J. Biosci., Vol. 19, Number 2, June 1994, pp 267–275. © Printed in India.

Abdominal pigmentation and growth temperature in Indian Drosophila melanogaster : Evidence for genotype-environment interaction

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MS received 8 September 1993; revised 13 January 1994

Abstract. Phenotypic variability for abdominal pigmentation in females of an Indian natural population of *Drosophila melanogaster* was studied using isofemale lines and by rearing the larvae and pupae at 4 different temperatures ranging from 20–30°C. The dark pigmented area was found to increase in all the three segments when the growth temperature decreases. A significant positive correlation was detected for the occurrence of dark pigmentation in the 5th and 6th segments in each growth temperature but for other comparisons the correlation was not regular. Analysis of variance (ANOVA) was carried out both for individual segments over different growth temperatures and also for each temperature over the three abdominal segments and in all cases found to be statistically significant. The results are quite different from the earlier observation in French *Drosophila melanogaster* and suggest that genes controlling pigmentation are temperature dependent; temperature could affect post-transitional events involved in pigmentation. The present findings also clearly indicate that significant genotype-environment interaction exists, responsible for the production of desired phenotype at the opportune moment during the life span of a species.

Keywords. *Drosophila melanogaster,* abdominal pigmentation; growth temperature; reaction norms; genotype-environment interaction.

1. Introduction

Phenotypic plasticity has long been recognized and studied in plants. It has been seen that the same genotype reacts to different environment in different ways to give rise to different phenotypes (Schmalhausen 1949). The diverse phenotypes that may arise from the interplay between a given genotype and various environment in which this genotype may live, constitute the norm or range of reaction of that genotype. Therefore, any phenotype, healthy or pathological arising under the influence of any environment found in nature or created artificially by man is necessarily within the range of reaction of the genotype that produces it. In a quantitative genetic approach a significant genotype-environment interaction is generally observed (Via and Lande 1985).

Considering the above factors, the fruit fly *Drosophila* appears most convenient because of the well-known life history traits and also because of a large amount of available data concerning its response to various environmental factors (David *et al* 1983). Among the various environmental factors, temperature is most convenient because it is easy to control and is also of great significance for any ectothermic organism. In *Drosophila,* genetic variability for the raction norms to temperature

has already been studied for various traits including bristle numbers, wing length, thoracic lenght, duration of development and viability (Gupta and Lewontin 1982; Coyne and Beecham 1987; Scheiner and Lyman 1989). In *D. melanogaster* the extention of black pigment in the female abdomen is a trait which exhibits a broad range of variation in response to growth temperature and for which several genetic effects have been described (Zucker 1958; Roberston and Riviera 1972; Robertson *et al* 1977; David *et al* 1990). Also, it has been demonstrated that variation in body colour in natural populations of *Drosophila* have some adaptive significance (David *et al* 1983, 1985; Payant 1986; Capy *et al* 1988).

India is a tropical country that spans a high latitudinal range and the temperature varies greatly from place to place. In this subcontinent many species of *Drosophila* are found including *D. melanogaster* which is greatly abundant in the months of October till April. Population genetical work taking several aspects in this species has been worked out in India (Das 1993 for a review). The present paper describes the results obtained for the first time in Indian *D. melanogaster* on the effect of growth temperature on the abdominal pigmentation in females which establish the fact that growth temperature indeed affects the abdominal pigmentation which provide evidence for a significant genotype–environment interaction.

2. Materials and methods

A local population sample of *D. melanogaster* from Kalpana area of Bhubaneswar city was collected by keeping fermented banana baits inside the houses during December 1992. The females were isolated at 25°C in culture vials containing a killed yeast medium to initiate isofemale lines. The female progeny of the successful lines *i.e.* those which produced at least 10 females and 10 males were scored for abdominal pigmentation. Twelve lines were then chosen at random and a group of 10 pairs was used to produce the next generation. After a few days each group was allowed to oviposit for a few hours at 25°C in four successive culture vials. One progeny vial of each isofemale line was then transferred to one of the four experimental temperatures which were chosen to cover almost the full range of temperature generally encountered in India, compatible with sufficient viability. With this method the larval density was kept below 100 per vial (David *et al* 1990). On emergence adults were transferred to fresh food and were examined a few days later. The pattern of abdominal pigmentation is determined during the second half of the pupal stage and remains completely stable at the time of adult emergence (David *et al* 1990). Thus, the females to be examined were kept at 25°C before scoring.

It was observed on a close inspection that the extention of black pigment was more variable on the posterior segments than the antrior ones, therefore, the last 3 tergites corresponding to the abdominal segments 5, 6 and 7 were considered for pigmentation study. For a given segment the extention of the black pigment may range from nill up to the complete surface. Each segment was divided into 11 classes ranging from zero (no pigment) to 10 (completely pigmented; figure 1) for pigmentation scoring. The value of pigmentation was estimated from a lateral view of the female abdomen (figure 1). In order to exclude observer's bias from data, the pigmentation values were determined by two independent observers. About 20 individuals from each isofemale line in each temperature were scored.

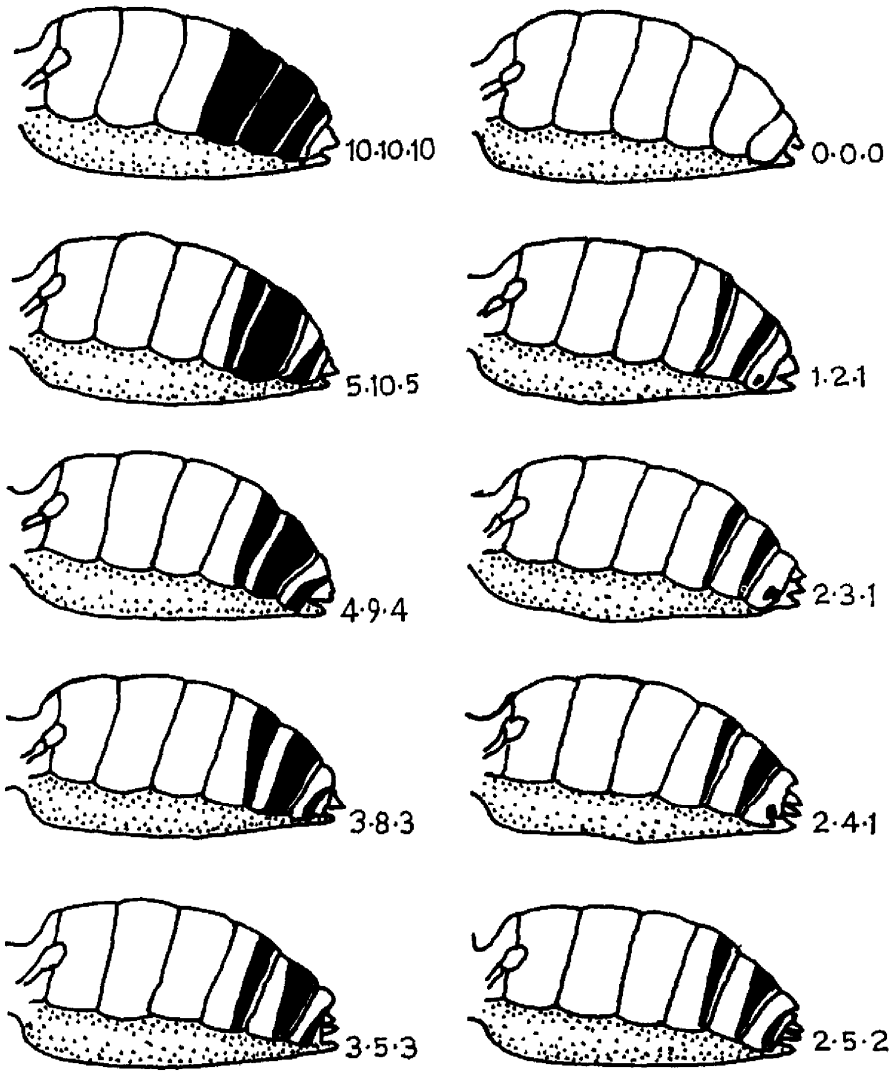

Figure 1. The phenotypic classes identified during the present study and used to estimate the pigmentation score in the last three abdominal segments of the females of Indian *D. melanogaster*. The figures indicate the value for each individual segment.

3. Results

The results of the present study indicate that individuals of *D. melanogaster* are highly polymorphic for the abdominal pigmentation and a general trend could be identified that when the growth temperature decreases, the pigmentation at the female abdomen increases (table 1). This is true for all the three segments over all the temperature range in *D. melanogaster*. Among the segments, the 6th one is more succeptible to growth temperature as could be marked from the figures 2 and 3. Intra-populational variation to this phenomenon seems to occur among the

Table 1. Average value of pigmentation score in 12 isofemale lines subjected to different growth temperature in three abdominal segments of Indian *D. melanogaster*.

Isofemale lines	20°C			25°C			27°C			30°C		
	5th	6th	7th	5th	6th	7th	5th	6th	7th	5th	6th	7th
1	3·67	9·00	3·00	2·20	4·80	1·80	1·20	2·60	1·20	1·50	2·30	1·00
2	3·00	10·00	2·00	3·00	5·38	1·13	2·20	4·40	1·50	2·00	4·44	0·70
3	2·50	7·00	4·00	1·80	4·60	1·30	2·10	4·00	1·00	1·60	2·70	0·60
4	2·83	9·00	3·17	1·40	3·80	1·40	1·90	3·50	1·30	1·50	2·60	1·00
5	3·33	9·00	3·00	2·30	3·30	1·30	1·86	3·29	1·14	2·10	3·50	0·40
6	3·00	9·25	2·00	2·20	5·70	1·00	2·17	3·29	1·00	1·70	2·90	0·70
7	2·90	8·50	3·30	2·10	4·40	1·80	2·00	3·00	1·00	1·70	2·90	0·40
8	3·67	9·67	3·67	2·20	5·00	1·10	2·18	3·50	1·20	2·00	3·40	0·40
9	2·60	7·60	2·80	2·20	4·00	1·50	1·88	3·75	1·00	1·90	2·90	0·80
10	2·70	8·10	2·90	1·90	4·00	1·50	1·88	2·50	1·30	1·60	2·20	0·90
11	4·67	10·00	2·17	1·80	5·30	1·40	1·50	4·00	1·00	1·70	2·70	0·90
12	3·50	9·00	2·00	2·00	4·80	1·20	2·29	3·57	1·00	1·88	3·57	0·86
Mean± SE	3·20 ± 0·18	8·84 ± 0·26	2·83 ± 0·19	2·09 ± 0·11	4·59 ± 0·21	1·37 ± 0·07	1·93 ± 0·09	3·45 ± 0·16	1·14 ± 0·05	1·79 ± 0·06	3·01 ± 0·16	0·72 ± 0·07

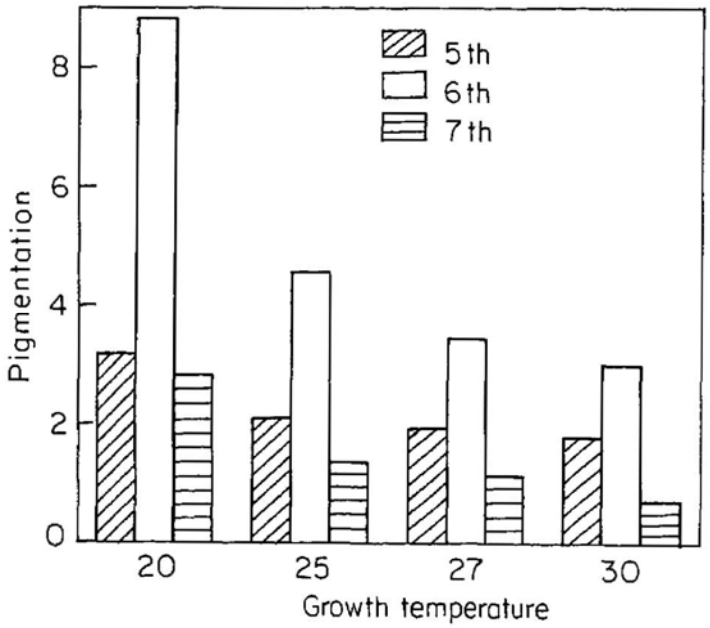

Figure 2. Histogram showing pigmentation value at different growth temperature based on the mean value at each growth temperature of table 1.

isofemale lines but the variation is not statistically significant (insignificant F values, not given).

In order to know the influence of one segment on the other for the occurrence of pigmentation, correlation coefficient (r) was calculated between each two segments and values are shown in table 2. It is evident from the table that there is a strong

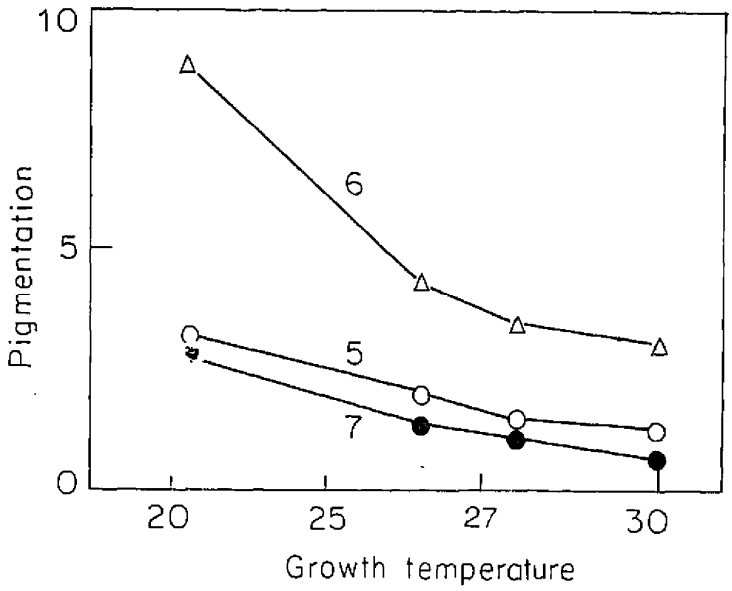

Figure 3. Values of abdominal segments for pigmentation scoring. The numbers 5, 6 and 7 refer to the abdominal segments.

Table 2. Correlation coefficient (*r*) between two abdominal segments for the occurrence of pigmentation in response to different growth temperature in females of *D. melanogaster*.

Growth temp. (°C)	5th vs. 6th	*P*	5th vs. 7th	*P*	6th vs. 7th	*P*
20	+0·70	<0·02*	−0·34	>0·10	−0·54	>0·05
25	+0·61	<0·05*	−0·21	>0·10	−0·33	>0·10
27	+0·58	<0·05*	+0·02	>0·10	+0·02	>0·10
30	+0·90	<0·001*	−0·80	<0·01*	−0·61	<0·05*

*Significant df= 10.

positive correlation between the 5th and 6th segments and the *r* is statistically significant for all temperatures. However, in certain other cases, the *r* was found to be statistically significant but in a negative way (*e.g.* for 5th and 7th and 6th and 7th at 30°C). Thus it could be assumed from this observation that interrelationship between the segments for abdominal pigmentation in response to growth temperature does exist at least for the 5th and 6th segment in the present experiment.

Analysis of variance (ANOVA) was carried out to measure the total variability (both for the same segment over different temperature and also for the same temperature over the three segments) and in all cases found to be highly significant statistically (table 3). These results show that in different temperatures, the same segment tend to have different pigmentation and also at a given temperature, different segments respond differently in different isofemale lines. However, when all the three segments were taken into consideration jointly, a general trend was

Table 3. Analysis of variance (ANOVA) for individual segments in four different growth temperature and for individual temperature in the three abdominal segments.

Variable (segment)	*F* value	Probability
5th	30·12	< 0·001*
6th	88·64	< 0·001*
7th	4·15	< 0·01*
Temperature (°C)		
20	382·47	< 0·001*
25	54·88	< 0·001*
27	243·04	< 0·001*
30	187·79	< 0·001*

*Significant $df_1 = 3$, $df_2 = 44$.

observed (figure 3) . The intensity of this variation also differs; the greatest difference for the segments was found to be the 6th (high *F* value) and for temperature at 20°C.

In order to know the extent of interaction between the three abdominal segments and temperature variations for the occurrence of pigmentation ANOVA (treatment-by-levels design) as suggested by Bruning and Kintz (1977) was carried out and presented in table 4. It is evident from the table that for $_{all}$ the variables *i.e.* temperature, abdominal segments and the interaction between these two, the *F* values are highly significant. This provides a clear cut inference in support of the fact that the interaction between temperature and abdominal segments gives rise to different phenotypic expression for abdominal pigmentation in different individuals.

4. Discussion

In a research programme involving the study of phenotypic plasticity and to analyse a reaction norm in *Drosophila* the isofemale line technique is generally preferred

Table 4. Analysis of variance (treatment by level design) for temperature, abdominal segments and the interaction of the two variables.

Source of variation	Sum of square	Degree of freedom	Mean square	*F* value	Probability
Total	818·93	143	–	–	–
Temperature	277·42	3	92·47	75·18	< 0·001*
Abdominal segments	213·63	2	106·82	86·85	< 0·001*
Temperature × abdominal segments	165·37	6	27·56	22·45	< 0·001*
Error	162·51	132	1·23	–	–

*Significant.

(Gupta and Lewontin 1982; Coyne and Beecham 1987; Gebhardt and Stearns 1988). The present study indicates that the growth temperature indeed influences the intensity of abdominal pigmentation in *D. melanogaster* females in India and a comparatively lower temperature increases the area of black pigmentation in all the three segments analysed. However, not all the segments respond identically to this temperature variations; in this study the 6th one is found to be much variable than the other two. In a similar experiment on French *D. melanogaster,* David *et al* (1990) have detected the 7th segment as most variable for the pigmentation and supposed the existence of an anteroposterior gradient in the phenotypic plasticity of the segments for which variability is maximum at the end of the abdomen. It has been identified that several homeotic genes control the antero- posterior gradient during embryogenesis (Scott and O'Ferrell 1986). Also, a gene in the fourth chromosome that has a significant effect on this gradient has been identified (Robertson *et al* 1977). Based on these facts it has been presumed that all these genes affect the differentiation of the adult epidermis during pupation and the extention of dark pigmented area (David *et al* 1990). However, the manner in which the pattern of pigmentation changes from one segment to the next as a function of tempeature is *not* consistent with the hypothesis that the segmental pattern is under the control of a global, "gradient-like" process. Thus based on the data on temperature effect it is not possible to say anything about which genes are involved in pigmentation. Further research is needed before drawing any further conclusion on this matter.

With the isofemale line technique, genetic variability may be calculated by estimating the coefficient of interclass correlation (Capy *et al* 1989). In the present study the correlation coefficient calculated was found to be significant positively between 5th and 6th and also negatively in some cases, The parallel variation of 5th and 7th segments in general over the whole temperature range and a more drastic change observed in case of the 6th segment (figure 2) is really difficult to explain and quite different from the results obtained in the French *D. melanogaster* (David *et al* 1990), Whereas, the pigmentation in these three segments when considered together was very high in French population of this species, it is quite lower in Indian *D. melanogaster* (see figure 4 for a comparison), India and France differ in climatic environmental conditions in several respect. Thus the observation of a comparatively darker pigmentation in French *D. melanogaster* seems to be due to a comparatively cooler environment experienced in France. Such a variation in natural populations was never been detected in *D. melanogaster* so far even though it is known that the pigmentation can be manipulated by selection (J R David, personal communication).

The evidence for genetic variation among different lines of *D. melanogaster* was provided by ANOVA (table 3) and significant interaction between genotype and environment (temperature) was detected. All these interactions are highly significant indicating the fact that the norms of reaction are genetically variable which is again different from one segment to the other and also from one temperature to the next. Thus, in other words, it can be assumed that different genetic elements become important at different environmental situations. In this respect, we are at present agree with the suggestions of Schnee and Thompson (1984) that the genes which control abdominal pigmentation are not exactly the same at all temperatures and some conditional expression may occur at different stages.

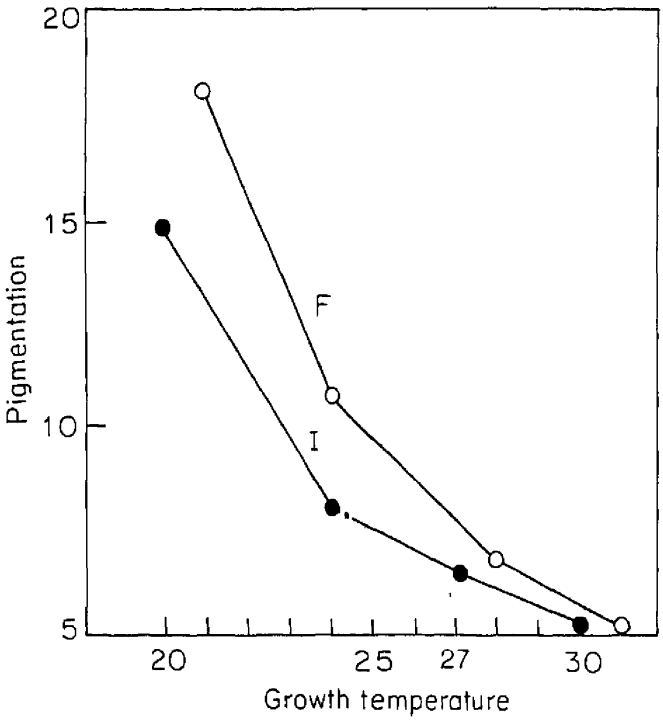

Figure 4. Sum of the mean values of pigmentation score in all the three abdominal segments. I refers to Indian (present study) and F to French populations of *D. melanogaster.*

The overall pigmentation either dark or light is expected to be the phenotype related to fitness. A number of animal species exhibits a pigmentation polymorphism and three main mechanisms provide a selective advantage in pigment variation; camouflage and protection against predation, mate recognition and some physiological advangages like regulation of the thermal balance *i.e.* darker individuals better absorb energy from light and infrared radiation (David *et al* 1985), If this is admitted, the distinct norms of reaction exhibited by successive segments could have another origin related to the construction of the body architecture in early embryogenesis. Whatever the cases may be, this hypothesis should be investigated on different populations of other *Drosophila* species as well. Since *D, melanogaster* is unique for its cosmopolitan distribution and known for its wide range geographic variations (David and Capy 1988) it provides an useful animal material for such studies. In addition, the data on Indian flies are really interesting and deserve further experimentations especially at lower temperature and also in other natural populations (J R David personal communication).

Acknowledgements

The authors are highly grateful to Professor J R David, Laboratorie de Biologie et Genetique evolutives, CNRS France for his comments on the results and suggestions.

AD thanks the Council of Scientific and Industrial Research. New Delhi for a Research Associateship. SM thanks the NCERT, New Delhi and the Principal, RCE, Bhubaneswar for facilities and encouragements.

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