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ORIGINAL ARTICLE

Analysis of Cold Stress Tolerance in *Drosophila Kikkawai*

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ABSTRACT
Thermal adaptation of insects consists of many factors, such as resistance to extreme temperatures, both high and low, and preference for optimal temperatures. For ectothermal animals, especially insects, temperature is a major abiotic factor, resulting in potential cold or heat stress. In the present study, after cold treatment at ^{0}C, recovery time was measured at six ambient temperatures and recovery time was found consistently larger for males then for females.
Keywords: *Drosophila kikkawai, cold stress, recovery time etc*

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INTRODUCTION

From the viewpoint of evolutionary ecological genetics, it is of interest to compare several principal components of thermal adaptation among sympatric species belonging to a related group of insects. The genus *Drosophila* contains many species. These species may have different niches according to their different seasons of emergence and their different food utilization although they occur in the same locality.

Ability to acclimatize to cold stresses was studied. Although these analysis were performed species which live under the same climatic conditions in nature, differentiation in characters concerning thermal adaptation were expected among ecologically diversified species. The normal thermal range, compatible with successive generations, is also variable among species. In the Drosophilid family, for example, a tropical species like *Drosophila ananassae* cannot be reared below 16°C, while for other species such as *D.takahashii* and *D.immigrans* 30° to 32°C appears an upper lethal limit. Tolerance to extreme environmental conditions is considered a major problem in evolutionary biology [1,2]. Extreme environments induce physiological stresses and thus a directional selection for stress tolerance. Up to now more attention has been paid to heat tolerance and to the protective effect of heat induced proteins. Cold tolerance has been estimated by the survival time at a non freezing temperature, for example –1°C [3]. At such a low temperature, *D.melanogaster* adults loose their mobility and feeding capacity, exhibit a knockdown which is also called a chill coma [4, 5]. Brought back at a higher temperature, adults will progressively recover a normal activity. Cold tolerance has been analyzed less and most investigations have considered the effects of very low temperatures, that is freezing tolerance and cryoprotection. Cryoprotection is, however, a small part of the whole story: for example in *D.melanogaster*, freezing occurs at about –18°C but adults set at 0°C will die in less than 2 days, and the developmental zero is about 10°C [6]. Cold treatment is often used as an alternative to CO_2 or ether anesthesia. In the present investigations the effect of cold stress was analysed in *D.kikkawai.*

MATERIALS & METHODS

All experiments were done with *D.kikkawai* mass population founded by 30 isofemale lines. Development was possible at 12-31°C on killed yeast, high nutrient food. For controlling population density, either groups of parental flies oviposited directly in culture vials, or young larvae were transferred to fresh vials. Except where otherwise stated, larval population density was kept below 100 in our experiments. Upon emergence, adults from several vials were pooled in a bottle (250 ml) containing a cornmeal-sugar medium seeded with live yeast. This procedure provided a single sample of numerous flies in similar physiological condition. After 1 or 2 days, adults were slightly anaesthetized with ether and distributed in culture vials (killed yeast food) in-group of 20 flies. Males and females were taken at random for shortening anaesthesia duration. These adults were either kept in the same vial or transferred

periodically to fresh food. For cold treatment, we used a temperature of 0°C. Vials containing 20 adult flies each were put in melting ice within isolated boxes. In most cases, treatment was initiated at 5.pm. in the evening and vials taken out at 9 .a.m. in the morning the next day, a duration of 16 hours. A fly was considered as having recovered from chill coma when able to stand on its legs. Adults were then transferred into small Petri dishes at full thermal range and waking up time was monitored for successive intervals of 1 min.

Three groups of 20 flies were simultaneously treated for 16h at 0°C and recovery time was monitored at six ambient temperatures (two groups at each 12,15,17,21,25,28 and 31°C).

RESULTS AND DISCUSSION

Result (Fig. 1) showed a quicker recovery with increasing temperature, a minimum time between 28°C-31°C. The recovery was slightly slower in females than in males. The recovery time was higher at the extremes of the temperature range i.e. 13, 15 and 17°C while values were almost identical at higher temperature of 25 and 31°C. The correlation between sexes for recovery duration was significant r=0.98.

Recovery time was strongly influenced by recovery temperature, with shorter values between 25°C-28°C. Recovery time increased almost linearly with duration of cold treatment and it was consistently larger for males than for females. It was highly variable among groups and increased with flies age. Variability among flies of the same group was always very high. Chill coma and its recovery seem to imply a modification of the nervous system, analogous in several aspects to what is observed with usual anesthetics such as CO_2 and ether.

Recovery time was significantly related only to the fly's age. In the first experiments, flies of different age (3,8,12,16 and 20 days) were used. The relationship between age and recovery time is shown for the whole data set in (Table 3). In each group of flies, the mean recovery time, the standard deviation and the coefficient of variation (CV) were calculated for each sex (Tables 1-3).

The fact that recovery from cold treatment is dependent on the recovery temperature suggests that it is an active metabolic process. These observations, as well as the symptoms observed (narcosis and progressive recovery) suggest that cold directly modifies the characteristics of the nervous systems, in an as yet unknown way [7].

In *Drosophila* recovery time appears as a highly variable physiological trait with higher CV values and it is unlike any other trait, either morphological or physiological, which exhibits such a large individual variation. A broad and uncontrolled variability of the recovery time, was observed especially when equivalent groups were not treated the same day. This variability cannot be accounted for by fluctuations in the experimental treatment, since vials put in melting ice will get standard, highly reproducible stress. Variability must be explained by variations in the physiological conditions of the flies, and more precisely of their nervous system.

Table 1 : Data on recovery time in minutes (m±SE) of *Drosophila kikkawai* subjected to cold stress at 0°C for 16 hrs followed by recovery at different temperatures.

Species	Sex	12°C	14°C	17°C	21°C	25°C	28°C	31°C
D.kikkawai	F	55.70±1.00	46.70±1.19	36.30±1.14	30.20±.866	23.30±0.83	20.00±0.97	19.20.±1.17
	M	61.30±1.24	51.50±1.47	42.20±1.52	32.00±.881	27.00±0.97	24.50±1.06	22.00±1.07

Table 2 : Recovery time (in minutes) from cold treatment at 0°C as a function of duration of cold stress in two *Drosophila kikkawai*. Growth and recovery was considered at 21°C

Species	Sex Duration	m±SE 16 hrs	m±SE 20 hrs	m±SE 24 hrs	m±SE 28 hrs	m±SE 32 hrs
D.kikkawai	F	37.60±0.87	45.30±1.43	55.20±1.21	68.50±1.83	98.10±2.35
	M	40.50±0.88	49.40±1.35	59.00±1.36	72.10±2.32	101.30±2.03

Table 3 : Recovery time cold treatment at 0°C as a function of age in *D.kikkawai*. Growth and recovery was considered at 21°C and stress was given 16 hrs.

Species	Sex Age	m±SE 4 days	m±SE 8 days	m±SE 12 days	m±SE 16 days	m±SE 20 days
D.kikkawai	F	28.20±0.87	33.00±1.55	36.89±1.95	40.69±1.92	46.10±1.69
	M	29.58±0.88	35.20±1.93	38.50±2.53	42.80±2.18	47.25±2.27

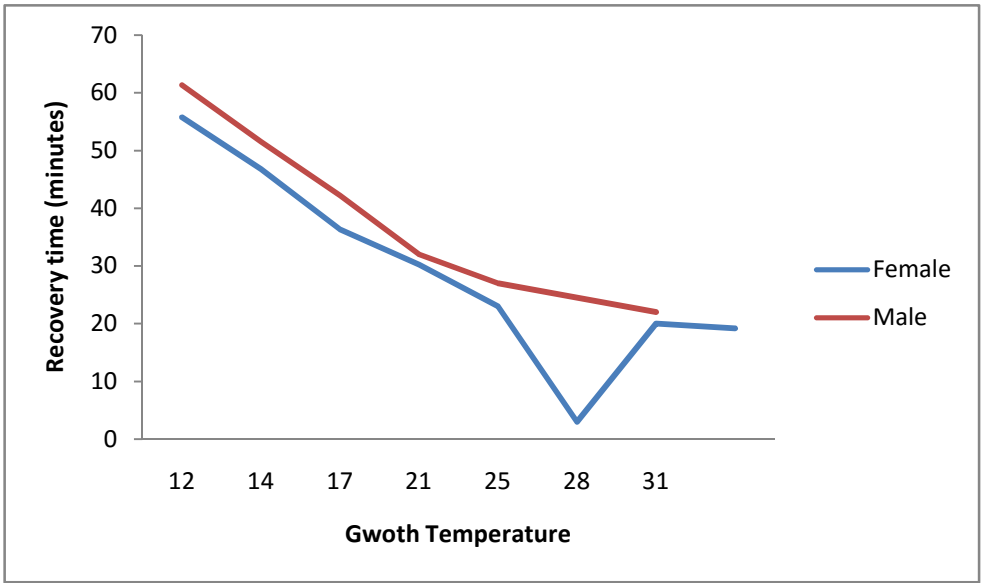

Fig.1 Relationship between recovery time (in minutes) from cold treatment at ^{o}C for 16 hrs followed by transfer of flies to different temperatures.

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