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Indirect thermal selection in *Drosophila melanogaster* and adaptive consequences

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Summary. Short-term indirect selection in *Drosophila melanogaster* for heat-sensitivity and heat resistance resulted in two strains, one heat sensitive and another heat resistant, and correlated responses were found for the rate of heat shock protein synthesis, behavioral patterns (asymmetrical sexual isolation) and fitness components (fecundity, fertility, viability, developmental time), as well as for several enzyme activities (MDH, G-6-PDH, ADH, ACHE). These responses associated with temperature selection may reflect the effects of differential inbreeding depression caused by homozygosity of temperature sensitive mutations with different pleiotropic effects. Selection even of a very short duration can induce significant adaptive and evolutionary changes.

Key words: Indirect selection – Temperature – Correlated responses – Reproductive isolation – Fitness components – Enzyme activities – *Drosophila melanogaster*

Introduction

Of particular importance for evolutionary changes are the correlated responses to selection pressure. Thus, it is generally accepted that in artificial selection experiments, strong selection on one character induces correlated responses in other characters (Falconer 1960; Lande 1979; Charlesworth et al. 1982). Such multidimensional responses, if they are adaptive, could accelerate evolutionary events.

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Short-term indirect thermal selection for heat sensitivity and heat resistance, applied to *Drosophila melanogaster* lines for ten generations, resulted in two strains which exhibit differences in terms of the quantitative trait "survival", when they are subjected to heat-shock (40 °C/25 min; Stephanou and Alahiotis 1983). These strains, named S_1 (sensitive, 6.50% survival) and R_1 (resistant to heat shock, 76.24% survival) also differ with regards to rate of heat shock protein synthesis (Stephanou et al. 1983). The cellular level of heat shock proteins induced after the temperature shock is positively correlated with survival (Stephanou et al. 1983; Alahiotis 1983). An analogous situation was observed when long-term direct temperature selection took place in *Drosophila melanogaster* cage populations (Alahiotis and Stephanou 1982).

Given this situation, in combination with the fact that the response to selection (over many generations) may also be reflected in changing patterns of variation (Waddington 1957), the selected S_1 and R_1 strains have been studied to determine if there is additional adaptive differentiation of these two strains, in terms of correlated responses in behavioral and biochemical traits as well as fitness components.

Materials and methods

The procedure followed in selecting the S_1 and R_1 strains has been described in detail elsewhere (Stephanou and Alahiotis 1983). Fifty lines, each derived from an inseminated female captured from a natural population in Gavros-Achaïa, Greece, maintained in mass culture under standard conditions (25 °C, 43 ± 4% mean relative humidity and using cornmeal-sugar-agar food medium; Alahiotis 1976) for about two years in our laboratory, were tested for their ability to survive when subjected to heat shock (40 °C for 25 min). From these lines two were chosen: one with the highest and another with the lowest tolerance to the heat shock treatment. Afterwards, indirect selection was performed for several generations as follows: from each of the two lines, approximately ten sublines were derived, each generated from a single pair (1♀ × 1♂). The parents of each subline were transferred three times to a new

food vial in order to increase the number of progeny obtained; from each subline at least 60 progeny were tested. Three-day-old progeny were anesthetized with ether and placed in empty glass vials (10♀ and 10♂ per vial) for 2 h before treatment. The vials contained no food, and moistened cotton plugs were forced into a position well below the surface of a Grant water bath into which the vials were immersed. After the heat shock, the flies were placed under standard conditions (25 °C) for 20 h, and the percentage of flies that remained alive was calculated. The untreated siblings of the most sensitive subline of the sensitive line or the most resistant subline of the resistant line were used to generate the next generation.

Sexual isolation index

The sexual isolation index was measured according to the method followed in our previous studies (Kilias et al. 1980; Alahiotis and Kilias 1982), i.e., random mating was tested by chi-square and the joint-isolation index of Malogolowkin-Cohen et al. (1965).

$$I = (X_{AA} + X_{BB} - X_{AB} - X_{BA})/N$$

where X_{AA}, X_{BB}, X_{AB}, X_{BA} stand for the four types of matings ♀A×♂A, ♀B×♂B, ♀A×♂B and ♀B×♂A, respectively, and $N = X_{AA} + X_{BB} + X_{AB} + X_{BA}$

$$\text{S.E. of } I = \sqrt{(1 - I^2)/N}.$$

A value of zero for this index indicates random mating; < 0, negative assortative; and > 0, positive assortative mating. Chi-square tests were used to determine if females and males of one strain mated more frequently than those of the other strain, and if assortative mating occurred.

Fitness components

The percentage of eggs that hatched was taken as the estimate of fertility, while the percentage of the adults emerging from the hatched eggs was used to estimate viability. To test for sterility, sets of 100 pairs (1 virgin female and 1 male in each vial) from the respective strains were put at 25 ± 1 °C and 43 ± 4% relative humidity. The mean sterility percentage was defined as the mean percentage of pairs failing to produce progenies. To estimate fecundity the mean number of eggs laid per fly per 24 h was used. The duration of development from deposition of eggs to emergence of adults was measured. Flies were allowed to lay eggs for 2 h and imagoes were scored every 4 h. For measuring fertility, viability and fecundity, 70 inseminated females (3 days of age) from the respective strains were used. For more details see Kilias et al. (1980).

Enzyme activity

These were measured in about six replicates in at least two separate experiments. The flies used were 3 to 4 days old in all assays, and the procedures used for the enzyme assays are given by Alahiotis and Kilias (1982) and Alahiotis (1979). Protein content was determined according to Lowry et al. (1951). The enzymes assayed in this study were: αGPDH; α-Glycerophosphate dehydrogenase (α-glycerol 3-phosphate: NAD^+ oxidoreductase, EC 1.1.1.8), G6PDH; glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: $NADP^+$ oxidoreductase, EC 1.1.1.49), 6PGDH; 6-phosphogluconate-dehydrogenase (6-phospho-D-gluconate: $NADP^+$ oxidoreductase, EC 1.1.1.44), ADH: Alcohol dehydrogenase (alcohol: NAD^+ oxidoreductase EC 1.1.1.1), ACHE: acetylcholinesterase (acetylcholine acetyl-hydrolase, EC 3.1.1.7), cMDH and mMDH, cytoplasmic and mitochondrial malate dehydrogenase (L-malate: NAD^+ oxidoreductase, EC 1.1.1.37).

The map position of the above gene-enzyme systems are as follows: G6PDH, 1–63.0; 6PGDH, 1–0.64; αGPDH, 2–20.5; ADH, 2–50.1; cMDH, 2–37.2; mMDH, 3–62.6; ACHE, 3–62 (O'Brien and MacIntyre 1978; Voelker et al. 1979).

In order to study the effect of heat-shock on the above enzyme specific activities, 3 to 4 day old flies from each strain (S_1 and R_1) were placed in empty glass vials for 2 h before treatment (37 °C/20 min); the same was true for the untreated (control) flies. The vials contained no food, and moistened cotton plugs were forced into a position well below the surface of a Grant water bath into which the vials were immersed. Starch gel electrophoretic analysis was based on techniques used elsewhere (Alahiotis and Berger 1977).

Results

Behavioral aspects

Table 1 shows the outcome of experiments done to estimate the sexual isolation index (SII) between the S_1 and R_1 strains. Experiments performed at 25 °C showed no statistically significant SII. Since the selective agent was temperature, and sensitive and resistant strains were obtained, we thought it of interest to see if temperature has an influence on this behavioral pattern. Consequently, experiments were performed at two additional temperatures (14 °C, 33 °C), chosen to cover the usual temperature limits of *Drosophila melanogaster* in the field. At all temperatures, the sexual isolation index was not significantly different from zero (Table 1). However, a particularly interesting finding was that the number (percentage) of the sensitive males mated decreased dramatically as the temperature increased, while the opposite was true for resistant males (Table 2). No such correlation was found for the S_1 and R_1 females. Thus, the increase in the sexual isolation index as the temperature is decreased might be due to the increased mating activity of the S_1 males and not to homogametic preferences.

Table 1. Mating preferences in crosses between the selected S_1 and R_1 strains of *D. melanogaster*

°C	♀S_1×♂S_1	♀S_1×♂R_1	♀R_1×♂S_1	♀R_1×♂R_1	*N*	Isolation index ± S.E.
14 °C	66	6	41	4	117	+0.197±0.091
25 °C	86	22	50	12	170	+0.152±0.076
33 °C	41	26	41	14	122	−0.098±0.900

Fitness components

Flies from both the S_1 and R_1 strains were tested for fecundity, fertility, viability, sterility and developmental time. Table 3 shows the results from experiments done under two conditions. First, under standard conditions of food medium (see "Materials and methods") and temperature (25 °C, control lines) and second, under heat-shock conditions. For the latter case, virgin females and males from both strains, aged 24 h, were shocked at 37 °C for 10 min. Females and males were maintained in separate food vials (each containing 10 flies) and shocked again (37 °C/10 min) when they were 72 h of age. Two hours after the second shock, sets of ten females and ten males were put in the same food vial for mating.

Analysis of variance followed by a posteriori comparisons (Newman-Keuls test; Winer 1971) for the data of each parameter listed in Table 3 showed that for fecundity, significant differences existed between the untreated R_{1c} and S_{1c} strains as well as between S_{1c} (control) and S_{1e} (experimental). The fecundity of the S_{1c} was higher than that of R_{1c} while if they received temperature shock the fecundity of the S_1 strain decreased (~ 50%) and that of the R_1 did not change. Differences were also found in terms of fertility between the S_{1c} and S_{1e}, where fertility decreased significantly when the flies were subjected to heat-shock. For viability, statistically significant differences were found between R_{1e} and S_{1e}, with the difference being in the opposite direction to those above. In the case of sterility, while it appears to be higher in the treated broods the differences are not statistically significant. For developmental time, differences were found between controls ($R_{1c} - S_{1c}$), between experimentals ($R_{1e} - S_{1e}$) as well as between R_{1c} and R_{1e} (all these differences being significant). In every case, the R_1 strain had longer developmental time.

Biochemical aspects

Specific activities for six enzymes were measured for the R_1 and S_1 flies which were or were not subjected to heat-shock (37 °C/20 min). The outcome from these experiments are presented in Fig. 1. Specific activities were determined before the heat-shock, soon after the heat-shock and 30 and 60 min after the stress. No general patterns in terms of the specific activity patterns were observed. However, in some cases after heat-shock (G-6-PDH, ADH, ACHE), the R_1 strain exhibited higher enzyme activities as compared with those for S_1. In the case of α-GPDH, specific activity was increased in the R_1 strain during the heat-shock and decreased as the recovery time increased. This was not true for the S_1 strain where specific activity of this enzyme was rather stable. For MDH, specific activity increased in both strains during the heat-shock. The specific activity of MDH measured refers to both cMDH and mMDH, because the crude extract used was electrophoresed and found to contain cMDH and mMDH. Visual examination of the gel did not enable us to determine whether the enzymic activity changes were due to cMDH, mMDH or both.

Table 2. Statistical analysis (χ^2 tests if females and males of one population mated more frequently than those from the other and if assortative mating occurs) of data from Table 1

	Temperature		
	14 °C	25 °C	33 °C
χ^2 assort. (1 d.f.)	0.011	0.025	2.44
χ^2 for random mating (3 d.f.)	91.17	77.71	16.81
χ^2_{δ} (1 d.f.)	80.42	61.20	14.44
χ^2_{φ} (1 d.f.)	6.23	12.44	1.18
$\%S_1$ ♀[a]	85.71	90.00	69.79
$\%S_1$ ♂[a]	127.38[b]	113.30[b]	85.41
$\%R_1$ ♀[a]	55.57	51.66	57.29
$\%R_1$ ♂[a]	11.90	28.33	41.66

[a] The percentage of females of each strain which had mating success

[b] Percentage > 1.00 because some males were successful in more than one mating

Table 3. Fitness components for the S_1 and R_1 strains of *D. melanogaster*. Values are means ± standard errors (c: controls, without heat shock; e: experimentals with heat shock)

Strains	Fecundity (means ± S.E. of eggs/fly/24 h)	Fertility (%)	Viability (%)	Sterility (%)	Developmental time (h)
R_{1c}	11.05 ± 1.06	91 ± 1.75	96.37 ± 1.70	7.07 ± 1.43	253.08 ± 0.64 (159)[a]
R_{1e}	10.85 ± 1.36	89 ± 1.38	89.43 ± 5.06	12.26 ± 1.44	259.92 ± 1.47 (45)
S_{1c}	16.80 ± 2.24	93 ± 3.18	92.80 ± 2.11	13.64 ± 1.76	250.38 ± 0.78 (102)
S_{1e}	8.65 ± 1.09	82 ± 4.07	96.42 ± 1.02	17.17 ± 1.42	249.42 ± 0.52 (193)

[a] Number in parenthesis refers to the number of flies used to estimate the mean ± S.E. developmental time

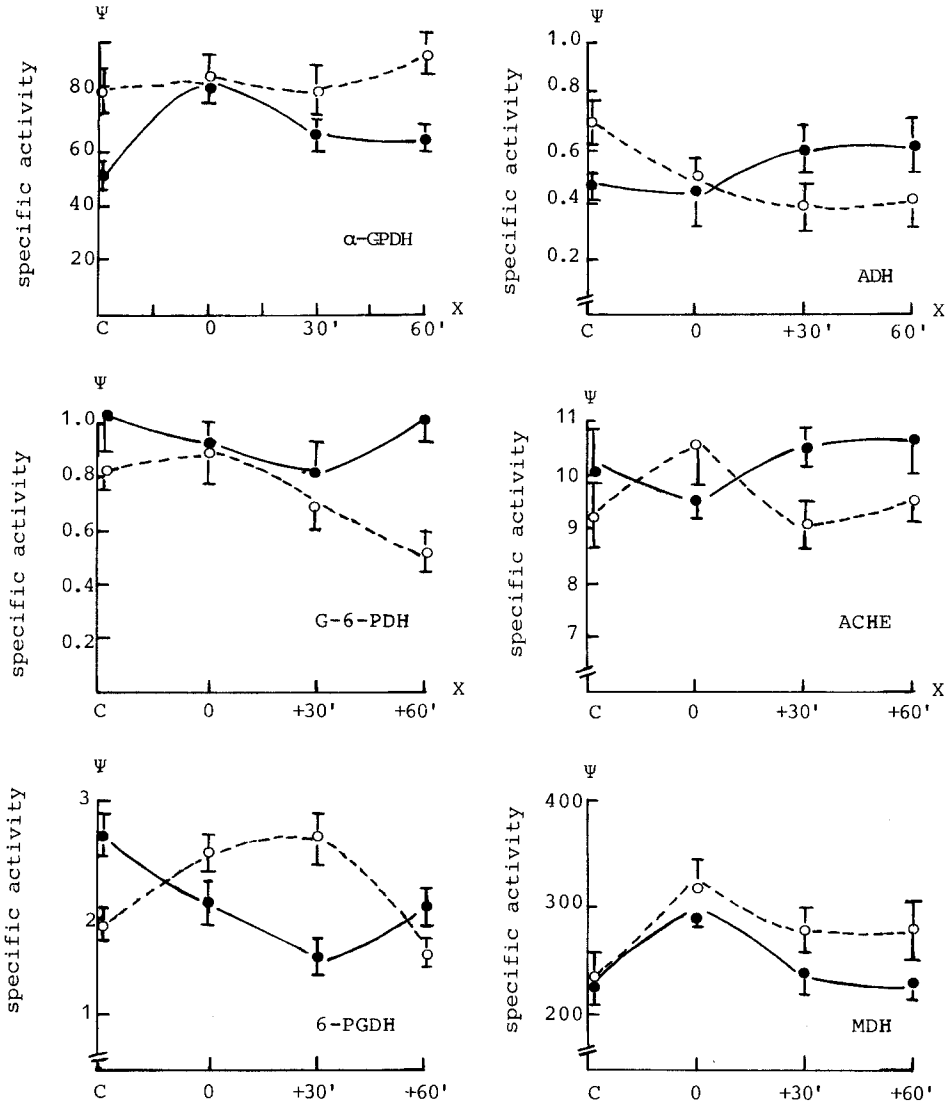

Fig. 1. Specific activities (M±S.E.) (ΔOD/mg pr^{-1} min^{-1}) of α-GPDH, G-6-PGDH, ADH, ACHE and MDH. *C* means before the heat shock; *O* means just after the heat shock; *+30′* and *+60′* minutes after the heat shock; ● R_1: ○ S_1 strains

Electrophoretic analysis (except for ACHE) did not reveal allozymic differences between strains except in the case of cMDH where 20% of the flies of the R_1 strain were heterozygotes (F/S) while the S_1 was monomorphic (FF).

Discussion

Short-term indirect temperature selection in *D. melanogaster* for heat sensitivity or heat resistance resulted in two strains which, when tested for reproductive isolation, showed a slight tendency to non-random mating at lower temperatures, a tendency not due to homogametic preferences. The results show that the mating preference between S_1 males and R_1 females is greater than that in the reciprocal cross (R_1 males with S_1 females). This kind of behavioral pattern is called asymmetrical isolation (Kaneshiro 1976; Ohta 1978; Watanabe and Kawanishi 1979) and had been used to suggest evolutionary sequences (e.g. Dwivedi et al. 1982; Wasserman and Koepfer 1980; Wood and Ringo 1980). This pattern of reproductive success may be a first stage of reproductive isolation, and in our experiments it developed after just ten generations of directional selection.

Taking into consideration the sib-mating scheme performed, one could argue that founder events also could affect the strain genotypes since the average coefficient of inbreeding in each line would have been high

at the 10th generation. However, we believe that the various differences observed between out lines might be attributed mainly to selective effects rather than to genetic drift for the following reasons: (1) As seen from the directional selection protocol, the single pair (1♀, 1♂) used to generate each generation was not chosen randomly, but on the expression of the specific character under selection (thermotolerance). (2) The selective assumption also is reinforced by additional observations on many pre- and post-mating fitness components (sexual isolation, survival, fecundity, fertility, developmental time) and biochemical traits (heat-shock proteins, enzymic activities), the differentiation of which also is related to temperature selection. (3) Taking into consideration the assumption that most genes with phenotypic effects are pleiotropic (Wright 1977), linkage disequilibrium and pleiotropy which determine the magnitude of correlated responses to selection (Falconer 1960) must be taken into account as forces which are not in favor of a major drift action in our system.

The observed temperature dependent mating ability of the males reinforces our previous observations that male parental investment is not negligible and under certain conditions sexual isolation can be a function not only of female behavior but also of male behavior (Alahiotis and Kilias 1982). Asymmetrical sexual isolation reported by other investigators for *Drosophila* strains that have undergone rapid speciation through bottleneck or founder events (Ahearn 1980; Carson and Kaneshiro 1976) has been attributed to inbreeding depression caused by homozygosity of partially recessive deleterious genes (see also Charlesworth et al. 1980). Taking this into consideration, we could also assume that the observed pattern of asymmetrical sexual isolation may reflect the effects of inbreeding depression caused by homozygosity of different temperature sensitive mutations with different pleiotropic effects. This assumption, although not based on specific evidence, is, however, strengthened by the fact that our strains exhibited differences in terms of cMDH genotypes (the S_1 being monomorphic and the R_1 polymorphic), as well as temperature-dependent differences in biochemical characters and fitness components, indicating that strong reorganization of the gene pool has been achieved as a result of selection pressure.

The ability of an organism to compensate for temperature change is based on the capacity of its biochemical and genetic machinery (Alahiotis 1983). In the present study, apart from the evolutionary and adaptive consequences observed for behavioral and fitness components, it appears that selection had a slight but significant tendency to induce differentiation in several enzyme activities, some of which contribute to energy production (e.g. G6PDH). Of particular interest is the finding that heat-shock increases the MDH specific activity in both strains. Previous studies (Behnel 1978) have shown that the increase in activity is due to de novo synthesis and the induction of heat sensitive puffs. However, Behnel (1978), referred to cMDH while it is quite possible that he measured the activity of both the cMDH and mMDH, since simple fly homogenization results in recovery of both enzymic forms (see "Results" section). While other studies (see review by Alahiotis 1983) have focused on the activities of mitochondrial enzymes and support the view that these activities are induced by heat-shock or anoxia, we cannot say yet, based on our electrophoretic data, that this is the case for MDH.

It is obvious that short-term indirect selection for heat sensitivity or resistance resulted in diverse correlated responses in behavioral, biochemical and fitness components. Selective regimes, even of a short duration (ten generations), can induce significant adaptive and evolutionary changes.

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