MEETINGS OF THE BOTANICAL SOCIETY

SYMPOSION ON "THE MECHANISM OF ECOLOGICAL ADAPTATION"

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Morphological and physiological adaptations to external conditions

In plants the roots are supplying many essentials for shoot growth among which water and minerals are quantitatively the most important. In addition specific substances (e.g. hormones) are produced by the root system. The function of the shoot is to supply the roots with essentials not produced by the roots themselves. Here the carbohydrates are quantitatively the most important, whereas also specific substances have to be transported to the roots. It can be demonstrated that in general the macro-nutrients, mentioned above, regulate the equilibrium between the transpiring photosynthesizing leaf surface and the absorbing root surface. External factors inducing high transpiration (high light intensity, low relative humidity) or reducing water absorption (dry soil, low root temperatures) tend to decrease this ratio, predominantly by either decreasing leaf growth more than root growth or increasing leaf growth less than root growth. Since leaf growth is more sensitive to adverse conditions than photosynthesis, the external conditions, listed above, all induce an accumulation of carbohydrates in the plants, which is favourable not only to the relative rate of root growth but also to various additional features of morphological differentiation. These features frequently have consequences for the equilibrium between transpiring and absorbing surfaces. Some of these are listed in the *tables 1–3.*

Table 1. Influence of 3.75 atm. NaCl-addition to a Hoagland solution on various features of bean plants

A. Direct effect on non-adapted plants:

		Hoagland	Hoagland + 3.75 atm. NaCl	
rate of net photosynthesis	per cm^2 leaf area	100	20	%
	per g plant weight	100	20	%
rate of transpiration	per cm^2 leaf area	100	18	%
	per g plant weight	100	18	%
B. Adaptations:				
leaf area/leaf dry weight	cm^2/g	460	272	
leaf area/plant dry weight	cm^2/g	253	138	
leaf/root ratio		4.1	2.5	
stomatal frequency	nr/0.8 mm^2	21	72	
dry matter content leaves		10.1	13.6	
soluble carbohydrate content leaves		0.55	1.14	
C. Rates after adaptation:				
rate of net photosynthesis	per cm^2 leaf area	100	72	%
	per g plant weight	100	61	%
rate of transpiration	per cm^2 leaf area	100	64	%
	per g plant weight	100	53	%

Salt addition to the root environment leads to a sudden decrease in transpiration and photosynthesis. Since the plants are not yet adapted this decrease is the same on a leaf area basis as on a plant dry weight basis.

Since leaf growth is more reduced than photosynthesis a gradual increase in carbohydrate content takes place leading to recovery of turgescence (osmotic adaptation) and of photosynthesis and transpiration. At the same time various morphological features show considerable changes and differences in leaf area/plant weight ratio result in differences between net assimilation rate and relative growth rate.

Table 2. Influence of light intensity on various features of maize

cal cm^{-2} day^{-1}		*242*	*39*	
A. Direct effects on non-adapted plants:				
rate of net photosynthesis	per cm^2 leaf area	100	7	%
	per g plant	100	7	%
rate of transpiration	per cm^2 leaf area	100	29	%
	per g plant	100	29	%
B. Adaptations:				
leaf area/leaf dry weight	cm^2/g	278	560	
leaf area/plant dry weight	cm^2/g	104	248	
leaf/root ratio		2.4	5.0	
shoot/root ratio		4.0	7.3	
dry matter content leaves		13.5	10.0	
soluble carbohydrate content leaves		13.1	7.2	
C. Rate of processes after adaptation:				
rate of net photosynthesis	per cm^2 leaf area	100	14	%
	per g plant	100	35	%
rate of transpiration	per cm^2 leaf area	100	28	%
	per g plant	100	70	%
	per g root weight	100	57	%

Lowering the light intensity results in a decrease in photosynthesis and transpiration. Since leaf growth is not directly reduced this results in exhaustion of carbohydrates which in turn reduces root growth more than leaf growth. The subsequent adaptations lead to thinner leaves and a high leaf/root ratio. As a consequence of these adaptations, considerable differences are obvious in comparing the activities on an area basis or on a plant weight basis. Even on an area basis light use is much better in adapted plants than in non-adapted plants (photosynthetic efficiency of shade leaves).

Table 3. Influence of overall temperature on various features of maize plants

		25°C	*15°C*	
A. Direct effects on non-adapted plants:				
rate of net photosynthesis	per cm^2 leaf area	100	75	
	per g plant weight	100	75	%
rate of transpiration	per cm^2 leaf area	100	42	%
	per g plant weight	100	42	%
water permeability roots	per g root weight	100	38	%
B. Adaptations:				
leaf area/leaf dry weight	cm^2/g	420	300	
leaf area/plant dry weight	cm^2/g	219	129	
root surface/root dry weight	cm^2/g	2430	1170	
dry matter content leaves		10.1	15.6	
soluble carbohydrate content leaves		6.3	16.8	
leaf weight/stem weight ratio		1.7	1.5	
leaf weight/root weight ratio		3.4	1.8	

C. Rates after adaptation:

rate of net photosynthesis	per cm^2 leaf area	100	78	
	per g plant weight	100	43	%
rate of transpiration	per cm^2 leaf area	100	41	%
	per g plant weight	100	22	%
water permeability	per g root weight	100	16	%

Lowering the temperature from 25 °C to 15 °C leads to reduction in photosynthesis, transpiration and water permeability of the roots. The latter two phenomena are important in reducing leaf growth and as a result carbohydrates accumulate. This leads to a relatively favoured root growth and to thicker leaves and roots. As a consequence photosynthesis and transpiration are more reduced on a plant weight basis in the adapted plants. The effect of root thickening on the permeability of the roots per gram root weight are especially clear.

These examples which can be elaborated with results from other factors e.g. mineral deficiency and root temperature, demonstrate the possible role of carbohydrates in inducing morphological changes in plants which are genetically constant. This effect which has been recognized by Klebs, has been overlooked for a long time after the discovery of numerous growth regulating substances. The latter are certainly important prerogatives for growth but do not seem to be important in regulating the relative growth rates of the various organs in growing intact plants in the vegetative stage.

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Physiological adaptations to climatic conditions

It has been known for a very long time that regional populations show physiological adaptations to the climate. The first experiences originate from provenance research in forest trees. For example: *Pinus sylvestris* from Central Europe grown in Skandinavia shows a vigorous growth but a poor winterhardiness. Later research work has revealed that in southern populations the formation of dormant apical buds and the build-up of a sugar reserve is retarded under the influence of the relative long photoperiod of a northern climate (LANGLET 1936).

TURESSON (1922,1929) explained this genetical differentiation as the result of gene recombination by the direct action of natural selection in a particular habitat. The result is an adapted "ecotype".

The variation, however, is generally of a continuous type so that "clines", character gradients are formed (STEBBINS 1950).

The work of COOPER (1951–1954) on *Lolium perenne* strongly supports this concept. It shows a range of locally adapted populations, each selected for its phenotypical fitness. In each apparently uniform population a great potential genetic variability is hidden, providing the possibility of a rapid adaptation to new conditions. Genetic analysis reveals a polygenic inheritance of characters and a frequent heterozygoty.

Southern populations of long-day plants often flower early in the season, before the summer drought. Northern populations of the same species generally flower relatively late due to inhibitions of development by short day. Southern populations have a lower critical daylength or they are day-neutral.

Apart from timing of flowering for the right season photoperiodism is important for the synchronisation of flowering within one region and, therefore, for cross-pollination.

When flower initiation takes place during lengthening days the time-lag between early and late individuals of the same long-day species will be diminished. The same applies to a short-day species in shortening days. As a matter of fact most autumn-flowering species are short-day plants.

Northern populations of the North-American range grass *Bouteloua curtipendula* become generative in June, the southern populations in July-August. The high-latitude forms can be classified as long-day plants, the low-latitude ones are stimulated by short day (OLMSTED 1944/45).

Another example of a physiological adaptation is the occurrence of growth inhibitions in populations from a rough climate. According to Olmsted the northern populations of *Bouteloua* show such inhibitions under short-day conditions. This habit improves winterhardiness.

The same has been observed in red clover (VAN DOBBEN 1967). Wild populations from high latitudes or altitudes form very small leaves and extremely short petioles in the winter season, even under greenhouse conditions. These inhibitions can be removed by the artificial lengthening of the photoperiod by weak light, even when the vegetative stage is maintained. GA has the same effect as long day.

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Adaptation of the pathogen to its host

Agricultural crops and their pathogens lend themselves pre-eminently for studies on adaptation under almost natural conditions in the field. In the Netherlands the area cultivated with the varieties of a crop and their shift in the course of the years are exactly known. Moreover the degree of attack by pathogens is registered yearly, and in many cases the races of the pathogens are determined and studied in the laboratory. Therefore agricultural crops and their pathogens form a large scale experiment, in which the sequence of events is much better known than in natural plant-pathogen communities.

It is a common experience that varieties resistant to a pathogen become susceptible after a varying number of years. In all cases studied it is well established that the population of pathogenic races changes and that new races appear to which the originally resistant host varieties are susceptible. It is generally agreed upon that these new races occur as at random mutations which are selected on those varieties which are resistant to all races except for these mutations. However, there is quite a lot of evidence, for instance in the case of *Phytophthora infestans*, that the hostplant plays a role in the induction of new races. In this respect the stepwise building up of virulence effected by passage through senescent leaves of the resistant host (potato, tomato) must be mentioned.

Therefore, in order to explain the adaptation of the pathogen to its host, induced mutations beside at random mutations have to be taken into consideration. The occurrence of new races as a result of genetic recombination by sexual or parasexual processes or as a consequence of heterokaryosis may, to a certain degree, also play a role in this adaptation phenomenon.

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Adaptation as a molecular-biological problem

Before considering what data of molecular biology could be useful to explain the mechanism of oecological adaptation, the question arises whether we may approach this adaptation as a process on the molecular level. Or is this a typical problem of "organism-biology"?

Thus first of all we have to consider whether some familiar forms of adaptation take place according to a mechanism dependent upon changes in certain molecules, in particular in proteins and in nucleic acids.

1. Adaptation of microorganisms to a new substrate: the enzymatic induction. This is a classical example of adaptation: the formation of a new enzymatic protein. The investigation into this process even led to a culminating-point in the history of molecular biology: the discovery of the regulator genes by JACOB and MONOD in 1961.
2. Adaptation of microorganisms to antibiotics. The simplest explanation of this phenomenon is based on the formation of enzymes which break down the antibiotics. There are, however, other explanations, of which we may mention: the impediment in incorporating an antibiotic in active cell elements, changes in the metabolism resulting in independence of the reaction inhibited by the antibiotic, expelling of the antibiotic by an increased production of enzymatic substrate etc. In all these cases we have to deal with changes of the metabolism which can be explained on the molecular level, in this case by altered enzymatic structures.

Sometimes the adaptation is due to a reduced permeation of the antibiotic as a result of a change in the cell membrane, though this may also be caused by a modified metabolism. Another possibility could be an alteration of the structure of the already for medmembrane.

3. Adaptation to high and to low temperatures. The most frequent cause of damage caused by high temperatures is the denaturation or inactivation of proteins. Often the inability to grow at a higher temperature is due to the inactivation of one enzymatic protein.

In this case an adaptation may be due to a changed structure of the protein concerned, which now is less liable to denaturation. If the resistance of proteins, among other things, is dependent on the formation of intramolecular hydrogen bridges, sometimes one single substitution of an amino acid may already lead to important changes in sensivity to temperature.

In many cases a high temperature causes more general modifications in the structure of protoplasm. This still may be explained by structural changes in a special protein or by changes in the metabolism, like e.g. a decrease in ATP production. It is necessary, however, to pay attention to changes on a supramolecular level. Changes in the supramolecular structure of the protoplasm could lead to an adaptation to higher temperatures e.g. by a change in the water-binding capacity of the protoplasm.

4. Adaptation to desiccation. It appears to be more difficult to explain both the sensivity and the adaptation to desiccation by a simple molecular change. Here we must rather think of changes in the supramolecular structure of the protoplasm than of the submicroscopical one. This structure, however, is also the resultant of the properties of the composing molecules and of the nature of the reactions by which these molecules are formed and broken down.

Summarizing we may state that a great partof the adaptational phenomena can be explained in a way based on changes in the structure of the enzymatic or structural proteins, in addition we have to pay attention to alterations in more complicated structures, formed by these proteins.

In what respect can the molecular biology be helpful to explain the adaptational phenomena? The starting-point is the central dogma of molecular biology:

$$\text{DNA} \longrightarrow \text{RNA}_{\text{mess.}} \longrightarrow \text{protein}$$

This is a rigid scheme: as long as DNA (the genes) remains constant, the same proteins will be formed. So when and where is it possible to introduce a variation in the structure of these proteins (if we state it in this way the adaptation problem is in fact identical with the differentiation problem)?

We may distinguish several possibilities:

1. Selection out of a genetically heterogeneous population. Characteristical examples we come across in the development of resistance to the antibiotic concerned, because of a somewhat deviating DNA composition, only this special cell will be able to develop in a milieu with the antibiotic.
2. Mutation. If the resistance also develops in a population, originated from one single cell, the above mentioned explanation is not adequate unless we assume that some aberrant cells

are formed by mutation, i.e. cells with an altered DNA. The replica-method by Lederberg is an excellent method to demonstrate this.

3. Mutagenal activity. The development of resistance as a result of mutation and selection will be facilitated if the antibiotic itself exerts a mutageneous influence. In this case, however, it always remains a stimulation of mutability in general without a special preference for mutation leading to resistance. No indications are present for any special directed mutation.

N.B. Although we have mentioned some striking examples of bacterial resistance caused by mutation, it is self-evident that also for the higher developed organisms genetical adaptation must be mainly explained by mutation and selection. It is characteristic that all these adaptations are primarily attributable to a change in DNA somewhere, thus to a change in hereditary properties.

The following adaptational phenomena are more interesting to explain physiological adaptations. This form of adaptation differs from the preceding one because the adaptation takes place more rapidly and often disappears when the original milieu is restored; all the cells of a population present the adaptation, the genetic identity, i.e. the DNA structure remains unaltered during the adaptation.

4. The role of genetic regulation. The investigation into the lactose fermentation by *Escherichia coli*, carried on by JACOB and MONOD in 1961 is already classical in this respect. The examined coli strain forms enzymes, necessary for the fermentation of lactose only if lactose is present in the milieu as a substrate. It appeared that certain mutants formed these enzymes under all circumstances, consequently also when lactose was not added. These mutants had mutated in so-called regulator-genes, differing from the structural genes, necessary for the formation of the enzymes concerned. JACOB and MONOD now drew up the hypotheses that this regulator-gene produces the repressor which normally prevents the transcription of some structural DNA's into RNA mess. This repressor is inactivated by lactose.

The reverse process also occurs i.e. the activation of the repressor by a special material, the enzymatic repression. After an assiduous search for the hypothetical repressor, this year GILBERT and MULLER HILL have succeeded in isolating a protein not only possessing the capacity to bind lactose but in addition its presence in normal and regulator mutants covers everything which may be expected in reference to the repressor. The problem may be conceived as follows: the regulator-gene forms a protein adhering to the DNA of certain structural genes, in this way preventing their transcription into RNA mess. If lactose is bound to this protein the properties change in such a way that a bond to DNA becomes impossible.

This view enables us to understand the physiological regulating mechanisms which play a role in the differentiation and in the physiological adaptation. It explains how the present genes can be activated or repressed, dependent on the circumstances, so that different proteins and different enzymatic structures can come into being.

N.B. Also a genetic adaptation via mutations in regulator-genes is possible. Several cases are known where bacteria adapted themselves genetically to a new substrate, not because of a new structural gene but because by mutation in a regulator gene, a formerly repressed structural gene was enabled to produce enzymes.

It is self-evident that this does not mean that this regulation is the only possible one. It has been stated again and again that it may be possible that in higher organisms with more complicated chromosomes an entirely different regulation may exist, including a different type of adaptation. But also the simple scheme offers several other possibilities.

5. Adaptation by regulation on the level of protein synthesis. It is conceivable that also with the next step, the proper translation of the genetic code into the code of the amino acids of the proteins in the ribosomes, a regulation could be possible, involving a physiological adaptation mechanism. It is obvious that the genuine protein synthesis is not only determined by the code but also by the constituting elements, the amino acids. If one of them would be lacking, this would affect the whole protein synthesis.

It is well known that the amino acids must be ligated to a specific transfer RNA. An indication for the existence of regulating mechanisms on this level is to be found in the data pointing to the fact that certain phytohormones, the cytokinines, have something to do with special forms of transfer RNA. Other observations point to a possible regulatory role of the ribosomes.

6. Adaptation by a change in the structure of the formed proteins. It would be incorrect to consider the changes in the formation of proteins only. A protein is not simply a chain of amino acids. The structure of a protein is also defined by secondary bonds of amino acids, when the folds of a chain contact each other in several places.

First of all this secondary and tertiary structure is determined by the sequence of amino acids itself, thus genetically determined. But in addition other factors play a role, affecting the nature of the secondary and tertiary bonds: temperature, pH, ions and special organic elements like hormones. It is not necessary that such changes are accompanied by a loss of activity, but they are very important e.g. for the thermo-resistance of a protein. On the one hand proteins may dissociate into smaller parts, the subunits, on the other hand they may form conglomerations of greater units. To a certain extent the enzymatic activity may not be affected by this process; with the dissociation into subunits enzymatic activity can be destroyed or changed (e.g. the glutamic acid dehydrogenase passing into aspartic acid dehydrogenase).

It has to be borne in mind that the genetic base of the sequence of the amino acids remains unchanged; changes of properties and of functions are the result of dissociating and uniting factors (among others the concentration of protein).

7. Changes on a supramolecular level.

We have already seen in what way proteins can form conglomerations with different properties. Also bonds with other parts of the cell occur, as a result of which the thermoresistance can be greatly affected. Here we draw near to the submicroscopic and the microscopic organisation of cellstructure. A molecular explanation does not seem to be adequate, although supramolecular structures and possible changes are also dependent upon the nature of the constituting molecules.

Now we have returned to the question in the beginning of this article, namely the value of molecular consideration of our problem. The controversy between molecular and organism biologists, between reductionists and anti-reductionists, is a seeming controversy, if we are aware of the fact that eventually everything is dependent upon genetic information and upon the molecules formed by this and also that the higher structures originate and adapt themselves by interactions between these molecules. These interactions can only be understood by considering the nature of these molecules while on the other hand we should not have known anything about these structural interactions if we had not investigated the intact organism, because only this enables us to put the biological questions.