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Cold temperature resistance in *Drosophila lutescens* and *D. takahashii*

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ABSTRACT

Differentiation in low temperature resistance was investigated in two closely related species, *Drosophila lutescens* and *D. takahashii*. Drosophila flies at various developmental phases from an egg to an adult fly stage were chilled by low temperature of −3°C for six hours and the percent of hatching or hatchability for eggs, the percent of emergence for nonadult individuals and the percent of flies remaining alive after the treatment or the survival rate for adult flies were measured.

It has been found from this experiment that cold resistance is in general higher in *D. lutescens* than in *D. takahashii*. For instance, adult flies 10 days after emergence of *D. takahashii* were all killed by the chilling, while in *D. lutescens* nearly all flies of the same age survived the low temperature treatment. The intraspecific variation of cold tolerance in both species was in the order of pupae>aged eggs>young eggs>larvae, leaving the larvae the most susceptible.

Cold tolerance of adult flies in *D. lutescens* showed little variation in relation to ageing of flies, but in *D. takahashii* older flies proved to be more susceptible than younger ones.

Genetic investigation by means of the chromosome substitution has proved that the cold tolerance is closely associated with the ratio of *D. lutescens* chromosomes involved in hybrid flies, suggesting that the relevant genes may be distributed in either sex chromosomes and autosomes of *D. lutescens*. Thus, the successful propagation of *D. lutescens* in the northern parts of Japan islands in contrast to *D. takahashii*, distribution of which is strictly limited to the southern parts, may most probably be due to the genetic differences in cold tolerance, mainly of adult flies, during the winter time.

1. INTRODUCTION

The ability of *Drosophila* flies to resist cold is considered to be one of the factors which determine their geographical distribution. There are a few studies on cold resistance of *Drosophila* flies conducted with special reference to their developmental stages. For instance, Crumpacker and Marinkovic (1967) in *D. pseudoobscura* and Kimura (1982a) in *D. takahashii* and *D. lutescens* investigated the ability of flies to survive when they are exposed to low temperatures in the laboratory at various developmental stages. They found adults to be much more resistant to cold temperatures than individuals at

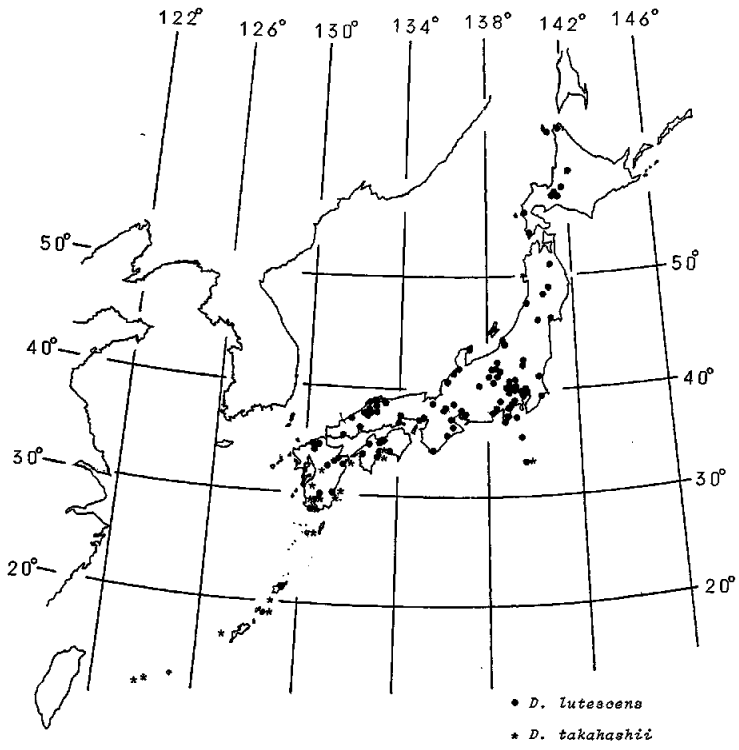

Fig. 1. The geographic distribution of *D. lutescens* and *D. takahashii* in Japan.

other stages of the life cycle. Jefferson *et al.* (1974) also measured the degree of resistance to cold in different developmental stage of *D. pseudoobscura*. Marinkovic *et al.* (1969) thought that certain gene arrangements of *D. pseudoobscura* appeared to bring about very high resistance to cold. Tucic (1979) studied the genes located on major chromosomes of *D. melanogaster* to determine to what extent they contributed to cold resistance. Kimura (1982a) suggested that cold hardiness at the imaginal stage in *D. takahashii* and *D. lutescens* was controlled by a gene or genes supposed to be located on the autosomal chromosomes.

Habitats of two closely related species, *D. takahashii* and *D. lutescens*, belonging to the same *takahashii* subgroup of the *melanogaster* group, are clearly separated into northern and southern areas of Japan by some unknown reasons (cf. Fig. 1: Kikkawa and Peng 1938; Okada 1956; Wakahama 1964; Bock and Wheeler 1972; Watanabe *et al.* 1978), although both species overlap in the border region of these two areas and can be caught together.

From crosses between *D. lutescens* females and *D. takahashii* males, fertile F_1 hybrid females and less fertile F_1 hybrid males are produced (personal observation). Kimura (1982a) also reported that fertile F_1 hybrid females and

sterile F_1 hybrid males were produced in reciprocal crosses between these two species.

This study has been undertaken for two reasons: (1) to detect differences in cold resistance between the two species by comparing their survival following exposure to cold at various stages before or after eclosion, and (2) to detect relationship, if any, between genes controlling the cold resistance and chromsomes.

2. MATERIALS AND METHODS

Progenies of 25 wild strains of both species collected in Japan and since propagated in our laboratory for about one to three years were used for establishment of cage populations for the study. After the cage populations were maintained for about a half-year, random samples were extracted from them and transferred to many culture vials filled with Bowling Green medium (water 1000 ml, agar 5 g, glucose 100 g, cornmeal 90 g, Ebios 40 g, and propionic acid 4 ml) and kept at 20°C.

Females were removed from the vial to large bottles and allowed to oviposit for 4 hours at 20°C on food placed in small cups which had been constructed by setting rings of glass (20 mm in diameter, 12 mm in height) on a glass slide. After oviposition, the eggs were counted and these, together with food in the small cup, were transferred to a new culture vial containing food medium and cultured at 20°C, until exposure to cold temperature. Number of eggs was adjusted to be from 20 to 110 per cup in order to avoid too much crowding.

Low temperature treatment was applied to either eggs, preadult individuals or adult flies only once by −3°C for 6 hours. Eggs and preadult individuals were taken at the age of 0 (collected within 4 hours after oviposition), 1 through 11 days for exposing to the cold temperatures without acclimation. After the treatment, they were grown in the laboratory kept at 20°C until adequate time did elapse for estimation of the resistance. The number of adult flies emerging from each culture vial was counted and then the survival rate for each developmental stage was calculated as an index of ability to survive. The temperature of the cold treatment adopted here, i.e., −3°C, was the mean of the minimum temperatures in the winter months in the border area of the southern and northern animalia in Japan (Kamimura 1981).

For the treatment of adult flies, they were sampled within 24 hours from the population for each species and these were transferred to new vials containing food medium, and kept at 20°C until exposure to cold. Adult flies at the age ranging from 0 (collected within 24 hours after eclosion), 1 through 10 days after eclosion were tested. About sixty to one hundred flies of each age were transferred to an empty vial without anesthesia just before low temperature treatment, and then exposed to −3°C for 6 hours without acclimation. After the exposure to cold, the number of adult flies were immedia-

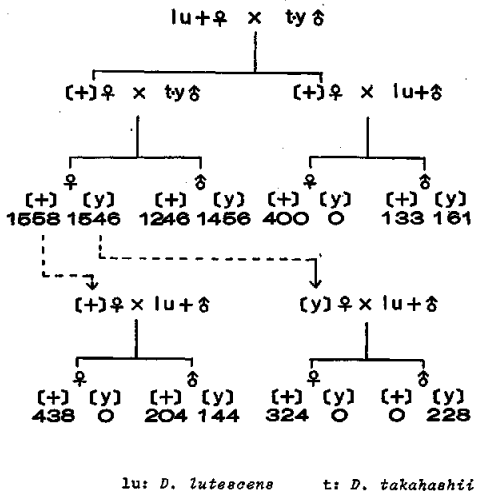

Fig. 2. Homologous test between X chromosomes of *D. lutescens* and *D. takahashii*.

tely counted and these were transferred to a vial containing food medium and kept for two days at 20°C, after which flies were examined for survival.

A yellow body color mutant fly (*y*) was obtained from one of our laboratory stocks of *D. takahashii*. By genetic analysis of F_1 and backcrosses between the mutant and the wild type flies, it was concluded that the yellow gene was located on the X chromosome of *D. takahashii*.

In order to ascertain whether sex chromosomes of *D. takahashii* and *D. lutescens* are homologous with respect to the yellow mutant gene locus, many kinds of crosses were carried out (Fig. 2). These crosses made it possible to conclude that the yellow gene locus was also on the X chromosome of *D. lutescens*.

Then, yellow mutant flies of *D. takahashii* were backcrossed six times to wild type flies of the same species. Consequently, the yellow mutant line having theoretically about 98 percent of genes of the wild strain except for the yellow gene was established. This yellow fly was used to detect to what extent the X chromosome and autosomes could contribute to cold resistance of flies.

Five kinds of flies were prepared: yellow flies of *D. takahashii*, wild flies of *D. lutescens*, F_1 hybrids between *D. lutescens* wild females and *D. takahashii* yellow males, backcross progenies with wild males of *D. lutescens*, and backcross progenies with yellow males of *D. takahashii*. These 8~11 day old flies were tested for their cold resistance by the same method mentioned above, since the most remarkable difference between both species in cold resistance was observed in flies older than 8 days in the earlier experiments. Rates of their survival were calculated and compared.

3. RESULTS

Table 1 and Fig. 3 show the survival rates of the control and tested flies of *D. lutescens* and *D. takahashii* at each developmental stage. It is found from them that the survival rate of *D. lutescens* is higher than *D. takahashii* in almost all developmental stages. The resistance of aged eggs to the low temperature was higher than young ones in both species. Resistance of larvae was the lowest irrespective of their developmental stage. Resistance of individuals in the larval stage gradually increased with the lapse of time, and it attained the maximum in the pupal stage just before eclosion, so high as to be comparable with the control.

Table 2 gives the survival rates of each ageing flies of both species after exposure to cold. In *D. lutescens*, most flies cold-treated at all ages have been found surviving, whereas in *D. takahashii*, resistance of adults decreased gradually from 93 percent at 0 day to zero at 10 days of age. In other words, nearly 93 percent of flies survived if cold-treated within 24 hours after eclosion, but very few flies could servive when older than 8 days flies were cold-treated. Thus, the proportion of survival of *D. takahashii* suddenly decreased when they became older than 8 days.

Thus, it is concluded from these experiments that adult flies older than 8 days of both species were distinctly different in resistance to low temperature

Table 1. *The effect of low temperature treatment at various developmental stages of D. lutescens and D. takahashii*

D. lutescens				*D. takahashii*			
Age* (days)	No. of eggs	*n***	survival rate $\bar{x}\pm$S. E.	Age* (days)	No. of eggs	*n***	survival rate $\bar{x}\pm$S. E.
0	685	18	.380±.041	0	1083	22	.114±.013
1	1397	31	.750±.028	1	1801	31	.681±.043
2	1230	28	.652±.043	2	623	13	.111±.018
3	1160	21	.317±.028	3	842	18	.064±.013
4	841	18	.298±.037	4	704	16	.064±.009
5	563	15	.270±.022	5	832	14	.182±.017
6	852	15	.547±.069	6	649	14	.452±.041
7	917	21	.714±.045	7	661	15	.838±.022
8	782	17	.883±.021	8	743	14	.709±.044
9	732	20	.917±.024	9	505	13	.840±.030
10	736	17	.931±.020	10	861	17	.823±.020
11	706	19	.888±.034				
Control	2656	58	.937±.008	Control	4113	66	.892±.010

* Age expressed in terms of number of days after oviposition.

** No. of culture vials.

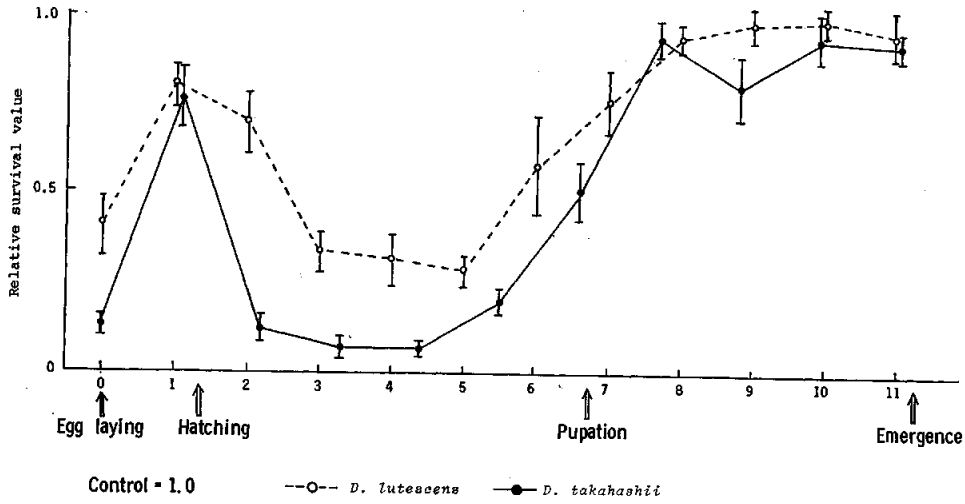

Fig. 3. The effect of low temperature at various developmental stages of *D. lutescens* and *D. takahashii*. Developmental periods of two species are expressed as being equivalent. Survival rates of individuals at each developmental stage of both species are calculated as the relative value taking the control as 1.0.

Table 2. *Survival rates after cold treatment of flies at various ages of D. lutescens and D. takahashii*

D. lutescens				*D. takahashii*			
Age	No. of flies	Mean freq. of survival	S.E.	Age	No. of flies	Mean freq. of survival	S.E.
0 day	1257	0.999 ±	0.001	0 day	1267	0.931 ±	0.017
1	767	0.983 ±	0.006	1	1056	0.882 ±	0.064
2	861	0.986 ±	0.006	2	1120	0.593 ±	0.131
3	850	0.990 ±	0.005	3	1027	0.383 ±	0.153
4	819	0.967 ±	0.012	4	725	0.348 ±	0.078
5	835	0.973 ±	0.009	5	658	0.626 ±	0.099
6	921	0.973 ±	0.011	6	884	0.434 ±	0.144
7	676	0.921 ±	0.055	7	721	0.324 ±	0.189
8	606	0.981 ±	0.010	8	618	0.050 ±	0.048
9	126	0.992 ±	0.008	9	690	0.007 ±	0.003
10	120	0.967 ±	0.030	10	120	0.000 ±	0.000

treatment. Apparently an age effect existed in the adult flies of the susceptible *D. takahashii*.

Table 3 shows the results of an experiment in the flies of the substitution lines, that is, the survival rates of adult flies of both species, F_1 hybrids between *D. lutescens* wild females and *D. takahashii* yellow males, backcross

Table 3. *Survival rates of parents, F_1 and backcrossed hybrid flies exposed to cold in the laboratory*

	No. of flies	Mean freq. of survival	S.E.
D. lutescens	852	0.980 ±	0.007
D. takahashii	1428	0.030 ±	0.026
F_1 (lu ♀×t ♂)	511	0.917 ±	0.012
B_1F_1 (F_1♀×lu ♂)	2720	0.878 ±	0.015
B_1F_1 (F_1♀×t ♂)	2541	0.437 ±	0.022

progenies from F_1 females crossed with *D. lutescens* wild males, and the same from F_1 females crossed with *D. takahashii* yellow males. Adult flies exposed to cold in this experiment were 8–11 days old. As is seen in Table 3, *D. lutescens* showed the highest resistance to cold and *D. takahashii* the lowest, while the survival rate of F_1 hybrid flies was so high as *D. lutescens*. In the backcross progenies, the survival rate of flies expected to carry 75 percent of genes or chromosomes of *D. lutescens* was 87.8%, while that of flies carrying 25 percent of genes or chromosomes of *D. lutescens* was 43.7%. It is thus considered that genes responsible for resistance to cold are dominant to their allele genes.

Table 4 gives survival rates after cold treatment of flies with wild and yellow phenotypes from the backcrosses. If no crossing over did take place between X chromosomes in the F_1 female, the difference between wild type flies and yellow ones of each sex in resistance to the cold temperature may be due to the sex chromosome they carry. Fig. 4 shows the average survival rates of B_1F_1 flies which carry eight combinations of sex- and autosomal chromosomes of *D. lutescens* and *D. takahashii*. In B_1F_1 progenies from crosses with F_1 females and *D. lutescens* males, 97 percent of the wild males carrying X and Y chromosomes of *D. lutescens* survived, but only 66 percent of the yellow males carrying heterogeneous X chromosome of *D. takahashii* and Y chromosome of *D. lutescens* did. In the B_1F_1 progenies between F_1 females and *D. takahashii* males, survival rates were 0.55, 0.30, 0.54 and 0.44 in wild females, yellow females, wild males and yellow males, respectively. The survival rate of wild females coincided with that of wild males, and survival rates of wild type flies were always higher than those of yellow types.

4. DISCUSSION

Resistance to cold treatment at various developmental stages of *Drosophila lutescens* and *D. takahashii* varied in somewhat a similar pattern, although *D. lutescens* had a higher survival rate than *D. takahashii* in all stages. This observation agrees well with the results obtained by Kimura (1982b).

Table 4. *Survival rates after cold treatment of B_1F_1 progenies from backcrosses between F_1 females and parental males*

Cross	Phenotype	No. of flies	Mean freq. of survival	S. E.
F_1♀×lu ♂	wild female	1586	0.893 ±	0.012
	wild male	751	0.968 ±	0.007
	yellow female	0		
	yellow male	391	0.660 ±	0.064
F_1♀×t ♂	wild female	658	0.545 ±	0.033
	wild male	397	0.543 ±	0.039
	yellow female	578	0.297 ±	0.050
	yellow male	702	0.436 ±	0.038

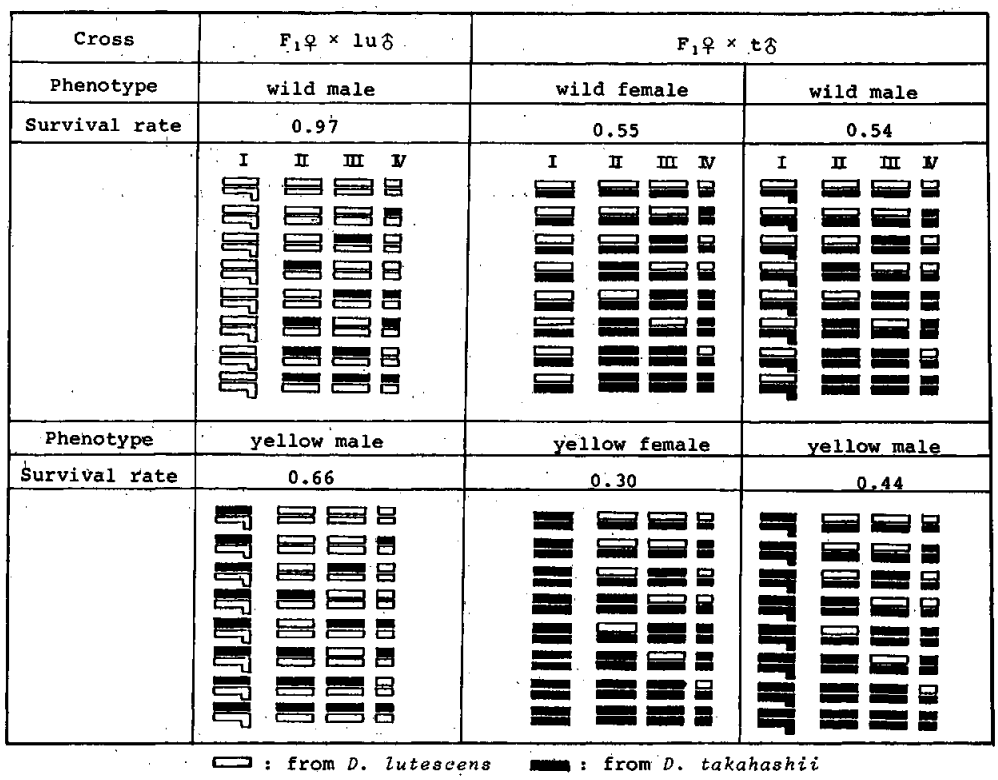

Fig. 4. Survival rates of adults which carry one of six combinations of the sex- and autosomal chromosomes of *D. lutescens* and *D. takahashii*.

It has been found in the present experiment that young eggs and larvae were rather low in the resistance in both species, though high survival was observed at late pupal stage. Eggs unhatched of one day old age after oviposition were also observed to have a high resistance. This may most probably be due to physiological changes in the eggs which might occur before hatching. Crumpacker *et al.* (1967) reported that eggs of *D. pseudoobscura* exposed to −3°C for one day in their experiment showed very high survival rate. Haven't they used eggs which are just before hatching, because eggs of *Drosophila* just before hatching are considered in general to have high cold resistance.

If rates of survival in three developmental stages are 0.38 in eggs, 0.30 in larvae and 0.90 in pupae in *D. lutescens*, and 0.11, 0.07 and 0.75 in *D. takahashii*, the number of eggs needed to have one adult fly emerged is 10 in *D. lutescens* and 170 in *D. takahashii*. Furthermore, a great difference between the two species was observed in the survival of adult flies. Almost all adult of *D. lutescens* survived regardless of ageing, while in *D. takahashii*, the rates of survival decreased with ageing and became zero at 10 days. This may suggest that the difference between both species in the cold resistance in adult flies has brought about the differentiation in the geographical distribution between the two species.

Tucic (1979) found that the cold resistance of *D. melanogaster* was controlled by genes which were spread over all chromosomes. Kimura (1982a), with *D. lutescens* and *D. takahashii*, reported that their cold resistance was controlled by a gene or genes located on autosomal chromosomes. In the present study, B_1F_1 progenies having a marker gene yellow, obtained from the chromosome substitution technique between both species have been investigated for their cold resistance in relation to sex and phenotypes. From these experiments, it has been found that wild type flies of two B_1F_1 progenies which carried the autosomal chromosomes of *D. lutescens* with the probability of 75 or 25 percent have been shown to have always higher resistance than those of yellow type flies. The yellow progenies in B_1F_1 have been shown to have some resistance to cold temperature, although no survival was observed in the flies homozygous for *D. takahashii* chromosomes. This suggests that genes located on sex- and autosomal chromosomes of *D. lutescens* may control cold resistance. From these results and the survival rate of F_1 hybrids, it is considered that some of these genes are dominant.

The results presented here indicate that the capacity for cold resistance is apparent at those developmental stages of an aged egg, pupa or early adult for the two species. However, the largest difference between two species for cold resistance is found at the late adult stage, *D. lutescens* being apparently higher than *D. takahashii*. It is clear, furthermore, that this difference depends on the genes located on the chromosomes of *D. lutescens*. Thus, it

appears that this situation in the species makes it a good candidate for overwintering, causing higher survival of adult flies of *D. lutescens* under severe winter temperature conditions and bringing about a distinct differentiation in geographical distribution of the two species.

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