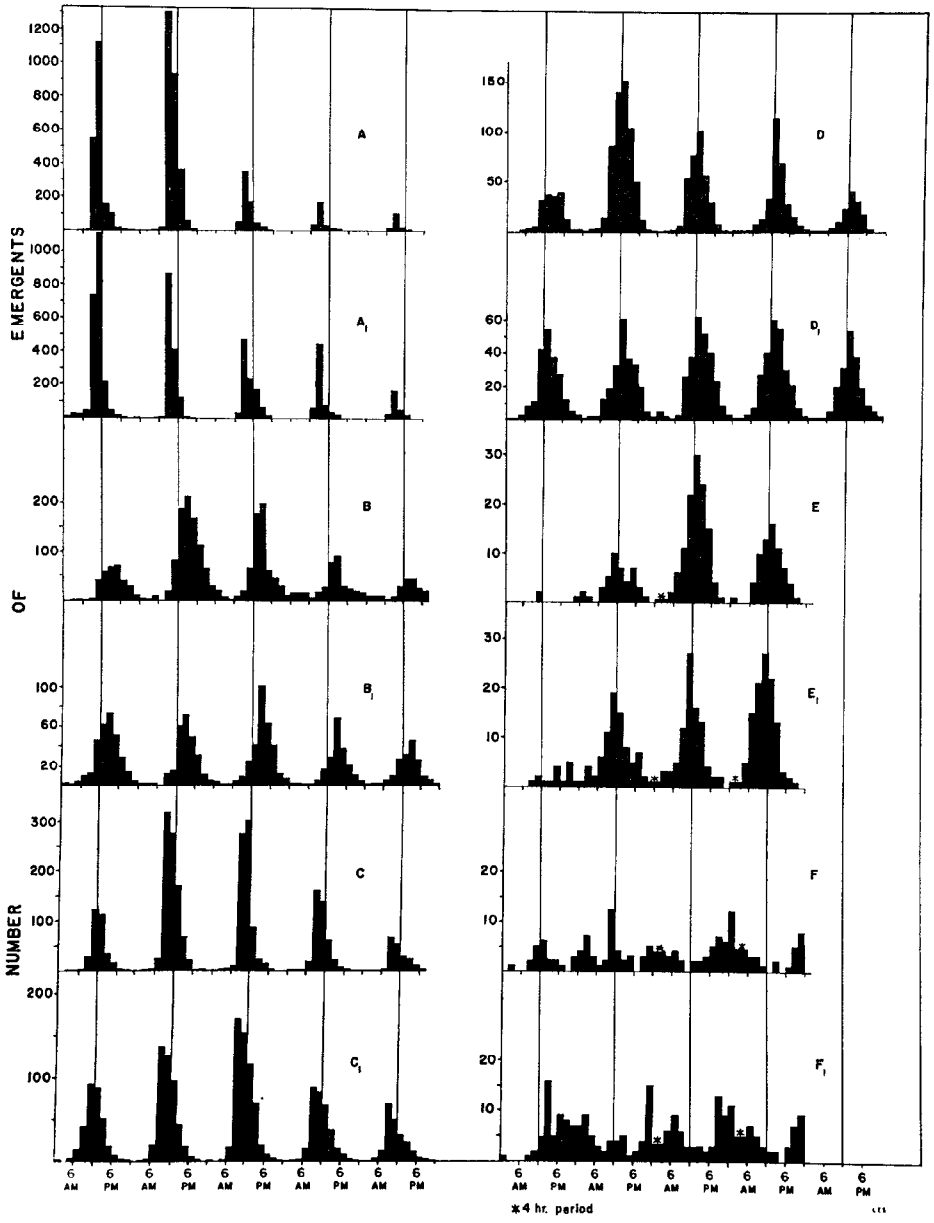

Fig. 1. Emergence in *Hippelates pusio* cultures exposed to various conditions. A & A_1, field conditions; B & B_1, continuous light; C & C_1, 10 hours light-14 hours dark; D & D_1, continuous dark; E & E_1, reared in 10 hours light-14 hours dark but 12 hours out of phase with the natural photoperiod and returned to the normal phase two days before emergence began; F & F_1, same as E & E_1 except colony in out-of-phase photoperiod during emergence as well as during rearing. Dates of emergence are as follows: A, A_1 June 5-10; B,C,D, June 13-18; B_1, C_1, D_1, E, E_1, F, F_1 July 4-8. The collections which are starred are for 4-hour periods, and in each such case the data are represented as two, equal, 2-hour collections.

were inverted over the two test containers and were given several twists to plant the can rims firmly in the soil. One-quart mason jars were turned into each of the threaded rings. On the fifteenth day after egg setting, gnats began emerging, and the first collection was made at 8:00 PM of that day. Collections were made every two hours for 48 hours. The remaining collections were made at two hour intervals from 8:00 AM until 6:00 PM. Gnats that emerged after 6:00 PM were released at 6:00 AM on the remaining days. All collections were made by removing the glass cover and draping the screened tops of the large cans with black cloth. Since eye gnats are positively phototactic, they went into the collection jars. The collected gnats were preserved in alcohol and later sexed and counted.

Results and Discussion. Emergence followed a fairly regular rhythm (Fig. 1, A & A_1). Maximum emergence occurred at 10:00 AM each day except on the first day, when it occurred at noon. This lag is difficult to explain but an hypothesis follows. Maximum emergence was delayed the first day because the rate of maturation in the population was rapidly increasing. Therefore, many flies became physiologically prepared to emerge during the "normal period of emergence" (8:00 AM to 4:00 PM) and peak emergence throughout this period was between 10:00 AM and noon. This normal period of emergence is determined by the collective "biological clocks" of the gnat population. Those gnats with their clocks out of phase with the majority of the population emerge at times other than the normal period of emergence (i.e., late in the afternoon and during the night). Since the number of gnats maturing per unit time was rapidly increasing the first day, the peak of emergence occurred when this rate was the highest. The peak was supplemented by the number of gnats that had matured earlier but whose biological clocks would not allow them to emerge until the normal period of emergence. Since on the remaining days the number of gnats maturing per unit time did not increase during the normal period of emergence, the peak of emergence occurred earlier.

Over 90 percent of the gnats emerged each day between 8:00 AM and 2:00 PM. Collections were discontinued five days after emergence began because the maximum emergence for a two hour period dropped to less than 10 per cent of the largest two-hour maximum previously reached. The total daily emergence had also declined to less than 8 per cent of the maximum daily emergence.

Confirming the findings of earlier workers, Pittendrigh (1954) showed that *Drosophila melanogaster* had an emergence peak soon after dawn. Brett (1955) confirmed Pittendrigh's work and gave 6:00 AM to 9:00 AM as the time of peak emergence. The adaptive significance of a dawn emergence is clear since emerging flies lose water at a rate of at least double that of mature flies and fail to expand their wings properly when the humidity is too low. My experiment suggests peak emergence in eye gnats under field conditions occurs 2 to 3 hours later in the morning than *Drosophila,* and the adaptive significance of such an emergence time is not clear.

Working with *Aedes taeniorhynchus,* Nielsen and Haeger (1955) noted the peak emergence of these mosquitoes was near sundown. Horsfall (1955) noted that mosquitoes in general emerge near sundown and pointed out that the water is ordinarily calm at that time.

EMERGENCE RHYTHM UNDER THREE ARTIFICIAL LIGHT REGIMES

Materials and Methods. At 1:00 PM on the day before expected initial emergence the bains-marie were placed in a bioclimatic chamber with temperature maintained at 80 ± 1° F and relative humidity maintained at 70 ± 5 %.

Three light regimes were attained by keeping one bain-marie in continuous artificial light, a second in continuous dark, and a third in 10 hours dark and 14 hours light. The two emergence cages used in the field tests were used for the continuous light regime and the simulated field conditions. A new 4-gallon emergence cage with the outer ring of a mason jar soldered in the center of the bottom was used for the continuous dark test. The emergence cage was lined with black cloth and inverted over the bain-marie. A mason jar lid placed in the outer ring excluded light except during collections, when the lid was removed and a 1-quart mason jar was turned into the threaded ring in the top of the emergence cage. An 8-inch diameter black cardboard disc was supported one inch above the bain-marie by four pieces of wire, and this disc in conjunction with the black cloth lining of the emergence cage allowed little or no light to reach the medium during collections.

The container in simulated field conditions was completely darkened from 8:00 PM until 6:00 AM, while the one in continuous light was only darkened for a 5-minute collection period every two hours.

The light intensity at the surface of the medium in the cans that were allowed light was 25 foot candles (measured by a General Electric 80W40Y-16 light meter).

Collections were taken in continuous light by covering the screened top of the container with black cloth, thereby forcing the gnats into the one-quart collection jar. The cage exposed to 10 hours dark - 14 hours light when in the light phase was treated the same way as the continuous light cage; however, when the can was covered for the dark phase, a center disc was placed in the mason jar ring. The disc was removed only during the 5-minute collection periods when a collection jar was placed in the ring. The continuous dark cage contained a disc in the ring at all times except during collection when a jar was turned into the ring.

Results and discussion. In this experiment eye gnats followed a circadian rhythm of emergence even under constant temperature and humidity with light conditions different from nature. There was approximately a 4-hour lag in peak emergences for gnats maintained in continuous dark (Fig. 1, D & D_1) as compared to those in 10 hours dark and 14 hours light (Fig. 1, C & C_1). The peak emergences in continuous light (Fig. 1, B & B_1) were 6 hours after the peak emergences in simulated field conditions. This demonstrates that eye gnats might have the phase of their emergence rhythm altered within one day of emergence (at least in the case of those that emerged on the first day).

The largest emergence peak of gnats in continuous light represents only 30 per cent of the total emergence for the day as compared with a 42 per cent emergence peak for the gnats in 10 hours dark - 14 hours light. The pupae exposed to continuous light and continuous dark emerged over a more extended period each day than those in the simulated day-night cycle. The extended period of emergence and the less pronounced rhythm in the continuous conditions are obvious in Fig. 1, B, B_1, D, D_1.

When cultures that were reared in alternating light and dark were placed in constant conditions, their emergence rhythm was evidently already set. The rhythm persisted in the constant conditions slightly out of phase with the rhythm in alternating light and dark.

Bremer (1926, in Brett 1955), working with the Mediterranean flour moth *Ephestia kuhniella,* and Lewis and Bletchly (1943, in Brett 1955), working with the dung fly, *Scopeuma,* reported the emergence rhythm was spread out in cultures maintained in constant conditions. This spreading effect was also demonstrated by Brett (1955) in Drosphila.

Brett (1955) also noted that cultures maintained in constant light from the time of oviposition exhibited no circadian rhythm of emergence. This is contrary to the findings of Bunning (1935, in Cloudsley-Thompson 1961), who reported that an emergence rhythm still persisted after fifteen generations in constant light. Work by Pittendrigh (1954) casts doubt on Bunning's work by showing no discernable rhythm in continuous conditions.

Many workers have shown that activity rhythm of animals are entrained by light and to a much less marked degree by temperature and humidity. Recently Brown (1962) has postulated that rhythms are also influenced by cosmic rays and the earth's magnetic field.

If one assumes that light is the stimulus that entrains the circadian rhythm of emergence, then my experiment clearly shows that the light stimulus establishing the emergence rhythm was effective earlier than the thirteenth day of development (at 75° F), otherwise, no rhythm would have been obtained in continuous dark or continuous light.

Emergence Rhythm of Eye Gnats Reared in Light Twelve Hours Out of Phase with the Natural Day-Night Cycle

Materials and Methods. Eye gnat eggs were placed in two bains-marie two-thirds filled with rearing medium. The containers were placed in a bioclimatic chamber maintained at 80 ± 1°F and 75 ± 5% relative humidity. The light was turned on at 6 PM and off at 8 AM for 12 days; then the light remained on until the experiment was concluded. The different light regimes were attained by covering and uncovering the emergence cages with four layers of black heavy cordurory cloth at the proper times. On the twelfth day (two days before the first emergence) at 9 PM the medium in each of the two bains-marie was divided into two equal portions. One-half of each of the two original containers was held in the same conditions in which they were reared; while the other half of each was placed in the light at 6 AM and dark at 8 PM (similar to natural light conditions). The gnats were collected every two hours as in previous experiments.

Results and Discussion. The emergence followed a regular rhythm for those gnats that were changed to the natural day-night cycle approximately two days prior to the first emergence (Fig. 1, E & E_1). The peaks of emergence for these gnats occurred at 2 PM for three consecutive days. The peaks of emergence for those gnats that remained in light conditions 12 hours out of phase with natural conditions (Fig. 1, F & F_1) occurred between 10 PM and 4 AM for three consecutive days, but the peaks were much less pronounced. The first 24 hours of emergence was erratic in both light conditions. The low numbers of gnats emerging in this experiment is unexplained; however, even with these low numbers a rhythm is evident.

When the gnats that had their light cycle 12 hours out of phase with the natural cycle began to emerge, the light cycle conflicted with that experienced by the eggs at oviposition in the laboratory, and perhaps this caused the less pronounced peaks and the somewhat erratic emergence. Certainly, if the exogenous mechanism that regulates the emergence rhythm is light or the change from light to dark or vice-versa, then the only explanation I can give is that the rhythm was partially entrained in the eggs either while they were in the laboratory before being placed in the medium or through the short periods of light and dark during collecting.

The results of this experiment might help to explain why the emergence peak in the field test was as late as 10 AM. The eggs placed in the field may have already been partially entrained to a later-than-normal dark period (laboratory lights often burned late). This might have resulted in a later-than-normal hour of peak emergence.

SUMMARY

The eclosion or emergence rhythm of a laboratory strain of eye gnats was determined under field conditions. The maximum emergence occurred at 10 AM on all days except the first day.

Eye gnat pupae were placed in constant temperature (80° F.) and humidity (75 per cent) under the following three light regimes: continuous light, continuous dark, and 10 hours dark and 14 hours light (similar to field conditions in June at Gainesville, Fla.). A rhythm of emergence occurred in both continuous dark and continuous light, but the peaks of emergence were delayed four to six hours and were less pronounced than in the simulated field conditions.

When one-half of the eye gnats that were reared at 80° F and 75% relative humidity and with the light and dark 12 hours out of phase with the natural day and night were changed two days prior to emergence to a light and dark cycle in phase with the natural cycle, their emergence followed a regular rhythm with peaks at 2 PM. The remaining half of the gnats were allowed to emerge in light and dark conditions 12 hours out of phase with the natural cycle, and their emergence showed a poorly defined rhythm with peaks between 10 PM and 4 AM.

These experiments suggest that the rhythm of emergence of eye gnats may be partially entrained in the egg stage by light conditions immediately after oviposition.

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