

Mutations: The Law of Recurrent Variation

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ABSTRACT

In spite of an overwhelming acceptance of mutations as the ultimate source of evolution, the worldwide study of induced mutations – once hoped to include the potential to speed up evolution and *inter alia* revolutionize plant and animal breeding (Muller) – has led to an entirely different (overall) result for breeding research in particular and biology in general: the law of recurrent variation. It states that “treating homozygous lines with mutagenic agents generates large, but clearly finite, spectra of mutants. This consistently occurs when the experiments are carried out on a scale adequate to isolate the potential of alleles causing phenotypic and functional deviations (saturation mutagenesis). However, due to almost invisible residual effects of changes in redundant sequences and/or of further chromosome rearrangements, the corresponding saturation curve is asymptotically approaching its limit for the micro-quantitative part of variation.”

1. INTRODUCTION

Hermann Joseph Muller (1890-1967), winner of the 1946 Nobel Prize in Physiology or Medicine “for the discovery of the production of mutations by means of X-ray irradiation” was convinced “that for the first time he had willfully changed the hereditary material and that evolution could then be speeded up” (Linskens 1982). Already in his ground-breaking paper of 1927 on the *Artificial Transmutation of the Gene* he had pointed out that “most modern geneticists will agree that gene mutations form the chief basis of organic evolution, and therefore of most of the complexities of living things” and on the basis of this agreement he finally remarked that “for the practical breeder, it is hoped that the method [of mutation induction] will ultimately prove useful”. In his Nobel lecture he emphasized both points again: the accumulation of mainly tiny changes “was the way in which natural evolution has occurred, under the guidance of natural selection” and hence this was seen to be also “the chief means of artificial animal and plant improvement”. Moreover, he was convinced that “under conditions of artificial breeding larger mutations also can be nursed through to the point where they become suitably buffered” – referring to Huxley’s proposal on the possibility how also larger mutations could be established in natural populations (Muller 1946).

These statements of Muller should be evaluated in connection with a more general revolution in biology in his lifetime: the initiation of the synthetic theory of evolution (neo-Darwinism), launched in 1937 by Dobzhansky’s book *Genetics and the Origin of Species* (for a discussion of this point, see Lönnig 2002b). From about that time on, at the latest, the majority of evolutionists viewed mutations (as defined by Muller) and selection to be the fully sufficient driving forces of the origin of all species and higher systematic categories of the plant and animal kingdoms alike. Thus being persuaded that mutations had, in fact, produced the raw materials for the origin of all genes and proteins, all physiological processes and anatomical structures of both the animal and plant kingdoms alike, the most surprising successes had to be expected by applying induced mutations and selection to animal and plant breeding research: three different time-lapse methods complementing each other for a complete success in a rather short period of time were thought to be at the disposal of the breeders: (a) multiplication of mutation rates, (b) well-aimed recombination and (c) intelligent selection.

Making the leap to the 21st century to evaluate these two topics anew – evolution by gene mutations and the meaning of induced mutations for animal and plant breeding – a most surprising divergence of opinion has to be noticed for the present thinking of most biologists: (1) the significance of gene mutations for evolution is viewed to be even more basic and substantial than ever before, (2) however, the importance of induced mutations for breeding research has almost vanished into insignificance and is generally considered today to be “occasionally useful in enlarging the genetic base of a programme in a limited and highly specific fashion” (Simmonds, see below). On the other hand: had the original expectations of Muller and most of his contemporaries become true, mutation breeding would still belong to the main topics, if not *the* main topic, of present breeding research.

As is usual in many historical treatments, the historical and scientific reasons for this separation and difference of judgement are briefly presented in the form of a series of representative quotations in the following text.

Moreover, the question is raised whether it is possible to deduce a generally valid rule or law from the immense input of 40 to 50 years of worldwide research on gene (and other) mutations in animal and plant breeding, and whether this could have any repercussions on the viewpoint of gene mutations of the modern synthesis as quoted above.

2. THE CRISIS OF MUTATION BREEDING IN AND AROUND THE 1980s

In the following paragraphs we will condense the general results for mutation breeding after several decades of intense research of this branch by directly quoting the authoritative statements of some of the world's best agronomical and botanical scientists, most of which have actively taken part in mutation breeding themselves.

Simmonds sums up some decisive points in his *Principles of Crop Improvement* (1979):

"Earlier overoptimism, to the effect that induced mutations were about to revolutionize plant breeding, has given place to a more sober appreciation of the technique as a valuable supplement to more conventional techniques in certain, rather restricted circumstances. ...[V]ery many programmes failed, especially in the early days of overoptimism, to produce anything useful because they were not fulfilled. Nowadays we see mutation-induction simply as one technique which is occasionally useful in enlarging the genetic base of a programme in a limited and highly specific fashion."

Leibenguth (1982) concurs by stating in his *Züchtungsgenetik (Genetics of Breeding)*:

"Almost all mutants distinguish themselves by negative selection values. According to observations in cereals and legumes the proportion of mutants being suitable for breeding amounted to 0.5 to 1 percent of the genotypes selected in these experiments. Besides, often a negative effect on other components of the pleiotropic spectrum of characters has been found that diminishes the breeding value of a positive mutant. Thus, nowadays the original aim to substitute the time-consuming and expensive methods of recombination breeding by "mutation breeding" is not up-to-date anymore. Mutation breeding is viewed to be less an autonomous method of breeding than an occasionally used supplement to traditional methods."

And the author adds that mutation breeding cannot be successfully applied to animal husbandry at all, because: "In contrast to plants, animals are genetically more severely balanced. Hence, all kinds of mutations are even more frequently lethal and more strongly diminishing vitality and fertility in animals."

For a similar comment on mutations in animal breeding, see Wegener and Gärtig 2002. As far as the author is aware, there is no mutation breeding of animals any more and one will search in vain for any contemporary 'animal mutation breeding newsletters' or reviews. In fact, even for plants the only available magazines on mutation breeding, the *Mutation Breeding Newsletter* (MBNL) and *Mutation Breeding Review* (MBR), have just recently been fused to *Mutation Breeding Newsletter & Reviews*. Among other reasons (partial replacement by another newsletter and the FAO/IAEA database), the editors mention that "we are facing a dwindling number of suitable submissions from outside for these two periodicals, and MBNL and MBR have become irregular publications since 2001" (Shu and Lagoda 2005).

In accord with Leibenguth's comments on plants, Micke (1976) stated: "[O]ne has to accept the fact that only a very small fraction of induced mutants (certainly less than 1%) has ever been found suitable to enter yield trials and eventually only 1% of those evaluated passed the official tests and obtained approval for commercial utilization".

In plant breeding the average proportion of negative or useless mutants to positive ones is smaller than 10,000:1. Making calculations on the basis that only 0.5% of all induced mutations were suitable for further investigations and that again only 0.5% displayed a positive selection value for the breeder, this proportion is about 40,000:1. An approximate mean value of 25,000 negative (or useless) mutants to 1 being positive should therefore not to be an unrealistic calculation for plant breeding.

As to the genetically even more severely balanced animals, the state of affairs has been so arduous that no realistic numbers can be given providing the basis of similarly approximate calculations regarding the proportions of negative (or useless) mutants to positive ones in animal husbandry. If, as an educated guess, one multiplies the proportionate number of disadvantageous mutations by a factor of 10, the result would already be some 100,000 to 400,000 negative (or unavailing or neutral) mutants with phenotypic effects to 1 useful for breeding research.

It was on the basis of such experiences, often made over dozens of years, that almost all commercial breeding stations in the USA and Europe have deleted mutation breeding from their research programmes.

A significant concrete example may back up this point: at the end of the 1960s it was still widely believed that it was possible to improve crop proteins by mutation breeding. After some one and a half decades of intensive efforts and extraordinary financial input, Micke and Weindl (1983) comment: "Our programme on the improvement of grain protein has now come to an end. ...[D]uring the 14 years of the programme it had to be recognized that the matter is more complicated and that there are some mutual limitations of quantity and quality."

Poehlmann (1987) has summed up the overall results of mutation breeding in agreement with the authors quoted above as follows: "One can only conclude that the results from mutation breeding in varietal development of the major field crops have been rather meager in relation to the efforts expended."

Peter von Sengbusch (1989 2003) concurs by the following observation: "In spite of an enormous financial expenditure, the attempt to cultivate increasingly productive varieties by irradiation, widely proved to be a failure."

Similar observations had been made all over the earth. Kiyosawa and Nomura (1988) observed: "These phenomena [amphidiploidization and mutations] undoubtedly played an important role in evolution, but the use of these methods for plant breeding is not so successful as expected at the beginning of the use, although some new varieties were developed."

Den Nijs and van Dijk (1993) comment on the expectations of mutation induction in apomictic plants: "Induction of variation by mutagenic treatment appears at first sight very promising, as useful mutations could directly be fixed by apomixis."

Yet – after providing some details – the authors close the topic as follows: "The success of mutation induction has not been evident (Bashaw *et al.* 1983), and breeders have turned their attention to other ways of inducing variation such as tissue culture."

Also, the distinguished German plant breeders Fischbeck, Röbbelen and Stutzer (1987) are in accord with these statements: "The objectives of practical plant breeding, to achieve new opportunities of a gradual and continuous amelioration of tried and tested breeding varieties could...not be realized."

And especially concerning the neo-Darwinian concept of "micro-mutations" these three authors continue: "Also, the modified concept of a direct use of so-called "micro-mutations" remained unsuccessful, because achievable breeding progress by this method distinctly lagged behind

useful variation, which could be developed from the broad stream of conventional recombination breeding.”

Yet, perhaps one of the most astounding facts in the history of genetics appears to be the enormous gulf between the optimistic descriptions of mutants by so many authors active in plant breeding research during that period of time and the later "widely spread disappointment regarding mutation breeding" (Micke 1970) due to the disconcerting reality, i.e. the meagre results obtained. Confirming the observation of a rather strange distance between hypotheses and reality, Micke continues his assessment after his calculations quoted above (explaining the relatively few useful mutants achieved in mutation breeding) as follows (Micke 1976): "In contrast to such rare achievements there have been innumerable 'promising mutants' reported in innumerable publications, which never seem to appear again on the stage after their first presentation. Nevertheless, there remains a respectable number of mutants which even the self-critical breeder or geneticist have seriously considered as progressive and of which only very few so far have contributed to the development of better crop cultivars. This experience has been disappointing to many, to those who worked with mutations and expected optimistically fast 'break-throughs' as also to those who watched the many mutation activities sceptically but nevertheless hoped that results would make the difficult task of plant breeders easier, at least in particular areas.”

In the same paper Micke also pointed out that neither the application of different mutagenic agents, nor various degrees of dosages, nor diverse modifying measures were able to revise the overall results: "The ultimate hope of obtaining more of the 'better' mutants has not been fulfilled".

3. SOME POSITIVE “SIDE EFFECTS” OF MUTATION BREEDING

Although the enormous successes and world-wide revolution firmly expected in plant and animal breeding in connection with the assumptions of the synthetic theory did not materialize, science nevertheless profited from the intense efforts of mutation breeding “by a rapid increase of the information on the localization of genetic effects in the genome of important cultivated plants” (Fischbeck *et al.* 1987). “A major contribution of mutagenesis, which can hardly be overestimated, is its use for the advancement of genetics” (Micke and Donini 1993).

Thus, basic scientific research has substantially benefited from this enterprise. In other words: “Although the production of plant mutants was economically unprofitable, it probably proved to be the most effective experimental instrument of modern basic research” (Sengbusch 1989 2003).

However, as to a deduction for a generally valid rule or law (the terms are used interchangeably here as in the case of the Mendelian rules or laws; for further points, see Lönning 2006), allowing predictions for the present and future work, the author is not aware of any endeavours comparable to the ensuing ones so far.

Regarding mutation breeding and basic questions on the origin of species, I have called the most important result of this branch of basic scientific research *the law of recurrent variation* (Lönning 1993 1995 2002a 2002c 2006), which will be the topic of the next paragraphs.

4. DEDUCING THE LAW OF RECURRENT VARIATION

A basic experimental discovery made by virtually all workers of mutation breeding and mutation research consisted of the fact that after repetitive mutagenic treatment of the lines and species tested, the spectrum of mutants will only slightly be increased by further experiments. In other words, there is a regularity in the appearance of the overall mutant phenotypes. After some 40 years of intensive mutation research in *Antirrhinum* no less a geneticist than Hans Stubbe has summed up his studies as follows (Stubbe 1966): “The continually improved knowledge of mutants in *Antirrhinum* has provided some essential experience [or results]. During the years each new large mutation trial showed that the number of really new mutants recognized for the first time, was steadily diminishing, so that the majority of the genetic changes was already known.”

Similarly, Werner Gottschalk, another of the world’s leading mutation geneticists stated (Gottschalk 1989 1994): “The larger the mutant collections are, the more difficult it is to extend them by new mutation types. Mutants preferentially arise that already exist.”

In other words, the number of mutants with new phenotypes asymptotically approaches a saturation line in persistently large mutation experiments.

The results of mutation breeding in barley achieved by Udda Lundqvist from the breeding station in Svalöf (Sweden) in decades of experiments clearly illustrate the phenomenon of recurrently appearing mutants. She reported at the end of the 1980s that during the last 50 years about 9,000 barley mutants have been isolated. Including lethal mutants, *at least* 100,000 mutants were identified (pers. comm. 1987).

The following examples of the recurrent appearance of special types of mutants have been compiled from Lundqvist (1986) in **Table 1**:

Table 1 Examples of repetitive appearance of certain types of barley mutants compiled according to data published by Lundqvist (1986).

Mutant	Appeared	No of Gene loci
Erectoides (dense spike mutants)	205 times	26
Praematurum (early maturity mutants)	110 times	9
Eceriferum (waxless mutants)	1,527 times	76
Breviaristatum (short awn mutants)	140 times	17
Exrubrum (anthocyanin-free)	61 times	18
Macrolepis (lemmalike glume mutants)	40 times	1
Hexastichon (six-row)	41 times	1
Intermedium (between two row and six-row)	144 times	11*
Powdery mildew resistant (including all kinds**)	154 times	?***

* 103 of these cases investigated; 11 *int* gene loci.

** 77 mutants were resistant against race D1, 48 had complete resistance, and 29 displayed brown necrosis.

*** Of 72 investigated resistant mutants, 54 were found to be distributed on 8 genes (the 28 recessive mutants belong to one single locus); for the remaining 18 mutants the number of loci does not appear to be fully established.

Thus, these 9 types of mutants appeared altogether 2,422 times. According to Lundqvist the 9,000 barley mutants isolated during some 50 years of extensive mutagenesis experiments, could be grouped into exactly 93 distinguishable types or classes. Many of these mutant types have appeared more than 100 times, some even more than 1,000 times (for additional details, see Lönnig 2006).

Closely comparable results have been achieved for all other crop plants ever included in mutation breeding research – e.g. pea (*Pisum sativum*), rice (*Oryza sativa*), corn (*Zea mays*), soybean (*Glycine max*) and many others (Lönnig 2002a).

Taking the work of all research stations and breeding companies around the world together, probably millions of barley mutants were induced. Of these only 25 were found to be acceptable for the list of commercial barley cultivars and 33 were added due to recombination (Hockett and Nilan 1985). However, since the average cultivation time of a commercial barley line is only about 10 years, most of these lines will not be cultivated any more.

Although in barley “mutants for just about every recognizable trait exist” (also Hockett and Nilan 1985), the results in barley may clearly illustrate Poehlmann’s comment that, as quoted above, “One can only conclude that the results from mutation breeding in varietal development of the major field crops have been rather meager in relation to the efforts expended”.

It may also be pointed out in this connection that – as far as the author is aware – neither plant breeders nor geneticists have ever reported the origin of any new species, or just any new stable races or ecotypes either surviving better or at least as well in the wild in comparison with the wild-type, in which the mutation(s) have been induced (Lönnig 1993 2001 2002a 2006, Lönnig and Becker 2004).

As to the recurrently appearing mutants, two pioneers of plant breeding research had emphasized the following key point already in the midst of the last century (Kuckuck and Mudra 1950): “As extensive experiments have shown especially in barley, the entire array of lines of the world seed bank (Weltsortiment) can be mutatively induced by X-rays...In part, these induced mutants proved to be genetically identical with similar lines of the world seed bank. In other cases the same phenotypes are due to different genes; thus in the latter cases so-called heterogeneous groups of features have been detected.”

Similar observations have been reported for several other crop plants as rice and maize. Instead of the regular and perpetual formation of new useful culture varieties, subspecies and species, incessantly the same spectrum of mutants is reproduced, so that after a certain number of experiments the method is hardly useful for plant breeding anymore.

My own studies during the last 40 years (including experiments with *Pisum*, *Antirrhinum*, and *Misopates* - altogether more than 2 million plants investigated) are in full agreement with the results of the authors quoted above (Kunze *et al.* 1997, Lönnig and Saedler 1994, Lönnig 1995 2001 2002a 2006, Lönnig *et al.* 2005).

5. FORMULATING THE LAW OF RECURRENT VARIATION

In agreement with the facts referred to above we can formulate the law of recurrent variation as follows:

Treating homozygous lines with mutagenic agents generates large, but clearly finite, spectra of mutants. This consistently occurs when the experiments are carried out on a scale adequate to isolate the potential of alleles causing phenotypic and functional deviations (saturation mutagenesis). However, due to almost invisible residual effects of changes in redundant sequences and/or of further chromosome rearrangements, the corresponding saturation curve is asymptotically approaching its limit for the micro-quantitative part of variation (Fig. 1).

Because mutations at different loci often cause similar or identical phenotypes (most traits are polygenic), the curve asymptotically approaching the saturation line for the mutant *genes* is distinct from that of the mutant *phenotypes*. In absolute terms, the distance between the curves is also aggravated by the redundancy of (1) the genetic code, (2) of gene functions, and (3) of conservative amino acid substitutions, allowing mutant proteins to approximately fulfill their original tasks and functions.

However, researchers must constantly remind themselves that the law of recurrent variation focuses only on DNA variations displaying measurable effects on the phenotype and usually disregards the rest (see also transposons, below).

In practice this means that in repetitive mutagenesis experiments the number of new mutants differing phenotypically from each other is steadily diminishing until a saturation limit is reached and progress under artificial selection comes to an end (Lönnig 1993 1995 2001 2002a 2002c).

Given similar genetical preconditions, the spontaneous mutation process in the wild will produce the same large but limited spectra of mutants, which have appeared in mutagenesis experiments. Although the mutation rate under natural conditions is usually decidedly lower, species consisting of large populations realize their potential of point mutations virtually in every generation: e.g. for the present generation of humans this means that each gene has recurrently mutated more than 100,000 times (>6,2 billion individuals, ca. 30,000 to 40,000 genes, mutation rate 10^{-5} per gene per generation).

In wild species most of the mutants will disappear shortly after their arrival because of their negative selection values.

Also, the law is valid for heterozygous lines and allogamous species. However, due to uninterrupted gene flow the curves will approach the saturation limits more slowly than in homozygous lines and autogamous species.

As for a discussion of the micro-quantitative part of variation, see Lönnig (2002c).

6. THE GENETIC BASIS OF THE LAW OF RECURRENT VARIATION

The genetic reasons for the law are rather simple. There are only a limited number of genes, which, upon mutation, can produce a restricted number of alleles, or in the words of Micke and Donini (1993): “It is important to understand the unique potential offered by induced mutations but also to realize the limitations of this tool. The first limitation is imposed by pre-existing genomes: genes that do not exist can neither be mutated nor [be] eliminated.”

Stig Blixt has summed up the point especially from the plant breeder’s view as follows (1972): A large – probably the major – part of the genes functioning in a plant are most certainly of no concern for the plant breeder, inasmuch as, being essential for the basic function of the cell and differentiation mechanisms, all genetic variation in such genes represent ‘forbidden mutations’, in the sense that the resulting mutants are completely non-functioning and immediately eliminated. A certain part of the genome may thus exist in probably one specific combination only,

representing what may be called the ultra-conservative part of the system. Another large part of the genome, which may then be called the conservative part, seems, although less rigidly, still to be required to be present in the developed form to produce a 'normal' organism able to carry out the plant functions in such a way as to result in a reproductive, competitive individual. Mutations in this part of the genome result in lethals, in sterile individuals, maybe in different kinds of severe chlorophyll deficiencies, and so on. Thus what has to be dealt with is certainly not the entire genome, but only a part of it, the variable or redundant part, and in all probability this constitutes a minor part."

As to the statement about a minor redundant part, the question of the biological meaning of transposable elements (TEs) might be raised, since up to about 90% of a plant's genome can consist of different families and classes of TEs. These problems have been extensively discussed during the last decade (e.g. Kunze *et al.* 1997, Becker and Lönnig 2002, Becker *et al.* 2002, Lönnig 2002a, Lönnig and Saedler 1994 1997 2002, Sternberg 2002) If the hypothesis is correct that most TE multiplications constitute weakly parasitic events without doing too much harm for the lines and species thus affected, such relatively huge DNA masses might be largely irrelevant for the plant breeder in the field (there are, however, several pertinent TE-effects, which appear to be similar to normal mutations like the production of alleles, including total inactivations of genes, and possibly some further ones not to be discussed here).

Nonetheless, since the DNA mass (pg) can vary strongly between closely related forms (species of the genus *Vicia*, for instance, vary between 1.8 and 13.3 pg per haploid genome (Murray *et al.* 1981, Nouzová *et al.* 1999, Nouzová *et al.* 2000), and because even within the same non-polyploid plant species, the C-value can vary substantially (Bennett and Leitch 1995 1997) – generally without known significant effects relevant for the plant breeder – we might conclude that the assessment of Blixt quoted above, is essentially still correct for any practical purposes even in our age of molecular genetics (for further details see the authors referred to in the last paragraph as well as Bennetzen 1998, Kellog and Bennetzen 2004, Bennetzen *et al.* 2005, Bennet and Leitch 2005).

So focusing on that variable or redundant part of the genome, in which mutations induce phenotypically deviant, but still rather viable, competitive, and fertile individuals and lines, what kind of variations do we detect upon closer inspection as to quality?

The variations induced are mostly loss-of-function-mutations (often including many alleles with a series of gradually reduced functions) and they are often slightly disadvantageous for the organisms thus affected, yet in some cases they are useful for the breeder (e.g. losses of gene functions coding for undesirable secondary plant metabolites harmful for human consumption) as well as micro-evolution (organ- and pigment reduction in cave animals, losses of flying abilities in insects and birds on islands around the world, losses of scales in fish species in closed lakes, losses of dispersion systems in island plants etc.).

In accord with this statement, Reinhold von Sengbusch, perhaps Germany's most successful breeder of the 20th century, summed up the essence of plant breeding by affirming that the transformation from the wild to the cultivated plant is genetically characterized by the fact that the features of the wild plants are dominant and those of the cultivated lines are recessive (Sengbusch 1980). Now, recessiveness generally means losses of gene functions (for a documentation of this point, see Lönnig 2002a).

However, the creation of entirely new functional DNA-sequences constituting new genes and new gene reaction chains for novel synorganized anatomical structures and/or physiological functions has never been achieved by induced random mutations in plants or animals. Thus, in accordance with the laws of probability, examples and cases relativizing the law of recurrent variation have not been observed so far (as for the limits of the origin of species by mutations, see Lamprecht 1974, Grassé 1977, Wittlich 1991, Scherer 1993, ReMine 1993, Margulis and Sagan 1997, Junker und Scherer 2001, Lönnig 1993 1995 2001 2002a 2002c 2002d 2004 2006, Swift 2002, Lönnig and Becker 2004, Lönnig *et al.* 2005, Meyer 2004, Schwabe 2001 2004 and many other authors). In fact, also all the models and data recently advanced to solve the problem of completely new functional sequences and the origin of new organs and organ systems by random mutations proved to be grossly insufficient in the eyes of many researchers upon close inspection and careful scientific examination (for the details, see Nilsson and Pilger 1994, Gene 2001, Behe 1996 2002 2003a 2003b 2004, Behe and Snoke 2004, Dembski 1998 2002 2004, Kefe and Szostak 2001, Berlinski 2003a 2003b, Lenski *et al.* 2003, Campbell and Meyer 2003, Trueman 2004a 2004b).

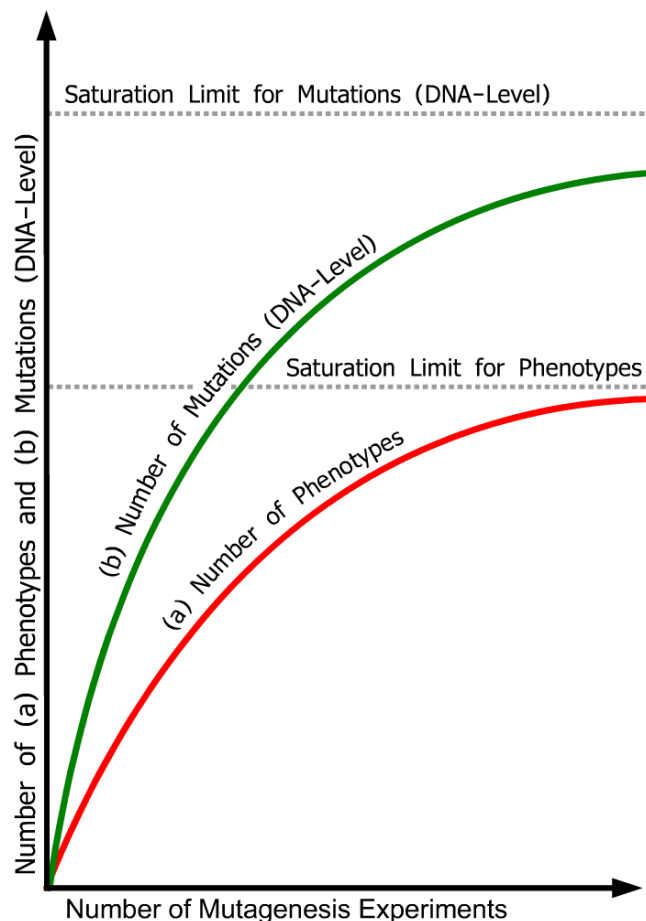


Fig. 1 Idealized saturation curves illustrating decrease of the number of new mutant phenotypes and new mutant genes in increased number of experiments until saturation limits are reached. Abscissa: increasing number of mutation experiments. Ordinate: (a) number of mutant phenotypes, and (b) number of mutations (DNA-level) with effects on the phenotype. Because mutations at different loci can cause similar or identical phenotypes (see **Table 1** above), the curve for the number of mutant genes is distinct from that of the number of new phenotypes. The redundancy problem – for example, some phenotypes appear only when 2 or more genes have been mutated (see further points in the text) – widens the distance between the two curves. The real curves will be different for different organisms, depending, among other things, on the genetic complexity of the species involved as well as on the scale and specificity of the experiments realized (different kinds and quantities of radiation, chemical mutagens, transposons, T-DNA). The common ground of all curves is the finite number of mutant phenotypes and mutant genes with effects on the phenotype (apart from a micro-quantitative rest of variations due to, for example, environmental and epigenetic factors, position effects, and 'junk DNA', which, however, does not change the basic situation).

7. PREDICTIONS AND RETRODICTIONS OF THE LAW OF RECURRENT VARIATION

The law is valid for plants and animals alike. As for plants, all the mutants which have ever been detected in and/or found to be useful for plant breeding research lie on the curves presented above (for a list, see FAO/IAEA 2004, and for a critical appraisal, see Lönning 2006). Even for very rare mutants it can usually be assumed that they have repeatedly occurred during the last decades. Just to mention two exceptional examples: as to the unstable polymorphism conveying powdery mildew resistance in barley (Pifanelli *et al.* 2004), it might be considered that the world barley production was some 155 million tons in 2004 consisting of about 3.8×10^{15} individual grains (one grain ca. 0.04 g). Hence, the occurrence of a grain carrying a mutant gene even with the low probability of only 10^{-12} per gene per generation due to a spontaneous mutation will still amount to several thousands of independent occurrences and thus grains carrying the mutant gene worldwide.

A similar calculation can be made for *Tunicate* in maize (molecular work on this mutant has been done by Thomas Münster, MPIZ, Cologne, pers. comm.), also lying at the more distal part of the saturation curve for phenotypes (Fig. 1): ca. 705 million tons of maize have been produced in 2004 (one grain ca. 0.2 g), i.e. some 3.525×10^{15} kernels. Even with the low probability of only 10^{-13} per gene per generation such a mutant could have occurred hundreds of times in 2004 alone (not to calculate the possible number of occurrences during the last decades).

Apart from polyploidy, almost all induced mutants useful for the plant breeder belong to the class of loss-of-function-mutations, as mentioned above.

The law, however, clearly excludes the origin of new complex *functional* sequences (entirely new genes and new gene reaction chains for novel synorganized anatomical structures and/or physiological functions) by random mutations. The low probability of the origin of new functional genes is a fact that also explains the necessity of genetic engineering transferring specific functional DNA sequences from one plant species to another – sequences which would never have been obtained by even the most massive mutation induction (Wittlich 1991, ReMine 1993, Dembski 1998 2002 2004, Behe and Snoke 2004).

Generally, losses of gene functions and the probability barriers to the origin of entirely new DNA sequences and functions as well as anatomical features are the window in which the law of recurrent variation describes its phenomena – the possibilities and limits of induced mutations for breeding and evolution. The limits especially explain the end of mutation breeding as an autonomous branch of breeding research in and around the 1980s and its present status of “an occasionally used supplement to traditional methods” or, as to present and future research, “this method is still used but will be replaced by directed engineering in the future” (Kahl 2004).

In all likelihood there will be no problem for the majority of contemporary biologists to accept and conceptually work with the law of recurrent variation as relevant for the factual possibilities and limits in actual mutation research and plant breeding. However, in spite of the three different time-lapse methods mentioned above (multiplication of mutation rates, well-aimed recombination, and intelligent selection), the possibility to extrapolate from the results of mutation genetics summed up in the law to any boundaries of gene (and generally genome) evolution and thus the origin of species will probably be a different topic for the majority of biologists mentioned in the introduction. Yet, an in depth discussion of the possible rational objections and scientific alternatives would necessitate a special extensive further paper beyond the scope of the present book, so that for the time being I will prefer to leave the topic open for biologists unable to accept the more inclusive inferences from the law of recurrent variation for the time window of evolution.

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