As the skeleton matures it also changes shape: this is concomitant with an increase in size. Previous studies on size-selected mice [1-4] suggest that changes in muscle development, fat deposition and cell size and number in lung, liver, spleen and kidney are produced by an accelerated passage through the growth process. Is this also true for shape changes in the skeleton? If so mice selected for large size should resemble full grown unselected mice and those selected for small size should resemble more juvenile stages of growth.

We tested this hypothesis by performing Fourier analysis on videodigitised images of the first and second thoracic vertebrae of mice selected for body size and of a pseudo-longitudinal series of unselected mice. We [5] have previously described a method for converting videodigitised images of mouse vertebrae to polar coordinates, adjusting them to a standard area, finding a mean shape and converting this to Fourier components. Multivariate analysis of the first n Fourier components (where n is chosen to give good discrimination between mean shapes) gives a generalised distance between shapes, i.e. a measure of their overall difference. The vector of the line joining the centroids of the shapes may be used to discriminate between similar and dissimilar changes. The mice used were a) the QA series of Falconer [6] which were made up of 29 unselected control animals, 30 selected for large size and 28 selected for small size over 13 generations [7] and b) 142 F_1 individuals from a cross of Balb/c and CBA inbred strains aged 25–60 days and sacrificed at 5-day intervals [8].

The first 40 Fourier sine and cosine components from the mean shapes of each series were entered into a canonical analysis. For T1 vertebrae (fig. 1) it is quite clear that the major part of the shape difference is along axis 1, with the bones arranged in rough order of age, and the 25-day-old vertebrae well separated from the rest [8]. The large–control–small axis is also linear but almost orthogonal to that of the age series. For T2 (fig. 2) a similar picture is obtained, with size variation due to selection for size and that due to normal growth again being almost orthogonal.

It is thus clear that our measure of vertebral shape behaves differently from the other parameters which have been studied in mice selected for size. Whilst muscle development, fat distribution, and cell number and size in a number of organs respond to selection by an accelerated passage through the growth process vertebral shape responds quite differently: presumably those features of shape which differ between 25- and 60-day-old mice are not those which differ between large and small mice. Exactly where these differences lie cannot be examined by Fourier analysis. We are presently developing techniques which will allow us to accurately localise such differences in shape.

1 Bryne, L., Hooper, J. C., and McCarthy, J. C., Anim. Prod. *17* (1973) 187.
2 Hooper, J. C., and McCarthy, J. C., Anim. Prod. *22* (1976) 131.
3 Clarke, J. N., PhD thesis, University of Edinburgh, 1969.
4 Falconer, D. S., Gauld, I. K., and Roberts, R. C., Genet. Res. *31* (1978) 291.
5 Johnson, D. R., O'Higgins, P., McAndrew, T. J., Adams, L. M., and Flinn, R. M., J. Embryol. exp. Morph. *90* (1985) 363.
6 Falconer, D. S., Genet. Res. *22* (1973) 291.
7 Johnson, D. R., O'Higgins, P., and McAndrew, T. J., Genet. Res. Camb. *51* (1988) 129.
8 Johnson, D. R., O'Higgins, P., and McAndrew, T. J., J. Anat. (1988) in press.

0014-4754/88/090776-02$1.50 + 0.20/0

Crystal cell pattern modification in a melanotic tumor strain of *Drosophila melanogaster*

A. Di Pasquale Paladino, P. Cavolina, G. Romano and R. Ribaudo

Department of Cell and Developmental Biology 'A. Monroy', University of Palermo, Via Archirafi 22, I-90123 Palermo (Italy)
Received 5 May 1988; accepted 13 June 1988

Summary. 3rd instar larvae of the melanotic tumor *tu-pb* strain of *Drosophila melanogaster* hold a lower number of free-circulating crystal cells in their hemolymph than the wild type ones. This pattern could result from an abnormal retention of mature crystal cells in the hematopoietic organs, as the strong hemocyte melanization inside the lymph glands of heat-treated *tu-pb* larvae seems to demonstrate. Melanotic tumor formation and modification of the crystal cell pattern may be related.
Key words. Crystal cells; hemolymph; lymph gland; melanotic tumor.

Crystal cells (fig. 1) comprise 5–10% of larval hemocyte population in *Drosophila melanogaster* [1]. They are the source of hemolymph phenoloxidase activity owing to the prophenoloxidases carried in their paracrystalline inclusions [2]. Like other blood cell types, crystal cells are produced in the hematopoietic organs (the lymph glands) and periodically released into the hemolymph [3].

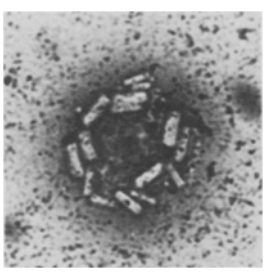

Figure 1. Crystal cell from larval hemolymph. ×4650

At present, *Black cells (Bc,2-80.6)* and two alleles of the lozenge locus, *lz(rfg)* and *lz(s)*, are the only reported mutants which specifically affect the crystal cells. Prophenoloxidases are activated in situ in *Bc* mutant larvae and, as a consequence, the crystal cells are blackened and lack paracrystalline inclusions; the pigmented hemocytes can be seen beneath the integument as individual cells and in the first pair of lymph glands [4].

Phenoloxidase activity is not detectable in cell free extract of *Bc/Bc* larvae (lethal condition) whereas *Bc/+* extract has less than half the activity of *+/+* [5].

Crystal cells do not differentiate in *lz(rfg)* or *lz(s)* larvae and the enzyme activity is absent in the hemolymph. It is likely that the *lz* locus influences the differentiation of mesodermal cell clones that give rise to the larval crystal cells [6].

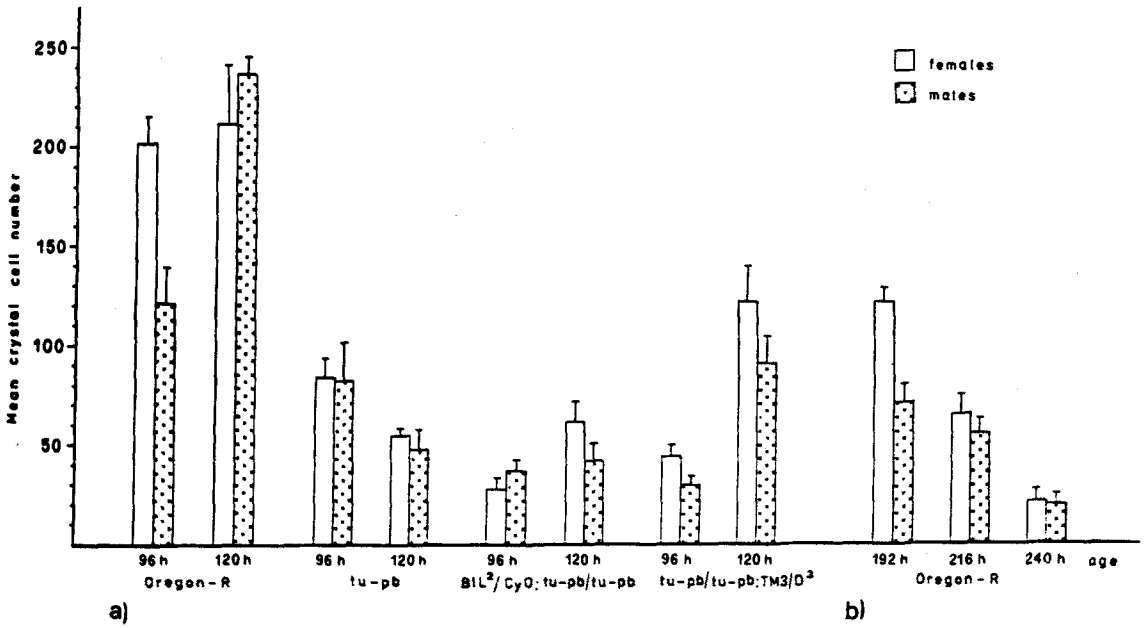

Figure 2. Results of mean crystal cell number counts in larval hemolymph of *Oregon-R*, *tu-pb* and genotypes including the 2nd or the 3rd *tu-pb* chromosome. Breeding temperature: *a* 23.5 °C; *b* 18.0 °C.

Furthermore, melanization of the crystal cells in wild type larvae can be induced by hot water (70 °C) treatment, and the distribution of heat-induced black cells in these larvae mimics the distribution of black cells in *Bc* mutant larvae[4].

Insect phenoloxidases are involved in adult cuticle sclerotization and pigmentation, but how the hemolymph phenoloxidases are related to these processes as well as the role of the crystal cells in wound healing and melanization of cellular capsules has not been established as yet. It may well be that activities in which melanization is involved, i.e. melanotic tumor formation, utilize precursors from these cells[4].

In analyzing larval hemolymph cellular populations we find that the crystal cells comprise only 0.9% of the blood cell types circulating in melanotic tumor *tu-pb* mutant third instar larvae and 3.5% of wild type *Oregon-R* larvae. *tu-pb* is an atypical melanotic tumor mutant in which tumor manifestation is restricted to the head of the adult; penetrance is also incomplete and different in the sexes[7,8]. Previous genetic analysis indicated that the *tu-pb* phenotype depends on at least two different loci, one recessive on the 3rd chromosome, the other one, apparently semidominant, on the 2nd chromosome: only genotypes including both the two large *tu-pb* autosomes elicit tumor manifestation[9].

The results obtained from crystal cell counts (fig. 2a) indicate that *tu-pb* 3rd instar larvae have fewer free-floating crystal cells in their hemolymph than *Oregon-R* wild type larvae. Both the two large *tu-pb* autosomes appear to affect crystal cell number, however the 3rd chromosome is more similar to the *tu-pb* strain in its effect, although neither of them, individually, seems to account for the further decrease in older larvae (120 h after the egg laying) that is evident in the *tu-pb* strain (fig. 2a). A few cells undergoing blackening can be seen scattered throughout the hemocoel in heat-treated *tu-pb* larvae, which, on the other hand, often display intense cellular melanization in the anterior pair of lymph glands (fig. 3c and table 1a). Furthermore, the pattern of heat-induced blackened cells is very different in *Oregon-R* larvae, paralleling the *Bc* phene (fig. 3d), although melanization in the lymph gland is extremely rare (fig. 3a and table 1a).

These results raise the question whether the presence of so numerous fully differentiated crystal cells inside the lymph glands is an unusual feature. According to Shrestha and Gateff[10] primordial blood cells in the hematopoietic organs do not form functional phenoloxidases, while fully differentiated crystal cells are very rare. On the other hand, Rizki et al.[4] state that a few fully differentiated crystal cells can be generally found in the anterior pair of the lymph glands.

In view of these facts, we interpret our observations suggesting that the scarcity of circulating crystal cells in the hemolymph of *tu-pb* larvae may be the consequence of a retention of fully differentiated crystal cells in the hematopoietic organs. In agreement with this hypothesis are the

Table 1. Hot water (70 °C) treated third instar larvae

Genotypes	No or few circulating melanized cells (%)*		Numerous circulating melanized cells (%)*		Total larvae	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
a)						
Oregon-R	2.9	2.7	97.1 (1.1)	97.3 (1.2)	280	260
tu-pb/tu-pb; tu-pb/tu-pb	100 (20.9)	100 (34.9)	0.0	0.0	302	269
BIL²/CyO; tu-pb/tu-pb	96.7 (14.0)	96.2 (19.6)	3.3	3.8	274	318
tu-pb/tu-pb; D³/TM3, Sb, Ser	59.2 (13.5)	47.4 (16.9)	40.8	52.6 (1.1)	287	175
b)						
Oregon-R	30.4	16.0	69.6	84.0	125	100
tu/pb/tu-pb; tu-pb/tu-pb	100 (11.6)	100 (21.9)	0.0	0.0	112	105

Breeding temperature: a) 23.5 °C; b) 18.0 °C. *Numbers in brackets indicate the percentage of larvae showing melanized lymph gland.

Table 2. Effect of *Bc* on melanotic tumor *tu-pb* incidence: comparison between genotypes including different 2nd chromosome markers

Genotypes	♀♀ % *tu-pb*	No.	♂♂ % *tu-pb*	No.	Total No.
Bc, Elp, px/tu-pb; tu-pb/tu-pb	0.09	2144	0.09	2260	4404
BlL^2/tu-pb; tu-pb/tu-pb	14.9	652	4.8	556	1218
tu-pb/tu-pb; tu-pb/tu-pb (tu-pb strain)	48.6	1226	12.9	1232	2458

results of heat-induced crystal cell melanization in lines bearing the 2nd or the 3rd *tu-pb* autosomes individually which show a clear correlation between crystal cell numbers and blackening patterns (table 1 a). Additional evidence that crystal cell functions are extremely reduced in the hemolymph of *tu-bp* larvae comes from the observation that melanine diffusion in heat-treated larvae, which, as Rizki has pointed out[4], is characteristic of the wild type, does not take place in *tu-pb* larvae.

In any case, the 3rd *tu-pb* chromosome, in homozygous condition, seems principally to contribute to crystal cell pattern modification; its effects are clearly visible in genotypes including *Bc*. In fact, the typical *Bc* phenotype nearly disappears in *tu-pb/Bc; tu-pb/tu-pb* adults, while *Bc* expressivity is very strong in *tu-pb/Bc; tu-pb/TM3, Sb, Ser* individuals.

The correlation between the modifications in crystal cell patterns and the genotype controlling the *tu-pb* manifestation could also account for a possible interrelationship between *tu-pb* and crystal cells.

Moreover, *tu-pb/Bc; tu-pb/tu-pb* individuals nearly fail to express melanotic tumors (table 2), indicating that functional integrity of crystal cells is required for *tu-pb* manifestation.

In attempting to find further evidence of these interrelationships, as *tu-pb* is a temperature sensitive mutant (tumor manifestation is suppressed at 18 °C)[8], we investigated temperature effect on crystal cell pattern in *Oregon-R, tu-pb* and *Bc,Elp,px/CyO* strains. A strong reduction of the crystal cell number is evident in samples of hemolymph (fig. 2 b) and in heat-treated larvae of the *Oregon-R* strain developing at 18 °C (fig. 3 b and table 1 b); likewise, the weak *Bc* phenotype observed in larvae and adults *Bc* individuals reared at a lower temperature (fig. 3 a) seems to be attributable to temperature effect.

This reasoning suggests that low temperature reduces the number of crystal cells circulating in the hemolymph. However, cell releasing from the lymph glands does not seem to be affected since no melanization is detectable inside the hematopoietic organs of heat-treated *Oregon-R* larvae (fig. 3 b).

tu-pb larvae maintain their typical distribution pattern of heat-induced melanized crystal cells also when reared in experimental conditions suppressing tumor manifestation in the adult fly (table 1 b). In this regard, it is interesting to note that the temperature sensitivity period for *tu-pb* manifestation falls at a later stage, coinciding with emergence[8]. Therefore we propose that the primary defect in *tu-pb* tumorigenesis may involve the normal crystal cell releasing from the lymph glands; one or more components, however, presumably intervening after 3rd larval instar, should cooperate to produce melanotic masses in the adult fly. It is worth while to note that tumor manifestation in the *tu-pb* mutant occurs at the emergence, simultaneously with the processes of tanning and melanization which are largely dependent on phenoloxidase activities.

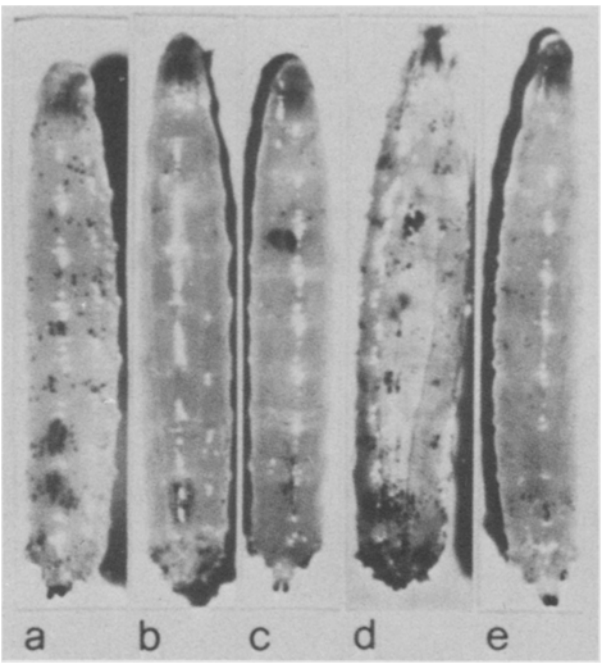

Figure 3. Distribution of blackened crystal cells in late third instar larvae treated with hot water (70 °C): *Oregon-R* reared at a) 23.5 °C and b) 18.0 °C respectively, and *tu-pb* reared at c) 23.5 °C (the same feature is observed at 18 °C also). Distribution of spontaneous black cells in *Bc*/+ larvae reared at d) 23.5 °C or e) 18 °C. ×16

Acknowledgment. We are grateful to Mr A. Polisano for his photographic assistance. This work was supported by a grant of M.P.I. Quota 60% (1985).

Abbreviations. BlL^2 and CyO = 2nd chromosome balancers; TM3 = 3rd chromosome balancer; Elp = 2nd chromosome dominant marker; D, Sb, Ser = 3rd chromosome dominant markers. For a detailed description see Lindsley and Greel[11].

1 Rizki, T. M., in: Genetics and Biology of Drosophila, vol. 2b, p. 397. Eds Ashburner and Wright. 1978.
2 Rizki, T. M., Rizki, R. M., and Bellotti, R. A., Molec. gen. Genet. *201* (1985) 7.
3 Gateff, E., Adv. Cancer Res. *37* (1982) 33.
4 Rizki, T. M., Rizki, R. M., and Grell, E. H., Wilh. Roux's Arch. *188* (1980) 91.
5 Rizki, R. M., and Rizki, T. M., Genetics *91* (1979) 103.
6 Rizki, T. M., and Rizki, R. M., Genetics *110* (1985) 98.
7 Di Pasquale Paladino, A., and Cavolina, P., D.I.S. *59* (1983) 31.
8 Di Pasquale Paladino, A., and Cavolina, P., D.I.S. *60* (1984) 84.
9 Di Pasquale Paladino, A., Cavolina, P., Romano, G., and Ribaudo, R., D.I.S. *66* (1987) 47.
10 Shrestha, R., and Gateff, E., Devl Growth Diff. *24* (1) (1982) 65.
11 Lindsley, D. L., and Greel, E. H., Genetic Variations of *Drosophila melanogaster*. 1967.

0014-4754/88/090777-03$1.50 + 0.20/0