Provisional chapter

Toward a New Paradigm for the Evolution of Developmental and Growth-Pattern Systems in Plants and Animals

Michael M. Lieber

Additional information is available at the end of the chapter

Abstract

Genetically controlled and environmentally responsive mutation as a significant feature of evolution has very likely occurred on different genomic levels. The evolution of developmental and growth-pattern systems in plants and animals could have occurred through a karyotypic mutator system creating controlled, frequent genomic changes on the karyotypic level in response to environmental stresses, such as temperature changes. Such a mutator system generating controlled karyotypic changes at very high frequency in response to stress was discovered. in the fungus, *Aspergillus nidulans*, once classified within the plant kingdom. This mutator system is itself representative of a basic, responsive developmental system producing changes in growth-pattern, morphology, and changes ensuing in a new pattern of differentiation, which are adaptive. Such a developmental, karyotypic mutator system may itself have evolved, through its own self-controlled evolution, into types of complex developmental systems that, through controlled, specific, and minute karyotypic changes during ontogeny, could control patterns of development in plants and animals, integrating different levels of organization. The deeper implications for development and evolution are illustrated, suggesting a new paradigm.

Keywords: model, organism, fungus, environmentally responsive, inner-controlling, karyotypic mutator, mutation, high, frequency, genome, instability, level, stress, accelerated, evolution, evolving, developmental, differentiation, growth, pattern, plants, new, paradigm, consequences

1. Introduction: environmentally responsive mutagenesis on different levels of the genome

Experimental investigations of mutations in various unicellular or simple colonial organisms have revealed the beginnings of a paradigm shift in biology. During the past 27 years, environmentally accommodating or adaptively, reactive, enhanced mutation connected to stressful conditions has been found in bacteria and yeast. In 1969, these were also evident within the unicellular green alga, *Chlamydomonas*, a eukaryote. (See references in [1]. Additional references can be accessed via http://www.googlescholar.com) However, with regard to yeast, there was also a far earlier report [2] of reactively, accommodating mutation to environmental stress. Such enhanced mutations, connected dynamically to stress, allowed for the sudden adaptation or accommodation of the single cells of the organism to changed, stressful situations. In various investigations, such adaptive accommodation via enhanced mutation to a particular nutritional stress allowed single cells in non-growing bacterial colonies to produce adapted, growing clones or sectors, which are referred to as papillae. While in many other investigations pertaining to other types of nutritional stress, such adaptation or accommodation allowed the growth of whole colonies from single cells during the stressful conditions. In 1989, 1990, and 1998, the author showed, through his own work on bacteria under nutritional stress, that the occurrence of a reactively accommodating mutagenesis to stress, which ensues in growing colonies that have accommodated to nutritional stress, is under internal, genetic control or regulation. This demonstrated that such mutation is nonrandom, connected dynamically to the stress, and also demonstrated that the mutagenesis displayed developmental features [3–5]. As pointed out, this indicated the evolution of an inner, mutator capacity, which could have defined the rate of evolution itself.

Earlier in 1967, it was also pointed out by the author [6] that the enhanced occurrence of many types of mutation was nonrandom, being under genetic regulation through internal mutator processes, whose existence in the past could have enhanced the degree of evolution from within. The nonrandomness of enhanced mutations within organisms under stress has become clearly manifested repeatedly in the last 27 years of mutation research. However, during this 27-year period of investigations, the particular, nonrandom mutations studied were only adaptively responsive mutations to nutritional requirements and to the stress of antibiotics. And, in the case of the green alga, the accommodating, reactive, or responding mutagenesis of high degree to stress, occurring within cells on the culture medium, permitted the growth of many colonies of joined cells in the presence of a chemical growth inhibitor within the culture medium. Such a growth inhibitor was another type of chemical stress. These accommodating reactively, enhanced mutations, connected to environmental stress, permitted accommodation or adaptation to stress on the molecular level of genetic organization in unicellular or simple colonial organisms. This would be in contrast to mutations that allow accommodation involving higher levels of organization, such as on the morphogenic level in a multicellular, differentiated organism. This would be a living organism that is comparatively far more complex than bacteria colonies and the colonial algae.

In unique contrast, very frequent mutations at the chromosomal or karyotypic level connected to or in response to physical stress can occur under inner control that lead to adaptive changes in the differentiation of pattern and morphology in olive-green, multicellular, internally organized fungal colonies having a central, crinkled morphology, sparsely populated with conidiophores/conidia, and of reduced growth rate. (See [5] for a brief description and earlier, relevant references.) An adaptively responsive, innerly controlled, greatly enhanced mutation on a higher genomic level determining development was shown to exist many years previously to most of the mutation studies referred to above, with significant implications for evolution, especially for the environmentally responsive evolution of developmental systems.

Investigation with the multicellular, differentiated eukaryotic fungus, *Aspergillus nidulans*, an ascomycete, once considered a lower plant, revealed that very frequent mutations on the karyotypical or chromosomal level of organization were an adaptive response to high-temperature stress. These environmentally responsive, adaptive, karyotypic mutations, a type of controlled instability, resulted in the production of many yellow sectors in each sparsely conidiated, olive-green colony. The sectors were composed of yellow, asexual reproductive structures, the conidiophores, made up of yellow conidia or spores, the means of asexual production. The ensuing production of such mutant sectors manifested itself phenotypically at a higher level as a new type of pattern differentiation through such sectors and morphological change within fungal colonies [7, 8]. This new pattern of differentiation and alteration in morphology relies upon inner-controlled, though environmentally reactive genomic changes. This would be a responding or reactive, inner-controlled hypermutation to stress on the level of the karyotype. These mutagenetic processes were adaptive or accommodating to stress in the following ways: Such inner-determined genomic alterations permitted or developmentally allowed, under temperature stress, large increases in vegetative, yellow spore production within the various, differentiated, mutant yellow sectors. The phenotypic, developmental consequence of or connection to such mutation also greatly increased the growth rate of such, flat, yellow sectors of normal morphology. (Note the photographs at the end of this article.) As will be illustrated, this mutator system is in itself an example or a model of an early developmental system that could have evolved into more complex, developmental systems through its own inner-controlled, adaptively, responsive mutability or instability at the karyotypic level of the genome.

2. Details of the investigation with *Aspergillus nidulans*, a plant-like organism

The fungus investigated, *A. nidulans*, is a normally haploid, eukaryotic ascomycete with eight chromosomes. Its colonies have internally septate hyphae made up of multinucleated cells divided by the respective septa. Without chromosomal rearrangements or new chromosomal configurations within the haploid genome, the fungus produces flat, grass green colonies due

to green conidiophores emerging vertically from the multinucleated hyphae composing the complex colonies. The colonies display high growth rates at various temperatures [7, 9]. Colonies with a single, new chromosomal configuration in each of their nuclei have a crinkled morphology and a reduced growth rate, especially at high temperature. The new chromosomal configuration responds self-mutagenically to various temperatures [9]. This fungus produces asexually reproductive spores, which are the source of new fungal colonies. Each spore, a conidium, has a single nucleus.

One particular strain of *A. nidulans* investigated at a high temperature (and at a lesser temperature) has two chromosomal configurations in the haploid genome. The configurations are nonuniform. These configurations have, respectively, partial duplications in trans of chromosomes I (Duplication I) and III (Duplication III). (These resided on respective chromosomal translocations.) *Aspergillus* colonies with these two karyotypic configurations or structures in the haploid genome are much smaller than normal colonies. Unlike normal colonies, those colonies have a morphology which is crinkled. This is especially pronounced at high temperature. These colonies produce far less vegetative spores or conidia at higher temperature, for example, 39.5°C [7, 8]. One of these two karyotypic configurations, defined as Duplication I, has two alleles or genes for conidial color. One allele is for green pigment production, and the other allele is for yellow pigment production. The two color alleles are heterozygous at the same locus within Duplication I. The green allele is dominant to the yellow allele, hence the green or olive-green color of the colonies. That is, these are colonies having green or olive-green conidia and conidiophores within the crinkled area. In some nuclei, a specific region of Duplication I containing the green allele is subject to deletions, resulting in yellow sectors of increased growth rate. The frequency of such deletion from Duplication I, and of corresponding yellow-sector production, is influenced by the other duplication, Duplication III, and by temperature.

Modulated by temperature and the age of the conidia from which colonies are obtained [7], Duplication III controls the degree and pattern of deletion including the green allele on Duplication I. As Duplication III becomes reduced in size as a result of deletions having occurred from it, the reduced Duplication III enhances (at normal culture temperature) the deletion of the genetic region including the green-allele region of Duplication I. A deleted or excised segment, a type of transposition element from Duplication III very likely inserted near the green allele on Duplication I, may trigger, under the control of Duplication III, such deletion. When this occurs under a temperature stress, that is a high temperature for culture growth, this mutagenic, deletional interaction of the two configurations, via a likely transposition process, is enhanced to even a far greater degree in newly regenerated colonies. Moreover, this mutagenic enhancement is clearly regulated, since the improved, yellow sectors, as a consequence of the deletions from Duplication I in many nuclei, all emerge at the same time, as one can see in the photographs. Furthermore, this temporal control of deletion, clearly under the control of the reduced Duplication III, becomes far more pronounced or effective at the stressful, higher temperature [7, 8]. The dampening, epigenetic influence of age-affected conidia on the degree of mutagenic interaction, in cultures obtained from those conidia, is also suppressed epigenetically through this higher temperature [7].

Specifically, at that higher temperature, irrespective of the age state of the conidia producing the fungal colonies, the environmentally accommodating or adaptive results of this extremely high mutagenesis involving controlled deletion, possibly at mitosis, on the karyotypic level of organization, are adaptively responsive fungal colonies at various levels of organization involving the genomic level. In this regard, each of those colonies respectively and symmetrically produces through the inner-controlled deletions many yellow sectors of increased growth rate. These are sectors also having an abundance of conidia or conidiophores, as well as having a relatively smooth or non-crinkled morphology. These are consequences that are very much accommodating or adaptive to the new temperature situation or induced epigenetic stress. This is especially the situation in the long term within the context of the evolution of new, adaptive differentiation patterns displayed by new adaptive strains of *Aspergillus*. Also, the configurationally, partial duplications, controlling such adaptation, are in effect an adaptively responsive, complex mutator system on the chromosomal or karyotypic level. This is a system that has internal regulation, and one which is environmentally sensitive or reactive. It is a system whose mutagenic behavior is asexually inheritable by means of conidia and sexually transmittable to an F1 generation by means of ascospores [7, 8]. This mutator system has its origin in genomic reorganizations on the karyotypic level. Many types of mutator systems, environmentally sensitive, can be traced to past genetic reorganizations [7, 8].

This situation with *Aspergillus* shows that inner-determined, internally regulated, greatly enhanced karyotypic changes can nevertheless be caused epigenetically by a physical stress, namely high temperature. Such induction occurs in such a manner that controlled karyotypic changes result in adaptive or environmentally accommodating changes on the differentiation/morphological level. This would also be the organizational level of the phenotype. This would be an example of a mutagenic, reactive connection of the karyotypic mutator to physical stress that ensues in a connected, reactively accommodating, differentiation/morphological change to that very physical stress, which permits adaptation. This is a situation that has not been demonstrated before. Such is highly significant as it now shows that morphological and differentiation patterns can be adaptively reactive and connected to environmental stress by means of a stress-induced, influenced mutagenesis involving genomic configurations. These would be developmental, controlling elements on the karyotypic level. Such elements would appear to be regulating the deletion and insertion of smaller transposition elements within the configurations. The physical stress affects, possibly through cytoplasmic and membrane distortions, the inner-controlled mutagenic interaction of the genomic configurations. This occurs in such a way that the regulation becomes enhanced, leading responsively (within one generation) to very frequent, karyotypic-based, controlled alterations in differentiation and morphogenesis in fungal colonies. Such reactive enhancement of mutation on the karyotypic level to environmental stress permits effective, environmentally accommodating alterations on the phenotypic level, which is on the organismal level. The resulting karyotypic alterations have become coextensive with the many yellow mutant sectors within each of a large number of olive-green colonies. And thus, such alterations have become coextensive with a new, adaptive pattern of differentiation and morphogenesis. This represents a reactively or responsively induced, new karyotypic analog of an adaptive differentiation and morphogenesis within a short period, and one connected dynamically to stress.

3. An adaptive phenomenon apparently unique in the history of such investigations

During the many investigations into environmentally accommodating, connected, reactively enhanced mutagenesis, the adaptively responsive phenomenon involving *Aspergillus* was not previously observed. This is especially and specifically the process whereby controlled, very frequent karyotypic change under and through physical stress can be manifested adaptively in a short period as very frequent, adaptive alterations in morphology, growth, and patterns of differentiation within growing, multicellular fungal colonies under stress. It is noted that such responsive adaptation by means of karyotypic mutator systems, whether or not transposition elements are involved, may not be perfect. This is because some karyotypic changes or instabilities could be deleterious. Nevertheless, the types of environmentally responsive mutator systems within *Aspergillus* could have themselves evolved into more effective mutator systems. These would have been systems with developmental features, leading through their evolving, inner-directed changes to more effectively adaptive developmental or morphological solutions to various types of environmental and internally related epigenetic stress.

This phenomenon of environmentally responsive phenotypic change is similar to the phenomenon of the genetic assimilation of induced morphological changes involving environmental stress in *Drosophila*. This was first discovered and investigated by C. H. Waddington in the 1950s [10–12]. In this regard, when developing *Drosophila* embryos are exposed to ether vapor stress treatments or shocks during a specific developmental period, a portion of the *Drosophila* develops two thoraxes with two pairs of wings in adult flies. Within each fly generation exposed to ether stress, developed bithorax flies were inbred or crossed. When after a small number of generations of this inbreeding under stress, a significant proportion of the subsequent progeny resulting from repeated inbreeding for the new morphology, and now free of ether stress, nevertheless still developed the bithorax phenotype as adults. In response to stress, the new morphogenesis has become genetically assimilated or inheritable in a relatively short period. In additional experiments involving inbreeding through a small number of adult generations, other types of morphological changes occurred. These were alterations in wing morphology, eye morphology, and in anal excretory papillae. These were also genetically assimilated following the inbreeding of the adult fly generations, whose embryos responded morphologically to the stress. These were flies whose developing embryos were subject to other types of imposed environmental stresses. These were temperature shocks with regard to wing and eye development and salt treatments of culture media with regard to papilla size.

Not generally investigated was whether or not many of such responsive, genetically assimilated, environmentally responsive morphological changes were adaptive to the environmental stresses in question. However, the genetically assimilated increase in papilla size as a response to salt stress may suggest an inheritable adaptation to the increased salinity in a relatively short period and to any future saline increases. Also, it was not determined whether or not new mutations on the gene and karyotypic level were induced through the imposed environmental stresses during embryogenesis. Though this situation cannot be ruled out, it should be further investigated. Also, these morphological alterations might have permitted the development of less obvious, internally adaptive and enabling features in complementarity with the evident, genetically

assimilated morphological changes. This possibility should also be investigated. In this connection, see [1] regarding what could be enabling mutations in developmental processes.

In later years, additional stress-involved assimilation experiments were performed. This was with a black caterpillar species. Developing embryos of such were subject to heat shocks within each developing, caterpillar generation. As a result, green adults developed during each of a small number of embryo generations subject to heat shock. Subsequently, developing caterpillars eventually became inheritably green without heat shock after a small number of generations through repeated inbreeding of green progeny caterpillars that had developed from exposed embryos in each of those generations [13]. As the authors of this research point out, it is feasible that such inheritably acquired color via heat stress would be adaptive as an effective camouflage in an environment of green, leafy vegetation during the warm season, and thus evolutionally adaptive in a relatively very short period in the context of evolution.

Genetic assimilation of morphological and pattern changes may have played a significant role in evolution. During evolution, such developmental, genetic assimilation of features at the organismal level could have involved some types of environmentally responsive, frequent genomic change on the karyotypic level. They and their effects could have become repeatedly combined through a relatively short period of inbreeding. Hence, this would have accounted for an adaptively responsive assimilation during a relatively, very short period, enabling thereby an accelerated evolution. (In this regard, the adaptive, *Aspergillus* mutator system was generated through inbreeding involving reorganized chromosomes.) With regard to such genetic assimilation of environmentally induced characters, an alteration in genomic organization is indicated [14]. This inheritable or genetic assimilation of environmentally influenced morphological alterations, and less evident, enabled features, could have been the dynamic source for the accelerated, nonlinear evolution of developmental systems in various organisms.

The role of karyotypic mutators in this is quite feasible. This becomes especially feasible in view of the following found with the *Aspergillus* mutator system: one can generate, through an asexual selection from an extremely high mutant-sector, colonial producer at high temperature, a group of colonies with a significantly, further-increased mean frequency of mutant sectors at high temperature compared to the mean mutant-sector frequency of another group of colonies at high temperature [7]. This would certainly suggest a genetic assimilation of a further increased karyotypic mutator effect at high temperature, possibly involving the stabilization of an epigenetic change, itself stressful. And the high-temperature stress would be mutagenic in the context of the inner mutator process, in a way, a nonlinear, epigenetic extension of the mutator process. Occurring in other situations, this could have affected the rate of morphological evolution itself.

4. The evolution of developmental systems due to environmentally responsive genomic changes

Relevantly, a nonlinear rate or burst of karyotypic evolution has been correlated with a high, nonlinear rate of morphological evolution in higher plants and mammals [15]. Karyotypic mutator systems similar to those described in *Aspergillus* may have played a significant role in this [16]. Also, these karyotypic mutator systems may have been mutagenically responding

to various environmental and internal stresses. These stresses could have been extremes in temperature and premature aging. The results of these processes may have been corresponding, nearly immediate morphological changes that adaptively accommodated to the new stresses through the environmentally responding mutator system involving the karyotype. This could account for the high, nonlinear rates of morphological evolution of the mammals and of higher plants.

Relevantly, frequent duplications of karyotype leading to polyploidy and corresponding morphological change during plant evolution have been shown to be associated with periods of environmental stress [17]. Polyploidy in plants and general karyotypic change have been very adaptive and have greatly contributed to plant speciation. It cannot be ruled out that such changes in ploidy or karyotype have had, or involved, a developmental, mutator effect, determining in a controlled, specific, and refined manner genomic changes on the karyotypic level. Such mutator systems could have had their origin in those very karyotypic reorganizations. And it is predicted that evidence or indications of this will be discovered in current plants. As long ago as 1940, the geneticist, Richard Goldschmidt, argued that evolution, especially macroevolution, could have involved the responsive or directed generation of mutation on the karyotypic/chromosomal level of organization, ensuing in the sudden occurrence of organisms with new inheritable, developmental, primary patterns [18].

Karyotypic mutator systems may have contributed to and may have themselves become part of the evolution of developmental systems in various organisms. By doing so, they could have determined the very rate or degree of such an evolution [1, 7, 8, 19], consequently enhancing the evolvability of developmental systems. It is likely with regard to the adaptive *Aspergillus* system that those developmental systems would have been the result of an adaptively or environmentally responding, evolving mutator system on the karyotypic level. This would have been a system evolving through its controlled, responding or reactive, connected instability to environmental stress. A consequence of this would have been the evolution in various organisms of even more effectively adaptive, mutator-based developmental systems, wherein inner-controlled, minute karyotypic changes, possibly involving transposons, would have occurred as features of ontogeny. Moreover, such an evolving and integrative mutator system, involving the karyotype, would have determined the very inner evolvability of the evolution of development in various organisms, including and especially in higher plants. In effect, the responsively evolving karyotypic mutator system would be the responsively evolving capacity to evolve developmental systems, the inner-evolving evolvability of evolution. Another avenue for evolution involving mutators might have entailed a modern version of pangenesis proposed in 1967 [6].

5. Likely consequences and possibilities from the evolution of karyotypic mutator systems

Though originating several years ago, experimental studies of *A. nidulans* have nevertheless made explicit, through further examination, a new type of environmentally responding,

inner-controlled adaptive mutation of high degree occurring on the karyotypic/chromosomal level. This is an inner-controlled, mutator process, connected to and arising through environmental stress, manifested phenotypically as adaptive changes in growth, differentiation, and morphology. This phenomenon displayed a temporal control reactive and connected to an environmental stress, and it seemed to enable a near-sudden or accelerated adaptive response to a physical stress. Such eventuates when a flexible or plastic accommodation to physical stress becomes necessary. By means of its timing, the phenomenon is adaptively developmental through different levels of organization, from karyotype to pattern differentiation and morphogenesis on the level of the organism. Its inner-regulated temporality is a critical adaptive characteristic of this reactive or responsive phenomenon. This inner capability to eventuate such an adaptively responsive phenomenon and the adaptive, developmental consequences or features is also inheritable.

This adaptively responding process or phenomenon may also be indirectly connected to other environmentally responding alterations in development, which have temporal features and which have become inheritable. Genetic assimilation may be one example of this. There may be other types of environmentally responsive mutator systems that are not evident, yet to be discovered. These may have also played a significant role in the developmental evolution of organisms. Nevertheless, it is likely that many developmental and growth-pattern systems in plants and animals have evolved from a basic, known developmental, karyotypic mutator system, such as the one discovered in *Aspergillus*. Such evolved systems could involve, refined, somatic intrachromosomal recombination. In fact, the process of deletion and transposition in the *Aspergillus* mutator system was proposed to involve specific, somatic intrachromosomal recombination implicating heterochromatin [7, 8].

In various invertebrate animals, controlled, frequent karyotypic changes, such as deletions of heterochromatin, do occur within somatic cells as opposed to germ cells, during development [20–22]. Such deletions or excisions may occur through intrachromosomal recombination [21]. And in certain amphibians, development is known to involve the creation of inheritable, irreversible nuclear (or chromosomal) changes within somatic tissue [see [23]], these changes possibly being deletions. During lymphocyte differentiation in mammals, there is a regulation of genomic rearrangement events in those cells [24]. It is well known that very high-frequency, genomic changes involving somatic hypermutation/intrachromosomal recombination in developmental, immunological tissues (B lymphocytes) occur as a controlled, adaptive response to internal environmental stresses, such as bacteria and viruses or other foreign antigens [25–27]. The developmental consequence is diverse antibody production, which is adaptive. In various plants, there are controlled changes in ploidy in different cells during development [28, 29]. In *Nicotiana*, controlled deletions of heterochromatin in somatic cells, possibly involving intrachromosomal recombination, occur frequently during development, which results in color variegation of the flowers [30]. In maize, some features of development are based on a transposition-insertion-deletion, controlling-element system, with many variations of such [31, 32]. Dr. McClintock, the author of this paradigm-altering research, proposed that many other aspects of maize development could be so based, as well. As in *Aspergillus*, such a system in maize is derived from a chromosomal or karyotypic reorganization. The system in maize and its variations is temperature sensitive. These developmental systems have

characteristics suggesting their evolution from responsive karyotypic-based mutators. In view of this, it is likely that other developmental systems occurring through environmentally responsive, changing karyotypes and based on innerly controlled, refined genomic changes will be discovered.

Hence, it is predicted that more and various karyotypic-based mutator systems, responsively generating or leading to frequent adaptive, inheritable changes in differentiation and morphology within short periods, will be detected in various organisms. These mutator systems, through their own inner-controlled instability, may form the basis for the future and rapid evolution of more complex and refined developmental and growth pattern systems, leading to more adaptive and, in many cases, productive organisms. This would include cultivated and nurtured plants used in agriculture and horticulture, but among the harmful, could include organisms that are pathogenic to such plants, as well. The likelihood that the environmentally responsive mutator systems in bacteria, *Aspergillus* and maize, are genetically related through evolution [5] makes this prediction even more likely. The developmental, Ac-Ds controlling-element system in maize [31] is similar to the mutator system in *Aspergillus* [5]. The adaptively responsive phenomenon exhibited by *Aspergillus* (once classified as a lower plant) provides further evidence for the occurrence of various types of adaptively responsive or reactive mutagenesis in many, various organisms. It gives greater likelihood to the conclusions stemming from those previous investigations of adaptively responsive mutagenesis. The developmental, adaptive system in *Aspergillus* makes it more predictable that environmentally responsive, inheritable mutator systems of various types, including those with developmental features, have been a significant parameter in a responsively accelerated, adaptive, developmental evolution of lower organisms, animals, and plants. This would have included the evolution of the progenitors of cultivated crops and of pathogenic organisms.

What appears to be an example of a predicted situation was recently described [33]. When a soil fungus pathogenic to rice was exposed to increasing copper concentrations, which increases are normally toxic to the fungus, and to temperature shocks in other experiments, frequent genomic rearrangements occurred in response to both types of stresses through the agency of transposition elements (TEs). With increasing concentrations of copper in the culture medium, the fungal colonies became resistant to the copper and grew. This was correlated with increased genomic alteration due to the insertion of certain TEs. Furthermore, increased copper resistance was correlated with frequent color changes of the colonies from gray to white. The alterations appeared as white sectors in photographs; morphological alterations were also generated. The fungal colonies adapted to the highest copper concentration displayed dense aerial hyphae. Those colonies were completely white. In earlier investigations by these authors, temperature shocks affected fungal growth and resulted in morphological transitions. These included pigment changes and the production of aerial hyphae. (Personal Communication.)

These responsive, frequent genomic changes to stress occurred over a short period, as indicated by the data. Under field conditions, where there are high concentrations of copper in the soil where the fungus resides, and the soil is very warm due to a tropical environment, this fungus exhibits a high degree of genetic diversity or genetic rearrangements, "suggesting [according to the authors] that high copper content of soil and temperature stress are among

the important environmental factors responsible for the high genetic diversity of the pathogen under field conditions." Another implication is that such adaptive, genetic diversity was responsively induced via TEs over a short period.

Though, as noted by the authors, "extensive research over the last several decades has elucidated numerous molecular responses to stress, it is much less known how these translate into organismal–level responses." The authors argue that environmentally responsive TEs indicate such a translation. Might the color and morphological change of the colonies in connection with copper concentration also reflect such a translation? One should recall in this regard that a process very likely involving transposition elements may also have been involved in the adaptively responsive mutator situation in *A. nidulans*. To reiterate, this is a situation where frequent adaptive changes involving color-pattern differentiation, growth, and morphology occurred over a short period. Experimentally supporting such involvement of controlling elements in *Aspergillus*, the transposition of genetic elements that occurred from chromosome to chromosome in *A. nidulans* resulted in morphological and pigment changes within short periods [34]. These transposing elements responsible for those phenotypic changes had their source in a duplication derived from Duplication I. As to whether or not such phenotypic changes, based on such small, mobile, karyotypic segments, were adaptive was, however, not looked into. Yet, experiments conducted by the author showed that high temperature could significantly increase, within a period just over a week, the frequency of generation of this genetically based phenotype [7]. In connection to environmental stresses, karyotypic mutators could have activated and controlled the deletion, transposition, and insertion of genetic elements throughout the genome with developmental effects during the course of their evolution.

The inner-controlled, responsively adaptive processes as described in this article may only be markers or shadows of a deeper, more encompassing adaptive dynamic, whose principle may be independent of scale or level of organization. The elucidation of this process may give a better insight into the translation mentioned above. In this regard, the following questions occur: How and why would the environmentally responsive and innerly controlled karyotypic changes, mediated by TEs, develop into adaptive phenotypes? What are the underlying connections that translate environmental cues or stresses into adaptive, organismal, developmental responses, from phenome to genome and through genome to phenome? The authors, regarding the pathogenic fungus [33], show that the TEs investigated do behave in different ways and are highly specific, responding differently to different environmental clues or stresses. Yet, what occurs through such specificity of action across different levels of organization that ensues in a correct phenotypic adaptation? Further studies of adaptively responding karyotypic mutator systems, which appear to integrate dynamically and specifically those levels, may provide insights into this.

6. Conclusion: toward strengthening the new paradigm with constructive results

Though it appears that karyotypic mutator systems through their own environmentally responsive, inner-controlled instability could have adaptively evolved into many current

developmental systems based upon inner-controlled genomic changes, such as those involving transposing genes, it is still not clear in many ways as to how specific adaptive changes on various levels could have been mediated during that evolution. In this regard, could a type of dynamic, epigenetic imprinting due to various stresses, via cellular states, cellular membranes, the cytoskeleton and nuclear matrix, on chromosomal behavior be involved in such specifically responsive adaptations? And could such an imprinting account for a likely responsively accelerated evolution of pathogenic organisms and higher plants through an epigenetic imprinting process regulating and determining lasting karyotypic mutator influences on the very developmentally involved epigenesis itself? Most relevantly, and predictable in this regard, inheritable epigenetic modifications in plants occur due to environmental stresses [35]. Such inheritable, adaptive epigenetic modifications, which the authors refer to as epimutations, are associated with an increased frequency of genomic rearrangements, whose generation appears to be nonrandom.

Such a further evolved, environmentally responsive process could be considered as a transgenerational, environmentally responsive, developmental system, perhaps a variation of genetic assimilation. It would be one manifesting and occurring through dynamic connections across different levels. As far as elucidating the dynamic underlying such specific connections and interconnected adaptations on various levels of organization, including the environmentally responsive, transgenerational epigenetic-karyotypic level, one must look for more interconnected, holistic and imaginative explanations, based on new assumptions. One such assumption could be external forces imprinting stable specificity through instability within and between cellular epigenomes. These explanations and assumptions could and should be elaborated and tested by experiment in order to gain a more complete, empirically based picture and so enable scientists to arrive at a heuristic, universal principle in biology.

Knowing such a principle may enable scientists to counter or reverse the generation and evolution of pathogenic organisms and promote the evolution of pathogenic resistance in crops, as well. Be this as it may, and pointing to aspects of such a principle, environmentally responsive and innerly controlled, adaptively changing karyotypic mutator systems, involving transposons, could have provided the inner dynamic and capacity for various, enhanced macro- and microevolutions of various organisms and their developmental processes over relatively short periods. Using tissue culture methods, the creation and application of such mutator systems in an epigenetic context, involving transmitted energies and stresses, may even become a significant parameter in a near-future evolution. This would occur through the genetic engineering of more productive, age-resistant plant cultivars with altered, adaptive developmental and growth-pattern systems. These would be developmental changes and features analogous to those generated by the mutator system in *Aspergillus*, a model and primary system. The *Aspergillus* mutator system is an early and significant example (effectively in 1972) of an internally regulated hypermutator system in a relatively complex, multicellular organism enabling quick adaptive responsiveness, on various levels of organization, to new environmentally induced conditions in the organism, and thereby innerly and developmentally evolutionary. The *Aspergillus* mutator system can certainly be seen as being an epigenetic system within a more inclusive one guiding, and cyclically being influenced by inner mutator processes, and one most likely prone to inheritable imprinting.

This would be a type of mutator-based, multilevel, epigenetic system probably forming the evolved basis for many, present-day developmental and growth-pattern systems, at least significant features of such, where controlled genomic change through responsively regulated genetic deletion, transposition, and insertion is involved. Certainly in many cases, gene activation and suppression occur as features of development. Yet, such genetic behavior is dependent on chromosomal configurations or states, such as heterochromatin and methylation. And, predictably, these could very well be epigenetically controlled, as well as controlling, through the environmentally influenced deletion, insertion, and expression of genetic factors, such as transposons, a process representing a type of position effect variegation through regulated intrachromosomal behavior. Modern genetic research has provided supporting evidence of this [e.g., [36]]. It clearly shows that transposition elements play a regulatory role in the development of various organisms, affecting gene expression [37].

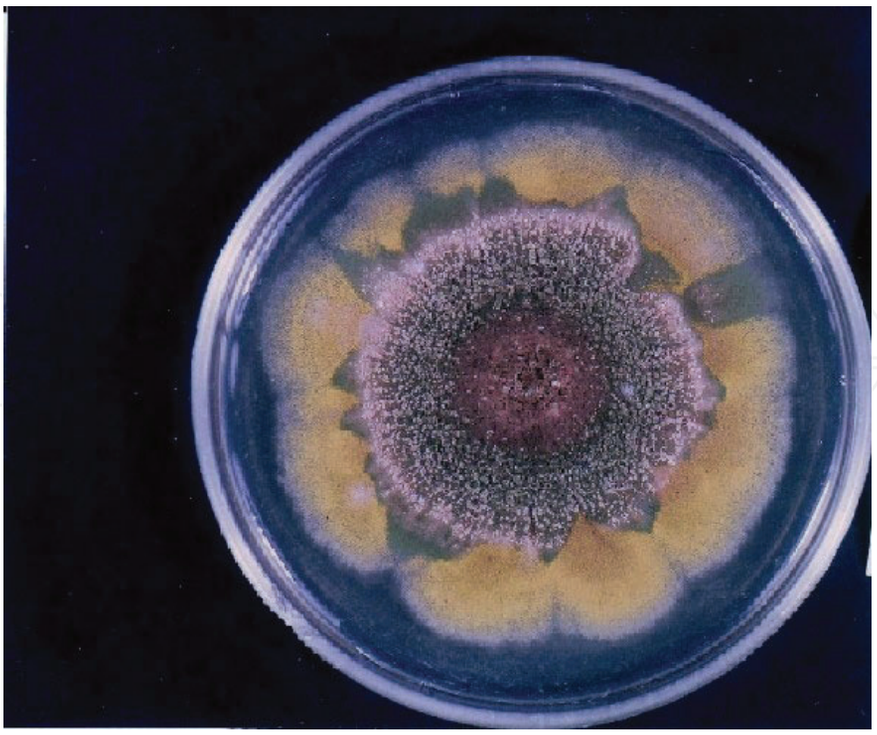
As shown in [36], an epigenetic system in a higher plant can induce enhanced, inheritable, and adaptive mutation, through responsive transposon insertion in specific genes, enabling seed germination in response to a chemical stress in culture that inhibits such germination in culture. This is an evolved, mutator-based system controlling development in which transposon activity must also be induced or enabled by the heat treatment of the parental generation prior to and necessary for the chemical-stress induction of the beneficial, adaptive mutations through the stress-responsive insertion of transposons into specific genes of the seed progeny, enabling seed germination. Thus, heat stress itself would be seemingly acting or being utilized within the system in a potentiating-mutagenic, epigenetically adaptive fashion. However, an implicated, controlling methylation of the inserted transposons—where methylation is under the regulation of another genetic region within the system—can inhibit the expression of the adaptive mutations, ensuing in resensitivity to the chemical stress. Subsequent heat treatment reactivates the expression of the beneficial mutations, as well as the expression of adjacent genes, through demethylation of the inserted transposons. The regulated methylation could mask the growth effect of the mutant genes in vivo when conditions would require plant dormancy. Under such cold conditions, as the research implies, the effect of the mutant genes would be nonadaptive but adaptive under warm conditions. The chemical stress is in fact a plant hormone that induces dormancy under cold conditions. In view of this, the evolved, inclusive, and responsive epigenetic control of mutant induction and expression would quickly be able to accommodate plants transgenerationally to changing environmental conditions, allowing for and inhibiting development when, respectively, necessary, and in a heritable fashion. Even though all the adaptive dynamics across different levels of organization have not been clarified, the systems or processes such as these can nevertheless be seen as also contributing to the beginning stages of a new paradigm for mutation and evolution.

A new paradigm encompassing mutation and evolution not only becomes creditable but very feasible. As viewed through this paradigm change, responsively, adaptively, enhanced genetic mutation on various genomic levels can occur, while defining or structuring levels of biological evolution so guided responsively via epigenesis by that mutation. This would be, through mutator processes, an inner-regulated, transgenerational, environmentally responsive, enhanced mutation on different levels to stresses. Thereby, this would have been a mutator-defined, responsive mutation controlling and structuring the rapid and responsively accelerated

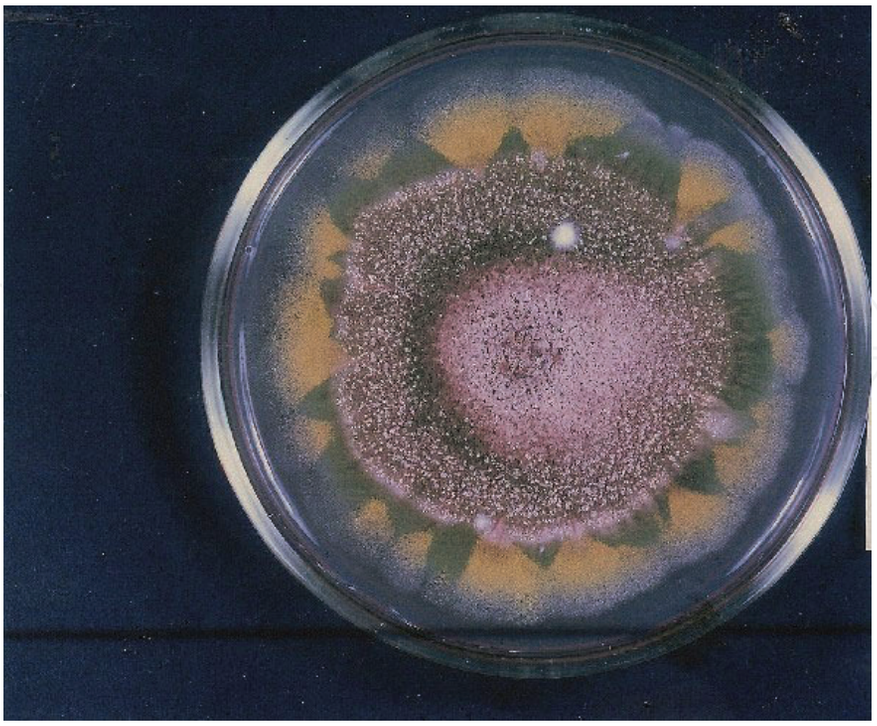
evolution of organismic, adaptive, developmental capabilities, and their expression. On a deeper, integrated level, the evolution of developmental and growth pattern systems would appear to have an inner, ordering, stabilizing dynamic or component capable of quickly accommodating adaptively to environmental and internally related epigenetic stresses, which tend to destabilize, and which in this context are mutagenic. Thus, evolution itself would appear to be a stabilizing, transgenerational, evolving developmental process, countering destabilization via mutator-controlled, multilevel, responsive mutation, through space-time. This perspective would not only have significant implications for agricultural research, such as crop improvement but could guide medical research, as well.

7. Photographs of *Aspergillus* colonies

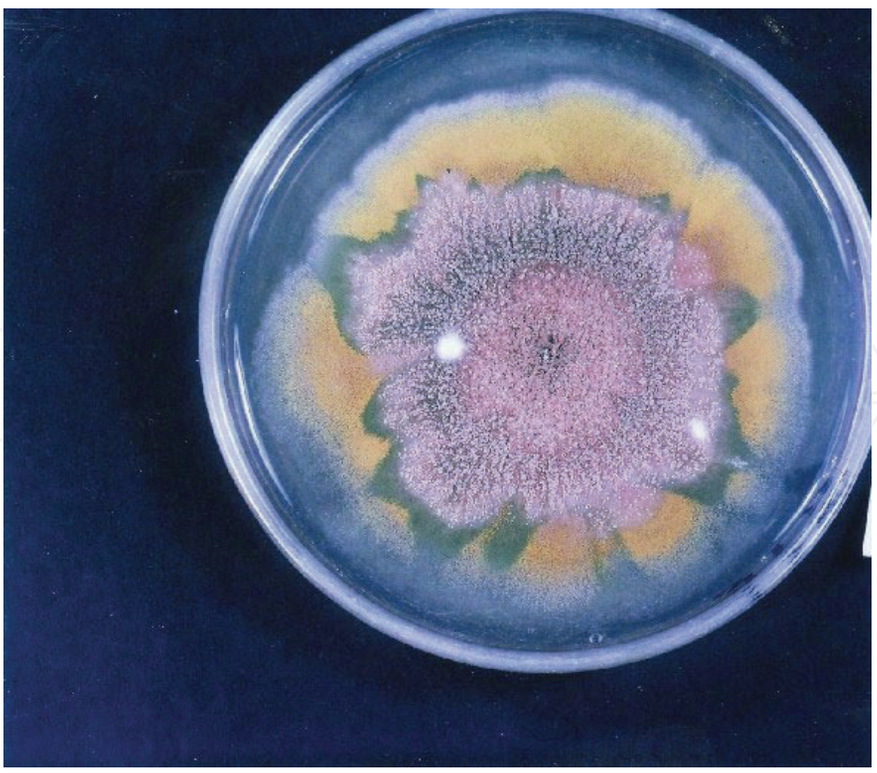
The four photographs each show an *Aspergillus* colony having produced through a karyotypic mutator system many mutant yellow sectors in response to a temperature stress. These colonies came from a large number of colonies displaying the consequences of such mutability producing the adaptive differentiation through the pattern of yellow sectors. Note in photograph IV the two white, mutant sectors displaying a normal morphology and improved growth rate. Their production in this situation, which only occurs in the mutator strain, might have been the result of the insertion of a small genetic element from Duplication III into an epistatic gene on chromosome II that influences pigment production.



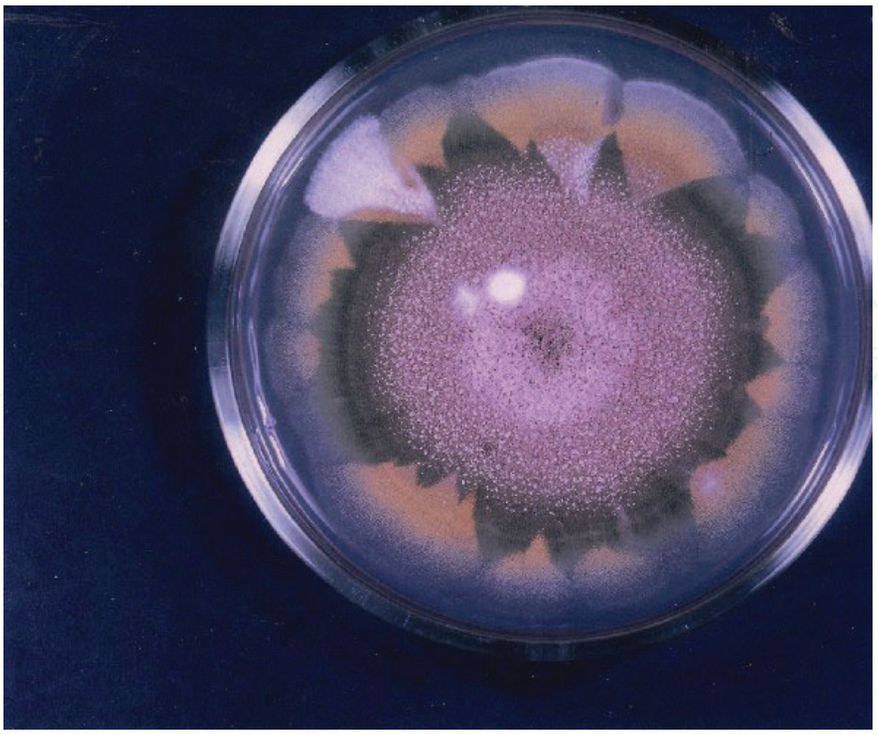
I



II



III



IV

Author details

Michael M. Lieber

Address all correspondence to: michaellieber@juno.com

Genadyne Consulting, Berkeley, CA, USA

References

[1] Lieber M. The problem of antibiotic resistant bacteria. The important role of environmentally responsive mutagenesis, its relevance to a new paradigm that may allow a solution. Theoretical Biology Forum. 2011;**104**(1):91-102

[2] Lindegren C. The Cold War in Biology. Ann Harbor, Michigan: Planarian Press; 1966

[3] Lieber M. New developments on the generation of mutations in Escherichia coli lysogens. Acta Microbiol. Hungarica. 1989;**36**(4):377-413

[4] Lieber M. Mutagenesis as viewed from another perspective. Rivista Di Biologia-Biology Forum. 1990;**83**(4):513-522

[5] Lieber M. Environmentally responsive mutator systems: Toward a unifying perspective. Rivista Di Biologia-Biology Forum. 1998;**91**:425-458

[6] Lieber M. Mutation, development and evolution. Thesis. Institute of Animal Genetics, University of Edinburgh; 1967

[7] Lieber M. Environmental and genetic factors affecting instability at mitosis in Aspergillus nidulans. Ph.D. Thesis, University of Sheffield; 1972

[8] Lieber M. The genetic instability and mutagenic interaction of chromosomal duplications present together in haploid strains of Aspergillus nidulans. Mutation Research. 1976;**37**:33-66

[9] Lieber M. The effects of temperature on genetic instability in Aspergillus nidulans. Mutation Research. 1976;**34**:94-122

[10] Waddington CH. Genetic assimilation of an acquired character. Evolution. 1953;**7**:118-126

[11] Waddington CH. Genetic assimilation of the bithorax phenotype. Evolution. 1956;**10**:1-13

[12] Waddington CH. Canalization of development and genetic assimilation of acquired characters. Nature. 1959;**183**:1654-1655

[13] Suzuki Y. and Nijhout HF. Evolution of a polyphenism by genetic accommodation. Science. 2006;**311**:650-652

[14] Piaget J. Biology and Knowledge. Chicago, Illinois, USA: University of Chicago Press; 1974

[15] Wilson AC. et al. Biochemical evolution. Annual Review of Biochemistry. 1977;**46**:573-639

[16] Cherry L, Lieber M, Wilson A. Phylogenetic analysis of chromosomal evolution in vertebrates. A Report submitted to the Proceedings of the National Academy of Sciences, USA. 1978. Unpublished

[17] Vanneste K, Maere S, Van de Peer Y. Tangled up in two: A burst of genome duplications at the end of the Cretaceous and the consequences for plant evolution. Philosophical Transactions of the Royal Society B. 2014;**369**, No. 1648 20130353

[18] Goldschmidt R. The Material Basis of Evolution. New haven, Connecticut, USA: Yale University Press; 1940

[19] Lieber M. Environmental and genetic factors affecting chromosomal instability at mitosis and the importance of chromosomal instability in the evolution of developmental systems. Evolutionary Theory. 1975;**1**:97-104

[20] Goday C, Estaban MR. Chromosome elimination in sciarid flies. Bio Essays. 2001;**23**(3): 242-250

[21] Beerman S. A quantitative study of chromatin diminution in embryonic mitoses of Cyclops furcifer. Genetics. 1966;**54**:567-576

[22] Waddington CH. Principles of Embryology. London: G. Allen and Unwin Ltd; 1956. p. 352

[23] Fischberg M, Blackler AW. How cells specialize. Scientific American. 1961;**205**(3):124-132

[24] Alt FW et al. Regulation of genomic rearrangement events during lymphocyte differentiation. Immunology. 1986;**89**:5

[25] Teng G. Papavasilion N. Immunoglobulin somatic hypermutation. Annual Review of Genetics. 2007;**41**:107-120

[26] Ziqiano L et al. The generation of antibody diversity through somatic hypermutation and class switch recombination. Genes & Development. 2004;**18**:1-11

[27] Mange A, Mange E. Genetics: Human Aspects, Sinauer Assocs. Sunderland, Mass; 1990

[28] Bino RJ et al. Flow cytometric detection of nuclear replication stages in seed tissue. Annals of Botany. 1993;**72**:181-187

[29] Galbraith DW. et al. System endopolyploidy in Arabidopsis thaliana. Plant Physiology. 1991;**96**:985-989

[30] Burns JA, Gerstel DU. Flower colour variegation and instability of a block of heterochromatin in Nicotiana. Genetics. 1967;**57**:155

[31] McClintock B. Chromosome organization and genic expression. Cold Spring Harbor Symposia on Quantitative Biology. 1951;**16**:13-47

[32] McClintock B. The control of gene action in maize. Brookhaven Symposia in Biology. 1965; **18**:162-203

[33] Chadha S, Sharma M. Transposable elements as stress adaptive capacitors induce genomic instability in fungal pathogen Magnaporthe oryzae. PLoS One. 2014;**9**(4):1-14

[34] Azevedo JL, Roper JA. Mitotic non-conformity in Aspergillus: Successive and transposable genetic changes. Genetics Research. 1970;**16**:79-93

[35] Boyko A, Kovalchuk I. Genome instability and epigenetic modification—heritable response to environmental stress. Current Opinion in Plant Biology. 2011;**14:** 260-266

[36] Ito H et al. A stress-activated transposon in Arabidopsis induces transgenerational abscisic acid insensitivity. Published online in Scientific Reports. 2016;**6**. Article ID: 23181

[37] Pray L. Transposons or jumping genes: Not junk DNA? National Education. 2008;**1**(1):32