CHARACTERISTICS OF THE DAILY BEHAVIOR AND ACTIVITY PATTERNS OF THE ADULT HOUSEFLY WITH SPECIAL REFERENCE TO TIME-KEEPING DEVICE

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SUMMARY: Characteristics of the daily behavior and activity patterns of the adult housefly were investigated with special reference to time-keeping device. (1) Two of three strains and four of eight colonies of flies showed similar resting behavior patterns to those of the standard Takatsuki colony, whereas two colonies exhibited markedly different patterns. The remaining one strain and two colonies of flies displayed intermediate resting behavior patterns. Flight activity patterns exhibited by all strains and colonies of flies were essentially similar to those of the Takatsuki colony. The general behavioral pattern exhibited by male adults in groups was different from that of females due to the increased mating activity. Female flies exhibited decreased stability in settling on the ceiling of the container as they grew older, while the number of landing on the ceiling increased during the first few days of adult life and then stayed rather constant. (2) The flies were entrained to a 24-hr photoperiod with a 12-hr photophase. The circadian rhythm with 12-hr active phase persisted in individual flies for more than two weeks in continuous darkness, although in some cases changes in both the period of the rhythm and duration of the active phase occurred simultaneously. The flies in groups also continued to keep rhythmic behavior in continuous darkness at least for a week. Most individual rhythmic flies displayed only once the characteristic nighttime resting behavior at the expected onset of scotophase, while the individual flies held under continuous illumination from their emergence did not exhibit any sign of circadian rhythms. Individual flies kept in the actograph chamber also showed a rapid disappearance of the activity rhythm under continuous illumination. (3) In the flies subjected to a 24-hr photoperiod with a 14- to 20-hr photophase, the resting phase of the rhythm was moved ahead by about 1-4 hr. The time change correlated to the duration of the previous photophase. Majority of flies ignored the photoperiod with a 22-hr photophase. In the flies exposed to a 24-hr photoperiod with a photophase shorter than 6 hr, the resting phase was delayed by a few hours. This result accounted for the inexplicable resting behavior of flies in nature under long-day conditions. A single dark period of 6 hr or longer, interrupting continuous illumination, initiated the overt periodicity in the flies. The time of the onset of resting behavior was inversely related to the duration of a single dark period. (4) A single dark stimulus for the resetting was effective only when given after the critical time, viz. at 9 hr after the onset of illumination; whereas a cyclical change in the direct response to a single dark stimulus was manifested by the rhythmic flies, maximal response occurring at the onset of the resting phase and the minimal one immediately after the onset of light.

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INTRODUCTION

The housefly is a ubiquitous pest of medical and sanitary importance. Nevertheless, the basic knowledge of the fly behavior has only meagerly been accumulated.

In a previous paper (Tsutsumi, 1966), the behavior and flight activity patterns of houseflies released in a small test container were reported under conditions of constant temperature and various light regimes. When flies subjected to a 24-hr photoperiod with a 12-hr photophase were placed under continuous illumination, they showed the resting behavior only once and then became arrhythmic. This fact suggested that the characteristic nighttime resting behavior was caused by some internal factor.

In a subsequent paper (Tsutsumi, 1968), the effect of some environmental factors on the behavior and activity patterns of rhythmic flies was reported. The occurrence of the resting behavior was inhibited under bright light (over 2,000 lux) or extreme temperature condition (higher than 40 C or lower than 10 C), and greatly influenced by hunger.

Such biological factors as the strain, sex and age may also modify the typical rhythmic behavior and activity patterns of the flies.

Since direct observation always involves errors made by the observer it seemed to be more advantageous to use an actograph capable of recording rhythmic activity of a single housefly over a long period of time.

When the result of some observation under natural conditions (unpublished) was compared with the laboratory data described previously (Tsutsumi, 1966), some difference became apparent in the time of the onset of the resting phase. Therefore, studies were made to find factors affecting the time of the onset of the resting phase.

In the majority of studies (Chiba, 1964, 1966; Gunn, 1940; Lipton and Sutherland, 1970; Lohmann, 1964; Minis, 1965; Nayar and Sauerman, 1970; Nowosielsky and Patton, 1963; Roberts, 1960; Youthed and Moran, 1969), persistent rhythms have been described in nocturnal or crepuscular insects, in which duration of active phase was shown to last only for several hours, or two peaks of activity were usually found.

Thus, the paucity of information concerning properties of daily periodicity of diurnal insects prompted a series of experiments designed (1) to study biological factors regulating daily behavior patterns, (2) to obtain actograph records of activity patterns of individuals, (3) to examine the effect of photofraction in a 24-hr photoperiod on the resting behavior, and (4) to find the function of the resetting system of the time-keeping device of this typically diurnal insect.

In the present paper, the results of these experiments will be described together with interpretation of the observations made in the field.

MATERIALS AND METHODS

In the present experiments, strains and colonies of the housefly, *Musca domestica* L., maintained in this laboratory, were used. The fly larvae were reared in continuous illumination at 25 ± 1 C on a diet of the following composition: wheat bran, 100 g; granular diet for rearing insects (Oriental Yeast Co., Tokyo), 100 g; fish meal, 5 g; baker's dry yeast (Oriental Yeast Co.), 0.5 g; water, 100 ml. The pupae were

transferred into a cage (30×30×30 cm); the emerged flies were supplied with 0.2M sucrose solution during their first day.

All the experiments were conducted at 26 ± 0.5 C in an experimental dark room illuminated by three 20-W "White" fluorescent lamps recessed into the ceiling of the room and covered with a frosted resin plate.

To examine the behavior of the flies, the following methods were adopted.

1) The test container

Apparatus. The test container was 56×61×115 cm (height), built up of five plywood plates and a transparent plastic plate. Three test containers were placed in

TABLE I

Light intensities in lux on each inner surface of the three test containers

Container	1	2	3
Ceiling	2-5	2-4	2-4
Wall A*	10-86	7-65	8-74
Wall B	3-32	1-15	3-15
Wall C	2-15	4-26	2-29
Wall D	2-10	2-12	2-12
Floor	20-35	10-35	15-38

* Wall A: front; Wall B: right; Wall C: left; Wall D: back

Light intensity was measured by Toshiba Illuminometers No. 5 made by the Tokyo Shibaura Electric Co., Ltd.

a row on a desk in the experimental dark room. Light intensities on each inner surface of these containers are presented in Table I.

Procedure. On the second day from their emergence, flies narcotized with ethyl-ether were counted and a batch of 50 was placed in a small screened cage of 11 cm in diameter by 27 cm in height containing fresh cotton pads moistened with 0.2 M sucrose at 26 ± 0.5 C, and maintained under this condition for one or two days. The flies were then transferred into a test container.

To examine the behavior, flies on each surface of the container, viz. ceiling, floor and walls, and those on the food were counted every 15 or 30 min. When counts in complete darkness were needed, a noctovision NVD 2001 attached with a Nikkor 50-mm objective lens (NIPPON ELECTRIC CO., Tokyo) was used. To estimate the activity, flying insects during a 30-sec period were counted every 30 min. The mean of three counts was recorded to express the flight activity.

The behavior patterns of individual flies were recorded with marked flies. Thoracic mesonota of seven narcotized flies of the Takatsuki colony were marked with lacquer paint. To recognize individual marked flies quickly at a distance of about one meter, such light-coloured paints as white, yellow and greenish white were preferable.

In Exp. 1 (a), the resting behavior patterns of the following three strains and

nine colonies of flies were examined.

The Lab em 7 em strain: derived from the CSMA colony and further selected by the repeated one-pair mating method.

The SRS strain: a standard insecticide-susceptible strain.

The *brwb* (Brown body) strain: a mutant of yellowish-brown body.

The Takatsuki white-eye colony: a white-eye mutant.

The CSMA white-eye colony: a pale yellow-eye colony.

The Mix 6b colony: collected in Denmark in 1964 as a high resistant fly to diazinon and selected with Bromophos pressure.

The 153 e2b colony: discovered in Denmark in 1963 as organophosphorus-insecticide resistant and selected with further malathion pressure.

The Hokota colony: originated from Hokota, Ibaraki Prefecture, Japan in 1960.

The RP-dia colony: collected in a district in Switzerland, where diazinon had been applied as a residual spray, and selected by Geigy Co.

The Nebi-dia colony: captured at Nebi, Ibaraki Prefecture, Japan in 1962 as diazinon and DDT resistant.

The Kokura-BHC colony: derived from a progeny selected by BHC pressure for two years after collected in Kokura, Fukuoka Prefecture, Japan in 1961.

The Takatsuki colony: originated from Takatsuki, Osaka, Japan.

2) The actograph recorder

Apparatus. An actograph recorder was made based on the principle of the capacitance sensitive device described by Grobbelaar et al. (1967) (Fig. 1). The instrument consisted of five parts; viz. a sensor chamber (Fig. 1A, a), an oscillator (Fig. 1A, b), a frequency detector (Fig. 1A, b), an amplifier (Fig. 1A, c) and a recorder (Fig. 1A, d). The basic principle was as follows: the oscillator frequency (approximately 10.7 Mc/sec) was changed by a variation in the capacitance between two spiral wire electrodes when the insect moved in the sensor chamber. This variation in frequency was amplified and converted into a pulse-like voltage variation, which would drive a pen-oscillo-recorder. The sensor chamber was a ring-like space (Fig. 1B, e) of 30 mm in depth by 75 mm outer or 55 mm inner diameter. An inner electrode (Fig. 1C, h), fixed on the acrylic resin plate, was made by winding polyvinyl formal coated copper wire (PVF wire) about 23 times around an acrylic resin pipe (54 mm outer diameter by 100 mm in length) and was connected to the preamplifier (Fig. 1C, b). The same PVF wire spiral wound about 22 times around a glass cylinder (75 mm inner diameter by 50 mm in length) served as an outer electrode (Fig. 1C, i). This outer electrode was easily separable from the connecting terminal as shown in Fig. 1C. A sheet of ring-like polyethylene mesh screen (Fig. 1C, j) was glued to the inner wall of the glass cylinder, serving as the floor of the sensor chamber. A transparent acrylic resin ring plate (Fig. 1C, k) was plugged as the ceiling of the chamber, in which the insect was allowed to move. Electronic circuits such as the oscillator, frequency detector and amplifier, including a pen-oscillo-recorder, were those manufactured by Nikkei Densoku Co., Ltd., Tokyo. A glass capillary tube containing water (Fig. 1B, f) was projected vertically into the sensor chamber through a slit cut in the ceiling plate and secured to the inner acrylic resin pipe with plastic tape. Sucrose crystals (Fig. 1B, g) were placed on the floor for food. To avoid accidental noise, each sensor chamber was placed in an individual box [260×310×220 mm (depth)], which was made of black acrylic resin plates. Under lighting conditions,

Fig. 1. The capacitance-sensitive-type actograph.

A: the actograph recorder for a single housefly; B: a part of the actograph;

C: a part of the sensor chamber.

a: sensor chamber; b: preamplifier; c: main amplifier; d: pen-oscillo-recorder; e: small ring-like space of sensor chamber in which a single fly is confined; f: a capillary tube containing water; g: sucrose crystal; h: inner electrode; i: outer electrode; j: floor of sensor chamber; k: acrylic resin lid plate.

the box was covered with a transparent acrylic resin lid; the maximal light intensity in the box was 12 lux. For complete darkness, it was covered with a black acrylic resin lid and black light-proof cloth.

Procedure. A single virgin female, lightly narcotized with ethylether, was transferred from a breeding cage into a sensor chamber of the actograph. Since the recording paper proceeded at a speed of 2 mm per min, it was possible to read the record that the fly was completely inactive only when her inactive state lasted for 18 sec or longer. The machine count was corroborated by simultaneous observations in preliminary tests. The activity was expressed in percentage of time spent in overall activity, including locomotor, cleaning and proboscis extension etc. per unit interval (30 min) (Fig. 2), since the full-scale deflection of the recorder needle did

Fig. 2. Activity records of houseflies obtained from the capacitance-sensitive actograph.

Periods of locomotion of the fly on the side wall of the chamber (Lw), locomotion on the floor (Lf) and rest (R) are indicated.

not necessarily indicate the state of the maximal activity of the insect because of the local difference in sensitivity within the sensor chamber. The calculation of the period of the free running rhythm was made by the method described by Roberts (1960).

More detailed procedures for specific experiments are described in the pertinent places in the text.

In the paper, the following abbreviations are used: LD signifies conditions of alternating light and darkness; LD 12:12 more specifically indicates a regime in which 12 hr of light alternate with 12 hr of darkness; DD means continuous darkness; LL continuous illumination.

RESULTS

*Experiment 1. The Strain, Sex and Age as Factors Regulating the Behavior and Activity Patterns of the Fly**a) The strain*

Averages of six counts at 30-min-intervals of flies resting on the ceiling after the anticipated onset of the resting phase are presented in Table II. Two strains and four colonies of flies showed similar resting behavior patterns to those of the

TABLE II

Comparison of the resting behavior of different strains and colonies of the housefly at the onset of the resting phase

Strain (or Colony)	Average no. of flies resting on the ceiling (%)	Lower limit (%)	Upper limit (%)
Lab em 7em	66.8	60.1	72.6
SRS	57.0	48.8	64.8
<i>bwb</i> (Brown body)	28.3	23.2	35.0
Hokota	70.3	62.0	76.9
Rp-dia	61.9	55.3	68.2
Nebi-dia	61.6	53.9	69.5
Kokura-BHC	50.0	41.9	58.1
CSMA white-eye	17.8	12.7	25.2
153 e2b	15.8	11.0	23.1
Takatsuki white-eye	5.3	3.1	9.3
Mix 6b	5.3	3.1	9.3
Takatsuki	50.8	44.0	57.3
	52.7	46.0	59.2
	49.3	42.7	56.0

Average of six counts beginning from the anticipated onset of resting phase was divided by the total number of flies released.

Takatsuki colony described previously (Tsutsumi, 1966). The number of flies resting on the ceiling increased rapidly towards the anticipated onset of the resting phase, whereas two colonies, i. e. the Mix 6b and the Takatsuki white-eye, exhibited markedly different patterns. The remaining one strain and two colonies of flies displayed intermediate resting behavior patterns. The behavior of the Mix 6b colony is illustrated in Fig. 3A, in which only less than 20 % of the total flies at most are shown to rest on the ceiling. When flies of the Takatsuki colony were used, such an unusually small value has never been obtained (Fig. 3B). The activity patterns exhibited by flies of the Mix 6b colony and those of the Takatsuki colony were essentially similar.

Fig. 3. Hourly change in percentage of the flies resting on the ceiling in LL after subjected to a 24-hr photoperiod with a 12-hr photophase.

Each line is from a separate experiment.

A: the Mix 6b colony (female adult); B: the Takatsuki colony (female adult);
C: the Takatsuki colony (male adult).

b) The sex

Male adults of the Takatsuki colony were kept in a light cycle of LD 12:12 for three days from the third day of their emergence. Observations and counts were made on the sixth day from their emergence.

The behavior patterns of flies are shown in Fig. 3C. Although the behavior of

male flies seemed to vary slightly with the time and there was some increase in number of flies on the ceiling towards the onset of resting phase, the general pattern was markedly different from that of female flies. In the absence of female adults, male flies very frequently attempted to mate with those of the same sex. Such behavior was also recognized throughout the anticipated resting phase.

c) The age

Ten female flies of the Takatsuki colony were released into a test container immediately after their emergence. To estimate behavioral change in relation to age, the following two indices were adopted; (1) the total flies landing on the ceiling for 10 min—Index L, and (2) the total cumulative number of flies resting on the ceiling every minute for a 10-min period—Index R. These counts were made at intervals of an hour from 10:00 to 22:00 hr Japanese Standard Time (J. S. T.) every other day.

Flies changed their resting behavior with the lapse of days, as shown in Fig. 4. Resting on the ceiling (expressed as Index R) gradually decreased, whereas landing onto the ceiling per unit interval (Index L) increased during the first few days of adult life, and then stayed rather constant.

Fig. 4. Effects of fly age on the stability of resting on the ceiling (Index R) and on the landing performances onto the ceiling (Index L).

Index R: the cumulative number of flies resting on the ceiling every minute for a 10-min period (circle). Index L: the total number of flies landing on the ceiling for 10 min (triangle). Countings were made at intervals of 1 hr from 10:00 to 22:00 hr J.S.T. every other day.

Experiment 2. Behavior and Activity Patterns of Individual Flies

a) Patterns in DD and LD

Three different female flies of the Takatsuki colony were simultaneously recorded with three sets of the actograph on the three channel pen-oscillo-recorder. Two (fly "A" and fly "B") were kept in DD after subjected to two cycles of LD 12:12

(Fig. 5A, B). As shown in Fig. 5A, activity rhythm of the fly "A" remained typically circadian; the period was estimated to be about 24.5 hr with an active phase lasting for about 12 hr.

A somewhat different pattern was recorded in the fly "B", in which activity rhythm remained, though its activity was initially rather low. The period was nearly 24 hr with an active phase much shorter than that in the fly "A". However, in the middle of the second week, the fly exhibited an increased activity and the period changed to about 25.3 hr (Fig. 5B).

The fly "C", subjected to LD 12:12 cycle throughout the experiment, was entrained to the environmental LD cycle (Fig. 5C).

Fig. 5. Activity patterns of three individual adult flies recorded simultaneously. A and B: in DD after subjected to two cycles of LD 12:12; C: in LD 12:12 throughout the experiment.

The records for each day are placed one below the other. The circle denotes the daily light condition. ◐, LD 12:12; ●, DD.

Since in DD the activity rhythm exhibited by a single fly in an actograph chamber appeared to continue for a considerably long period of time, another observation was made to ascertain if female flies of the Takatsuki colony would also exhibit such persistent rhythmic behavior in groups in a test container. The flies resting on the ceiling were counted in complete darkness at intervals of one hour with the noctovision a week after the onset of DD. The results are presented in Fig. 6. The periodicity seemed to continue without damping out, although peaks were slightly flattened in appearance.

b) Patterns in LL

It was shown earlier (Tsutsumi, 1966) that a group of 50 flies released in a small

Fig. 6. Hourly change in the number of flies resting on the ceiling in DD. The flies were subjected to light cycles of LD 12:12 for three days and then kept in DD for a week. The arrow indicates the time when scotophase was set a week before.

container and kept in LL after three light cycles of LD 12:12 displayed only once the characteristic nighttime resting behavior at the anticipated onset of darkness. To ascertain if individual females in a container also exhibit this behavior pattern, a series of experiments was conducted. Marked flies were placed in a small screened cage until the third day of their emergence. After release, the flies were checked for exhibiting normal behavior and subjected to three cycles of LD 12:12 and then to LL.

Fig. 7. Behavior patterns of seven individual flies (A-G) in LL after subjected to light cycles of LD 12:12 for three days.

The tallest bar indicates that the fly rests on the ceiling, the medium-height bar on the walls, and the shortest bar on the floor. Countings were made at 15-min intervals.

The results are presented in Fig. 7. Four of seven females visited and then stayed for a few hours on the ceiling in the anticipated resting phase in LL.

With 14 marked flies without subjecting to dark period from their emergence, no sign of the approximate 24-hr cycle characteristic of circadian rhythms was detected (Fig. 8).

Fig. 8. Behavior patterns of 14 individual flies (H-U) in LL.

The flies had been subjected to LL since their egg stage. For other explanations, see Fig. 7.

A similar result was obtained with the actograph. Eleven of 22 female flies placed in LL for days after two cycles of LD 12:12 exhibited only once the characteristic nighttime resting patterns (Fig. 9A). Only one female showed a second trough in activity at 36 hr after the onset of LL. Other 10 exhibited considerably high levels of activity from the onset of LL showing no overt rhythmicity (Fig. 9B).

Experiment 3. Behavioral Response to Various Photofractions

a) Behavior and activity patterns under various photofractions

The result of a preliminary observation on the resting behavior of the flies in a large outdoor cage is presented in Fig. 10. Since the observation was made on June 18, the flies were exposed to a natural photoperiod with a 15 hr 52 min photophase (3:46 to 19:38 hr J. S. T.). In the outdoor cage, flies resting on the ceiling increased in number at 18:00, when the light intensity was still high enough to allow them to fly freely. The fact suggested that the flies were not entrained to this light environment in contrast to the results obtained in the laboratory. It seemed necessary to find factors responsible for this inexplicable phenomenon. Since the light appeared to

Fig. 9. The activity pattern of an adult fly in LL after exposed to two cycles of LD 12:12.

A: an incomplete nighttime rest occurred only once; B: no nighttime rest occurred.

Fig. 10. Hourly change in the number of flies resting on the ceiling and the floor of an experimental hut placed in a large outdoor cage.

Countings were made on June 18. The arrow denotes the time of the sunset.

be the most important environmental variable, behavioral patterns of flies under various photofractions in a 24-hr photoperiod were examined in the laboratory. Female flies of the Takatsuki colony were subjected to a 24-hr photoperiod with a 2-, 4-, 6-, 8-, 10-, 14-, 16-, 18-, 20- or 22-hr photophase from the third day of their emergence.

In Fig. 11, the activity and behavior patterns of the flies subjected to the photoperiod with a photophase longer than 12 hr are presented. Under these "long day" conditions, the flies exhibited the characteristic resting behavior before the onset of scotophase. The time of the beginning of resting phase seemed to be correlated with the time of the onset of photophase. For instance, if the light was turned on 4 hr before the anticipated onset of the active phase, the commencement of the resting phase advanced by approximately 2 hr.

Fig. 11. Behavioral response of the housefly to various photofractions of a 24-hr photoperiod.

Abscissa: time in hours after the onset of LL after three LD cycles with a photophase longer than 12 hr.

When placed in light cycles of LD 22:2, the majority of the flies tended to ignore this unnatural environmental light cycle. No cyclic pattern was obvious.

Experimental results with a photophase shorter than 12 hr are shown in Fig. 12A and B. In the flies kept in LD 10:14 or LD 8:16, the beginning of the resting phase coincided with the onset of scotophase. However, when the flies were subjected to a photoperiod with a photophase shorter than 6 hr, the onset of the resting phase was delayed by a few hours.

b) A single dark period as a factor inducing the periodicity

In the foregoing experiments, flies had been entrained to a 24-hr period by at least two LD cycles before the observations in LL. Since the flies subjected to only

Fig. 12. Behavioral response of the housefly to various photofractions of a 24-hr photoperiod.

Abscissa: time in hours after the onset of LL after three LD cycles with a photophase shorter than 12 hr.

TABLE III

Behavioral response of female flies of the Takatsuki colony to a single darkness for various lengths

Length of a single darkness (hr)	Time C*	Time A**
12	11 hr, 35 min	11 hr, 20 min
10	13 hr, 2 min	13 hr, 10 min
8	13 hr, 42 min	14 hr, 2 min
6	14 hr, 37 min	14 hr, 57 min
4***	—	—
2***	—	—
1***	—	—

The flies were kept in LL from their emergence.

* Time C indicates the time when percentage of the flies resting on the ceiling exceeded 35 in LL after a single darkness.

** Time A denotes the time when percentage of the flies flying per unit time (30 sec) decreased to less than 20 in LL after a single darkness.

*** A single darkness of 4 hr or less did not induce obvious rhythmicity in the flies. Therefore, Time C and Time A could not be determined.

a single LD cycle exhibited similar behavior and activity patterns to those exposed to two or three LD cycles, the arrhythmic flies in LL were placed in a single dark period of 1-, 2-, 4-, 6-, 8-, 10- or 12-hr and returned to LL again. Observations and countings were initiated at the onset of LL. The experiment was repeated twice and results were expressed as "Time C" and "Time A" (Table III).

A single dark period of 1 hr induced no obvious rhythmicity in the flies. In the flies placed in the darkness for 2 hr, an equivocal cyclic pattern was recognized. A peak and trough were considerably evident in the activity curve and the behavior pattern of the flies subjected to the darkness for 4 hr. However, the cyclic pattern exhibited by the flies was not so obvious as that displayed by the flies subjected to three LD cycles.

The behavior and activity patterns of the flies placed in a single dark period of 6 hr or longer closely resembled those of the flies exposed to two or three LD cycles with scotophase of the same length. The critical time of the onset of the resting phase in LL determined by the "Time C" and "Time A" was varied with the period of the single darkness (Table III).

Experiment 4. Behavioral Response of the Rhythmic Flies to a 6-hr Dark Pulse

As shown above, a single dark period interposed in LL initiated an activity rhythm in the arrhythmic flies provided the darkness was at least 6-hr long. With this dark "pulse", it seemed possible to analyze the resetting mechanism of the rhythm of the fly. A series of experiments was, therefore, carried out to find what behavior and activity patterns would result if the rhythmic flies at various phases are exposed to a single 6-hr dark pulse. Female flies of the Takatsuki colony were subjected to two light cycles of LD 12:12 from the third day of their emergence and then kept in LL. They were then exposed to a single 6-hr dark pulse at 6, 9, 14 or 19 hr after the onset of LL, when observations and countings were initiated. The noctovision was used for counting the flies in darkness (Figs. 13 and 14).

When the rhythmic flies were subjected to a single pulse at 6 hr after the onset of LL, the number of flies resting on the ceiling increased considerably (Fig. 13A). However, there was neither the second peak in the number of flies resting on the ceiling (Fig. 13A) nor the second trough in flight activity (Fig. 14A) at 27 hr after the onset of LL.

A quite different pattern was obtained when the flies were subjected to a single pulse at 9 hr after the onset of LL (Fig. 13B and Fig. 14B). Both the second distinct peak in the number of flies resting on the ceiling and precipitous reduction in flight activity appeared at 30 hr after the onset of LL. Similar results were obtained when the light-to-dark transition occurred at 14 or 19 hr after the onset of LL (Fig. 13C, D and Fig. 14C, D).

To ascertain complete absence of response for resetting during an earlier part of the active phase, the flies were exposed to a single pulse at 1 hr after the onset of LL (Fig. 15). The number of flies on the ceiling was relatively small during the dark period; the first normal peak appeared at the expected onset of the resting phase, followed by no second peak.

DISCUSSION

1. Biological factors regulating the behavior and activity patterns of the housefly

As pointed out by Arevad and Mourier (1963), no significantly different activity patterns among various strains or colonies of flies were detected. Although most flies exhibited similar behavior patterns to those of the Takatsuki colony, a tendency not to rest on the ceiling was apparent in two colonies of flies derived from Denmark,

Fig. 13. Effect of a single 6-hr dark 'pulse' on the resting behavior of the rhythmic flies.

The hours of illumination and darkness are represented respectively by white and black bars along the horizontal time axis.

Abscissa: time in hours after the onset of LL after two cycles of LD 12:12.

Fig. 14. Effect of a single 6-hr dark pulse on the flight activity of the rhythmic flies.

For explanations, see Fig. 13.

where residual spray of insecticide to the ceiling of the piggery and cowshed was conducted and many flies would rest on the animal, especially the sow (Keiding, 1965). Therefore, it seems necessary to use flies of a certain strain or colony to study the diurnal behavior.

Because of the absence of overt reproductive cycles, male adults have preferably been used in experiments on circadian rhythms in insects. However, Hardeland and Stange (1971) reported that, in male adults of *Drosophila melanogaster* grouped

Fig. 15. Effect of a single 6-hr dark pulse on the resting behavior (A) and the flight activity (B) of the rhythmic flies.

For explanations, see Fig. 13.

together in a small cage, an unusual peak of activity occurred at about the middle of the dark period due to an increased mating activity. A comparable situation appeared in the housefly (Fig. 3C). Male flies tried to mate with other resting individuals so frequently that accurate estimation of activity was difficult especially during the resting phase. On the other hand, there was little activity in female flies throughout the former half of the resting phase (Fig. 3B). Therefore, it is pertinent to use virgin female flies supplied with sucrose solution alone.

Little is known concerning the aging effect on the daily periodicity of diurnal insects. Under natural field conditions, reproductive adults of the emperor dragonfly, *Anax imperator* (Leach), tend to fly during midday, whereas young dragonflies fly mainly during a few hours after dawn (Corbet, 1957, 1960). Hardeland and Stange (1971) found that the overall activity attained a maximal level three days after emergence in *Drosophila virilis*, while in adults of *D. melanogaster* activity did not vary with the age. In the present experiments, female flies exhibited decreased stability to settle on the ceiling as they grew older, while landing on the ceiling increased rapidly in frequency during the first several days of adult life and then stayed rather constant. In the blowfly, *Phormia regina* Meigen, deterioration in rhythmicity occurred when moribund from starvation (Green, 1964). In the housefly, even if food was always sufficiently supplied, the duration of inactivity gradually reduced, especially in the resting phase, and hence periodicity became covert with advancing adult age (Fig. 4). Therefore, it is relevant to use young adults a week after their emergence for studying the diurnal behavior of this insect.

2. Behavior and activity patterns of individual flies

With the American cockroach, *Periplaneta americana* L., Lipton and Sutherland (1970) pointed out that different recording methods may partially be responsible for variation in the female rhythm, and that a mechanical instrument (the wheel cage) generally records only locomotory activity, but the capacitance monitor records such incidental activities as self-cleaning movements and rubbing against upper surfaces, as well as ordinary running activity. Tilting-type actographs were used to record locomotory activity of houseflies (Parker, 1962) and a blowfly (Green, 1964). A sort of capacity-sensing contrivance was utilized to record the activity of the fruit fly, *Drosophila* (Hardeland and Stange, 1971). Since a single female housefly fed to repletion was at most 20 mg in weight, an electronic device was used to record the overall activity of a single housefly in the present experiment.

The activity rhythm of the housefly was studied by Parker (1962), who found that the rhythmic activity displayed by four flies in groups in an actograph cage continued for four days under constant dim light. To calculate the period of free running rhythm, a single fly should be used. In the present experiments, overt rhythms persisted for more than two weeks without damping out. It was presumed that these rhythms would continue further. Each rhythm was slightly longer than 24 hr and no single fly showed a period shorter than 24 hr. However, the flattened peak in the behavior patterns of flies in groups one week after the onset of DD may suggest that the period of free running rhythms varies from one individual to another, and there may be some rhythms exhibiting free running periods shorter than 24 hr.

According to Green (1964), the mean period of free running rhythm of the blowfly, *Phormia regina*, was 24.3 hr in DD. Flight activity of the female mosquito, *Culex pipiens pallens* Coquillett, in DD was recorded by an actograph and the period

was about 24 hr (Chiba, 1964, 1966). From recent studies on the nature of circadian rhythmicity of other species of insects (Aschoff, 1963; Beck, 1968; Brown, Jr., Hastings and Palmer, 1970; Bünning, 1960, 1967; Corbet, 1966; Danilevsky, Goryshin and Tyshchenko, 1970; Harker, 1964; Pittendrigh, 1960; Sollberger, 1965), the fact that the periods of free running rhythms were not exactly 24 hr and that different individuals recorded concurrently did not exhibit similar activity pattern seems to support the existence of the biological clock also in the housefly.

Lipton and Sutherland (1970) demonstrated the variability of activity rhythms in a laboratory population of the American cockroach, *Periplaneta americana*, classifying them into three criteria, viz. rhythm, pattern and random. Konopka and Benzer (1971) isolated three mutants of *Drosophila melanogaster*, in which the normal 24-hr rhythm of eclosion and locomotor activity had drastically been changed. In the present study of endogenous activity rhythms it was found that changes in both the period of free running rhythm and the length of active phase occurred in the fly "B" under constant environmental conditions (Fig. 5B). Such a spontaneous change was also shown in the cockroach, *Blaberus* (Harker, 1964) and *Periplaneta* (Roberts, 1960). Although the change in periodicity may possibly be produced by some physiological changes within the insect, factors responsible for this phenomenon are not clear at present. It seems premature to conclude that all properties of rhythmic phenomena are under genetic control, since little is known about the way in which rhythm is produced.

Although some rhythms persisted in LL (Roberts, 1960; Nowosielsky and Patton, 1963), it is generally accepted that persistent rhythms are lost more frequently in LL than in DD. In the test fly, *Glossina morsitans* Westwood, a clear circadian rhythm emerged and continued in the activity of flies in DD, although in LL this rhythm damped out after two to three cycles (Brady, 1972). Occurrence of secondary arrhythmicity of this kind was recognized in the eclosion rhythm of *Drosophila pseudoobscura*, in which the individuals were themselves arrhythmic (Zimmerman, 1969). According to Tychsen and Fletcher (1971), such arrhythmicity was also produced in the Queensland fruit fly, *Dacus tryoni*, in which the endogenous rhythm of "ready to mate" was disturbed under constant high light intensity, all fluctuations being completely damped out by the third cycle. Since most rhythms hitherto reported continue at least for a few days in LL, the rapid disappearance of rhythms of the housefly in LL constitutes a major interest. Earlier experiments (Tsutsumi, 1966) revealed that most flies in groups exhibited only once the characteristic nighttime resting behavior in LL after three LD cycles and thereafter displayed arrhythmic behavior. Similar results were obtained in the present experiments by actograph. Slightly higher percentage of the flies exhibiting arrhythmicity from the onset of LL after LD cycles in the actograph record may probably have resulted from an augmented activity due to relatively a high light intensity. The first resting period in LL after LD cycles continued for only a few hours. This fact also suggests that illumination affected considerably the individual resting flies. Individually marked flies exhibited no sign of a cyclic pattern in LL after exposed to LD cycles. The arrhythmicity in a population of flies, therefore, may not result from asynchrony among individuals that were still rhythmic. This was also recognized in an experiment in which individual flies confined to an actograph chamber exhibited arrhythmic activity patterns in LL for relatively a long period of time.

3. Behavioral response to various photofractions

In laboratory experiments, a light cycle of LD 12:12 is used as a model of natural day and night. Since the housefly in DD exhibited a circadian rhythm with active- and resting-phases of nearly the equal lengths, it seemed that they could be entrained to the environmental light cycle of LD 12:12. On the other hand, light suppressed rapidly the endogenous rhythm, especially when the fly was exposed to light in the resting phase. Therefore, the entrainment of the phase of the rhythm to a 24-hr photoperiod with a photophase longer than 12 hr would be incomplete, while flies might possibly be entrained to a 24-hr photoperiod with a photophase shorter than 12 hr. The result obtained seemed to support the above assumption. However, an unexpected phase shift was detected when the onset of the photophase corresponded with the latter half of the active phase of the fly. Although the cause of this shift is not certain, it appears to be closely associated with the so called "phase response" phenomenon (Pittendrigh, 1960; Aschoff, 1960, 1965; Minis, 1965). The present result explains the inexplicable behavior of the flies in the field, especially under long day conditions.

The behavior and activity patterns of the houseflies exposed to a single dark period of longer than 6 hr, interrupting LL, were nearly identical with those of flies subjected to a 24-hr photoperiod with a scotophase longer than 6 hr for three days. This fact suggests that duration of dark period, as well as light-to-dark transition, is an important factor to initiate periodicity. This is in contrast to the data presented by Pittendrigh and Bruce (1959) for the fruit fly, in which flash light (1/2000 sec) initiated the emergence rhythm in DD. The time of the onset of resting phase in the fly in LL was dependent upon the length of a single darkness (Table III). From this, the resetting mechanism of the time-keeping device can be examined.

4. Response of the rhythmic fly to a 6-hr dark pulse

When the flies in their usual daily activity pattern were exposed to bright light at night and then kept in continuous very dim light, the reversed rhythm developed by only 12 hr of nighttime illumination (Parker, 1962). This fact may suggest that the rhythm is reset when given a dark stimulus to the fly one day after the onset of LL.

There has been no systematic study on the response of rhythmic insects to a single dark stimulus as for resetting. The two problems warranted special attention. The first was the direct behavioral response of the flies to a single dark pulse. The rhythmic flies showed a cyclical change in response to a single pulse applied in various phases of the rhythm (Figs. 13-15). Maximal direct response seemed to occur at about the beginning of the resting phase and minimal one immediately after the onset of LL.

Resetting of the rhythm by a single pulse was regarded as an "all or nothing" phenomenon. No resetting was successful if a stimulus was given before the latter part of the active phase of the flies. If the stimulus was given to the flies after the critical time, resetting could be made. Disappearance of a rhythm is not a very convincing evidence for the stopping of the internal clock, since coupling mechanisms may have been affected. The fact there exists a critical time for resetting, may suggest that elimination of the activity rhythm in LL will be closely related to some functional change of the resetting system of the time-keeping device in the housefly.

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