

oocytes and seem to lose their certain parts. Also the cytoplasmic RNA is feebly labelled as may be revealed by very few grains in the cytoplasm.

The conclusion of early workers with regard to the nucleolar origin of 'yolk nucleus' fails to be confirmed by my present investigations, and I fully agree with NATH's et al.<sup>10</sup> view advocating that the nucleolar extrusions in fish oocytes are artefacts; and such a conclusion derived by earlier workers from the irregularity of nuclear membrane (sometimes rupturing also) having nuclear pouches is erroneous and may be the effect of fixation and the mechanical disturbance.

*Résumé.* L'expulsion de la matière nucléolaire dans les œufs du poisson est étudiée ici par autoradiographie. Employant la cytidine-H<sub>3</sub>, nous avons vu que le RNA nucléolaire passe dans le cytoplasme sous forme diffuse, et non comme un nucléole complet appelé «noyau-jaune».

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Temperature and Mutant Expression

The uniform adaptive phenotypic reactions to environmental change performed by populations of wild-type organisms are thought to be originated by natural selection. Out of a heterogeneous population, with individuals reacting in various directions, only those genotypes showing a useful reaction would be preserved.

There is support for this idea in the fact that wild populations do not react uniformly to such unnatural variations in environment as provided by temperature shock, X-rays and various chemicals (review GOLDSCHMIDT<sup>1</sup>). Moreover, by artificial selection for a special type of reactivity to such abnormal conditions in *Drosophila*, BATEMAN<sup>2</sup> obtained stocks showing predominantly the reaction selected. On this basis one might expect that in an experimental situation, where a mutant gene is introduced in an otherwise wild genotype, a given change in environment would not result in a uniformly directed reaction of the mutant expression. However, in the many experiments done on the reactivity to temperature change of mutant expression in *Drosophila melanogaster*, only uniform reactions were reported. They were in fact described as a property of the mutant gene. We mention the work of ZELENY and his group (a.o. HERSH<sup>3</sup>) on Bar, STANLEY<sup>4</sup>, HARNLEY<sup>5</sup> and RIEDEL<sup>6</sup> on vestigial and STERN<sup>7</sup> and HOUSE<sup>8</sup> on cubitus-interruptus. In those experiments, mostly inbred laboratory stocks were used to ensure a uniform response, since the main interest lay in the precise quantitative relations between temperature and mutant expression. These relations were expected to yield information about the nature of gene action (PLUNKETT<sup>9</sup>).

In contrast, for the experiments reported here, the mutant ci<sup>D</sup> (cubitus-interruptus dominant) was crossed into three recently caught wild stocks from Colmar (France, courtesy of Prof. H. BURLA, Zürich), Leiden, and Gouda (Netherlands) in such a way that together with ci<sup>D</sup> only chromosomes 4 and Y were introduced, amounting to about 0.2% of the total genetic map. As ci<sup>D</sup> is homozygous lethal it was in all cases balanced over another 4th chromosome lethal, sparkling-Cataract (spa<sup>Cat</sup>, abbreviated below as Cat).

The ci<sup>D</sup> mutant shows among other features a terminal interruption of the 4th longitudinal wing vein. The expression of ci<sup>D</sup> was measured as the percentage ratio of the

length of the 4th to that of the 3rd longitudinal vein distal to the anterior crossvein (a/b in Fig. 1). All cultures were set up with 10 cm<sup>3</sup> of a constant food medium (2% agar, 15% sugar, 4% dead dried yeast, 0.1% nipagin) and with a constant population density of 100 larvae. All larvae had been reared at 25° up to 48 ± 1½ h of age. In each culture the left wings of 20 ♀♀ and 20 ♂♂ were measured.

In the first experiment, the three stocks were compared at temperatures 20° and 25°. The results in Table I show clearly that the shift in expression of ci<sup>D</sup> is directed differently. The Leiden stock shows a direct relation between temperature and mean expression, the Colmar stock no change and the Gouda stock an inverse relation.

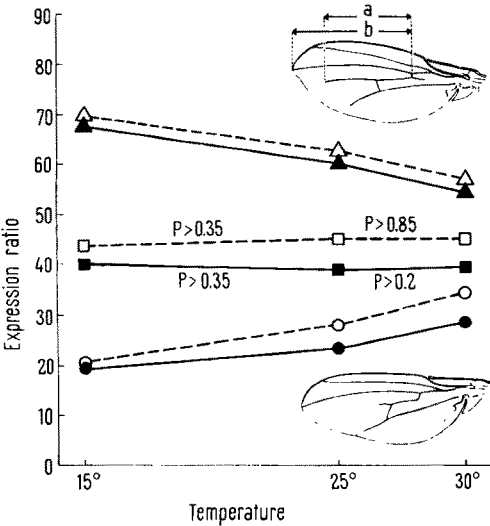


Fig. 1. The mean expression ratio at 3 different temperatures of the Colmar ci<sup>D</sup>/Cat base population (□, ■) and the lines selected for high (Δ, ▲) and low (○, ●) expression ratio. Open figures and broken lines represent females, solid figures and lines the males. The differences between the samples were tested following Wilcoxon and probabilities exceeding 0.001 are given in the Figure. The means of the high line are based on 40, all other means on 60 individuals.

Tab. I. The mean expression ratio of ci<sup>D</sup> in 3 different wild stocks at different temperatures. From the Gouda stock for each mean only 40 individuals were measured, the other means are based on 60 individuals. P values are calculated after Wilcoxon.

Stock		Mean expression ratio		Difference 25°-20°	P
		25°	20°		
Gouda	ci <sup>D</sup> ♀	52.3	56.6	- 4.3	<0.001
	Cat ♂	46.6	50.4	- 4.2	<0.001
Colmar	ci <sup>D</sup> ♀	49.1	48.0	- 1.1	>0.4
	Cat ♂	40.5	39.8	+ 0.7	>0.14
Leiden	ci <sup>D</sup> ♀	43.7	34.7	+ 9	<0.001
	Cat ♂	32.2	29.3	+ 2.9	<0.001

<sup>1</sup> R. B. GOLDSCHMIDT, Physiological Genetics (New York 1938).  
<sup>2</sup> K. G. BATEMAN, J. Genet. 56, 443 (1960).  
<sup>3</sup> A. H. HERSH, J. exp. Zool. 57, 283 (1930).  
<sup>4</sup> W. F. STANLEY, Phys. Zool. 4, 394 (1931).  
<sup>5</sup> M. H. HARNLEY, Genetics 21, 84 (1936).  
<sup>6</sup> H. RIEDEL, Arch. entw. Mech. 132, 463 (1935).  
<sup>7</sup> C. STERN, Genetics 28, 441 (1943).  
<sup>8</sup> V. HOUSE, Genetics 40, 576 (1955).  
<sup>9</sup> C. R. PLUNKETT, J. exp. Zool. 46, 181 (1926).

Further experiments were done with the Colmarci<sup>D</sup>/Cat stock (no change in mean) and two stocks obtained from it by selecting for 6 generations for respectively high and low expression ratio at 25°. Three cultures each of the base population and the 'low' line and two cultures of the 'high' line were reared at 15°, 25° and 30° (Fig. 1). The mean expression of the low line has a direct relation with the change of temperature and the mean expression of the high line an inverse relation. The frequency distributions (Fig. 2) reveal that each selection line reacts uniformly in one direction. The mean of the base population does not change significantly with the change in temperature. However, the frequency distributions suggest an increase in the variation at lower temperatures on either side of the mean, resulting in significantly higher variances at lower temperatures (Tab. II). This can only mean that the Colmar ci<sup>D</sup>/Cat base population is heterogeneous and consists of individuals reacting to a change of temperature in different directions. Selection for high or low expression ratio at 25° separates the genotypes with different reaction norms.

Our results demonstrate that the conclusion of HOUSE<sup>7</sup>, that 'ci<sup>D</sup> ... shows a linear increase in L-4 interruption with increasing temperature' has no general validity. The direction of temperature response is not determined by the properties of the ci<sup>D</sup> gene. The reaction is governed by the whole developmental system of wing venation including the influence of the ci<sup>D</sup> gene. Therefore it is completely dependent on the genetic background.

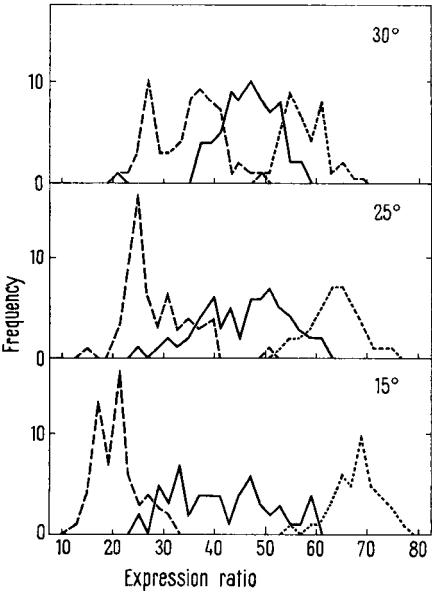


Fig. 2. Frequency distributions at 3 different temperatures of the Colmar ci<sup>D</sup>/Cat base population (solid lines) and the lines selected for low (broken line) and high (stippled lines) expression ratios. Only the distributions of ♀♀ are given.

Tab. II. Variances of the Colmar ci<sup>D</sup>/Cat base population at 3 different temperatures. The variances are pooled from 3 cultures, and *P* was calculated following Bartlett's test.

Variances				<i>P</i> for homogeneity
	30°	25°	15°	
♀	30.1	59.3	113.6	< 0.001
♂	27.3	60.5	87.0	< 0.001

The question arises whether this conclusion has general validity. Only after performing the experiments, did I find that KAMSHILOV<sup>10</sup>, as early as 1939, had published some experiments done with the same theoretical background, which seem to have been overlooked. Using the phenotypic expression of the *Drosophila* mutant eyeless as influenced by humidity of the culture media, he succeeded in selecting for a change in direction of the phenotypic reaction. Moreover KAMSHILOV<sup>10</sup> in eyeless, and recently WADDINGTON<sup>11</sup> in several other mutants, found that selection can change the quantitative relationship between temperature and expression.

The results reported here indicate that temperature experiments on morphological mutant traits have no more value in elucidating gene action than experiments on any normal morphological character. Environment acts on a development system arising as an integrated result of a complex interplay of many genetic factors. This kind of experiment can only tell something about the properties of such systems as a whole. In addition, these experiments provide a clear demonstration of interaction between genotype and environment in the sense in which this term is used in quantitative genetics (ROBERTSON<sup>12</sup>).

**Zusammenfassung.** Durch Versuche mit der Mutante ci<sup>D</sup> von *Drosophila* wurde nachgewiesen, dass die temperaturabhängigen Veränderungen in der Ausprägungsweise ausschliesslich durch den Restgenotypus bestimmt werden. Diese Feststellung hat Bedeutung für Genphysiologie, Evolution und quantitative Vererbung.

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Genetisch Laboratorium Leiden (The Netherlands), December 22, 1960.

<sup>10</sup> M. M. KAMSHILOV, C. R. Acad. Sci. URSS 23, 302 (1939).  
<sup>11</sup> C. H. WADDINGTON, Genetical Research 1, 140 (1960).  
<sup>12</sup> F. W. ROBERTSON, Genetical Research 1, 288 (1960).  
<sup>13</sup> I should like to thank Prof. H. GLOOR for his critical interest and Prof. H. R. VAN DER VAART of the Instituut voor Theoretische Biologie, Leiden, for advice in statistical matters.

Action of Reserpine on the Submicroscopic Morphology of the Pineal Gland<sup>1</sup>

In a previous paper<sup>2</sup>, it was demonstrated that the main morphological characteristic of the pinealocyte, the parenchymal cell of the pineal gland, is represented by club-shaped perivascular expansions connected to the cell edge by thin pedicles. These expansions contain mitochondria and two types of vesicles: one having an homogeneous light content and the other an heterogeneous one, with a central osmium deposit (Fig. 1). The mean diameter of the two types of vesicles is of 410 Å while the dense granules have a mean diameter of 210 Å. The existence of a single distribution curve for the two types of vesicles was considered as an indication that there may be intermediary forms between them (Fig. 3). The name plurivesicular secretory processes was proposed for these cellular expansions emphasizing their probable function in the pineal gland.

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<sup>2</sup> E. DE ROBERTIS and A. PELLEGRINO DE IRALDI, sent for publication.