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THE CONCEPT OF RESISTANCE IN THE LIGHT OF MOLECULAR BIOLOGY

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Phytopathologist & Geneticist

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THE CONCEPT OF RESISTANCE IN THE LIGHT OF MOLECULAR BIOLOGY

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A problem commonly faced in plant or animal pathology is the one dealing with relations between hosts and pathogens in connection with physiologic races in the latter. It has been a fact established long ago that plants that have shown resistance to a disease for a number of years may suddenly be wiped out by this disease. We have been knowing for quite some time that the reason why such a thing occurs is that a population of a given species of pathogen may appear uniform phenotypically though it might be most heterogeneous genotypically. Such a population could also be uniform genotypically and this uniformity can be of two types; homozygous and heterozygous. The homozygotes themselves can be haploid, diploid or even polyploid; the heterozygotes can be diploid or polyploid but not haploid, for the heterozygous condition requires at least two alleles for each gene while in the haploid the alleles are unique. Those terms need some explanation, they are related to genetics: when an organism contains a certain number of chromosomes the latter can be found under different conditions: it may present two, three, four or more sets of the chromosomes. It is then said to be in the monoploid or haploid, diploid, triploid, tetraploid and popyploid condition respectively.

A chromosome in one set together with its like in any other set are said to be homologous. Thus in diploids, triploids and tetraploids, there are pairs, triplets and quadruplets of homologues respectively. Of course, two unlike chromosomes in any set are non-homologues.

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Genes are located in the chromosomes. Each gene is in fact a molecule of nucleic acid, mostly deoxyribonucleic acid or DNA (most plant viruses and a few animal and human viruses have ribonucleic acid or RNA as their genetic material. Cauliflower mosaic and bean golden mosaic viruses are composed of DNA). In these nucleic acids, the genetic information for proteins synthesis which is the main fonction of the gene, is assured by the four bases Adenine, Thymine (replaced by Uracile in RNA), Guanine and Cytosine.

The genes are single on a chromosome set, therefore, there are as many copies of a given gene as there are sets (haploids) in an organism (there are sometimes several like genes that are very close together, thus almost completely linked in what can be considered as one locus (seat of the gene). This complex is called a polygene). The copies of a given gene whatever their numbers are called alleles or allelomorphs. Alleles or genes are commonly designated by the letters of the common alphabet; they can be dominant or recessive and are then represented by capital and small letters respectively. An allele is said to be dominant on another one when both being together it is its expression that shows up in the phenotype. For example if "A" designates the red colour and "a" the white one AA and Aa will both appear red. The white colour will show only when the allele is double that is "aa" (in diploids).

If we have the dominant alleles A,B,C, D,E,F, the corresponding recessive ones are a,b,c,d,e,f. But a group of alleles such as A,A^1, A^2, A^3, a, a^1, a^2, a^3, represent copies of the same gene slightly modified by mutations. AA are two identical alleles, so are "aa, bb, BB....."A" and "a" can be called homologous alleles though this term is mostly used for chromosomes and not for genes or alleles. But remember that identical or homologous alleles represent the same genes this is why they are represented by the same letter, capital, small or with an index.

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when two alleles are identical on the homologous chromosomes, the cell or the individual which contain them is said homozygous for these alleles; examples are: $\frac{A}{A}\,\frac{b}{b}\,\frac{c}{c}\,\frac{d}{d}$. When they are homologous, that is $\frac{A}{a}\,\frac{B}{b}\,\frac{c}{c}$, the cell or the individual is heterozygous. If all the alleles are identical or homologous on all the homologous chromosomes, the cell or the individual is totally homozygous or totally heterozygous respectively. Such conditions are extremely rare in an organism, so that homozygosity and heterozygosity are rather approximate conditions. An individual which shows homozygosity or heterozygosity in 80% of all its genes may be considered as homozygote or heterozygote respectively.

In a little book I wrote (in French) on coffee rust caused by Hemileia vastatrix and H. Coffeicola. I have tried to clear up the matter about "degree" and "category" of homozygosity and heterozygosity. "Degree" in my mind, refers to the "number" of genes in any one condition and "category" to the "kind" of genes. Therefore, two different categories of genes may present the same degree of either condition, so that a population of plants may be composed of one or several categories of homozygous or heterozygous individuals. If it is of one category it will be uniformely homozygous or uniformely heterozygous. Homozygotes can be haploid, diploid or eventually polyploid; heterozygotes can be diploid or polyploid but not haploid, for heterozygosity requires at least "pairs" of alleles, while the alleles are single in haploids; this is the reason why haploid individuals or haploids gametes are always homozygous or pure; whence the law of the purity of gametes in diploid organisms. The following examples will give a better idea of the situation:

I HOMOZYGOTES

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a) dominant haploid : ABCDEF

b) recessive haploid : abcdef

c) dominant and recessive haploid : AbcdEf

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HOMOZYGOTES	a)	dominant diploid	:	AABBCCDDEEFF
2	b)	recessive diploid	:	aabbccddeeff
	c)	dominant and recessive diploid	:	AAbbCCddEEff
	a)	dominant tetraploid	:	AAAABBBBCCCCDDDDEEEEFFFF
3	b)	recessive tetraploid	:	aaaabbbbccccddddeeeeffff
	c)	dominant.recessive tetraploid	:	AAAAbbbbCCCCddddEEEEffff

HETEROZYGOTES

They are at least diploid and never pure though they can be uniform if they are composed of one category. Their characteristic is that they are formed of homologous (not identifical) alleles

1) diploid: AaBbCcDdEeFf
2) tetraploids
   a) AAaaBBbbCCccDDddEEeeFFff
   b) AaaaBbbbCcccDdddEeeeFfff
   c) AAAaBBBbCCCcDDDdEEEeFFFf

As you see we can have many categories and many degrees of heterozygosity. There can be also many categories of homozygosity but strictly only one degree (or no degree) in homozygosity. But as we said formerly absolute homozygosity is extremely rare. One way or perhaps the only way to obtain it would be to multiply (with Colchicine for example, in plants) the chromosomes (and consequently the genes) in an haploid individual. Even then, mutations in the long run will alter the genes.

A high percentage of homozygosity can be obtained also by self-fertilization for a number of generations. But this way, the condition is not complete.

A heterozygous population composed of a single category of individuals represent a clone. If homozygous, the population is a pure line. The latter situation will be maintained for ever whether the individuals are reproduced asexually or sexually by inbreeding.

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As said above such a population may gradually become heterozygous by mutations and also by cross breeding even with another homozygous population of a different category. Needless to mention crossing with one or several heterozygous populations.

An homozygous population of one category may change, though remaining homozygous. This may happen through the phenomenon of gene amplification according to which genes may multiply to over 400 copies of each in a single cell. Of course, mutations can occur also during and after the processes, leading to all kinds of variations. We shall speak in more details later of this phenomenon which is of the utmost importance in the development of physiologic races and biotypes in pathogens and hosts.

Physiologic races can be found in any pathogenic organism, viruses, bacteria, fungi... Viruses possess only one nucleic acid either RNA or DNA, in one molecule.

Nucleic acid are composed of the bases Adenine (A) Guanine (G), Thymine (T) (replaced by uracile (U) in RNA), Cytosine (C). Viruses can be considered as being haploid. Point mutation may occur in viruses, changing one base into another. A virus can pin on its nucleic acid a segment of nucleic acid of a cell and transport it with itself or independently on the nucleic acid of another cell. It can graft end to end a segment of another nucleic acid on its own, thus lenghtening the latter. It can also pin or graft itself on the nucleic acid of a cell. They probably can attach themselves to one another in many ways. All those mechanisms can change a virus to a more or less different one, that is a new more or less stable race.

All other biological entities besides viruses are cellular (Mono or pluri-cellular) and possess the two nucleic acids, DNA mostly in the nucleus or nuclear material and RNA mostly in the cytoplasm (We should mention viroids which are small viruses devoid of their protein envelope or capsid

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and Prions which are supposed so far to be just protein molecules without nucleic acid of any kind). Moreover, messenger RNA is primarily found in the nucleus and DNA is encountered in certain cytoplasmic bodies such as mitochondria, plastids and others.

Bacteria are mostly single cell individuals. They are said procaryotes because their genetic material is not surrounded by a nuclear membrane and is therefore somewhat free in the cytoplasm. Bacteria contain a single circular chromosome bearing several thousands genes (DNA molecules); thus they are haploid. Through a phenomenon called conjugation two bacteria may join side by side and exchange or give one to the other segments of their chromosomes with the genes. Bacterial viruses or phages can transport segments of RNA of a bacterium on the DNA of another bacterium. Of course, mutations can alter also the genetic material and are probably the main causes of variations in bacteria, leading to new races.

In many bacteria and some yeasts, there is a small circular chain of DNA distinct from the bacterial chromosome which contains a few genes, particularly genes of resistance to antibiotics. It is called plasmid.

Plasmids play a very important role in the development of resistance in bacteria. The same plasmid may carry on different loci of its DNA several genes, each resistant to a given antibiotic. Such a plasmid can thus confer to a single bacterium resistance to all those antibiotics.

Five classes are found in fungi: Phycomycetes, Ascomycetes, Basidiomycetes, Deuteromycetes or Adelomycetes (Fungi imperfecti), Agonomycetes or Mycelia Sterilia (Sterile fungi). The latter class is usually considered as an order of the Deuteromycetes.

The first three classes show both asexual and sexual reproduction. They present an haploid and a diploid phase (Ascomycetes and Basidiomycetes present also a dicaryotic phase in which two nuclei are associated but not fused; it is equivalent to a diploid condition). Only asexual reproduction occurs in Deuteromycetes by bits of mycelium or true asexual spores called conidia, the same encountered in Ascomycetes and less frequently in Basidiomycetes.

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They are in fact considered as Ascomycetes in which the sexual stage is lost or has not been discovered so far.

Agonomycetes have no spores of any kind either asexual or sexual. They reproduce themselves asexually by bits of mycelium or asexual structures, such as sclerotia, setae, tubers and the like. Deuteromycetes and Agonomycetes are thus haploid fungi for life aside from possible fusion of mycelia which could bring about di-or polycaryotic conditions.

It looks as though variation is the privileged condition of nature. This is not surprising, for ecological conditions are likely to change at any time in the universe; so living beings must have the possibility to adapt themselves to any new condition otherwise they might perish. They must then have the faculty of constituting a stock of genes in some individuals of any given species allowing them to resist any adverse situation and build up a new population of adapted individuals. Virulence and resistance are in fact consequences of this policy of Nature. When one thinks of the two phenomenons the tendancy is to visualize them as opposed, separate one in the host, the other in the pathogen and intercorrelated. This is the reason why the theory of Flor states that for each gene that governs resistance in the host there is a corresponding gene that control virulence in the pathogen. But the way I conceive the matter is that virulence and resistance are the two poles of a condition that expresses itself by degrees of susceptibility. Therefore, this condition should be visualized as being independent in the pathogen as well as in the host, that is: the pathogen is more or less virulent when it manifests more or less resistance toward the host, and the latter is more or less resistant when it shows more or less virulence toward the pathogen. In this view point the plant and the pathogen would represent two classes of entities belonging to two different worlds. Higher green plants are privileged for they are self-sufficient and possess both RNA and DNA with a large number of nucleotides constituting the genes which may undergo favorable or unfavorable mutations (a nucleotide is the name given to the complex base-sugar-phosphate in a nucleic acid but this term is often used to designate the base alone).

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Pathogens are of two kinds: biogenic which possess nucleic acid (either RNA or DNA or both) and abiotic, that is, devoid of nucleic acid of any kind. The latter cannot undergo mutations but may induce mutations in biogenic pathogen or higher plants and animals.

Two different plants such as tomato and potato possess genes that are different in number and kind. However, they can be attacked by the same viruses or the same fungi. They can also develop resistance to those viruses and fungi which themselves may increase or decrease in virulence through mutations. Abiotic pathogens may induce in biological entities mutations conducive to the same kinds of physiologic disturbances as biogenic ones. This is well illustrated in animal and human cancer as reported by Weinberg (1984): Hamster embryo cells treated with methylcolanthrene a cancerogen chemical, developped tumor, and any cell from this tumor transferred elsewhere to an appropriate environment would develop a form of cancer similar to or different from the original one. Whence the conclusion by the biologists that cancer is not an ensemble of a hundred distinct affections but a single disease induced by just a few molecular mechanisms common to all types of tumor (Weinberg, 1984).

It follows that any mechanism inducing chromosome or gene modifications may lead to the formation of a new physiologic race: equal or unequal crossing-over, all kinds of chromosomal changes or mutations particularly duplications and translocations, gene (including point) mutations, cellular fusions, hybridization in sexually reproducing pathogens.

In equal crossing-over the homologous chromosome are alined in such a way that the loci of their respective alleles are on a vertical line. Thus in the exchange of segments each transferred allele occupies the locus of its partner. In unequal crossing-over the alignment of the homologues compels the partner alleles to dispose themselves on oblique lines; thus one is shifted on the right or on the left with respect to the other, at crossing-over. In this way, duplication of alleles is likely to occur.

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Gene duplication and amplification mostly occurs through translocation. In translocation broken segments of chromosomes may get inserted in homologous or different chromosomes. It results that several copies of a particular gene may be found on one or more chromosomes of a single cell. Cell fusions and hybridization can transfer the same genes even in very dissimilar organisms and in different parts of the world. Further translocations everywhere might increase the number of copies of each gene and mutations followed by successive translocations will modify and multiply these genes which become new ones. It is this way that continual changes may occur in one original entity.

Let us lay some emphasis on the phenomenon of mutation: Unequal crossing-over and translocation that lead to duplication and amplification of genes may be considered as chromosomal mutations. The other type of mutation is gene mutation which refers to changes that occur in the structure of the gene itself. They include:

1) breaks in the DNA strands conducing to lost, gain or new arrangements of nucleotides by insertion of the broken segments on other DNA

2) change of the bases one into another in one or several codons (a codon is a group of three bases which mostly determines a particular amino-acid to enter in a protein construction).

When the change involves only one base the mutation is called "point mutation." The organic bases are of two kinds, two purine (the molecule is composed of two rings) Adenine and guanine and two pyrimidine (the molecule is composed of one ring) Thymine (or uracile in RNA) and cytosine. When a base of one kind is replaced by the other of the same kind (a purine by a purine or a pyrimidine by a pyrimidine) the mutation is said to occur by transition. It is by transversion when a purine replaces a pyrimidine or vice versa. (Herskowitz 1965, Sarah Grau Hamerlok 1981).

The transformation of one base into another may be induced by chemical treatments. For example the treatments by nitrous acid of RNA of the tobacco mosaic virus may bring about the substitution of an amino group NH2 by an hydroxyl (OH). This may transform cytosine into uracile and adenine into hypoxanthine.

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The latter is a kind of artificial base which is similar to guanine and can therefore unite with cytosine (Fraenkel-Conrat, 1964). Other chemicals can induce the attachment of a bromine atom or a methyl group (CH3) to nucleotides. Ultra.violet rays may provoke the linking together of two identical bases or two bases of the same type: A-A, G-G, A-G (purine) C-C, T-T, C-T (pyrimidine). Those are called dimers, of which the most common are T-T. Dimers are often lethal.

Going back to the matter of virulence and resistance, the seeming correlation between the genes of the pathogen and those of the host is sensible only in the relations between biogenic pathogens and the host which itself is essentially biogenic. But so far as the modality of action itself is concerned there might be as we have seen no much difference between the biogenic and the abiotic pathogen. What needs consideration is the way the host is affected, whether it can react or not and how it will react if it can. All these depend on the condition of its nucleic acids particularly the DNA. This DNA can be affected the same way or differently by either type of pathogen (biogenic or abiotic). Its reaction, if any to either type also might be the same or different. Accordingly, so far as virulence and resistance are concerned as regard to a particular plant species, every pathogen must be considered as unique. With regard to biogenic pathogens, their genes vary in kind and in number compared to those of the host plant. Moreover there can be several identical or different genes for resistance or virulence concerning a given plant with regard to the same pathogen. For example, in different species of coffee, the resistance to the same strain of Hemileia Vastatrix may be due, for one species, to the production of a substance lethal to the fungus, for another, the strenghtening of the cellular wall or the occurrence of a particular structure of the stomata which prevents the entrance of the pathogen into the plant. Furthermore for a given variety of Coffea arabic for example in regard to the same strain of Hemileia, resistance may be due to two or more causes at the same time which suggests that at least two genes for resistance could be in play in the host plant.

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Is there in this Hemileia strain a gene of virulence for each of the gene of resistance of the coffee variety? This does not seem logical.

On the other side, if we consider the resistance due to the synthesis of a toxic substance by the coffee plant, plants of a given variety of coffee, even homozygous may present various degrees of resistance to the same race of Hemileia. The reason is that the gene which codes for the toxic substance may exist in many identical copies each of which determines a given quantity of the substance. Thus the degree of resistance of the plants in the homozygous population is related to the number of identical copies of the gene in the individual plants. Therefore, the population though uniform with regard to the other genes is not so quantitatively for the character of resistance. This is again a consequence of gene amplification.

It appears gradually (Schimke, 1981) that many biological phenomenons involving quantitative characters such as yield, vigour, gradual acquisition of virulence or resistance in general (gradual increase of resistance of plants to pathogens, to unfavorable climatic or soil conditions; of bacteria to antibiotics; increase of virulence in pathogens; of resistance of insects to insecticides etc) are related to gene amplification in these diverse biological entities.

It follows from the above considerations that when an homozygous population of a plant shows various degrees of resistance to a pathogen, one may think that there are several races of the pathogen into play while there is only one race and many biotypes, so far as resistance is concerned, in the homozygous population. It follows also that there might be, in reality, fewer races of a pathogen than it looks, many of them listed as different being in fact subraces of the same race.

Of course, as it has been found, a virus can insert partially or totally its DNA in that of the host. Study on crown gall disease have shown that

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Agrobacterium tumefaciens, the organism which causes the infection can insert in the DNA of the cell through a plasmid called Ti (for Tumor inducing) a portion of its DNA called transferred DNA. This transferred DNA carries a gene which codes for either octopine or nopaline, two substances of the Opine group which stimulate cell proliferation in view of the production of larger quantities of either substance necessary for the life of the Agrobacterium organism and its plasmid (Chilton, 1983). This is one kind of interaction encountered in the host-pathogen relations but this does not substantiate the gene for gene hypothesis of Flor.

At all events it has become evident that in such relations it is the DNA of the host that is primarily concerned and it might prove advisable to reconsider (revise) the concept of physiologic races, resistance, virulence and susceptibility in the light of molecular biology.

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REFERENCES

Chilton, M.D. 1983- L'introduction de génes étrangers chez la plante. Pour la Science No. 70, France

Fraenkel-Conrat, H. 1964 - The Genetic Code of a Virus. Scientific American No. 4

Hamerycks, S.B. 1981- Biologie-Génétique Département Education Nationale, Bruxelles, Belgique

Herskowitz, I.J. 1965- Genetics 2nd Edition-New York

Pierre-Louis, F. 1983 - La Rouille du caféier Publication technique du Département de l'Agriculture d'Haiti

Schimke, R. 1981 - Amplification des genes et résistance aux médicaments Pour la Science No. 39, France

Weinberg, R. 1984- Une base moléculaire du cancer- Pour la Science No. 75, France

For Flor hypothesis, refer to:

1) Agrios, G.N. 1978- Plant Pathology-New York
2) IICA 1977- Contribuciones al conocimiento de la roya del cafeto-San José, Costa Rica