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Effects of Circadian Eclosion Rhythm and Temperature on Estimating the Relative Rate of Development in *Drosophila mercatorum*

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ABSTRACT The present paper examines the problem of how to evaluate fitness between some genotypes. The rate of development was measured in six combinations of light (LL or LD) and temperature (22, 25 or 28°C) conditions using three strains of *Drosophila mercatorum*. The relative rate of development was not greatly affected by developmental temperatures in LL where no eclosion rhythm was observed. However, the relative values were found to vary with temperature in LD where a definite peak of emergence was seen immediately after the light was turned on. This temperature effect on the relative rate of development may be due to the existence of a 'gate' for eclosion in LD as suggested by Skopik and Pittendrigh (1967) (*Zool. Mag. 174-179, 1983*)

The rate of development has been investigated as an important component of fitness from the point of view of population genetics. Considerable variations in this character were revealed in natural population of *Drosophila pseudoobscura* (Dobzhansky *et al.*, 1942). Buzzati-Traverso (1955) found that the genotypes leading to a faster developmental rate had a great selective advantage in experimental populations of *Drosophila melanogaster*. The actual length of developmental period is apparently affected by developmental temperatures (Pittendrigh, 1954; Ohnishi, 1976; Mckenzie, 1978) and larval densities (Barker, 1973; Ohnishi, 1976).

Drosophila biologists have given little attention to the effects of circadian rhythms on estimating the relative fitness among several different genotypes. It has been shown that the timing of an eclosion peak is determined by a circadian rhythm, resulting in the definite peak of emergence immediately after the shift from the dark to the light in the light-dark (LD) cycle (Pittendright, 1954, 1981; Brett, 1955); this timing is essentially independent both from developmental temperatures and from larval densities. Consequently a population is partitioned into several age-groups with about 24 hours intervals. On the other hand, in continuous lighting (LL), no eclosion rhythm is observed because of the circadian clock may not be operative.

Considering these facts, the present study will pay particular attention to experiments designed to evaluate interstrain differences in the rate of development as a fitness component by measurements in six treatment combinations of light (LL or LD) and temperature (22, 25 or 28°C) conditions.

Materials and Methods

Flies used: Two of three strains used were obtained after artificial selection for high (HRR) or low (LRR) pulse repetition rate (small or large interpulse interval, Aipi) of the A courtship sound for 16

generations in *Drosophila mercatorum* (Ikeda and Maruo 1982). Another strain (CRR) was derived from an unselected control line. These three strains had been kept without any selection for about 1.5 years in small mass cultures under laboratory conditions (LD = 12 : 12, 25 ± 1°C, RH = 60 — 80%) when the study was begun. Table 1 shows the interpulse intervals of the three strains which were measured at the 15th generation of selection (May, 1980) and at the end of this study (April, 1982). The significant difference in A_{ipi} was found between the two measures for the LRR strain ($P < 0.05$), but not for the other two.

Table 1. The average interpulse interval of the A courtship sound (A_{ipi}, in msec) of the three strains measured at the 15th generation of selection (May, 1980) and at the end of this study (April, 1982).

Strain	May, 1980		April, 1982	
	N	A_{ipi}±SE	N	A_{ipi}±SE
HRR	10	8.53±0.07	5	8.53±0.05
CRR	10	9.04±0.06	5	9.09±0.08
LRR	10	9.66±0.04	5	9.49±0.05

N, the number of males tested; SE, standard error.

Flies were cultured on the standard medium consisting of cornmeal (90 g), glucose (100 g), dry yeast (40 g) and agar (5 g) in 1000 ml of water.

Measurement of the rate of development: Well-fed adult flies at 6 days of age were allowed to lay eggs on the standard medium for two hours (7:00 — 9:00 p.m.) in the evening of the LD cycle, for *D. mercatorum* was known to show higher oviposition activity in the evening (Ikeda and Maruo 1982). About 40 hours after oviposition, 50 first instar larvae were sampled from the cultures and introduced into a food vial containing 15 ml of the standard medium, which kept the larval desnity constant throughout the present study.

These '50-larvae cultures' were placed in two different light conditions, LD = 12 : 12 (the light phase being between 9:00 a.m. and 9:00 p.m.) and the continuous light (LL), at three different temperatures, 22, 25 and 28 ± 1°C. Thus six treatment combinations were available to measure the rate of development. 10 replicates (500 larvae in a total) were set for each of treatment combinations. The number of adults emerging from the cultures were counted by hourly intervals from the first day to the last day of eclosion.

For experiments in LL, the rate of development was measured by the average time from egg to adult, then significance tests for group comparisons were made by a simple t-test. For experiments in LD, the average time from egg to adult was also obtained and a t-test was made for group comparisons, although adult emergence not continuously occurred for three to four days because of the fact that no eclosion was found in the dark phase. The rate of development was also mentioned by the number of adults emerging at each of the days of eclosion in LD. Thus group comparisons were made on the basis of the number of flies emerging on the first day of eclosion and those emerging on the successive days by means of the m x n table.

Results

Measures of the rate of development in LL are summarized in Table 2. Adult emergence occurred continuously for about 50 hours, resulting in a unimodal eclosion pattern without a rhythmic change depending upon the time of day. The mean times of development from egg to adult, which were

Table 2. The rate of development measured in the LL condition at three different temperatures.

Temp.	Strain	No. of flies emerged	Developmental time (hr)		
			Mean ± SE	Median	Range
22°C	HRR	418	375.7 ± 0.6	373.5	353-404
	CRR	442	353.0 ± 0.4	350.5	332-388
	LRR	424	361.6 ± 0.5	357.5	330-389
25°C	HRR	391	304.0 ± 0.4	301.5	285-333
	CRR	480	286.1 ± 0.4	283.5	272-321
	LRR	431	292.1 ± 0.4	291.5	275-321
28°C	HRR	390	283.4 ± 0.4	281.5	261-310
	CRR	433	262.6 ± 0.4	260.5	249-296
	LRR	449	269.5 ± 0.4	269.5	252-296

Table 3. The relative rate of development among the three strains at threee different temperatures. The values were standarized by CRR either on the basis of the mean time of development for LL and LD[a], or on the basis of the proportion of flies emerging on the first day of eclosion for LD[b].

Temp.	LL			LD^a			LD^b		
	CRR	LRR	HRR	CRR	LRR	HRR	CRR	LRR	HRR
22°C	1.00	0.98	0.94	1.00	0.98	0.98	1.00	0.80	0.70
25°C	1.00	0.98	0.94	1.00	0.99	0.96	1.00	0.90	0.59
28°C	1.00	0.97	0.93	1.00	0.94	0.93	1.00	0.62	0.22

usually employed as the rate of development, were significantly different among the three strains (P always less than 0.001). The fastest rate was observed for CRR, followed by LRR and next by HRR regardless of the developmental temperatures, although the developmental time evidently decreased as the temperature increased (P always less than 0.001).

Interstrain differences in the mean value were about 12 — 14 hours between HRR and LRR, whereas they were about 6 — 9 hours between LRR and CRR. These differences were not greatly affected by the temperatures. Thus no difference was found in the relative rate of development which was standarized by the average value of CRR among the three different temperature treatments (Table 3).

In LD, none of the total 3793 adults was found to emerge from the puparium in the dark phase, but mainly at the beginning of the light phase. Therefore, adults emerging can be clearly partitioned into three to four age-groups with about 24 hours intervals. The results are shown in Table 4.

basis of the mean developmental time.

The present results showed that the developmental time decreased as the developmental temperature increased both in LL and in LD, as has been previously reported (Pttendrigh, 1954; Ohnishi, 1976; Mckenzie, 1978). However it should be noted that the interstrain differences in the mean developmental time were not greatly affected by the temperatures in LL. Namely the relative rate of development among the three strains were almost constant regardless of the developmental temperatures in the continuous light. Some differences in the relative rates given on the basis of the hourly averages were seen between LL and LD. At 22°C, the values were more homogeneous under LD than under LL, but at 28°C, the relative differences were slightly augmented under LD vs LL. Hence, a LD cycle can either accentuate or diminish the differences in the free-running rates depending upon the temperatures.

The rate of development in LD can be well defined by the proportion of adults emerging on each of the days of eclosion rather than the values employed above. Adult flies were partitioned into three to four age-groups in accordance with the day of eclosion. Flies emerging on the first day may have a selective advantage as compared to those emerging later. Thus it is worth mentioning that the relative fitness as influenced by the rate of development may evidently vary with the developmental temperature in LD which is actually close to the natural light condition.

The temperature effects on the relative rate of development may be due to the existence of a 'gate' or an 'allowed zone' for eclosion in LD, which was suggested by Skopik and Pittendrigh (1967). That is, only individuals having reached a proper developmental stage by the time of gating are able to emerge from the puparium at a certain time of day. Otherwise, individuals which have not sufficiently completed developmental stage by the gating time are compelled to remain in the puparium until opening of the gate on the following day even if they develop enough to emerge during a 'forbidden zone' for eclosion.

In *Drosophila*, the gate for eclosion exists immediately after the shift from the dark to the light in LD, resulting in a peak of eclosion in the morning hours once a day (Skopik and Pittendrigh, 1967). The proportion of individuals passing the gate every day may depend upon the developmental time which is apparently sensitive to temperatures. Thus the interstrain difference in the developmental time, if any, may be enhanced differently between different temperatures in LD much more than in LL where no eclosion rhythm is observed.

Kyriacou and Hall (1980) suggested that three mutations of *per* gene altering circadian rhythmicity of eclosion and locomotor activity also affected oscillation of the interpulse interval of the courtship song in *D. melanogaster*. In the present study, interstrain differences in the developmental rate found in LD may possibly reflect differences in circadian rhythmicity of eclosion because the three strains used here were also obtained through artificial selection for pulse repetition rate of the courtship sound. However we unfortunately failed to examine the free-running period of eclosion.

According to the previous observation (Ikeda and Maruo, 1982) which had been carried out immediately after the end of selection for pulse repetition rate, the fastest rate of development was found for LRR, followed by CRR and next by HRR. This rank had been confirmed by other brief tests at that time. However, in the present study which was made after about 1.5 years without any selection, CRR developed faster than LRR regardless of the temperature condition. Similarly the A_{ipi}

Table 4. The rate of development of the three strains measured at three different temperatures in LD. The mean developmental time and numbers of adults emerging at each of the days after oviposition are shown. Proportions of flies emerging on the first day of eclosin are put in parenthesis.

Temp.	Strain	Mean develop. time (hr)	No. of flies emerging at each of the days							
			Day 11*	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Total
22°C	HRR	360.3 ± 0.6					268 (65.8%)	120	19	407
	CRR	352.0 ± 0.4					429 (93.7%)	19	10	458
	LRR	358.4 ± 0.6					342 (74.5%)	91	26	459
25°C	HRR	289.8 ± 0.7		206 (57.1%)	139	16				361
	CRR	277.4 ± 0.3		440 (96.5%)	14	2				456
	LRR	281.5 ± 0.6		364 (86.5%)	34	23				421
28°C	HRR	273.8 ± 0.7	71 (21.2%)	244	17	3				335
	CRR	254.6 ± 0.2	434 (96.2%)	16	1					451
	LRR	270.1 ± 1.0	266 (59.8%)	88	75	16				445

*Day 0 is the day of oviposition.

The rank of the rate of development was the same as that obtained in LL. The average developmental times were significantly different among the three strains at the three temperatures respectively ($P < 0.05$ between HRR and LRR at 22°C, $P < 0.01$ between HRR and LRR at 28°C, $P < 0.001$ for the other comparisons). Interstrain differences in the mean value changed depending upon the temperatures, differing from the results in LL. This reflects in the differences in the relative rates between those given in LL and LD (Table 3). This may be caused by the fact that adult emergence did not continuously occurred in LD because of the existence of a 'gate' for eclosion as will be discussed later.

The number of flies emerging on the first day of eclosion was significantly different among the three strains as a given temperature (P always less than 0.001), and also among the three temperature treatments both for HRR and for LRR (P less than 0.001) but not for CRR ($X^2_{(2)} = 5.1277$, $P < 0.05$). The relative proportions of flies emerging on the first day were standarized by the value of CRR at the three different temperatures respectively (Table 3). As can be seen, the differences in the relative value changed depending upon the temperatures in LD. However, in our experiences in the previous study (Ikeda and Maruo 1982) where a number of brief observations were performed, the relative values seemed to be be very sensitive even to slight changes in developmental temperatures. This temperature effect may be inevitable because it is very difficult to accurately control the temperature to a given degree throughout the entire developmental stages. This fact, indeed, prompted us to do this kind of experiment.

Discussion

In most of experiments reported so far, the rate of development was measured as the time from egg to adult in the continuous light, which showed a nearly normal distribution. Then comparisons of the rate of development, or calculations of the relative rate of development, between some genotypes in question were usually made on the

was also found to change only for LRR, but not for CRR and HRR during the two years (see Table 1). Thus this reversal in the rank may be possibly due to genetic homeostasis which was defined as "the property of the population to equilibrate its genetic composition and to resist sudden changes" by Lerner (1954). It is necessary to continue watching the progress of change in those characters to further discuss this in detail.

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