hibernation than at the beginning, while the greatest variations concern the polyunsaturated fatty acids that are lower before the hibernation than at the beginning, and again fall in the last period. About polyunsaturated acids the most evident increase is that of the linolenic acids; in these analyses arachidonic acid was not found.

As the hibernation coincides with the period of low temperature, our results agree with those of LYMAN.

Riassunto. Gli autori hanno studiato le variazioni degli acidi grassi dei lipidi del tessuto sottocutaneo del *Rinolophus ferrum equinum* in differenti periodi dell'ibernazione ed hanno riscontrato modificazioni quantitative nei vari acidi grassi e particolarmente nei polinsaturi.

E. TURCHETTO, M. G. GANDOLFI, and R. RONDININI

Istituto di Chimica Biologica and Istituto di Istologia dell'Università di Bologna (Italy), January 21, 1963.

Isolation and Characterization of a Polyhydroxy Compound in Adult *Tribolium confusum* Duval[1]

DEVI et al.[2] while studying the variation in the contents of DNA, RNA and nucleotide in *Tribolium confusum* Duval[3], during the various stages of growth and development suggested the presence of a hydroxylated aromatic compound (or hydroxylated aromatic amino acid) in the adult insects. The present paper is primarily concerned with the isolation and characterization of the hydroxylated aromatic compound (or compounds). Insects of 28–30 days old were taken from a pure stock continually reared for the last ten years on a diet composed of whole wheat flour, previously passed through sieve No. 80 and, 5% dried brewer's yeast. The cultures are kept in a room maintained at 28±1°C and at the constant humidity of 70±5%. The insects were homogenized for 5 min at 0°C in cold glass distilled water (10 ml/100 mg of insects) in an Elvehjem-Potter homogenizer, and then filtered through a sintered glass funnel in a cold room. The filtrate was freeze-dried. A spongy mass of light brown colour which changed to deep brown on exposure to air, was obtained. The yield was 5 mg for every 100 mg of the insect used.

For the identification of the compound a 5% solution of this material in water was used; (a) with $FeCl_3$ solution it gave a deep purple colour, (b) on warming with Millon's reagent it gave a red colour and (c) on heating with a secondary amine in the presence of sulphuric acid and on subsequent alkylation it gave a deep blue indophenol. These chemical tests indicated the compound under question was most probably an aromatic amino acid with free hydroxyl groups or a polyhydroxyphenol.

Since the material was not obtained at this stage to the requisite degree of purity, identification of the compound was carried out by two dimensional ascending paper chromatography on a sheet of Whatman No. 1 filter paper (16″ × 20″) in the solvent systems butanol: acetic acid: water (4:1:5 by volume) and phenol:water (4:1 by volume). Between application of the material on chromatogram, the spot was dried in a stream of N_2. The chromatogram was run until the solvent front reached the end of the paper (it usually takes 16–18 h). Solvents were removed by keeping the paper in an air oven maintained at 40–50°C. The dried chromatogram sprayed with 0.1% ninhydrin solution made in 95% ethanol, revealed the presence of nine amino acids. According to their Rf values they appeared to be cysteine, arginine, glycine, tryptophane, alanine, leucine, proline, phenylalanine and valine. Since only nine amino acids in water extractable material could be detected by ninhydrin test, we assume that there are only nine amino acids present as such in adult insect.

Since the use of ninhydrin is not promising to detect the hydroxylated aromatic compounds except those containing $-NH_2$ groups in the α position, one-dimensional paper chromatography was carried out by the ascending method in the solvent system butanol, acetic acid, water (4:1:5 by volume). The reference substances used were dopa (dihydroxy phenyl alanine), catechol and tyrosine, and the amount used was 10μl (0.1%) of the known substances and 50 μl for the test substance. The dried chromatogram was dipped into a solution containing 0.44*M* $K_3Fe(CN)_6$ in 0.1*M* phosphate buffer pH 7.8. Three coloured spots immediately appeared on a yellow background. In the Table the results are given. The colour of the ensuing spots and the Rf values gave easy identification as catechol. Tyrosine did not give any spot, owing to the absence of two hydroxyl groups. A similar chromatogram was also run thereafter and the area corresponding to catechol (based on the Rf value obtained from the previous chromatogram) was cut out, dipped into chloroform and allowed to stand for 4 h. The liquid was decanted off,

Rf values of catechol, DOPA and the compound extracted from adult insects with water, when subjected to one-dimensional ascending chromatography in a solvent mixture containing butanol:acetic acid:water (4:1:5); the characteristic colours as revealed on the dried chromatogram dipped into $K_3Fe(CN)_6$ solution prepared in phosphate buffer pH 7.8, identified the compound tested.

Rf values and colour reaction of catechol, DOPA, tyrosine, and the extract obtained from adult insects

Compound tested	Rf System: butanol/acetic acid/water 4:1:5	Colour, characteristic of the compound after ferricyanide reaction in phosphate buffer pH 7.8
Catechol	0.840	brownish black which changed to permanent blue
DOPA	0.250	orange brown changed to dark blue colour afterwards
Tyrosine	no spot	no colour
Extract of adult insect	0.820	light brownish black changed to light blue

[1] This work has been supported from a grant (A-1388) received from the National Research Council of Canada.

[2] ANIMA DEVI, A. LEMONDE, and N. K. SARKAR, Exp. Cell Res. *29*, 443 (1963).

[3] The life cycle of *Tribolium confusum* Duval is subdivided into five well defined phases such as (a) embryonic stage (—6–0 days), (b) larval stage (0–13 days), (c) prepupal stage (14–17 days), (d) pupal stage (18–22 days), and (e) adult stage.

allowed to evaporate at room temperature to a very small volume and then subjected to infra-red analysis. The presence of two hydroxyl groups adjacent to each other (*o*-position) in the compound was revealed.

A steep rise in optical density of an RNA solution at 260 mμ was noted upon the addition of an increasing amount of catechol. This information allows one to conclude that the material extracted from adult insects with water and responsible for giving a high value for free nucleotide concentration when measured at 260 mμ[2], is catechol.

Résumé. A côté de neuf acides aminés un composé polyphénolique est décelé dans l'extrait aqueux de *Tribolium confusum* Duval. Le chromatogramme sur papier traité par $K_3Fe(CN)_6$ et l'analyse I.R. révèlent qu'il s'agit du catéchol.

ANIMA DEVI, M. A. MUKUNDAN, F. V. PATIL, and N. K. SARKAR

Department of Biochemistry, Faculty of Medicine, Laval University, Quebec (Canada), November 26, 1962.

Mutant Expression in Inbred Lines and Crosses

When a quantitative character can be scored more than once on single individuals, its phenotypic variance may be regarded as made up of two components, V_c and V_i. V_c refers to the variance common to the repeated scores on an individual while V_i refers to the independent variance of each score alone[1]. For a character which can be measured on the right and the left-hand side of an individual, the variance from, say, the left side will be $V_L = V_c + V_i$ and the variance of the sum of the measurements on the two sides will be[2] $V_{L+R} = 4V_c + 2V_i$.

In genetically homogeneous populations, the common component of variance V_c is attributable to variation in the external environment. The independent component is variously described as 'chance or stochastic variability'[3], 'developmental error'[4], 'developmental noise'[5], and 'internal accidents in development'[6], terms in which the authors try to express the organism's inability to realize a complete, bilaterally identical course of development notwithstanding identical genetic and environmental conditions.

It has been shown in *Drosophila* that the relative contributions of the two components of variance differ according to the type of quantitative character. This is further reflected in characteristic differences between inbred lines and crosses with respect both to variance and to the value of the mean, relative to those of the parents. The causes and consequences of such differences are still obscure. We need similar analyses on a greater variety of characters in different environmental situations and in novel genetic conditions as in the expression of mutants of variable expression. There the properties of the system can hardly be attributed to a long established adjustment by natural selection.

The mutant used for this purpose was *cubitus interruptus* dominant (ci^D, 4th chromosome) of *Drosophila melanogaster*. In single dose it causes a terminal interruption of the 4th longitudinal vein of the wing. With the help of suitable, marked stocks this mutant has been introduced into several inbred lines so that the variance of expression can be studied in different genetic backgrounds. The lines were derived by long continued brother-sister mating from the Gabarros and Pacific wild stocks. The different inbred lines and crosses between them were reared in $^1/_8$ l creamers at 25°C and at low densities to eliminate possible effects of crowding. Both wings were scored for expression of ci^D on 20 individuals of each sex per culture. Expression is measured as the ratio of the length of the 4th vein present to that of the uninterrupted 3rd vein, both measured from the anterior crossvein[7,8].

The results are shown in the Table. Only those lines and crosses are shown in which the entire distribution of expression falls within that part of the scale where expression is approximately linear in relation to genetic and environmental changes. A description of the various tests and a discussion of this problem has been given earlier[8].

In all crosses the mean values of the scores in ♀♀ are close to that of the midparent value, thereby providing additional support for the linearity of this part of the scale. With respect to the variance, the independent component V_i is several times larger than the common component V_c. This is in agreement with unpublished experiments, carried out in collaboration with Dr. F. W. ROBERTSON, which showed that considerable changes in body size and developmental time on different chemically defined sterile media were not accompanied by alteration in the average expression of ci^D which is therefore insensitive to such gross changes in the environment. Also different levels of crowding and larval growth at successive periods in live yeast cultures did not affect the expression.

In the Table all estimates of V_i and V_c are combined in weighted estimates for all crosses and all inbreds respectively. The values of V_i are remarkably similar, but the V_c value for crosses is smaller. As, however, both V_i and V_c are not homogeneous within inbreds and within crosses (using Bartlett's test $P < 0.05$ for V_i values and

Means and variance components of the expression ratio of ci^D in inbred lines and crosses. Explanation in text

Inbred line or cross	mean ♀♀	N	V_{L+R}	$2V_i$	$4V_c$
Gabarros 2	39.8	120	27.32	17.65	9.67
Gabarros 4	41.7	80	32.98	30.63	2.35
Gabarros 6	37.1	160	41.54	22.85	18.69
Pacific 4	60.4	120	36.01	17.03	18.98
Gab. 2 × Gab. 4	41.9	160	20.14	19.35	0.79
Gab. 2 × Pac. 4	51.0	40	44.77	31.54	13.23
Gab. 2 × Pac. 10	49.8	160	29.10	23.35	5.75
Gab. 4 × Pac. 4	52.2	40	35.41	22.68	12.68
Gab. 4 × Pac. 10	53.8	160	34.36	21.10	13.26
Pac. 4 × Pac. 10	58.5	160	40.58	30.31	10.27
Total inbreds		480	35.30	22.55	12.75
Total crosses		720	31.40	23.52	7.88

[1] E. C. R. REEVE and F. W. ROBERTSON, J. Genet. *51*, 14 (1954).
[2] E. C. R. REEVE, Genet. Res. *1*, 151 (1960).
[3] E. C. R. REEVE and F. W. ROBERTSON, Nature *171*, 874 (1953).
[4] G. A. CLAYTON, J. A. MORRIS, and A. ROBERTSON, J. Genet. *55*, 131 (1957).
[5] C. H. WADDINGTON, H. GRABER, and B. WOOLF, J. Genet. *55*, 246 (1957).
[6] K. MATHER, Heredity *7*, 297 (1953).
[7] W. SCHARLOO, Exper. *17*, 121 (1961).
[8] W. SCHARLOO, Arch. Neerl. Zool. *14*, 431 (1962).