



Temperature sensitive gene expression: Two rules.

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Gene expression is composed of *two* words: *gene* and *expression*.

Gene: a gene is defined as the basic unit of the transmission of a biological trait from parents to their children (or in molecular terms, a segment of deoxyribonucleic acid (DNA), composed of a transcribed region and a regulatory sequence that makes transcription possible).

Expression: the word expression, *in genetics*, is defined as the process of expressing a gene.

Each gene functions like a book that stores the information. Consider several books in an academic library. It is evident that all the books are not alike in subject matter making some more popular than others. Most books are read a lot once taken off from the shelf while others are for *consultation* or *reference* purpose *only* and thus are used *very rarely*. Each book, in turn, contains different information and has a different function and purpose. Similarly, each gene stores the genetic information and has different genetic expression effect.

The gene is found in the cells of all living organisms, from bacteria to humans. For a molecular geneticist, a *gene* is a segment of DNA of which some is transcribed and that includes a promoter (a specific DNA sequence to which RNA polymerase binds and signals where RNA synthesis (transcription) occurs), coding sequences (the sequence of nucleotides in DNA or RNA that determines the specific amino acid sequence in the synthesis of proteins), and a signal for ribonucleic acid polymerase (RNA polymerase) to stop. The rationale of genes is to store the genetic information. Each gene contains the information required to make a protein, or in some cases a non-coding RNA (ribonuclease). Genes are expressed to produce functional RNA and protein molecules in the cell.

Gene expression occurs in two steps (Figure 1). The *first* step is *transcription*. In this process, the gene is copied to produce a RNA molecule (a primary transcript) with essentially the same sequence as the gene. Most genes (humans) are divided into *exons* (any segment of an interrupted gene that is represented in the mature RNA product). The protein-coding sequences of a

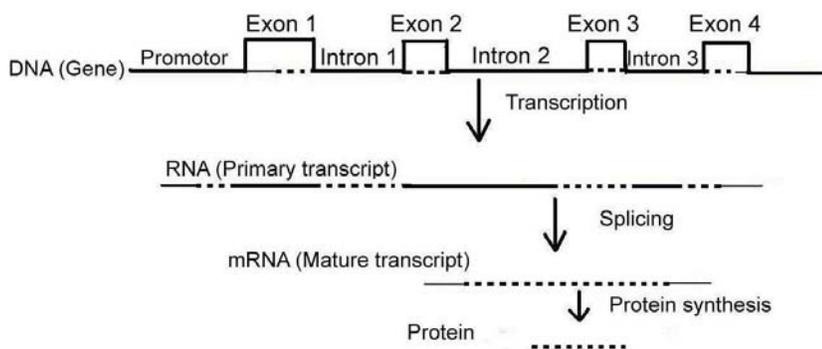


Figure 1. A model on gene expression: transcription and translation process.

gene) and the *introns* (the DNA base sequences interrupting the protein-coding sequences of a gene). These sequences are transcribed into RNA but are cut out of the message before it is translated into protein. Only the exons carry information required for protein synthesis. Most primary transcripts are therefore processed by *splicing* to remove intron sequences and generate a mature transcript or mRNA (messenger RNA) that only contains exons. The *second* step

is *translation*. Three nucleotides are required to produce one amino acid and the chain of amino acids must fold up to fabricate the final tertiary structure of a protein.

Some genes are expressed in each cell of an individual all of the time (known as housekeeping genes) and are essential for very basic cellular functions. Other genes are expressed in particular cell types or at particular stages of development. For example, in case of an individual human the genes that encode muscle proteins such as actins and myosin are expressed only in muscle cells, not in brain cells. Still other genes can be activated or inhibited by signals circulating in the body, such as hormones. This differential gene expression is achieved by regulating transcription and translation. All genes are surrounded by DNA sequences that control their expression. Proteins called transcription factors bind to these sequences and can switch the genes *on* or *off*. Gene expression therefore is controlled by the availability and activity of different transcription factors.

As transcription factors are protein themselves, they must also be produced by genes, and these genes must be regulated by other transcription factors. In this way, all genes and proteins can be linked into a regulatory chain of command starting with the *transcription factors* present in the *egg* at the beginning of the development. A number of human diseases, such as diabetes II that appears in time and space at a later age of an individual's life due to the high carbohydrate intake (environmental stimulus), are known to result from the absence or malfunction of transcription factors and the disruption of gene expression thus caused. A classical example for the demonstration of gene regulation in humans is the variation in the human skin color from one individual to another and that varies from light to dark black. Skin color is determined by the amount and type of the pigment in the skin termed as melanin. Individuals close to sun are darker (more pigment) than those far away from the sun (less pigment) and is an example of gene expression effect. The structural gene for example forms the hair on a human body, while the regulatory gene regulates the intensity of coloration of human hair ranging from white to dark black. Such studies, however, do not provide any information on how a contribution of an interaction of genes with the environment affects the phenotypic variation from one individual to another. That is whether a gene is turned "*on*" or it is turned "*off*" during the development of a phenotypic trait (how the gene expression effect is altered through the environmental stimuli).

In humans, upon fertilization of the mother's egg by a father's sperm, the genes are turned "*on*" and or turned "*off*" at each stage of the developmental process depending upon the environmental conditions through the transcription-translation process, thereby forming different parts of the body depending upon the size and shape.

Thus, the development of a phenotypic trait is a product of gene expression (genes interacting with each other and with the environment). There are innumerable studies on gene regulatory systems in plants, on *Drosophila*, bacteria and humans. Such investigations demonstrate only whether a given gene product is present (turned "*on*") or absent (turned "*off*") under a specific environmental condition. For example, Gupta (1983) investigated the presence and/or the absence for an enzyme adult acid phosphatase-6 (AP-6) in backcross progeny of *Drosophila pseudoobscura* and *D. persimilis*. AP-6 is present or absent in *D. pseudoobscura* but always absent in *D. persimilis*. Gupta and Lewontin (1982) published a paper that explains the influence of the environment stimuli on genes and vice-versa. Other examples are the markings on a cat's fur, and the shape of a tree's leaf.

The present paper deals with the importance of gene expression effect in developing the two rules.

As it is impossible to carry out an experiment on humans, Gupta (1978) used *Drosophila* to prove how the process of transcription and translation leads to the two hypotheses: 1) whether the genes are turned "*on*", and 2) the genes are turned "*off*" for a phenotypic trait under different environmental conditions. That is how the gene expression plays an important role in the development of a morphological trait from one environment to another.

Experimental Procedure

Eight iso-chromosomal lines for the second chromosome of *Drosophila pseudoobscura* from Santa Cruz Island, at sea level, California, were used for the experiment. These lines were maintained in half-pint milk bottles on Carpenters medium at 24°C before they were used for the experimental work. Heterozygotes (F₁'s) between lines were created by mating pairs of iso-chromosomal strains at random (1 × 2, 3 × 4, 5 × 6 ... 7 × 8) so as to reconstitute the variety of genotypes present in nature (The results for heterozygotes are not discussed in this paper). For our purpose, we have considered the data on viability % *only* in *parents*. [Viability is defined as the percent of fertilized eggs placed in a vial that reached adulthood (eclosion from the pupal case)] to provide the experimental evidence for the occurrence of gene expression phenomenon. The fertile eggs collected varied from 6 to 14 hours of age. For each parental homozygote, eggs were collected and cultured at two densities (40 and 140 eggs/vial) at the specified temperature. Ten replicates were made at two egg densities and at three temperatures (14°, 21°, and 26°C). Each vial contained 10-12 ml of Carpenters medium. The results obtained at 40 eggs/vial are not discussed here as both the egg densities showed the same pattern from gene expression view-point.

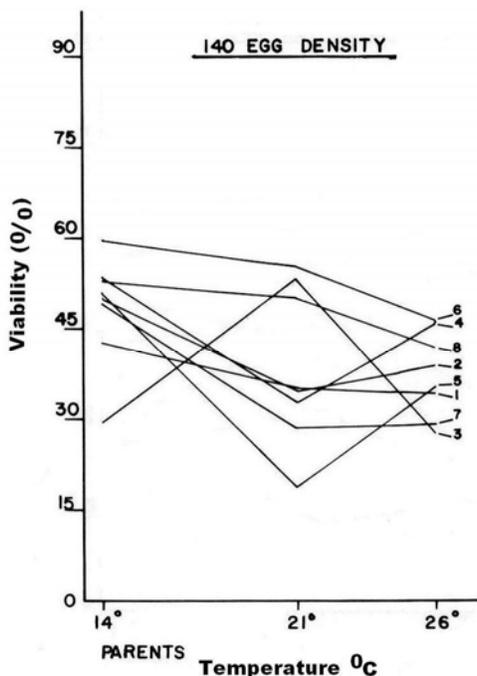


Figure 2. Viability of eight genotypes of *D. pseudoobscura* (numbered from 1 to 8), Santa Cruz, California. Each raised at three temperatures (14°C, 21°C, and 26°C). Each genotype interacts differently with the temperature and provides evidence for the gene expression effect (genes turned **on** and genes turned **off**).

Results and Discussion

Figures 2, 3, and 4 shows on the x-axis the three different temperatures (14°, 21°, and 26°C) while on y-axis is the viability (%) in parental strains of *Drosophila pseudoobscura*.

In Figure 2, there are eight different parental genotypes numbered from 1 to 8, each raised at the same time at three different temperatures. The genotypes numbered 6 and 8 produce more progeny at 14°C but less at the other two temperatures (21° and 26°C); genotype 3 produces highest viability at 21° but low at the extremes 14° and 26°C; genotype 5 yields highest viability at 14° but lowest at 21°C; genotype 4 produces more viable progeny at 14° and 26°C but lowest at 21°C; genotype 7 yield the highest viability at 14° but lower at 21° and 26°C. The Figure leads to the three important evolutionary approaches: 1) the norm of genotype 5 is of 'V' shaped; 2) while the norm of genotype 3 is of inverted 'Λ' shaped; 3) the two genotypes 3 and 5 *cross each other* when nurtured at a temperature range between 14° and 21°C; and between 21° & 26°C. It demonstrates the phenomenon

of genotype \times temperature interaction. Based on such facts one cannot predict the outcome of the phenotype developed from a genotype when moved from one temperature to another. Thus, the development of a phenotypic trait of phenotype from a given genotype is temperature sensitive. These data provide the gene expression effect on the development of an individual fruit-fly. This signifies that each genotype is different genetically and the genes differ in their expression from one temperature to another. That is how the genes are expressed considering and analyzing the viability data for a genotype in question from one temperature to another. Thus this figure demonstrates the presence of turning “*on*” for *higher* viability at one temperature, and turning “*off*” the genes for *lower* viability when raised at other temperatures for a given genotype.

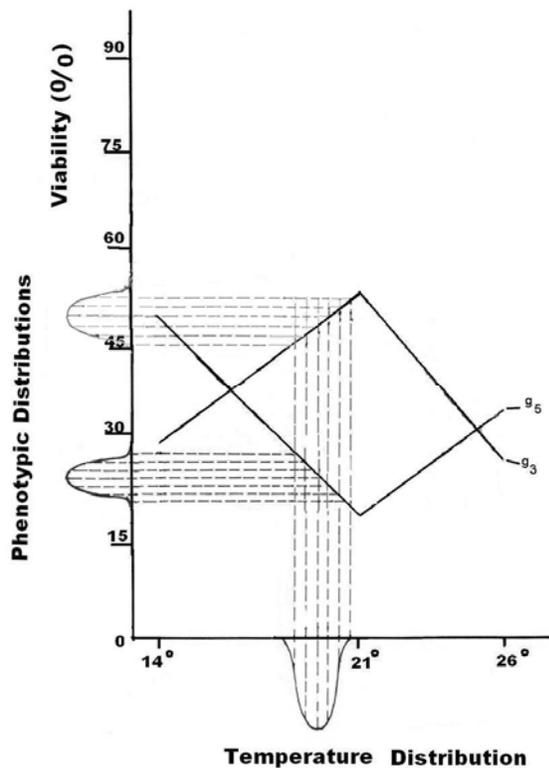


Figure 3. **Rule 1: Two phenotypic Distributions:** The viability data, illustrating the *two* phenotypic distributions, for two genotypes g_3 and g_5 taken from figure 2 at the temperature range of 18.5°C and 20.6°C provides the evidence of genes **turned on** (mostly gene expression effect).

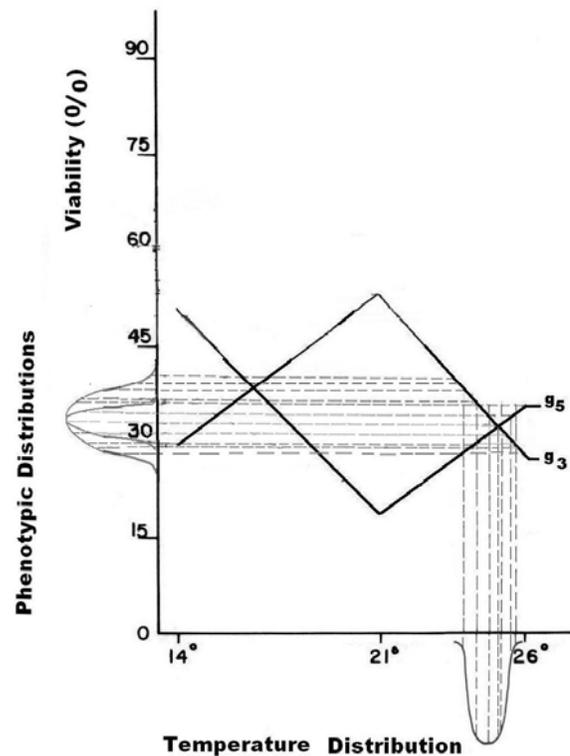


Figure 4. **Rule 2: One phenotypic Distribution:** The viability data, demonstrating the only *one* phenotypic distribution, for two genotypes g_3 and g_5 taken from figure 2 when the temperature range varies from 23.6°C and 25.6°C provides the evidence of genes **turned off** (mostly non-genetic or environmental stimuli expression effect).

For the present paper, the two genotypes 3 and 5 (g_3 and g_5) are selected to explain not only the gene expression as turned “*on*” or turned “*off*” but also the process of gene transcription and translation towards the development of the viable progeny (viability) as a phenotypic trait.

In Figure 3, when one takes into account raising these two genotypes (g_3 and g_5) at a temperature range between 18.5° and 20.6°C, one gets two different phenotypic distributions with a mean viability of 24.2 and 49.4 percent for the genotype g_5 and g_3 , respectively, indicating the significant impact of the gene action and less impact of the environment (temperature) in determining the viability. It is interpreted in terms of the gene expression effect as genes turned “*on*”.

When one cultures these two genotypes at a temperature range of 23.6° to 25.6°C (Figure 4), one finds that both have the same phenotypic distribution (both genotypes show the same mean viability of 34.2%). There is a little or no gene effect in determining the phenotypic trait (viability). It implies the significant contribution of non-genetic factors (temperature effect) and less impact of gene action. Thus, it is considered as the gene action turned “*off*”.

Formulation of Two Rules on Gene Expression

In order to invent the two rules on gene expression effect, the two different temperature ranges are considered: the first temperature range from 18.5° to 20.6°C to the left of 21°C where the two genotypes g_3 and g_5 **do not cross each other**, Figure 3; and the other temperature range from 23.6° to 25.6°C to the right of 21°C (Figure 4), where the same two genotypes (g_3 and g_5) **cross each other**. Based on the experimental data the two rules have been established as detailed below.

Rule 1: Two Phenotypic Distributions:

Here, a temperature range is considered where the two genotypes g_3 and g_5 **do not** cross each other. Thus, considering the temperature range of 18.5° – 20.6°C to the left of 21°C (Figure 3), the vertical lines were drawn from x-axis towards each of the two g_3 and g_5 genotypes and a temperature distribution curve was drawn below the x-axis line. From each of the two genotypes, drew horizontal lines towards the y-axis to get the mean viability. Based on these data, two different curves were drawn on facing out the y-axis line, and that outlined two distinct phenotypic distributions with a mean viability of 24.2% for g_5 , and 49.4% for g_3 genotypes, respectively. This demonstrates the phenomenon of gene expression effect as turned **on** because of the significant difference in the mean viability (25.2%) between the two genotypes. The gene expression effect is mostly genetic and thereby the transcription and translation process of g_3 and g_5 genotypes for viability is **normal**.

Rule 2: One Phenotypic Distribution:

Now considering the temperature range of 23.6° – 25.6°C to the right of 21°C for g_3 and g_5 genotypes where they **cross** each other (Figure 3), the vertical lines were drawn from the x-axis towards each of these two genotypes, and a distribution curve was drawn facing out below the x-axis line. In order to get mean viability for each of these two genotypes drew horizontal lines towards the y-axis from genotypes g_3 and g_5 . The two curves facing out the y-axis line were formed that overlapped each other. That is, only **one** phenotypic distribution curve was obtained, and that produced only **one** phenotype with a mean viability of 34.2% for each of two g_3 , and g_5 genotypes. This provides the experimental evidence of gene expression effect as turned **off** because there was no difference in the viability yielded by each of the two genotypes. The gene expression effect is mostly non-genetic and thus influenced by the temperature stimuli. That identifies the **disruption** in the gene expression effect results from the absence or malfunction in the transcription and translation process.

Thus, the data discussed above provide the evidence for the two hypotheses of transcription and translation that lead to: 1) the gene expression effect as turned “*on*”, and 2) the gene expression effect that are turned “*off*”, for the viability in *Drosophila* when raised under three different temperatures. It is evident that the gene expression effects are not only applicable to prokaryotes but

are also relevant to eukaryotes including humans on one-to-one basis (an extreme human example: an individual who commits suicide under a heavy environmental stress shows the *suicidal gene* expression effect as turned “*on*” while the *normal* gene expression effect for behavior is turned “*off*”. Such suicidal gene effect of an individual human being ranging from an intellectual to a politician is due to the profound stressful environmental stimuli having not been identified yet).

Thus, the experimental approach facilitated in formulating *two rules* on gene expression effect. However, both experimental and other hypothetical aspects discussed above provides the evidence that the normal gene expression activity of turning “*on*” (the two genotypes g_3 and g_5 *do not cross* each other) and/or turning “*off*” (two genotypes g_3 and g_5 *cross* each other) for an individual genotype depends upon the surrounding environment and that affects the phenotypic expression. That is the gene expression effect is temperature sensitive and the environment, thus, controls the transcription and translation processes in an individual’s daily life.

Acknowledgment: Appreciation is extended to Michelle Orr and Ruth Faye Richards.

References: Gupta, A.P., 1978, Ph.D. Thesis, Harvard University, Cambridge, MA; Gupta, A.P., and R.C. Lewontin 1982, *Evol.* 36(5): 934-948; Gupta, A.P., 1983, *Dros. Inf. Serv.* 59: 47-48.



Evolutionary significance of temperature on gene expression.

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The experiment is designed to understand how the environmental stimulus such as the temperature affects the gene expression for a phenotypic trait that involves the process of transcription and translation leading to an evolutionary approach, using *Drosophila pseudoobscura*.

Experimental Procedure

Ten iso-chromosomal lines for the second chromosome of *Drosophila pseudoobscura* from Strawberry Canyon, 200 feet above sea level, California, were used for the experiment (Gupta 1978). These lines were maintained in half-pint milk bottles on Carpenters medium at 24°C before they were used for the experimental work. Heterozygotes (F_1 's) between lines were created by mating pairs of iso-chromosomal strains at random (1×2 , 3×4 , 5×6 , 7×8 , 9×10) so as to reconstitute the variety of genotypes present in nature (the results for heterozygotes are not discussed in this paper). For our purpose, we have considered the data on absolute viability percent *only* in *parents*. [Viability is defined as the percent of fertilized eggs placed in a vial that reached adulthood (eclosion from the pupal case)] to provide the experimental evidence for the occurrence of gene expression phenomenon. The fertile eggs collected varied from 6 to 14 hours of age. For each parental homozygote, eggs were collected and cultured at two densities (40 and 140 eggs/vial) at the specified temperature. Ten replicates were made at two egg densities and at three temperatures (14°, 21°, and 26°C). Each vial contained 10-12 ml of Carpenters medium. The results obtained at 40 eggs/vial are not discussed here as both the egg densities showed the same pattern from gene expression viewpoint.