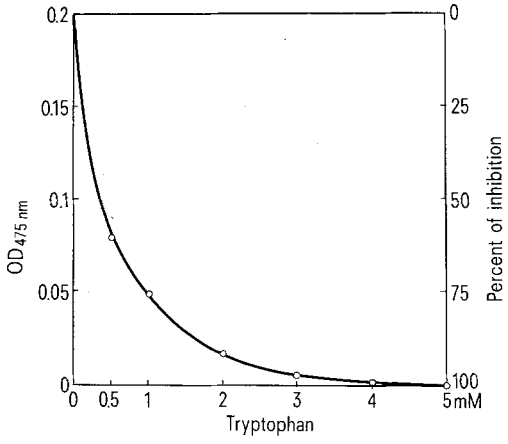
with the rise in tryptophan pyrrolase activity in these tissues, under the influence of tryptophan in vivo. The same effect was also obtained in the case of the mammalian system in vivo (table 2). The present results support our previous observations on the inverse relationship of tyrosinase and tryptophan pyrrolase in *Bufo melanostictus* during experimental pigmentation and depigmentation[4]. It further suggests that increased tryptophan level in the biological system can bring about disturbances in the genesis of melanin. According to Badway[9,10] increase in tryptophan level in body tissues is possible due to the effect of stressful agents like ethanol or catecholamine. Incidentally, stress has been considered to be a factor in the origin of vitiligo[11]. So it appears that the inhibition of tyrosinase and DOPA auto-oxidation and activation of tryptophan pyrrolase under the influence of higher concentrations of tryptophan may be the factors involved in the impairment of melanin biosynthesis in vitiligo.



Inhibition of DOPA auto-oxidation by graded amounts of Tryptophan. DOPA (1 mM) was incubated with different concentration of tryptophan (0.5 mM; 1.0 mM; 2.0 mM; 3.0 mM 4.0 mM and 5.0 mM) in a total volume of 1 ml at 37 °C, pH 7.0 for 8 h. OD of the solutions were determined at 475 nm in a Hilger-Watts Spectrophotometer after incubations, which represents DOPA auto-oxidations i.e. DOPA-chrome formation in a given time.

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Hemolymph proteins in an ascitic condition, induced by lethal *1(3)gl* tumorous tissue in *Drosophila hydei*[1]

Ž. Srdić and P. Borner

Laboratoire de Génétique, Université de Genève, CH-1224 Genève, and Zoologisches Institut der Universität Zürich, CH-8057 Zürich (Switzerland), 18 March 1980

Summary. Drosophila hydei larvae homozygous for the gene *1(3)gl* may survive to the age of 3 weeks, become bloated and be incapable of metamorphosis. Wild-type flies after the injection of a fragment of the *1(3)gl* disc, which has been previously culture in vivo for 40–500 days, also become bloated (ascitic reaction). In the hemolymph of both the advanced *1(3)gl* larvae and ascitic adult hosts the accumulation of a specific protein is observed.

The gene *1(3)gl (lethal (3) giant larvae),* a recessive lethal gene in *Drosophila hydei,* is located at the distal end of an autosome which is number 3 according to Berendes[2] and number 4 according to Spencer[3]. Homozygous *1(3)gl* die as advance larvae. Incapable of metamorphosis, they may survive to the age of 3 weeks whereas the normal pupation age is about 7 days. Such larvae are oversized, bloated and rather transparent, and show a characteristic syndrome[4] the most salient features of which are hypertrophy of the brain and degeneration of imaginal discs. The latter disappear during larval life, except for the dorsal mesothoracic and ventral metathoracic discs, which coalesce into a single poorly-organized mass of large dimensions. When fragments of this mass are injected into the abdomens of adult hosts, the tissue behaves as a benign tumor; it proliferates rapidly but does not reduce the lifespan of the host.

Transplanted *1(3)gl* brain tissue by contrast behaves as a malignant tumor, killing the host. These properties of brain and disc tissue, in conjunction with the chromosomal localization of the gene, leave no doubt that *1(3)gl* of *D. hydei* is homologous with *1(2)gl* of *D. melanogaster*[5], although certain features of the phenotype differ between the 2 species.

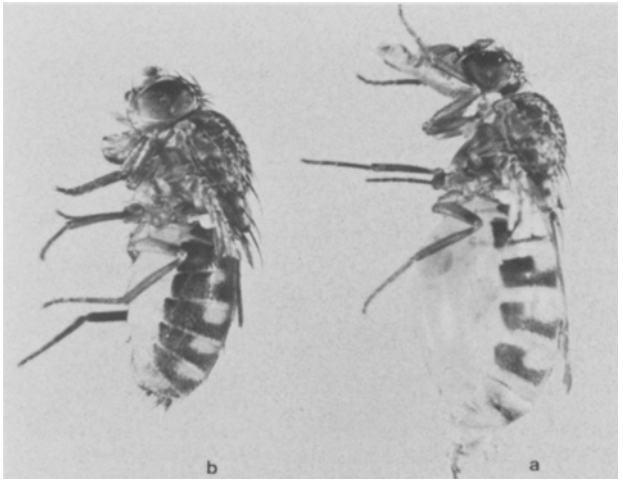

Fig. 1. *Drosophila hydei* females (strain Alicante wild-type) 20 days after the injection of an imaginal disc. (a) Fragment of disc from a *1(3)gl* larva, cultured in vivo for 40 days, (b) disc from a normal larva (control). In both specimens the wings were removed to facilitate photography.

In *D. hydei, 1(3)gl* disc tissue which has been cultured for some time, provokes in the host at each new transplantation an ascitic reaction which usually becomes very pronounced after 2 weeks. This phenomenon has prompted us to analyze the hemolymph proteins in ascitic flies, in comparison with normal hemolymph from lethal and normal larvae.

Material and methods. Larvae and flies were kept at 25 ± 0.5 °C in culture bottles on a standard diet containing cornmeal, wheatmeal, sugar, dried yeast, agar and propionic acid.

Imaginal discs were dissected from advanced 3rd instar larvae in sterile Ringer's solution according to Derksen and Berendes[6]. Discs were injected into the abdomens of adult females of a normal laboratory strain (Alicante wild-type). Hemolymph was collected from varying numbers of larvae or flies and pooled to make samples of about 0.1 ml. Normal and *1(3)gl* larvae of 7 days of age were used, a few hours before normal pupation time. In addition, over-aged *1(3)gl* larvae of 20 days were also bled. Ascitic flies were used 20 days after the injection of lethal imaginal disc material. The latter had been previously cultured in vivo for 40 days (2 transfers) or for 530 days (25 transfers). Since pure hemolymph from normal flies was difficult to obtain, we used as a control the mutant *net*[7]. Newly hatched flies of this mutant generally develop large blisters on their wings that contain pure hemolymph free of any cellular elements. For separation of the hemolymph proteins polyacrylamide gels were prepared according to the procedure of Davis[8] as modified by Borner[9]. Densitometrical tracing of the stained protein bands was carried out by a DD2 densitometer and a DB5 recorder (Kipp and Zonen).

Results and discussion. Wild-type adult hosts which have received a fragment of *1(3)gl* disc, begin to swell after a few

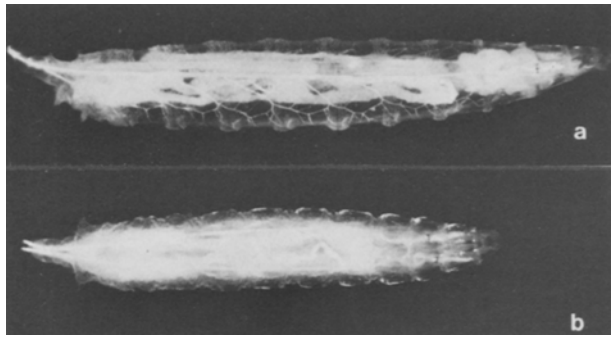

Fig. 2. A 20-day-old lethal *1(3)gl* larva of *Drosophila hydei* (a) with full-grown normal larva (b) for comparison.

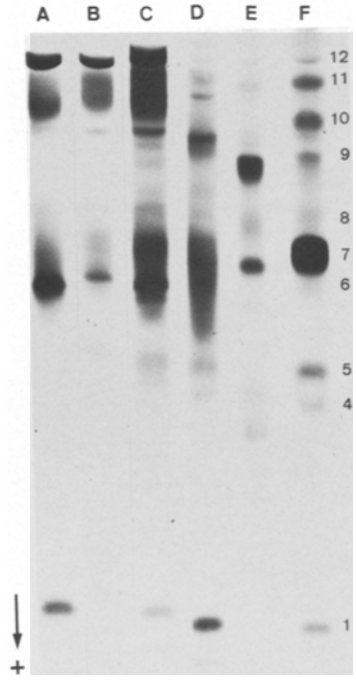

Fig. 3. Separation of hemolymph proteins on polyacrylamide gels. A, 7-day-old wild-type larvae. B, 7-day-old *1(3)gl* larvae. C, 20-day-old *1(3)gl* larvae. D, wild-type flies (controls). E, host flies after injection of *1(3)gl* disc tissue cultured in vivo for 40 days. F, host flies after injection of *1(3)gl* disc tissue cultured in vivo for 530 days. In each case a total of µg hemolymph protein was layered on the sample gel. The protein fractions are numbered beginning with the most anodal band. Fractions 2 and 3 are too faint to be visible on the photograph, but can be clearly detected by densitometrical tracing (see figure 4, B and C).

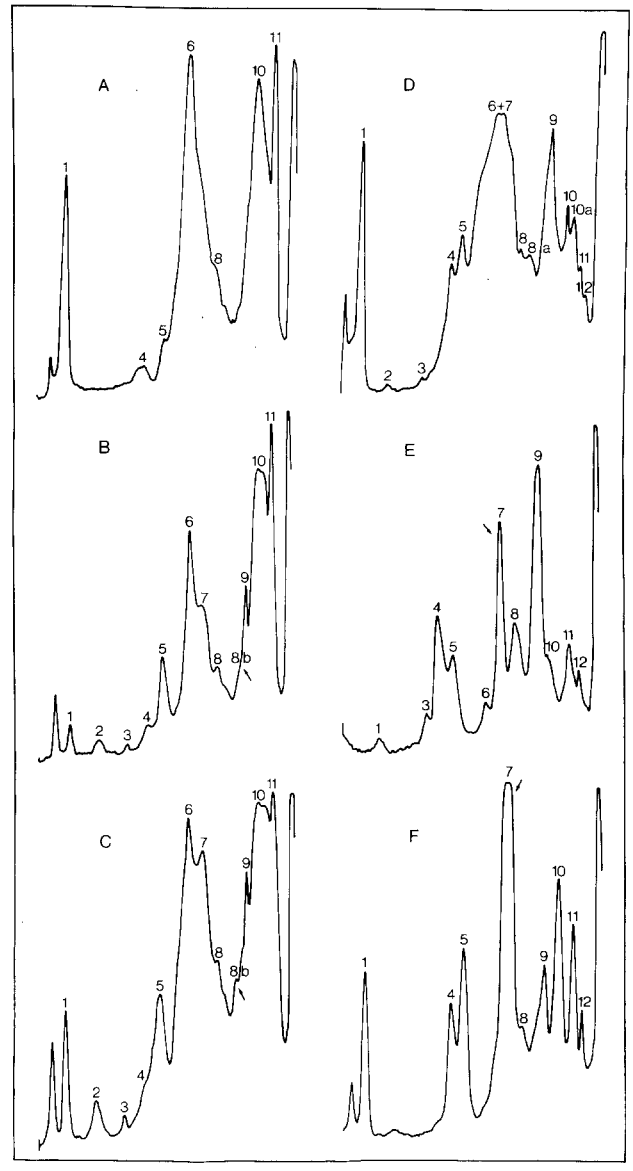

Fig. 4. Densitometrical tracings of the electropherograms illustrated in figure 3. Explanations are given in the legend to figure 3.

days. Within 10-20 days after the injection, a state of maximal bloating has been reached, whereby not only is the abdomen distended to capacity, intersegmental membranes being stretched to the limit, but also the labium, which cannot be retracted anymore, and even the neck are bloated (figure 1). The bloated flies never copulate, nor do they lay eggs. However, this pathological state does not affect viability: the mean longevity of ascitic flies is the same as that of controls. Although the tumor grows incessantly, it is clearly not the volume of tumorous tissue that causes the bloating, but rather a genuine ascitic state, generated either by an overproduction of hemolymph or by water retention. In ascitic flies the abdomen becomes transparent so that most of the internal organs are visible. The latter retain their normal aspect, except the fat body which is strongly reduced.

It is interesting to note that flies injected with a *l(3)gl* disc, taken directly from a larva, in general do not develop the ascitic reaction. By contrast, when disc tissue has been previously cultured in 2 successive hosts over a period of about 40 days, a single small fragment of the tumor will induce the reaction in 80–90% of the cases.

It is also noteworthy that the lethal *l(3)gl* larvae become ascitic towards the end of their life. At the age of 7 days when normal larvae are ready to pupate, the lethal larvae are distinguished by a reduced size of some internal organs, especially the salivary glands and the fat body. Imaginal rudiments, imaginal discs, gonads, and parts of the brain are also underdeveloped. Lethal larvae at this stage are more transparent than their normal sibs. They can survive up to 15 more days under favorable conditions, without a supplementary molt, whereby they become very large and transparent (figure 2). During this period, while organs and anlagen remain stationary or degenerate, with the exception of the brain and the one remaining disc, the volume of the lethal larva increases essentially due to an excess amount of hemolymph. This suggests the supposition that there may be a connection between the bloated state of *l(3)gl* larvae and the ascites induced in normal flies by *l(3)gl* tissue. It should however be recalled that certain other mutations also produce bloated lethal larvae, in particular *l(3)tr* of *D. melanogaster*[10] and *l(3)gl* of *D. hydei*[11].

Results of the electrophoretic analysis of hemolymph proteins in the wild-type and *l(3)gl* larvae as well as in the host flies following injection of the lethal disc tissue are illustrated in figures 3 and 4. As can be seen, protein fractions 2, 3, 7 and 8b are detectable only in the *l(3)gl* larvae, among which fraction 7 increases rapidly in concentration during their survival period (figure 4, B and C). It is of interest to note that the same protein fraction accumulates in the hemolymph of host flies carrying the *l(3)gl* tissue. In flies with disc tissue which had been previously cultured in vivo for 530 days, fraction 7 became the most prominent protein band (figure 3, F). Thus, it appears that both the development of the ascitic state in *l(3)gl* larvae and the induction of the ascitic reaction in host flies after injection of the *l(3)gl* disc are correlated with the accumulation of a specific protein in the hemolymph.

In *Drosophila*, as in other insects, the hemolymph proteins are known to be synthesized in the fat body[12,13]. As mentioned above, the fat body in the *l(3)gl* larvae becomes greatly reduced at the end of the survival period. Similarly, a large part of the fat body in the abdomen of the bloated host fly is destroyed. Consequently, the protein accumulated in the hemolymph probably has its origin in some tissue other than the fat body. To what extent this protein is directly involved in the induction of the ascitic state is not yet clear. Further experiments are needed to explain the causal relationships of the phenomena reported here.

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Genetic variability in 3 co-occurring forms of the starfish genus *Othilia (= Echinaster)*

Rebecca D. Tuttle and R. Lindahl[1]

Developmental Biology Section, Department of Biology, The University of Alabama, University (Alabama 35486, USA), 4 October 1979

Summary. High levels of genetic variability have been found in 3 (grey, brown, orange) co-occurring forms of the starfish *Othilia*. These high levels appear to correspond to the generalistic habit of this starfish. The biochemical data, coupled with morphological observations, indicate that the grey and orange forms are morphs of the same species and that the brown form is a species separate from the grey and orange forms.

To date, information concerning genetic variation in starfish has been limited[2-4]. Manchenko and co-workers have only recently initiated an extensive survey of allozymic variation among several starfish species from the Sea of Japan[5-10]. In the present study, we compare allozymic variation among 3 co-occurring forms (grey, brown, orange) of the starfish genus *Othilia (= Echinaster)* from the Gulf of Mexico. In addition, allozymic and morphological data have been coupled in order to determine whether these morphs are morphs within a species or diverging species.

Materials and methods: 26 grey, 19 brown and 24 orange specimens of *Othilia* were collected from off the coast of Panama City, Florida and maintained without food for 4–