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# Action as a Dynamic Property of the *Genotype* × *Environment* Interaction
## Implications for Biotechnology

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### Summary

The action resonance theory (ART), a hypothesis based on a logical extension of EINSTEIN's theory of Brownian movement, suggests that the *genotype* × *environment* interaction can be modelled as forceful encounters of the gene-products of an organism with its environment. This model has implications for molecular and cell biology, morphogenesis, evolutionary development *via* mutation, the mechanism of natural selection and overall function of ecosystems, extending SCHRÖDINGER's programme for molecular biology. Action, a thermodynamic property with the same physical dimensions as angular momentum and PLANCK's quantum of action, is proposed to be reversibly generated as a result of the molecular exchange of quanta, which become resonant at equilibrium, corresponding to an optimum degree of entropy and action for living systems. Because the theory can potentially predict solutions to unsolved problems such as the folding of proteins it has strong implications for successful genetic modification of organisms and for biotechnology in general; the design of a programme of research to test this theory is proposed. A key element in this research programme, improving productivity and sustainability, would be the need to select genetically modified strains in the ecological environment or niche in which they are required to function.

### Introduction – What is life?

This paper presents a simple set of hypotheses regarding the *genotype* × *environment* interaction and the likelihood of achieving sustainable genetic modification of organisms. These hypotheses can be subjected to experimental testing, particularly in microorganisms. They are worth testing because, if consistent with the results of new experiments and with existing data, they have the potential to improve the rate of biotechnological progress by avoiding projects with a low probability of success.
The hypotheses are predictions derived from the basic statements made recently in the action resonance theory (ART). This theory [1] was designed to provide unifying ex-

planatory mechanisms for a range of biological and environmental phenomena, involving the generation of ordered motion in sets of bio-molecules. Such ordered motion occurs in the replication of DNA in mitosis, in its translation to RNA, protein synthesis and cell division. Although the mass flows of the bio-molecules involved are usually taken for granted, we can profitably ask how are such concerted purposive flows of molecules generated.

The specific modes of action of each genotype in ecosystems are of primary interest, for these define their naturally evolved role in a living community. We recognize the different species primarily by their morphology, but also by what they do. This is true of the most microscopic disease organism bordering on the definition of living, such as the prions of "mad cow" disease, to the AIDS virus, and the photosynthetic bacteria on land and in the sea. This observation applies through the entire plant and animal kingdoms including the red-wood *Sequoia sempervirens* forests of California to elephants in Africa and the great whales of the Antarctic Ocean. Although evolutionary biologists usually prefer not to emphasize the *functions* of different species in ecosystems, to avoid any implication of global purpose, it is nevertheless often easy to indicate the complementary nature of the roles that particular genotypes have adopted in the action of ecosystems. We assume that the appearance of purposeful behaviour by different species in the function of ecosystems to be an adaptation, as a result of Darwinian natural selection [2].

What decides the possible modes of action in each living species? To what extent can these be modified using biotechnology and are there reasons why some objectives may succeed while others are bound to fail? To answer this question we need to inquire into the unique features of life itself, and to understand the nature of the forces involved in its sustenance. One remarkable response to this question was made over half a century ago by one of the pioneers of quantum mechanics, Erwin SCHRÖDINGER. In his short but fascinating book [3] *"What is Life?"* his answer was that a characteristic feature of living organisms is their capacity *"to feed on negative entropy"*. As Karl POPPER [4] pointed out, SCHRÖDINGER could just as well have said the same of any steam engine but SCHRÖDINGER's book also contains a key chapter that discusses the concept *of "the hereditary code-script"*. There is no doubt that he considered this an equally essential feature of life – one that clearly excludes the steam engine as a living organism. Furthermore, SCHRÖDINGER predicted that the logical basis of life systems would turn out to be the quantum theory itself, pointing to the potential role of quanta such as ultraviolet rays in inducing mutations in genes, thus introducing genetic variation, the possibility of natural selection and the improved fitness of species for their environment. These perceptive ideas were written nine years before WATSON and CRICK [5] had shown the potential for the four bases in DNA to function as a source of genetic information and the genetic code itself was understood.

Despite Erwin SCHRÖDINGER's confidence, over 50 years later the conquest of biology by the logic of quantum theory is by no means evident, whereas the science of molecular genetics predicted by SCHRÖDINGER has advanced to the front rank of biology. Indeed, most molecular biologists and many physicists would claim that quantum mechanics has no role in biology, since biological processes normally involve macroscopic processes where roles for individual quanta or of the uncertainty or correspondence principles are irrelevant.

This complacency may soon be shaken. ART proposes that, on the contrary, all forces between molecules and their consequent accelerations can be explained as quantum exchange forces [1] and that such quantum processes are normal in the function of biological systems. Therefore, the modern action theory suggests that, after all, the quantum theory as proposed by SCHRÖDINGER be of key significance in describing the mechanism of life. Furthermore, the thermodynamic consequences of this dynamic basis for forces between molecules are vital for the generation of biological order out of chaos. The ART provides the basis for a point of view that living systems evolve by selective adaptation to achieve an optimum degree of entropy. This requires a significant revision [1] of our current understanding of the second law of thermodynamics, a revision that recognizes that entropy is essential for life as a dynamic process.

Even cooperative interactions between molecules and organisms involve considerable entropy generation, reflected in the dynamically ordered relative motions of bio-molecules. This implies that the concept of the second law as proceeding uniformly from order to increasing disorder or chaos must be rejected. On the contrary, the action view of the second law predicts a continuous process in spontaneous systems from locally chaotic conditions as a result of the emission or absorption of radiant energy proceeding towards a dynamically ordered outcome, although of greater entropy. As PLANCK [6] pointed out, absorption of energy quanta by molecular systems always involves an increase in entropy corresponding to higher quantum states in electronic, vibrational, rotational or translational molecular motion. Rather than increasing entropy being uniformly associated with the idea of increasing disorder, it will now be necessary to recognize that an optimum degree of entropy is associated with life processes. This is because the very generation of motion and action is an essential feature of life, although it also amounts to an increase in entropy.

In considering the Darwinian evolution of species, DAWKINS [7, 8] has elegantly argued that molecular evolution is not a matter of blind chance, although chance is involved in natural selection. Instead, the probability of meaningful progress in evolution to higher forms of species is increased by the cumulative effect of positive mutations. DAWKINS noted that there were far too many possibilities of different gene products and insufficient time for blind chance to have generated living organisms. He invokes Darwinian natural selection so that organs of amazing complexity, such as the patterned peacock's tail, or even the human mind, could be generated in a cumulative fashion, improving the odds for success in the time available by building on pre-existing biological structures. So the disassembly and re-assembly of biological molecules and more complex structures such as proteins, DNA and organs are all performed under strictly contingent conditions. There can be no such thing as true statistical independence in the assembly of the basic molecules used as building blocks.

DAWKINS [7] has emphasized the point that he considers that natural selection is exerted independently at the level of "the selfish gene", implying that evolution is dictated by an unconscious will for genes to guarantee mechanisms for their own survival. However, he has recently modified his viewpoint [8] so that genes operate independently to ensure their own survival, but must be cooperative with other genes in order to operate successfully. This is a first step in recognizing that there is a genuine genotype $\times$ *environment* interaction. In fact, some may find it very difficult to

accept his previous position, or even his modified view, as actual scientific truth. Although mutation of genes may occur independently and at random, it seems indisputable that tests for fitness, and therefore the probability of survival of particular mutants, must take place by selection in whole genotypes or organisms rather than of isolated genes as specific lengths of DNA. Individual genes are selected, but only in a generational process depending on the relative success of the particular *genotype × environment* interaction, or of the phenotype that develops. These tests of fitness, leading to the increased frequency of particular genes, are obviously cooperative phenomena. One could legitimately argue that it is the relative success of the process or action in which the gene participates that is selected, rather than the gene itself. Actually, these two results are synonymous.

However, DAWKINS's arguments have highlighted an important point about the difference between natural evolution and genetic engineering. Molecular biotechnologists direct evolution by selecting out particular kinds of mutations and then forcing these into the genome of particular organisms more frequently than would otherwise have occurred in the species as a whole. Provided these mutations are non-lethal to the complete genotype, a particular genetic character will now be expressed in a deliberately propagated strain of the organism. Subjected to competition in the natural environment, tests for fitness still occur, but genetically modified strains are normally protected in some degree from such tests. Certainly, performance testing by "trial and error" is still possible (e.g. growth rate, total productivity, expression of particular gene products). However, an important new factor has now appeared that determines the likelihood of the gene's survival, which may have secondary consequences difficult if not impossible to predict. The nature of the *genotype × environment* interaction for the species as a whole has changed, perhaps in a less sustainable way. Selfishness is expressed here, not by the particular gene, but by their human propagators. To what extent is this result new or unnatural and what risks, if any, does it contain?

Are there implications of the ART for biotechnology? For example, the genetic modification of microbes in an ecosystem for specific roles such as bioremediation of organic contaminants (e.g. pesticides) has sometimes been proposed. Even assuming the necessary permissions needed to carry out this modification and to test it, it is by no means certain that such modified organisms can succeed in the environment. In this theoretical paper, by developing SCHRÖDINGER's programme regarding the nature of life, we will examine the prospects for success of such research programmes and suggest some precautions that may be needed.

## The Action Resonance Theory and Brownian Motion

In proposing that the Brownian motion of particles visible in a light microscope, both translation [9] and rotation [10], corresponds to macroscopic and cumulative effects of molecular collisions, EINSTEIN [9] stated *"In this paper it will be shown that according to the molecular-kinetic theory of heat, bodies of microscopically-visible size suspended in a liquid will perform movements of such magnitude that they can be easily observed in a microscope, on account of the molecular motions of heat."* In his set of

papers on Brownian motion up till 1917 EINSTEIN [9, 10, 11] (see FÜRTH [12] for English translation) consistently proposed that a thermodynamic force equal and opposite to the thermal or kinetic motion of the molecules was involved.

In his 1917 paper, EINSTEIN [11] was even more specific about the nature of this force, proposing that quanta absorbed and emitted by the molecules in the molecular field were responsible. *"We can thus consider the following as rather certainly proved. If a ray of light causes a molecule hit by it to absorb or emit through an elementary process an amount of energy hν in the form of radiation, the momentum hν/c is always transferred to the molecule, and in such a way that the momentum is directed along the direction of propagation of the ray if the energy is absorbed and directed in the opposite direction, if the energy is emitted"*, stating elsewhere in this paper that the resonator is thus subject to a force in the direction of the beam of radiation (*"Der letztere erleidet also eine Kraftwirkung in der Richtung des Strahles des Strahlenbündels"*). He emphasized this conclusion, based on the law of conservation of momentum, in his final sentence in a journal on Brownian motion, asserting *that "one can, therefore, consider a theory to be justified only when it is shown that according to it the momenta transferred by the radiation to the matter lead to such motion as is required by the theory of heat"*. EINSTEIN clearly considered that the "completely directional" force delivered by the absorption and emission of quanta must be responsible for the momentum (and its associated kinetic energy) of the molecules. Classical electrodynamics would have predicted that the emission of radiation would take place as an isotropic process, in the form of spherical waves. Consequently, no recoil could occur. By contrast, the quantum theory of radiation necessarily involved a directional recoil as a result of the corpuscular character of quanta, so that their impulse or momentum would be highly concentrated in its effect. This property of quanta, for which EINSTEIN was awarded the Nobel Prize almost 20 years after he proposed it in 1905, also explained the Compton effect discovered in 1923. Electrons could be ejected from a metallic surface by a beam of light, a result not predicted if the force exerted by the beam of energy was evenly distributed over the surface. The mechanism by which photosynthetic pigments operate, allowing the transfer of electrons from water molecules releasing oxygen, must also depends on the particle-like nature of quanta and the highly localized effect of their impulses.

This important theory about the relationship between the impulses imparted to molecules and their molecular motion seems to have been almost completely ignored since, with FÜRTH [12] even referring to EINSTEIN's thermodynamic force originally proposed in the 1905 paper as a "fictitious force". The 1917 paper [11] has often been quoted later, but usually for predicting the possibility of the *laser* (light-assisted stimulation of electromagnetic radiation – *Ausstrahlung* and *Einstrahlung*). It may have been a problem that few people actually read the original papers of outstanding scientists and NOBEL laureates like Max PLANCK and Albert EINSTEIN, probably assuming they would be too difficult to understand. This is a pity, because the clarity and quality of their arguments and the simplicity of their prose makes the ideas of both these scientists, mere mortals not gods, more accessible than some might imagine.

Perhaps surprisingly, there has been little or no interest in investigations of the basic cause of individual molecular motions. The assumption that Brownian motion must always be random has probably deflected interest. The ART [1] takes as granted

EINSTEIN's proposal for the generation of molecular motion and then extends the idea that quanta of a given frequency correspond to a series of impulses of particular frequency causing recoil in the individual molecules, both at absorption and emission. Quanta are proposed to be caused as spontaneous emissions associated with collisions between molecules, by energy exchange processes leading to the observed changes in linear momentum experienced by the molecules. The momentum of a quantum of energy is usually considered to be far too small in a single event of absorption or emission to affect the direction of motion of molecules. However, as a result of extremely rapid energy exchange processes transmitted over very short distances from all parts of the molecular field at the speed of electromagnetic radiation or light, a significant cumulative force is developed. It is the impulses from energy rather than electrostatic effects which generate the changes in the direction and speed of motion. Even the polarisation of asymmetric molecules is regarded in action theory as a redistribution of the elementary particles in molecules (i.e. of protons and electrons), as a result of the operation of these impulsive quantum exchange forces, rather than being the primary pre-existing cause of changes in motion.

A full account of this theory is given in the book *"Action in Ecosystems"* [1]. Suffice it to say that the ART based on EINSTEIN's proposals has been shown to extend to a surprisingly wide range of dynamic and thermodynamic phenomena of the biosphere. In particular, the capacity of one atom or molecule to shield or screen another from the impulses of action exchange forces has widespread application. This allows one to generate explanatory mechanisms for phenomena such as VAN DER WAALS forces, the thermodynamics of heat engines and their efficiency, the heat capacity of molecular systems and gradients in chemical potential resulting from gradients in concentration or of temperature.

EINSTEIN [11] surmised that the absorption and emission of quanta was a matter of chance with respect to time and direction, so that the molecular motions generated were liable to be random. ART, however, importantly suggests that the asymmetry of biological molecules developed in evolution may result in non-random motions, as a result of action exchange processes.

## Action as Scalar Angular Momentum

What is this dynamic property of action? Action is described and explained by KENNEDY [1] and the reader is referred to this book for further information. However, as a dynamic as well as thermodynamic property, action has the same physical dimensions as angular momentum ($ML^2T^{-1}$; $mr^2\omega$). Unlike angular momentum, it is a positive scalar quantity with no specified direction (i.e. it is not a vector). It should always be regarded as a positive attribute mathematically. In quantum theory, it corresponds to the quantum state as designated by the quantum number. Each increase in quantum number of one unit is accepted as adding $h/2\pi$ [J s] to the angular momentum or spin of elementary particles, such as electrons and nucleons or molecular assemblies of such particles. The ART is based on the idea that the action state of molecules is sustained by the energy the system of molecules contains. The greater the energy, the higher the

action or quantum number. As a result, macromolecules like proteins or DNA will have much more action per molecule at a given temperature than simple molecules of lower inertial mass although this would be much less than the action of its constituent atoms if these were not bound at the same temperature.

Obviously, a higher action state will usually correspond to a higher energy content, although kinetic energy of molecules will remain the same, characteristic of the temperature. We conclude that the increased energy of higher action states is indicative of increased potential energy and of total energy, but not of kinetic energy. Thus, the heat capacity per molecule of complex molecules like proteins, with much greater action, is greater. In general, the relationship between action state and energy content, because of cooperative interactions between matter and energy, is exponential so that less energy is needed to increase the action by units of $h/2\pi$ as the quantum number rises. Indeed, since increasing the energy content of a molecular system at a given temperature corresponds to increasing its entropy, there is conjectured to be a logarithmic relationship between the action state and the entropy [13]. This concept was first proposed in the context of the autoimmune disease of nerve-muscle function, Myasthenia Gravis. By binding together molecules of the acetylcholine receptor protein, increasing the action and entropy of the composite particles but decreasing their overall mobility, abnormal antibodies diminish the ability of the nerve-muscle junction to respond to the neurotransmitter, acetylcholine. Furthermore, these products of the disease, because of their greater inertia and energy content, damage the membrane systems involved as a result of action exchange forces generated in Brownian processes, eventually leading to irreversible degeneration of the tissues.

A direct relationship with action infers that entropy is a dimensionless capacity factor indicating the distribution of matter in a system, allowing it to have a characteristic energy and action content. Since there is a direct relationship between the action and the energy content of the system, and hence the entropy, it is now possible to unify three viewpoints of the nature of entropy.

These three viewpoints are:

i) the original idea of CLAUSIUS, proposed around 1860, as the integral of the heat absorbed reversibly $(Q)$, with respect to temperature $(T)$,

$$dS = dQ_{rev}/T; \text{ so } S = \int_0^1 dQ/T,$$

ii) the idea of BOLTZMANN and GIBBS, proposed around the 1880s, as a statistical distribution of molecules to physical states of different energy,

$$S \equiv -k\Sigma_i p_i \ln p_i \equiv -k\Sigma_i 1/\Omega \cdot \ln(1/\Omega) = k \ln \Omega,$$

where $k$ is BOLTZMANN's constant, $p_i$ is the probability of a particular state with $\Sigma_i p_i = 1$, but where different states may have different probability; for equal probability of each state, $p_i = 1/\Omega$ *and* $\Sigma_i p_i = 1$. The greater the number of states available for different molecular complexions, the greater the entropy.

iii) the newer idea advanced in 1983 [1, 13], based on a direct relationship between action $(@)$ and entropy $(S)$,

$$S = k\Sigma \ln(2\pi @/h)^{3N}, \text{ where } @ = mr^2\omega.$$

Both entropy and BOLTZMANN's constant, usually given in physical units of Joules per degree Kelvin, are recognized in ART as pure numbers with no physical dimensions. Temperature, unlike the extensive energy content expressed as Joules or ergs, is an intensive quantity. It has the dimensions of energy intensity or torque and indicates the rate at which action changes during transitions of state. When entropy is zero (e.g. at zero degrees Kelvin), the action has a minimum value, equal for each couple to PLANCK's quantum of action $(h/2\pi)$, corresponding to the zero point and ground state energy.

Thus, we now have three ways to calculate the entropy of a system. But the third action-entropy relation is proposed to be the more complete view, since it determines the energy content necessary for a particular temperature or torque and the resultant temperature-dependent equilibrium statistical distribution as a necessary result of the operation and balancing of action resonance exchange forces. The action exchange forces provide the mechanism for continuously re-distributing molecules between the available physical states, adhering to a principle of least-action, leading to a hierarchical distribution of the total energy corresponding to the distribution of action states. Thus, there is a strongly natural relationship between these three points of view, based on physical reality rather than as abstract mathematical ideas.

## The *Genotype* × *Environment* Interaction: The Flow of Information

SCHRÖDINGER pointed out two essential features of life, the hereditary-code script which we now recognize as genomic DNA and the flow of negative entropy. We may now interpolate the generation of action, based on genetic information, as a third essential feature of life systems. Increases in action are also increases in entropy. However, the asymmetric molecule, DNA, functions as a code and a script because it carries information. This means that the interaction of genomic DNA and its gene products with the environment is far from random. On the contrary, the transcription of DNA to RNA and then protein biosynthesis are both accurate systems with relatively few errors, leading to molecular products that can then modify the action field in which they operate.

The asymmetric DNA specifies the production of gene products, proteins, which themselves are asymmetric. These are characterized by specific properties or activities and, although colloidal particles, they catalyse highly specific processes. For example, the enzymes bind specific substrates, converting these to products with a high degree of certainty. All of this purposeful behaviour demonstrates the information content of DNA. In action theory, gene products are referred to as coupling agents, because these couple the exchange forces of the action field to the performance of essential biochemical work.

We now begin to see how the information content of DNA could be exerted, as a flexible force directed in the environment and *vice versa*. In action theory, the genetic code expressed in RNA is proposed to forcefully select the amino acid specified. A binding correspondence can be expected between the effect of the three bases on the action resonance field nearby and the specific amino acid. Selection of an amino acid can be thought of as binding or attraction. We would therefore expect the specifying code to

diminish the extent of action resonance with the specified amino acid, presumably by screening effects diminishing the energy content of this interaction. Fewer impulses from quanta exchanged between code and acid, would generate "attraction", since impulses received from outside the zone of interaction will tend to propel the amino acid towards the segment of code and thus control the accurate enzymic synthesis of the protein.

This feature of the hereditary code-script, or DNA, extends SCHRÖDINGER's programme. It adds a third feature to his description of life – *the flow of biological information and generation of specific action.* Living systems are more than static collections of interesting objects, such as biological molecules, organelles and whole organisms. We recognize that these structures are ordered, and are usually molecules with decreased entropy content per unit mass compared to the smaller molecules from which they are made up. For example, the entropy of sugars, starch, DNA and proteins is clearly less than that of the equivalent mass of $CO_2$, $H_2O$, $NH_3$ and phosphate molecules from which they are made in processes like photosynthesis. However, without action, these material objects cannot be regarded as living. It is their functional action that typifies their role in ecosystems and without action, clearly an increase in entropy, their existence would be meaningless and, indeed, impossible.

We need to understand very clearly the meaning of the word information. Indeed, perhaps by chance, the etymology of the word as *in-formation* is most apt. The code *in* DNA is responsible for the *formation* of various coupling agents as gene products which are characterized by being almost invariant in their primary structure. In other words, the information of DNA specifies exactly what should be made and hence the action that will be generated in the interaction with the environment. Critically, DNA's coded information specifies a degree of non-randomness. Complete randomness in the code would amount to maximum entropy, with no obvious characteristic behaviour. In information theory, a negative relationship between entropy and the logarithm of the information content was proposed by SHANNON [14], consistent with this viewpoint of the information content of DNA. Therefore, it is the restrictions that DNA imposes on the specific structure of each biological coupling agent that denotes its information content. This information expressed in the coupling agent in turn restricts the range of action states that the agent may adopt and its optimum average degree of entropy. However, it is essential for maximum efficiency that significant degree of freedom of action exists in all the products generated from DNA and RNA. Truly, the information contained in DNA is just as important for what it does not allow than what it allows.

This means that the very idea of life demands the expression of a significant degree of entropy. In principle, this entropy can be calculated as a function of action as well as energy content and probability statistics. However, this entropy is of a kind restricted by the degree of asymmetry, which generates a directed response to external forces. As a result, the motions developed in molecules, their characteristic flows and hence the modes of behaviour of the temporarily coherent sets of molecules of particular species, from amoeba to gazelle, will correspond to their adaptive speciation, and be characteristically purposive. Furthermore, these motions will be cognizant of the action of other species as a result of the process of adaptation resulting from natural selection, guided within flexible limits by action exchange forces.

**Asymmetry and Directed Motion in Biological Molecules**

The mid-19th century French chemist, Louis PASTEUR, who later established the disciplines of microbiology and immunology, first recognized that tartaric acid, a product of wine-making, had stereoisomeric asymmetric forms. These asymmetric molecules both have exactly the same chemical composition but different optical activity as shown by their property of their solutions of rotating the plane of polarized light in different directions. PASTEUR showed they also formed crystals with unique symmetry, as a related property. He postulated that certain molecules, as a consequence of the geometrical arrangement of their constituent atoms in space, were optically active and that such molecules could not be superposable on their mirror images (i.e. they must be dissymmetric). PASTEUR postulated further that the molecules of an optically active substance must be superposable on the mirror images of the molecules of the enantiomorph of that substance. So the enantiomorphs, dextrorotatory tartaric acid (as an analogy, equivalent to a right-hand glove) and laevorotatory tartaric acid (analogous to a left-hand glove) are mirror images of each other, but neither can produce a mirror image of itself. Since PASTEUR's time, an important role for asymmetry has been shown in biology, since organic molecules in living cells such as amino acids, organic acids and nucleic acids such as DNA are all asymmetric and optically active.

In action theory, the asymmetry of the structure of biological molecules dictates that, as a result of action exchange forces carried on quanta exchanging momentum between molecules, the motion developed will not be random. Although radiant energy may be transmitted randomly, the response of individual molecules of asymmetric structure is that both the absorption and the emission of quanta is vectorial with reference to the biological molecule. Consequently, the action or angular momentum developed in recoil reflects the information content of their asymmetric structure. The motions developed are therefore non-random and the Brownian movement of such biological molecules is equally not random. EINSTEIN's theory of random Brownian motion applies to particles of uniform composition and symmetrical structure, so that all forces developed as EINSTEIN proposed from the absorption and emission of quanta would be random. However, the information content of biological molecules means that they are of non-uniform, non-symmetrical composition. KENNEDY [1, Chapter 6] discusses how the CROOKES radiometer, can be set in motion by MAXWELL's pressure of light, as a result of having cross vanes with one surface black and the other reflective. The cross vanes are caused to rotate in a near vacuum in a particular direction, with the black surfaces receding. This result is despite the fact that the illumination of the vanes is randomized by scattering prior to illumination. The directed motion developed depends for its direction on the asymmetric structure of the radiometer rather than the direction of the incident radiation. The radiometer is proposed to provide a model for specificity in the most likely direction of motion of biological molecules absorbing and emitting quanta, whether of frequencies in the ultraviolet or of extremely long-wavelength radiation, essential to the functioning of living systems.

We can find examples of such directed motion in biological systems and many others will soon emerge now that we have a basis to anticipate it from knowledge of its

mechanism and significance. For example, the ATP synthase proposed by MITCHELL [15] to couple proton gradients across biological membranes of prokaryotes and eukaryotic chloroplasts and mitochondria to the synthesis of ATP by dehydrating inorganic phosphate *plus* ADP has been observed to rotate in the lipid bio-membranes in which the enzyme is embedded [1, Chapter 6]. Action theory proposes [1] that the action resonance exchange forces resulting from the dynamic exchange of quanta associated with the reversible ionisation of protons from water clusters provides a force accelerating individual protons into the ion channel of ATP synthase. The quanta of energy provided by the transition of 3-4 protons across a membrane with a gradient in pH of about 2.5–3.0 units provide the exchange forces needed to activate ATP synthase so that ATP can be synthesized from ADP and inorganic phosphate. The probable function of the generation of angular moments and rotation in bio-polymers associated with such ionisations was remarked on previously by KENNEDY [16].

## Some General Implications for Biotechnology

### *Protein Folding*

The *genotype × environment* interaction is proposed in the ART to be a realistic, forceful encounter between an organism and the environment that gives it sustenance [1, Chapter 7]. The most immediate expression of this forceful interaction is in the formation of proteins as gene products and coupling agents from the code in DNA. Yet, even the mechanism of folding of the polypeptide chains of amino acids is an unsolved problem of molecular biology, discussed recently by LESK [17]. Currently, sequence-structure relationships are studied qualitatively by comparing proteins from closely related species showing small variations in amino acid sequences. As LESK points out, *"we do not yet understand how to reason from the sequence to the structure"*. Yet, nature plainly has a simple algorithm that reliably specifies the three-dimensional structure of a protein in a particular environment from the amino acid sequence. Once this algorithm is discovered a computational method to relate the facts about structure as revealed by x-ray crystallography and magnetic resonance (NMR) studies will be available.

ART suggests that such an algorithm can soon be achieved. Elements of the theory that contribute to this view [1] are the relationships between screening effects and the enthalpy of formation and heat capacity of molecules and the balancing of action exchange forces. Most-favoured conformations of proteins will exist that optimize screening potential through dynamic alignments of all the atoms of the amino acids, thus minimizing the energy and the action of the macromolecule at the temperature of the system. In action theory, screening effects from the impulses of energy by matter provide the basis of molecular bonding and other quantum mechanical effects such as molecular spectra. The information required to achieve such conformations in a particular environment is completely contained in the amino acid sequence itself. However, even a mutation leading to a protein with a single amino acid altered can lead to drastic changes in the behaviour of proteins, as observed by PEREG-GERK *et al.* [18]. The heat stability of the tetrameric *Lac* repressor of *Escherichia coli* was altered from 53 °C to 93 °C by substituting a positively charged lysine molecule with

hydrophobic leucine, isoleucine or methionine. The lower stability of the wild-type is consistent with the prediction from ART that quantum exchange forces involved in the ionisation of lysine would lead to denaturation of the *Lac* repressor by dissociation of the tetrameric repressor, now unable to bind to the *Lac* operon.

### *Morphogenesis*

Every organism develops from its earliest germinal material within the environment and its morphogenesis and function is responsive to it. Indeed, the internally generated action exchange forces tend to act until the point at which they come into equilibrium (of a metastable kind, dependent on the rate of energy flow) with external exchange forces directed towards the organism. These define size and shape.

ART does not critically distinguish between interactions involving atoms and molecules and those between higher order structures with some degree of coherence such as cellular organelles (e.g. nuclei, chloroplasts and mitochondri), cells, organs and whole organisms. Each of these structures are dynamically ordered by the detailed balancing of internal and external action exchange forces similar to those proposed in electrodynamics by FEYNMAN [19]. This action approach would be inclusive of the TURING [20] model, in which he laid the basis for a linkage between the new science of molecular genetics, then in its infancy, and morphogenesis. He related the problem to the mechanical state of developing tissues describing the positions, masses, velocities and elastic properties of plant cells and the chemical state involving composition, the diffusibility. He proposed taking into account changes in position and velocity of cells as given by NEWTON's laws of motion, the stresses, including osmotic pressures, chemical reactions and diffusion and the need for a continual supply of free energy to maintain these wave patterns for growth was shown. Turing introduced some of the concepts related to development of catastrophe theory of the 1980s, such as metabolic oscillations, stationary and travelling chemical waves.

All of these factors involve the generation of molecular action from the impulses of quanta, resulting in dispersive field transfers of momentum between molecules. Using ART and approaches based on simultaneous minimization of energy and of action, it should now be possible to consider the development of suitable algorithms capable of expressing the dynamic spatial geometry and the rate of morphogenetic development in organisms. ART suggests that morphogenesis involves the interaction of molecules with a hierarchy of fields that we have traditionally segregated into a suite of forces or potentials (e.g. electromagnetic, electrostatic, VAN DER WAALS attractions, thermal, chemical (potential), inertial and gravitational) but which are all expressed in a common form as action exchange forces resulting from the quantized impulses of energy.

### *Productivity of Crops in Agroecosystems – Rubisco Carboxylase and Oxygenase activity*

Prior evolutionary action has given the organism the genes required for a particular set of responses to the environment, expressed in the form of coupling agents for these particular functions. This process of genetic adaptation to the environment is considered as the evolutionary result of the natural selection of suitable genes. These

genetically specified functions will allow each organism to grow and develop, to search its environment for nutritional materials and to carry out the biochemical and physiological actions needed to ensure it performs all its essential life processes associated with sustenance and reproduction. Severe changes in the environmental conditions will seriously perturb the degree of optimization in action states achieved, predisposing the species to a further process of adaptation modulated by the new matrix of action exchange forces.

Increasing the productivity of agricultural ecosystems by the application of genetic biotechnology has been proposed. There are already a number of successful examples, based on alternative means of pest control, such as genetically engineered cotton (Bt) incorporating *Bacillus thuringiensis* genes for *Lepidoptera* larvae toxins (Cry I), used now for several years in Australia [21] under license to Monsanto with little evidence of deleterious effects on growth such as severe yield penalties and allowing a significant reduction in the use of chemical pesticides (endosulfan). However, proposals to achieve more productive crop plants by direct modification of DNA have met with less success. A problem in such a goal can be unknown or hidden factors. For example, an international "brainstorming" workshop held in Hawaii in the mid-1970s identified a set of research imperatives [22] that might contribute to agricultural crop production, to help meet the challenge of providing sufficient food for the increasing human population. This included a proposal to reduce the extent of photorespiration in C3-plants by plant breeding. As a result of later research in biotechnology, the proposal to genetically engineer one of the most common enzymes and proteins on earth, ribulose-bisphosphate carboxylase (Rubisco) in order to enhance the ratio of its carboxylase activity *versus* its oxygenase activity emerged. In C3-plants, using the Calvin cycle to fix atmospheric carbon dioxide, Rubisco can either fix carbon dioxide by making 3-phosphoglycerate or fix oxygen in a reaction producing 3-phosphoglycollic acid. There is no obvious reason for the oxygenase activity and an extensive metabolic rescue process known as the photorespiratory cycle must be undertaken as a consequence. This appears to be an energetically costly and pointless process.

However, it was suggested [23, 24] that the oxygenase activity could be of importance in controlling the pH value of the chloroplast stroma, in producing acid to neutralize the alkalinity produced when nitrite is reduced to ammonia for amino acid production in chloroplasts. Two hydroxyls ($OH^-$) radicals are produced for each nitrite reduced in the chloroplast stroma. In C4 plants which lack photorespiration, the processes of nitrite assimilation and Rubisco activity occur in different chloroplasts and it was further suggested that the evolutionary pressure for the development of C4 plants provided a solution to this problem caused by inorganic nitrogen nutrition. Several attempts to obtain support from Australia's main basic research funding agency to test this hypothesis were not forthcoming. Perhaps this was because many plant physiologists have regarded the dual activity of Rubisco as "nature's mistake", and the agency would prefer to fund research to rectify this mistake.

In the interim, several research projects in Australia and overseas have sought to engineer the gene coding for Rubisco, substituting amino acids in the active centre in order to modify the ratio of carboxylase to oxygenase activity. These projects have all been reported as (abject) failures. No sustainable improvement in the ratio could be achieved, let alone an improved productivity from the C3 plants involved from energy

saving using the genetically modified Rubisco. The alkaline reaction hypothesis remains untested.

A problem for researchers in describing and explaining functions of plants and other organisms is that they do not have unique and independent functions or objectives. It is a feature of ART that a solution to one evolutionary problem is likely to be complementary to other problems. Because life is an overall process of accomplishment from the complex *genotype × environment* interaction and because the selective mechanisms involved in achieving least-action/least-energy solutions may be optimized for several results simultaneously, we should not be too surprised if the two meanings of the word, function, are expressed at once – namely function either as mechanism or as destined role.

Regarding energy needs, it is usual to consider the total energy as enthalpy and entropy terms and to relate each to biochemical mechanisms. The ART extends the potential role for energy in ecosystems. Energy is required as quanta covering a broad range of frequencies, characteristic of the temperature of the molecules of the system, all of which are essential in sustaining living systems. The resonant quanta providing the turgor pressure in plant cells, allowing the plant to stand erect, are clearly just as important as the quanta allowing protein or starch synthesis to proceed for storage purposes in the seed. Since all molecular motion is dependent on resonant energy and its associated impulses, we cannot omit any of these stages of dissipation because the transport of chromosomes in mitosis may require resonant quanta of a particular wavelength. Equally, the folding of newly synthesized proteins may be achieved by quanta made available by particular oscillations or chemical reactions. So the oxygenase activity of Rubisco and the resultant photorespiration might also assist C3-plants to generate specific quanta needed for cell processes, such as cell division and the function of microtubules in active processes. Such speculative suggestions require extensive experimental testing,

*Natural or Unnatural Selection*

We should also expect to find a degree of interactive harmony between the processes of an organism and those of the surrounding environment. Mutual advantage has rewards for both organisms, which can be selected simultaneously through the mechanism of action resonance fields. This possibility should be a warning for plant breeders. In the area of the breeding of legumes as new crops, it can happen that selection for agronomic traits is performed using inorganic nitrogen nutrition with chemical fertilizer, rather than supplied by $N_2$ fixing rhizobia after nodulation. Because there is now a *genotype × environment × genotype* interaction involving two organisms, ART would predict that the breeding process, or molecular engineering, should be conducted in an interactive mode, varying the genotypes of both the legume plant as well as the *Rhizobium* bacterium. The host plant genetic control of the efficiency of symbiotic nitrogen fixation has been rather neglected. The late Alan GIBSON [25] discussed research work performed in this area, drawing attention to the research of NUTMAN [26] indicating the potential for good results in this area and subsequent work by a few score of researchers. Significant *genotype × environment* interactions were observed in

the case of the tropical legume Spanish clover [27] and mean heterosis indicated that $F_1$ crosses fixed an average of 78% more nitrogen than inbred parents. All these results indicate that significant advantages could be gained by evolving legumes cooperatively with their microsymbiont. However, the desirable outcome of actually heeding this suggestion is often absent in legume breeding programmes [28]. *Phaseolus vulgaris* was bred for yield using fertilizer-N. An unintended result was that this modified legume lost its ability to nodulate promiscuously with a broad range of *Rhizobium phaseoli* bacterial strains found in most soils. Consequently, the advantage of symbiotic nitrogen fixation was lost and poorer yields often resulted. A great deal of resources has had to be expended in order to rectify this problem, which could easily have been avoided by doing the original plant breeding using rhizobia. The temptation to use urea instead should have been avoided.

The use of suitably adapted soil or rhizosphere microbes as biofertilizers, capable of speeding up the rate of mobilizing soil nutrients such as phosphate or even of fixing nitrogen from air, as inoculants when growing cereals such as rice or wheat or other gramineous crops such as sugarcane and maize seems to be potentially an environmental advantage [28, 29]. In this case, it is proposed that progress will be much faster if mutants are generated, either spontaneously or by the use of transposons or other mutagens with the selection of superior results as endophytic bacteria in the root system of the cereal plants concerned (e.g. wheat, rice, maize, etc.)

*Bioremediation*

The possibility of employing genetically modified organisms (GMOs) for purposes of bioremediation of environmental contaminants has appeal. Acting as main growth substrates or degraded in co-metabolism as secondary substrates, selected or engineered genotypes have the potential to decontaminate soils and water. However, similar constraints on the performance of such GMOs would be expected to exist as in the selection of potential biofertilizer strains. They would need genotypes providing a comparative advantage to ensure sufficient numbers of biomass to have a significant impact. There may be advantages in providing such genes for degradative reactions in a genetic background ensuring high numbers of cells. Plasmids carrying genes expressing degradative functions can often be transferred between microorganisms. This approach was employed [30] in providing strains of *Bradyrhizobium* able to nodulate leguminous plants and *Azospirillum brasilense* with the capacity to degrade the herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), as well as fulfilling other beneficial roles in the plant, such as fixing nitrogen gas biologically. Plants to which these bacteria were associated in the roots were then able to grow at concentrations of this synthetic auxin in nutrient solution that would normally be lethal. In principle, these modified strains can be used to protect susceptible plants such as cotton from residual inhibitory effects as a result of using 2,4-D to control weeds in rotation crops such as wheat.

However, benefits may not always be obtained. For example, negative effects on symbiotic nitrogen fixation have been observed on some species of legumes, indicating that insertion of plasmids or other genes into the microbial genome may affect other functions and ultimately plant growth rates. Can such effects be predicted from a better knowledge of *genotype* × *environment* × *genotype* interactions?

Such a complex interaction does not necessarily easily yield solutions. However, it is suggested that an action analysis may help ensure that suitable information is obtained as quickly as possible before obstacles are reached. Furthermore, more suitable genetic backgrounds may help ensure there are no yield penalties.

## Conclusion

Although this discussion could only consider a limited range of cases, these indicate that action exchange forces have a critical role in many genetic molecular processes. The cost of molecular engineering could be reduced many-fold if better predictions were possible of the relationships between gene sequences, folding of amino acid chains and of molecular function. For example, it is difficult to predict the consequences of particular mutations on the action of the altered proteins formed as a consequence of such mutations. Knowing how proteins fold, providing a basis for estimating changes in effectiveness of function, would clearly be an advantage in limiting the number of trials needed to achieve particular goals. The ART suggests that the folding pattern of proteins will represent a three-dimensional least-squares plot optimizing the relative positions of the atoms in the macromolecule. A test of the validity of ART can be made by its ability to predict protein structure from amino acid sequence prior to experimental information from crystallography becoming available. This could be achieved by participation in the Critical Assessment of Structure Prediction (CASP) [17] once suitable algorithms have been prepared.

Other predictions made from ART such as those involved with morphogenesis and attempts to alter productivity can also provide tests of the ART. For example, changes in temperature should lead to a re-adjustment in the position of the least-action, minimum energy, arrangements of molecules. It would be anticipated that all force fields affect the process of morphogenesis, including that of gravity, since the action exchanges will not distinguish between different classes of force. The suggestion of a need to select for superior mutant genotypes in the environment where the new strains must operate could also be beneficial in the areas of inoculant biofertilizer production and bioremediation of environmental contaminants. Provided adequate research funding is made available, it should be possible to use even these more complex processes, aimed at promoting the survival and effective operation of the coupling agents formed as further tests of the ART.

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