

Mutagenesis in *Physalis pubescens* L. ssp. *floridana*: Some Further Research on Dollo's Law and the Law of Recurrent Variation

Wolf-Ekkehard Lönnig*

Retired from Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Federal Republic of Germany

Correspondence: * loennig@aol.com

ABSTRACT

In a mutagenesis programme 240,000 *Physalis* plants have been investigated to further test Dollo's law and the law of recurrent variation during the last few years (2005-2008), including 10,000 M₁ plants and 8,600 M₂ families. In contrast to the predictions of Dollo's law, that "an organism cannot return, even partially, to a former state already realized in the series of its ancestors" (or, more generally that evolution is "irreversible"), in several mutants the inflated calyx syndrome (ICS, also known as "Chinese lantern") did, indeed, *partially revert* into the direction of normal sepals after fertilization, at least phenotypically. Also, in several individual cases the ICS proved to be even so strongly reduced that hardly anything of the former typical lantern structures could be identified. However, in line with Dollo's law so far, all these starkly pronounced cases turned out to be only modifications and thus could not be obtained as a distinct revertant character in a pure mutant line. Moreover, polyphyly vs. monophyly of the ICS is extensively discussed considering the different current views on the origin of species. In agreement with the predictions of another biologic law, the law of recurrent variation, a significant range of virtually identical phenotypes (including an imposing number of mutants with reduced lanterns) reappeared time and again – i.e. independently of each other – in many M₂ families, but no indications for the origin of a new *systematic* species (not to bring up the rise of a *primary* species) have been found in the range of mutants, which were probably all due to losses-of-function mutations. As to saturation mutagenesis, a plateau concerning different phenotypes may almost have been reached for some features as perhaps leaf margin structure, but certainly not yet for other characters including the ICS. Moreover, after briefly referring to some points in the introduction, several clarifications concerning (and objections against) the law of recurrent variation are addressed in the *Discussion* including the applicability of the term "law" and its relevance in biology in general as well as the principle of the "*Unendlichkeit im Kleinen*", that is microevolutionary infinity.

Keywords: Chinese lantern, Dollo's law, law of recurrent variation, mutagenesis

Abbreviations: Gy, gray (see definition below)

INTRODUCTION

During the time of "overoptimism" (Simmonds) in mutagenesis research and the climax of *mutation breeding* worldwide in the 1960s and 1970s, which – on the basis of evolutionary speculations in the wake of the modern synthesis (Muller 1927; Dobzhansky 1937; Huxley 1942; Mayr 1942; Muller 1946; Stebbins 1950; Mayr 1963; and several others) – was firmly predicting and eagerly expecting a basic revolution in plant and animal breeding due to induced mutations with the aim of substituting the conventional time and labour-consuming recombination breeding, thousands of detailed mutagenesis papers were published especially on the improvement of cultivated plant varieties (not to mention the few ones on livestock breeding).

However, in contrast to the strong anticipations and predictions, there were – to remind the reader of the fully justified and warranted verdict of Micke already referred to in an earlier paper (Lönnig 2006) – "innumerable 'promising mutants' reported in innumerable publications, which never seem to appear again on the stage after their first presentation". In fact, the overall results were "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" (both quotations according to Micke, 1976; for a more detailed discussion of the history and overall results of mutation

breeding, see Lönnig 2005; Sanford 2005, Lönnig 2006). Additionally, the inclusive results were also deeply disappointing to many protagonists and adherents of the synthetic theory of evolution (neo-Darwinism), which had provided the apparently powerful theoretical basis and had supplied strong positive predictions for the breeder's hopes and expectations from at least about 1937 onwards (an important historic fact hardly mentioned in the literature covering this era of plant breeding and mutation research).

Yet, on the other side of the coin, to explore genomes and to fully identify gene functions, gene (and further) mutations still seem to be an indispensable tool in genetics in addition to the exciting modern tools of molecular genetics like DNA sequencing, antisense RNA, siRNA, transformation, somatic embryogenesis, T-DNA etc. In fact, forward mutagenesis "probably proved to be *the most effective experimental instrument of modern basic research*" (von Sengbusch 1989, 2003, emphasis added), recently often complemented by reverse genetics.

Moreover, in sharp contrast to plant breeding where mutation induction is nowadays only "occasionally useful in enlarging the genetic base of a programme in a limited and highly specific fashion" (Simmonds 1979), it can be strongly relevant for studies of evolutionary questions like Dollo's law (Lönnig *et al.* 2007) and especially the law of recurrent variation (Lönnig 2005, 2006). In fact, by mutation induction – with saturation mutagenesis as its long-term goal – the basic statements and general implications of these biologic laws can be further investigated and tested

and either be corroborated, modified or (at least potentially) disproved. This is true not only in the better-investigated plant species like the fully sequenced *Arabidopsis thaliana*, rice (*Oryza sativa*), maize (*Zea mays*) and several others, but also generally for plants with new apomorphic features clearly distinguishing them from their nearest relatives and often also from the rest of the angiosperms. However, the issues of convergence and losses of function may also be recurrent topics in this connection (see, for example, Hu and Saedler 2007; Khan *et al.* 2009). *Physalis* with its special morphological feature of the sepal-derived and further developed "Chinese lantern" is such a case (as for similarities with and differences to *Misopates*, whose strongly elongated sepals constitute its most conspicuous apomorphic character, see below and Lönnig *et al.* 2007).

Before I am going to examine and review these questions in the *Discussion* of the present paper, it is perhaps not inappropriate to briefly address the term "law" (occurring twice in the headline of the current research article) by first referring to some of Mayr's comments on the applicability of this notion in biology. Among other points, he asserts: "Many biologists and philosophers deny the existence of universal laws in biology and suggest that all regularities be stated in probabilistic terms, as nearly all so-called biological laws have exceptions. Philosopher of science Karl Popper's famous test of falsification therefore cannot be applied in these cases" (Mayr 1999). Moreover, "there are no natural laws in biology corresponding to the natural laws of the physical sciences" (Mayr 2004a). Also, in 2001 Mayr argued as follows:

"One of the surprising things that I discovered in my work on the philosophy of biology is that when it comes to the physical sciences, any new theory is based on a law, on a natural law. Yet as several leading philosophers have stated, and I agree with them, there are no laws in biology like those of physics. Biologists often use the word law, but for something to be a law, it has to have no exceptions. A law must be beyond space and time, and therefore it cannot be specific. Every general truth in biology though is specific. Biological "laws" are restricted to certain parts of the living world, or certain localized situations, and they are restricted in time. So we can say that there are no laws in biology, except in functional biology which, as I claim, is much closer to the physical sciences, than the historical science of evolution."

I will come back and address the reasons for such statements in more detail in the *Discussion* of the paper, including some points concerning the most elementary "laws" in genetics, the Mendelian laws. However, let us briefly mention here that – as pointed out in earlier papers (Lönnig 2005, 2006; Lönnig *et al.* 2007) – the term "law" in the cases of "Dollo's law" of irreversibility and the "law of recurrent variation" is meant in its strict sense and thus Popper's criterion of falsification will in principle be applicable for both of them. As to the law of recurrent variation, one of its most basic predictions is "treating homozygous lines with mutagenic agents generates large, but clearly finite, spectra of mutants" in a saturation mutagenesis program, excluding the random generation of new complex functional sequences (entirely new genes and new gene reaction chains for novel synorganized anatomical structures and/or physiological functions) by induced or naturally occurring random mutations. Thus, the law would be refuted by any spontaneous, accidental formation of new complex functional genes and/or novel gene reaction chains with correspondingly new functional phenotypes. And Dollo's law is, of course, disproved when – in contrast to his prediction – structural or morphological reversal does occur (for an extensive discussion of this law, its history and its implications, see Lönnig *et al.* 2007).

Let us keep in mind for the empirical and theoretical gist of the present paper that the majority of present-day biologists is persuaded that the different types of random mutations (also aptly compared to "typing errors") and recombination (and some authors add the effects of trans-

posons and gene duplications) have been fully sufficient to generate all the raw materials necessary for macroevolution, including that of microbes and the totality of all species and *baupläne* of the plant and animal kingdoms past and present (to name but a few of the more prominent authors of an almost infinite list of scientists of the majority opinion, i. e. the neo-Darwinian or synthetic theory: Stebbins 1950; Simpson 1953; Heberer 1959; Tax 1960; Mayr 1963, 1970; Gottschalk 1971; Stebbins 1971, 1974; Dobzhansky 1975; Gottschalk 1976; Dobzhansky *et al.* 1977; Simpson 1984; Stebbins 1992; Gottschalk 1994; Dawkins 1997; Mayr 1999; Sauer and Rehfeld 1999; Mayr 2001, 2002; Dawkins 2003; Coyne and Orr 2004; Dawkins 2004; Mayr 2004a, 2004b; Futuyma 2005; Coyne 2009; Shubin 2009). However, against any expectations and predictions of neo-Darwinism, all the more so in the year 2009 – when this paper was written – being the 150th anniversary of the publication of *On the Origin of Species* (24 November 1859) and the 200th anniversary of Darwin's birth (12 February 1809) with commemorations around the world (including even more than 12,000 clergy; West 2007; Waters 2009; Zimmerman 2009), there is a significant and steadily growing minority of researchers remaining unconvinced of this vantage point, at least concerning gradualism (see, for example, Goldschmidt 1940; Nilsson 1953; Goldschmidt 1961; Lamprecht 1966; Eldredge and Gould 1972; Lamprecht 1974; Goldschmidt 1980; Schwabe and Warr 1984; Denton 1985; Lima de Faria 1986; Lönnig 1986; Schwabe 1986; ReMine 1993; Scherer 1993; Lönnig 1994; Lönnig 1995; Behe 1996; Gould 1996; Kunze *et al.* 1997; Lönnig and Saedler 1997; Margulis and Sagan 1997; Wesson 1997; Denton 1998; Schwabe and Büllesbach 1998; Kahle 1999; Lima de Faria 1999; Schwartz 1999; Axe 2000; Erwin 2000; Jablonsky *et al.* 2000; Lönnig 2001; Schwabe 2001; Becker and Lönnig 2002; Becker *et al.* 2002; Gould 2002; Junker 2002; Lönnig 2002; Lönnig and Saedler 2002a; Lönnig and Saedler 2002b; Schwabe 2002; Swift 2002; Berlinski 2003a, 2003b; Campbell and Meyer 2003; Conway Morris 2003a; Conway Morris 2003b; Lönnig 2003; Müller and Newman 2003; Valentine and Jablonsky 2003; Axe 2004; Behe 2004; Behe and Snoko 2004; Erwin 2004; Lönnig 2004; Lönnig and Becker 2004; Meyer 2004; Schwabe 2004; Valentine 2004; Lönnig 2005; Sanford 2005; Theißen 2005; Behe 2006; Junker and Scherer 2006; Lönnig 2006; Lönnig *et al.* 2007; Behe 2007; Gould 2007; Lönnig 2009; Meyer 2009; Theißen 2009).

For further authors, see the almost 900 scientists of the *Scientific Dissent from Darwinism* (see Discovery Institute). Among other points, many object that (1) we have yet to see the genuinely evolutionary relevant *induced* progressive phenotypes and new species able to survive in the wild, which have been postulated and predicted by the modern synthesis, asserting to explain the origin of the sum total of all life forms on earth for the last 3.8 billion years by its "two factor-theory" (Mayr 1970), i. e. by *mutations* and *selection*. (2) Many of these researchers also raise the question (among others), why – even after inducing literally billions of induced mutations and (further) chromosome rearrangements – all the important mutation breeding programmes have come to an end in the Western World (instead of eliciting a revolution in plant breeding, either by successive rounds of selected "micromutations" (cumulative selection in the sense of the modern synthesis), or by "larger mutations", which – "under conditions of artificial breeding...can be nursed through to the point where they become suitably buffered", Muller 1946, a point relevant for both, neo-Darwinism and Goldschmidt's hopeful monster hypothesis) and (3) why the law of recurrent variation is endlessly corroborated by the almost infinite repetition of the spectra of mutant phenotypes in each and any new extensive mutagenesis experiment (as predicted) instead of regularly producing a range of new systematic species by cumulative selection or otherwise, a point which leads up to the experimental part of the present paper.

MATERIALS AND METHODS

First several pilot projects with *Physalis pubescens* L. ssp. *floridana* (Linné 1753; often abbreviated to *Physalis floridana*) were performed in 2005 to test for homozygosity of the material available for this species and also to study its behavior under different environmental conditions, (a) distinct soil constitutions, (b) temperatures, (c) growth and lantern formation in the field in differently sized Jiffies, (d) documentation of the behavior of potential pollinators etc. Moreover, dry seeds were treated with gamma rays at the nuclear research center Jülich (Kernforschungszentrum Jülich, Germany) by graduate engineer Manfred Thomé. First, a series of treatments were performed to detect the most promising applications of gamma rays for the large mutagenesis project intended: 200 Gy, 300 Gy, 400 Gy and 500 Gy (one gray being equal to the dose of one joule of energy absorbed per one kilogram of matter). In this pilot project I obtained the following numbers of plants after different gamma applications in comparison to the untreated control:

Gy	№ of seeds	№ of plants obtained	Percentages of plants obtained from seeds
Control	352	188	53.40
200	994	715	71.93
300	965	118	12.23
400	842	13	1.54
500	648	4	0.62

As to the perhaps unexpected number of plants obtained from seeds after treatment with 200 Gy, it should be noted that it has been found time and again in mutagenesis experiments that a relatively low dose of different kinds of radiation can stimulate seed germination (see, for example Chicea and Racuciu 2008), an impression which was also gained from the work with *Antirrhinum*. However, 200 Gy seems to be already a rather high dose of radiation, even for dry *Physalis* seeds and reiteration of such procedures with corresponding results would be necessary to fully establish it in the above case. Regarding the overall germination results, applications between 200 Gy und 300 Gy proved to be most promising for the further large experiments and thus another series (with 200, 220, 240, 260, 280 Gy) was carried out. In the following text, the dosage numbers in Gray are given before the individual mutants, as for example "mutant 240 Gy/2412", meaning that the M₁-seed was treated with 240 Gy, the second number refers to the family studied. Usually these families consisted of 27 M₂-plants each, being the descendents of one of the thousands of consecutively numbered M₁-plants. The number behind the slash refers to the number of the family, in which the mutant appeared. In 2006 I evaluated 3,500 M₂-families and in 2007 I investigated 5,100 M₂-families in the field (so altogether 8,600 families), including 10,000 M₁ plants constituting altogether about 240,000 plants so far.

Since the results of mutagenesis relevant for Dollo's law and for the law of recurrent variation are strongly overlapping, both topics are sometimes briefly addressed in the following sections of the *Results*, but in the *Discussion* are referred to in more detail including some supplementary information of the impact and meaning of the overall experimental results in *Physalis* for these laws. Moreover, most of the mutants displayed pleiotropic effects on many organs so that often it was not possible to clearly group them, for instance, into well defined categories with perhaps some special effects only on lanterns, or the leaves or flowers or shoots. Nevertheless, let's start with mutants displaying aberrations in the most visible and most strongly apomorphic feature of *Physalis*, the ICS (inflated calyx syndrome) or Chinese lantern.

RESULTS

Chinese lantern mutants

1. Lantern mutant 240 Gy/962

The first lantern mutant discovered in the experimental field

(7 July 2006, 18.03 hour), was mutant 240 Gy/962 as shown in **Fig. 1A-M**.

The mutant displays 5 to 7 sepals, petals and stamens (in **Fig. 1A** it shows 7, in **1C** it has 6). In comparison to the wildtype (WT), the sepals are definitely larger before fertilization: 10 to 15 mm in the mutant, thus as long as its petals (**Fig. 1A-C**). The sepals of the WT are only 5 mm long at this stage (**Fig. 1D**). Yet, the sepals of the mutant display only a relatively weak growth after fertilization (up to 20 mm in the mutant, **Fig. 1I, 1J**) when the WT starts growing its sepals enormously to generate the large Chinese lanterns (ICS up to 50 mm in length (**Fig. 1L**)). Moreover, in most mutants – probably depending on the developmental stage of the plants and environmental conditions (concerning more details on the latter, see text for mutant 240 Gy/938 below) – the sepals regularly do *not* form a lantern but their distal parts frequently stay separated and, in fact, even wide open so that the berry can be seen (**Fig. 1G, 1H**), yet in some other phenotypic deviations (modifications) of the mutant, the sepals can form a relatively small lantern-like covering around the berry either closing tightly around it (**Fig. 1I**) or at a small distance from it (**Fig. 1J, 1L**). Another unusual feature, which has to be mentioned for this mutant are the strongly elongated pedicels (**Fig. 1M**).

Independently of the mutant just described, two further mutants with very similar phenotypes (i.e. almost identical phenotypes in agreement with the law of recurrent variation) appeared in the following year: 220 Gy/3922 and 280 Gy/46 (photographs can be retrieved in the supplementary www-document of Lönnig 2009a, see slide 15 with 7 figures). Locus identity test crosses were not successful so far. Thus, at present it is unknown whether losses of DNA functions of the same gene (hence producing three different alleles) or losses at different loci are involved.

2. Lantern reduction and variation in mutant 240 Gy/938

Lantern reduction was still more pronounced in mutant 240 Gy/938 (see **Fig. 2A, 2B**).

Most important perhaps is the fact that in this mutant the berries often grow larger than the growth-retarded lanterns, so that they can even break the small lanterns up (**Fig. 3C**, left side of the ICS). Nevertheless, in spite of the overall uniform appearance of the ICS in the M₃ population as shown in **Fig. 2A**, also some variation of the mutant's phenotype has to be noted (see **Fig. 3D**). For example, in the M₂ population there were lanterns, although being distinctly shorter than the WT, yet definitely not so strongly reduced as perceived in the rather uniform M₃ population in **Fig. 2A** above.

Thus, quite surprisingly, in the M₂ population the ICS of many mutant plants looked much more normal than the rather uniformly growth-retarded lanterns in M₃. The reasons for such phenotypic variation – which all may be categorized as modifications (being by definition non-inheritable), between (and in several cases also within) the two generations – could be manifold: varying environmental parameters like temperature, light intensity and spectral composition (especially UV-light present or absent), degree of moisture in soil and air, day length, intensity of fungal and other infections (of root, shoot and/or leaves as well as flower organs) etc. may be involved, solitary or in combination of two or more factors. My impression is that the mutant phenotypes are generally more strongly affected by varying environmental parameters than the WT plants, which appear to be definitely better buffered against such milieu differences and more resistant against fungus and virus attacks and also against pests – perhaps not unexpectedly so for loss-of-function mutations. Additionally, at least in a minority of the cases, heterozygosity (WT/mutant gene: Aa) could be relevant, also epigenetic factors.

Concerning the similar lanterns of mutant 220 Gy/3911, a mutant which also displays a range of different lantern phenotypes from smaller but otherwise almost normal ones to strongly growth-retarded examples exhibiting large parts of

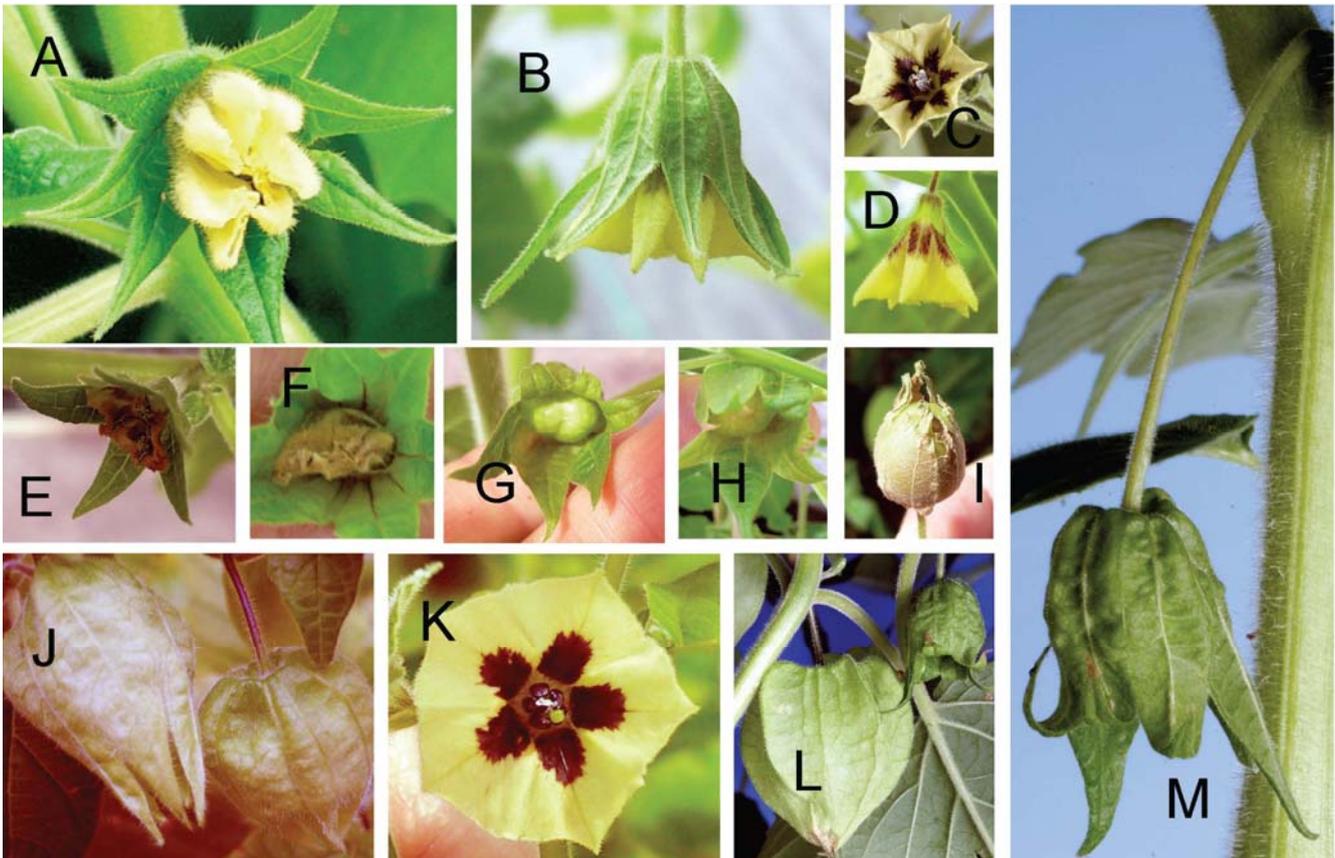


Fig. 1 (A) and (C) Front view of mutant 240 Gy/962 with varying organ numbers (in the present case seven). (B) Same mutant, side view (note that in all three figures the sepals are elongated before fertilization. This stands in strong contrast to the wildtype development with its small sepals as shown in (D)). (E) - (G) different stages of the mutant's development after fertilization: first the flower is withering away (E), (F), as in the wildtype after fertilization. However, in comparison to the latter, growth of the sepals is retarded and instead of forming a lantern they often bent outwards so that the fruit can directly be seen (G), (H). Yet many rather strong modifications of the phenotype can also be found: the relatively small sepals can also grow tightly around the fruit and open at the tip (I). (J) Another modification in two lanterns, in which the sepals surround the fruit at some distance, so that some space exists between the fruit and the sepals. (K) Normal (wildtype) flower. (L) Direct comparison of wildtype lantern (left) with small open mutant lantern (right above). (M) Regularly strongly elongated flower pedicel of the mutant.

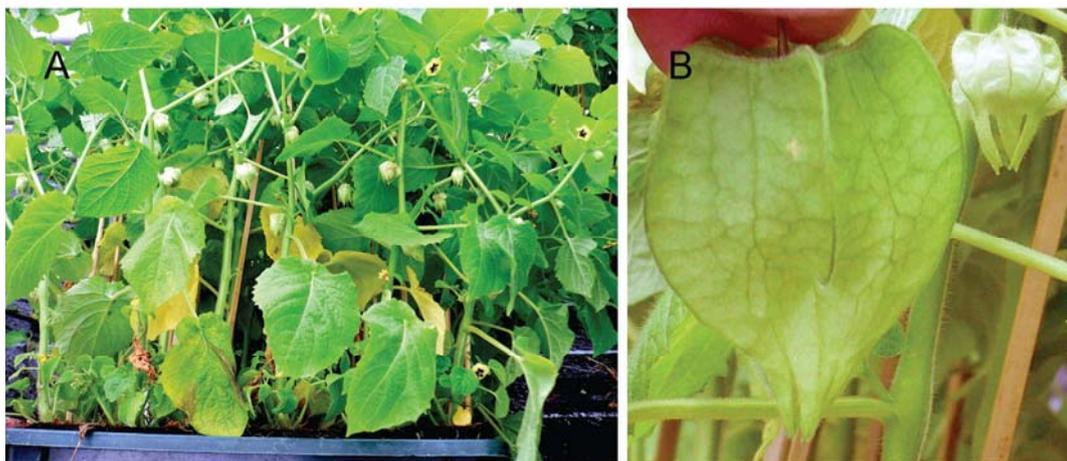


Fig. 2 Lantern mutant 240 Gy/938. (A) M₃ population of the mutant, all individual plants with lanterns of reduced size. (B) Comparison of wildtype (left) with mutant lantern (right above): note the extreme difference in size.

the berry and additionally showing strongly affected/abnormal leaf formation and overall distinctly smaller plants compared to the WT, see the phenotypes and variation under the supplementary material to the present paper in the www-document of Lönnig 2009a, slide 22 with 7 figures.

3. Even further lantern reduction in mutants 260 Gy/1820, 220 Gy/1676 and 220 Gy/801

The sepals of mutants 260 Gy/1820, 220 Gy/1676 and 220

Gy/801 are probably constituting the most strongly reduced heritable lanterns so far. Just to describe the flower and fruit of the mutant 260 Gy/1820 in some detail: First, the petals of the mutant are often deformed and not fused. Second, the male and female parts are usually sterile and only after many repeated trials there is any berry development at all. Third, in this case (i.e. fertilization and corresponding berry formation) the petals stay at and are, in fact, fused to the slightly growing calyx – in contrast to the behaviour of the WT where the fused petals forming the corolla are shed.



Fig. 3 Variation of the lantern in mutant 240 Gy/938. In (A), (B), and (C): The developing berry regularly grows larger than the growth-retarded Chinese lantern so that it can even break it up, as can especially be seen in (C) above on the left hand side of the fruit. (D) A much more normal looking phenotype of the mutant (right), which appeared in the M_2 . Some possible explanations for the differences are given in the text.



Fig. 4 Reduction of petals, stamens and lantern of the mutant 260 Gy/1820. (A) One of the small minority of successful pollinations and fruit development with seeds in it. The petals, which are fused in the *wildtype* from the very beginning of their development and already shed at the beginning of fruit formation, are separate in the mutant from the start and are staying at the calyx. (B) Calyx of the same flower, side view, showing the largest "lantern" so far. (C) Habitus of the mutant with its birch-like leaves.

Fourth, in comparison to the large lanterns of the WT (length of 50 mm), the calyx remains rather small (12 mm) and, in fact, the lantern is almost reduced to a calyx-like feature (see the details in **Fig. 4A, 4B**) reminding, perhaps, a bit of the calyx of *Corylus avellana* (hazelnut). Fifth, in contrast to mutant 240 Gy/938 (see **Fig. 3**) the small calyx remains wide open and does not even close tightly around the berry. Although this was true in many cases also for lantern mutant 240 Gy/962 (**Fig. 1J, 1L, 1M**), as a modification the latter can also tensely close around the fruit (**Fig. 1I**). In distinction to mutant 240 Gy/938, larger lanterns of mutant 260 Gy/1820 (as shown in **Fig. 4B**) have not been detected. However, it has to be admitted that only very few pollinations have been successful and hence variation within a necessary large population could not be studied so far. As to the overall habitus of the mutant, already from a distance one can recognize that the leaves are distinctly smaller than those of the WT, reminding of the leaves of a birch tree (**Fig. 4C**).

Below I will come back to the question whether such mutants as the ones just described as well as the following ones may be relevant for Dollo's law (see the *Discussion*).

In the mutants 220 Gy/1676 and 220 Gy/801 the lanterns start growing without fertilization, but then most of them – including the rest of the flower structures – are quite unexpectedly shed. Nevertheless, in some cases small lanterns are formed (**Fig. 5B**). Also, the sepals and petals look distinctly different from the WT: (1) Pedicels are elongated (**Fig. 5A**). (2) The tips of the sepals appear to be shortened until fertilization and the basal parts of the fused sepals appear to be more bloated (also **Fig. 5A**). (3) The number of

the petals is raised (up to 10) and they form a rather irregular petal whorl. (4) Additionally, the honey guides are irregular and more slender (**Fig. 5C**). (5) The style is elongated and protrudes from the rest of the flower. (6) Stamen formation is reduced. (7) When lanterns develop at all, they remain open with now elongated non-fused tips (**Fig. 5B**). (8) In contrast to mutant 220 Gy/1676, the independently arisen but very similar looking mutant 220 Gy/801 every now and then can and does form some small berries due to autogamy.

In the following examples the lanterns are not so strongly reduced but display some other characters which may also be relevant for Dollo's law and the law of recurrent variation.

4. Mutants with reduction of particularly the tips of the lanterns

Two phenotypically almost identical mutants appeared independently of each other in two different M_2 families, mutant 220 Gy/2666 and mutant 220 Gy/166, which displayed the following characteristics in distinction to the WT: (1) Smaller flowers, (2) honey guide enlarged, (3) sepals already smaller in the flower's petal stage (anthesis), (4) all lanterns open, (5) size reduced, (6) form more cylindrical than in the WT, (7) tips of lanterns starkly diminished (almost half as long as compared to the WT), (8) fertility strongly reduced (male and female), (9) yet occasionally can produce a fruit (**Fig. 6A-E**).

The two virtually identical phenotypes segregating independently of each other in different M_2 families remind

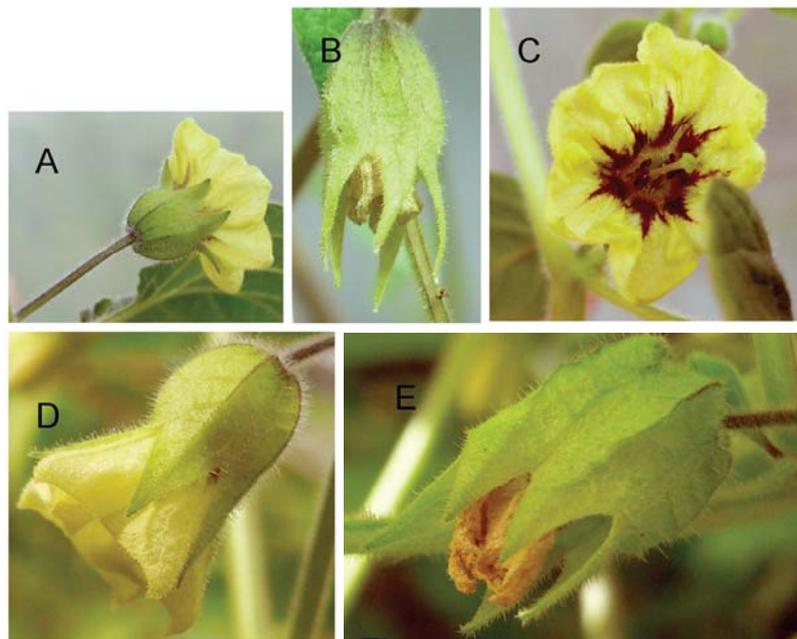


Fig. 5 Flower anomalies and lantern reduction in mutants 220 Gy/1676 and 220 Gy/801. (A), (D) The calyx appears to be more bloated and "bigger" than the wildtype calyx before and during anthesis (A: 220 Gy/1676, D: 220 Gy/801). (B), (E) When lantern-like structures are formed, the now elongating tips do not fuse as in the wildtype so that calyx stays open. (C) Petal number is raised and the irregular honey guides are smaller than in wildtype.

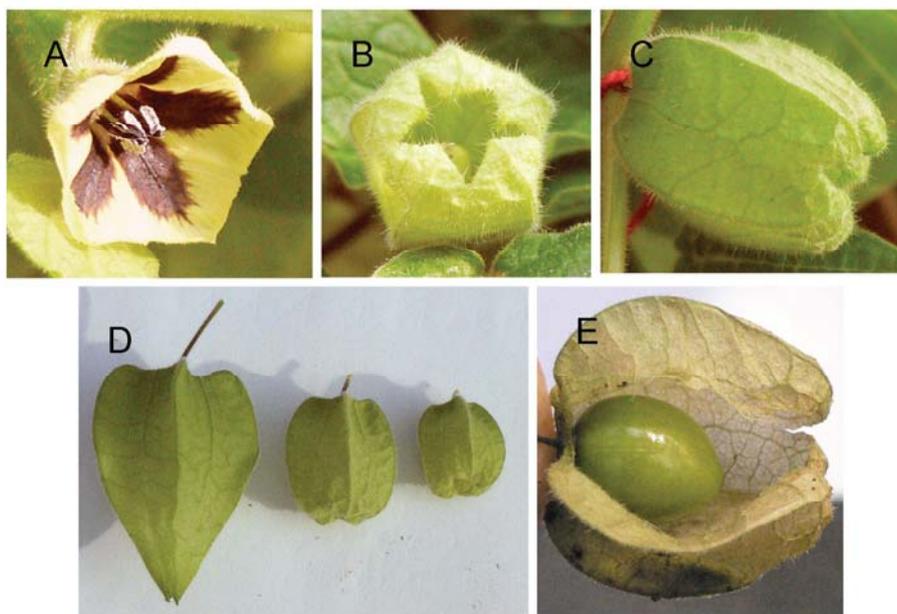


Fig. 6 (A) – (D) Mutants 220 Gy/2666 (A)-(D) and 220 Gy/166. (A) In contrast to the mutants 240 Gy/962 and others described above, the petals are distinctly smaller than those of the wildtype (for the latter see Fig. 1K), the tips of the petals are almost missing, but the honeyguides are vigorously enlarged. (B)-(D): The fully developed but reduced lanterns of the mutant. (B) Seen from above with developing berry to be partly recognized inside, (C) side view of the reduced nearly cylindrical lantern. (D) On the left of this figure: The typical wildtype Chinese lantern, and on the right beside it two lanterns illustrating variation in size of mutant 220 Gy/2666. (E) Phenotypically almost identical mutant 220 Gy/166 with berry. It was cut open displaying the berry within.

of the comparable cases mentioned above for mutants (a) 220 Gy/1676, 220 Gy/801 and (b) 240 Gy/962, 220 Gy/3922, 280 Gy/46, where a similar generation of the respective phenotypes has also occurred twice in the former and even three times separately in the latter case (so in the latter case similar phenotypes were found in the descendants of M_1 plants even after three different treatments, i.e. with 220 Gy, 240 Gy, and 280 Gy). From the technical feasibility perspective 8,600 families in *Physalis* is already very much in the experimental field, and, in fact – concerning our lines of research – a range of further groups of phenotypes very similar to each other or almost identical within a group have repeatedly (and, as mutation events, independently) occurred in the plant material in agreement

with the law of recurrent variation (see further points below). However, the phenotypes of two of the mutants reported above, 240 Gy/938 and 260 Gy/1820, seem to have appeared only once so far. Yet the law of recurrent variation predicts that in a regular repetition of such field trials of say altogether 1,000,000 and more M_2 families (hardly realizable in *Physalis* at present for one or a few geneticists for practical and financial reasons, but reverse genetics may assist here) also those phenotypes would recurrently appear independently of each other in different families in the experimental field (except, perhaps, in some extremely rare cases at the far right of the saturation curve, see Lönnig 2005, 2006). We will return to this topic in the *Discussion* below.

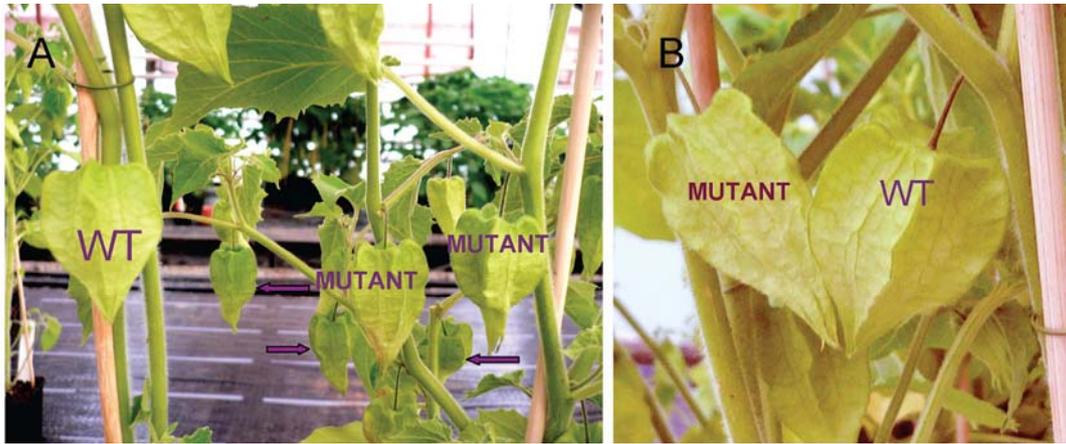


Fig. 7 (A) On the left the large wildtype lantern, on the right several lanterns of mutant 240 Gy/985, which are more slender than those of the wildtype. (B) Same mutant on the left of the figure and the wildtype now on the right hand side, wildtype and mutant almost touching one another. Mutants with more slender lanterns have also appeared many times independently of each other.

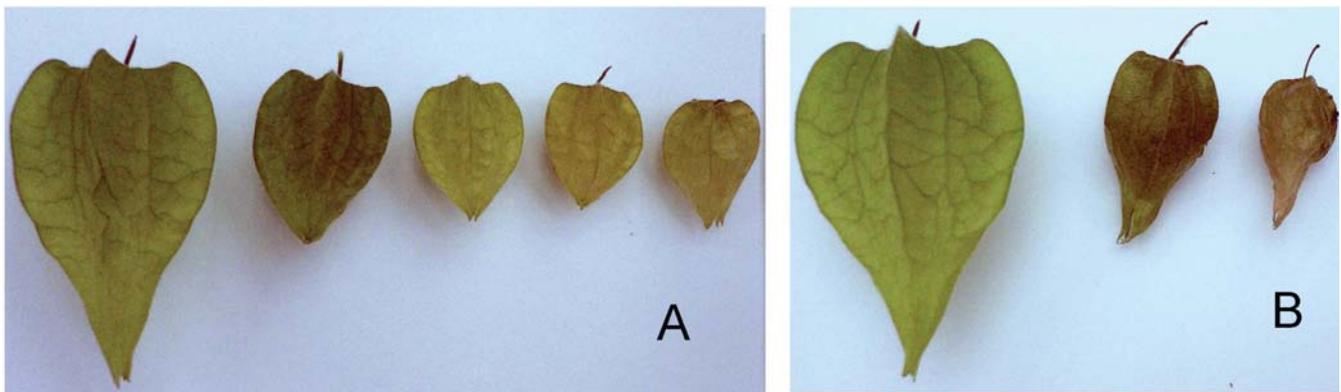


Fig. 8 (A) Mutant 260 Gy/1405. On the far left the wildtype phenotype, on the right four examples of the lanterns of the mutant displaying slight variation in size. (B) 260 Gy/1698, order as in (A): left the wildtype, right two lanterns of the mutant. In line with the law of recurrent variation, such smaller lanterns appeared independently of each other also in the following M₂ populations: (1) 220 Gy/ 711, (2) 220 Gy/ 740, (3) 220 Gy/1543, (4) 220 Gy/1577, (5) 220 Gy/1869, (6) 220 Gy/1899, (7) 240 Gy/ 941, (8) 240 Gy/1236, (9) 240 Gy/1958, (10) 240 Gy/2701, (11) 260 Gy/ 386, (12) 260 Gy/ 527, (13) 260 Gy/ 946, (14) 260 Gy/1521, and, in fact, several more cases (see the figures in the supplementary material under <http://www.weloennig.de/Physalis1a.pdf>, especially slides 16, 17, 21, 24, 28, 29, 30 and 41).

5. Further lantern mutants: (1) more slender ones and (2) overall smaller but otherwise rather normal phenotypes

A range of further lantern mutants has appeared in the field trials: several independent cases with very similar looking lanterns being as long as those of the WT but clearly more slender (Fig. 7)

Moreover, there were many mutants with smaller lanterns of otherwise more or less normal form, in most cases almost miniature versions of the WT lanterns, as shown for mutants 260 Gy/1405 and 260 Gy/1698 in Fig. 8.

An important point to be mentioned for these mutants is the fact that most of them were developing lanterns *without* fertilization, a fact in clear contrast to the finding that in the WT, lantern formation started *only* after fertilization (He and Saedler 2005, 2007a, 2007b). Hence, this tight correlation between lantern development and fertilization in the WT appears to have been abolished in most of these mutants, so that in such cases lantern formation is now definitely independent of and clearly decoupled from fertilization. I will briefly come back to this point in the *Discussion*.

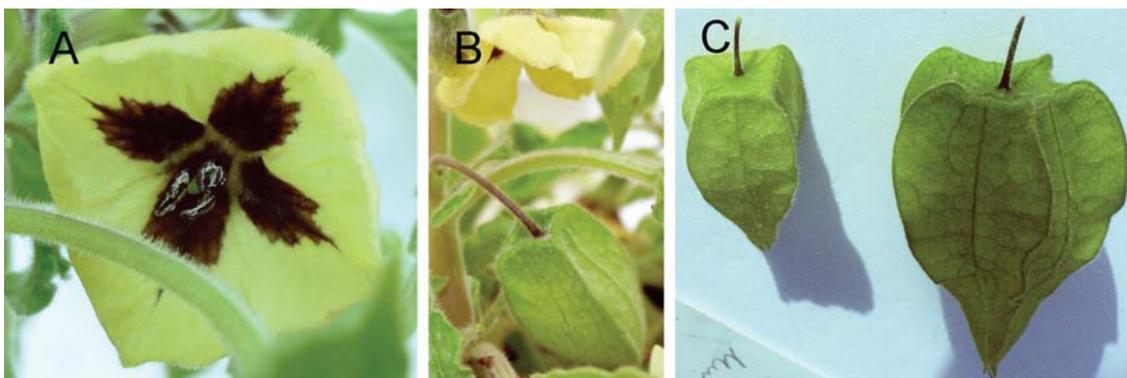


Fig. 9 (A) Tetrapetalous mutant of *Physalis* 240 Gy/3979: (B) Tetrapetalous flower (above) and correspondingly tetramerous Chinese lantern (below). (C) Left: 220 Gy/468 in comparison to wildtype lantern. Such phenotypes appeared seven times independently of each other in different M₂ populations.

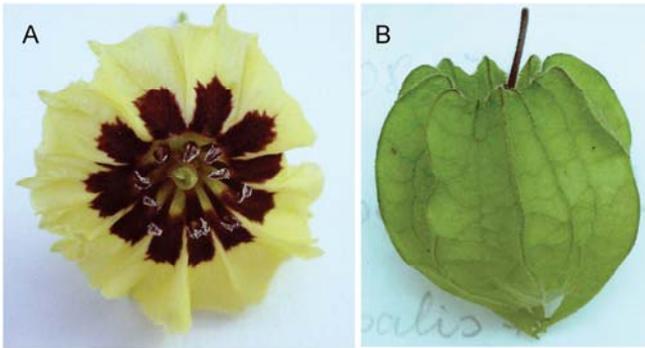


Fig. 10 (A) Dodecapetalous flower of mutant 240 Gy/3911. (B) Enneamerous lantern of same mutant.

6. Tetrapetalous flowers and one dodecapetalous/enneamerous phenotype

Mutants displaying tetrapetalous flowers and correspondingly tetramerous lanterns arose seven times independently of each other in different M_2 families: 220 Gy/468, 220 Gy/3008, 220 Gy/3205, 220 Gy/3902, 220 Gy/2503, 240 Gy/3645, and 240 Gy/3979. In **Fig. 9A** the flower of the latter is shown and in **Fig. 9B** the tetramerous lantern.

In contrast to the tetramerous mutants, mutant 240 Gy/3911 displayed flowers with up to 12 petals (dodecapetalous) and 9 clear rims (enneamerous) in the lantern (**Fig. 10A, 10B**). However, in the descendents of the next generation, the M_3 , almost only flowers with 7 to 8 petals and a corresponding number of rims in the lanterns were found (not shown here). Possibly environmental factors are involved in this phenomenon (see the details above).

Such mutants with variable phenotypes, which are not fully preserved to the next generation, or reappear only under very special environmental conditions, lead up to the next topic, namely to some even more extreme lantern modifications.

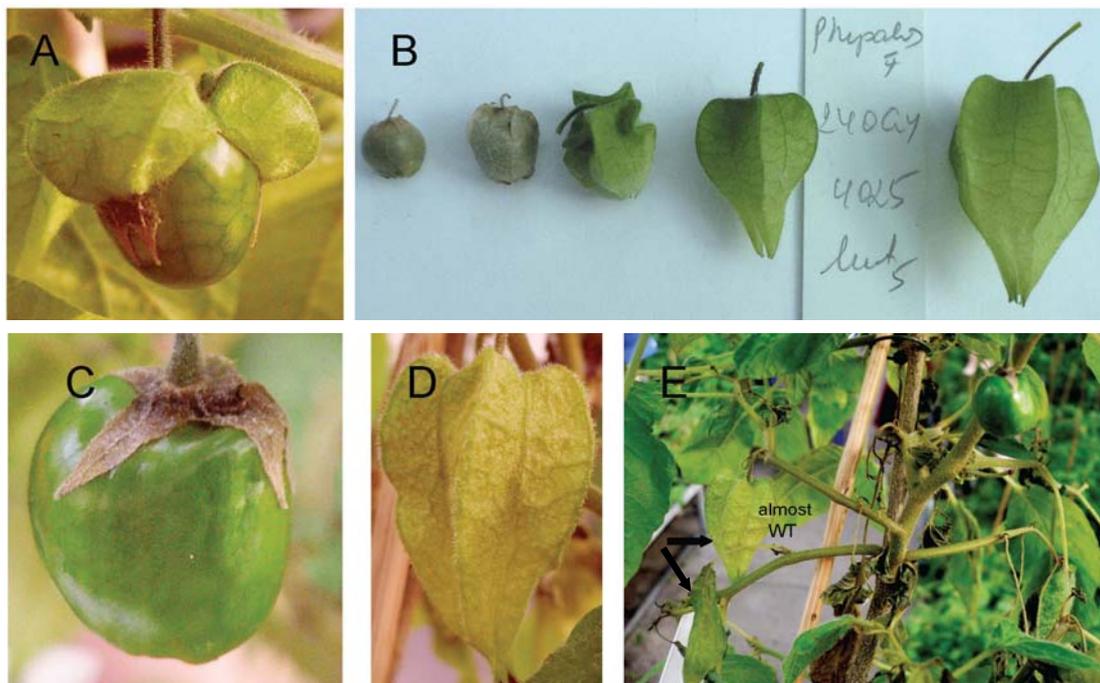


Fig. 11 Mutant 240 Gy/4025. (A) The residual structures of the lanterns stay regularly on top of the berries like the hats of some Chinese folk fashion wear. (B) However, also in this mutant strong variation could be detected: the four lanterns on the left of the tag showing different modifications from intense reduction tightly around the berry (far left) to nearly normal (fourth from left to right), so that the typical wildtype lantern is only about 1 cm longer than that of the mutant (right side of the tag). **Mutant 280 Gy/246:** (C) Utter reduction of lantern to some residual sepal-like structures in contrast to the almost normal form of the lantern in the same mutant and same plant individual as shown in (D). (E) This photo exemplifies that such extreme differences (modifications) can occur even at the same plant (note the arrows).



Fig. 12 The *globosa* mutant of *Physalis*. (A) Flower with two whorls of sepals, the inner sepal whorl shorter than the normal outer one and bent inwards. (B) Front sepals of the two whorls removed. In contrast to *Antirrhinum* and *Misopates*, several nonfused carpels with styles and stigmas can be distinguished. (C) Dry outer lantern cut open displaying second inner lantern.

7. Extreme lantern modifications

Before we turn our attention to the extraordinary phenotype of the *globosa* mutant in *Physalis*, let us first have another look at some of the extreme lantern modifications appearing in our plant material.

The lanterns of the mutant 240 Gy/4025 and 280 Gy/246 often display extreme phenotypic differences as evidenced in **Fig. 11**, from inordinate reductions showing only some residual sepal-like structures (**Fig. 11C**) to almost normal ICS form yet smaller in size.

At present it is an open question whether such extremely nonheritable variation as documented in **Fig. 11** will be continued in the next generations or whether the phenotypes of the different mutants would become less variable as appears to be the case for mutant 240 Gy/938 in the M₃ population (see **Fig. 2**).

8. The *globosa* mutant of *Physalis*

It was expected that losses in the homeotic B class genes, *Deficiens* and *Globosa* – whose homeotic phenotype was, as far as is known, first detected in *Antirrhinum majus* in 1917 (Baur 1924, 1930; Stubbe 1966), with *Deficiens* being the first homeotic plant gene to be cloned and characterized (Sommer *et al.* 1990; Schwarz-Sommer *et al.* 1992; shortly followed by *Globosa*, Tröbner *et al.* 1992) – would display an extraordinarily uncommon phenotype in *Physalis*. In the *deficiens* and *globosa* mutants of *A. majus* (and in other flowering plants with null mutations in the homologous class B gene(s)), the petal whorl is transformed into a second whorl of sepals and stamens to carpels. Thus, I assumed that such a mutant in *Physalis* developing two whorls of sepals in anthesis would also later develop a lantern within a lantern. The prediction was, in fact, fulfilled as expected (see **Fig. 12**). Note that, in contrast to the WT, in this mutant too, lantern formation – even double lantern formation – again occurred regularly without fertilization.

Even so, there are also some differences to be noted for this *globosa* mutant of *Physalis* in comparison to the known B mutants of other dicotyledonous plant species (apart, of course, from the fact that most of the latter do not form lanterns), the most prominent feature being several free carpels (**Fig. 12B**). Although carpel number is also raised in the B mutants of *Antirrhinum*, *Misopates*, *Arabidopsis* (Jack *et al.*

1992) and others, they are fused with each other in the homeotic mutants of the latter species.

Unfortunately no clear *plena* mutants have appeared in our M₂ plant material so far, but it can be predicted from the mutants in other dicots that a homozygous recombinant between a B mutant and a *plena* mutant of *Physalis* (representing a C mutant – transforming stamens and carpels into petals and sepals additionally repeating this process almost unlimited) – thus would produce a phenotype consisting of a nearly infinite series of lanterns within lanterns.

9. Leaf mutants

The typical WT leaf of the *Physalis* line is shown in **Fig. 13A** on the left. Interestingly, several independently arisen leaf mutants appeared in the M₂ families displaying entire leaf margins (**Fig. 13B**). Also, regularly mutants displaying smaller leaves segregated in the M₂ families reminding in form and size somewhat of the leaves of birch trees (**Fig. 13A** on the right). Moreover, at least two independent cases of mutants with extremely slender lanceolate leaves (**Fig. 13C** (enlarged in **Fig. 13D**) in contrast to the WT right) were found. Both proved to be totally sterile.

10. Series of mutants with reduced or enlarged honey guides

The regularity and number of the induced honey guide mutants were really exciting. **Fig. 14** presents an overview of such independently arisen mutants aptly illustrating the law of recurrent variation for this feature. Molecular investigations will reveal whether the discoveries of Noda *et al.* (1993) and Schwinn *et al.* (2006) on the control of floral pigmentation intensity and patterning in the genus *Antirrhinum* by a small family of MYB-regulatory genes are also relevant for comparable differences in *Physalis*.

Not only the space of a paper but the scope of a book would be required to describe all the different mutants found for *Physalis* in the mutagenesis experiments so far (just to illustrate, I have taken more than 19,000 photos of the plant material). However, we have to be content in the present journal paper to show several remarkable mutants relevant for our topic, i.e. for "some further research on Dollo's law and the law of recurrent variation". The reader who is interested in the phenotypes of further different

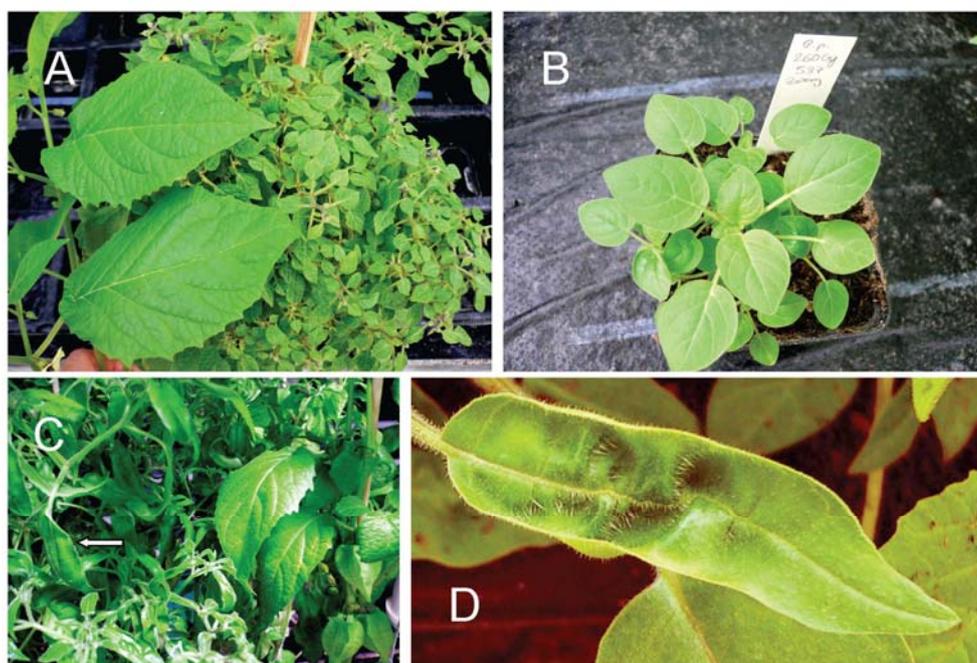


Fig. 13 (A) Wildtype leaves of *Physalis pubescens* ssp. *floridana* (left), in comparison with mutant 220 Gy/1503: leaves very small, margins almost entire. (B) Mutant 260 Gy/537 displaying entire leaf margins. (C) Two independently arisen mutants show extremely slender lanceolate leaves (left in contrast to the wildtype on the right), both proved to be totally sterile. (D) A slender leaf enlarged.



Fig. 14 A series of independently arisen honey guide mutants in *Physalis*. The size of the honey guides was either reduced or enlarged and the colour was varying strongly: (A) wildtype flower for comparison. The first four mutants (B-E) display strongly reduced colour intensity but the area covered was roughly the same as in the wildtype. In the mutants (F-N) colour intensity is normal or only slightly reduced but the area is mostly strongly diminished. In O only a very residual ("rudimentary") honey guide can be recognized and in P nothing seems to be left over of it. In Q-T the coloured area is enlarged (Q: on the left the wildtype again, on the right the mutant). In the last two mutants U and V the coloured area seems to be slightly diminished but of the same intensity as in the wildtype. (Numbers of the mutants from B – V: 220 Gy/149, 220 Gy/436, 220 Gy/1747, 220 Gy/2233, 220 Gy/3121, 220 Gy/2834, 220 Gy/3911, 240 Gy/4082, 260 Gy/1950, 220 Gy/2442, 240 Gy/4045, 220 Gy/1676, 220 Gy/3068, 220 Gy/281, Wildtype and 220 Gy/2085, 220 Gy/3100, 240 Gy/2344, 220 Gy/2031, 220 Gy/60 and wildtype, 240 Gy/3812, and further independently arisen cases not shown here: 200 Gy/47 and 200 Gy/249 like 220 Gy/149; 220 Gy/62 and 280 Gy/255 like 220 Gy/2834; 220 Gy/3648 like 220 Gy/3121.)

mutants and their independent occurrence (as, for instance, (a) also regularly appearing mutants with additional different leaf margins and form, (b) leaf colour variation, as well as (c) the strange "curly cale" mutant reminding of Brussel's sprouts, mutants without inflorescences reminding of the *bracteomania* mutants of *Antirrhinum* and *Misopates*; cf. Huijser *et al.* 1992, Lönnig *et al.* 2007; and many others) may be referred to the supplementary material in the wwdocument of Lönnig 2009a. So, after a preliminary general remark, we turn our attention first to the relationship between the *Physalis* mutants and Dollo's law.

DISCUSSION

Introductory remark

Recently an editor of the prestigious *EMBO Reports* complained about conformism in science and its stifling effects on progress: "It is rather ironic that most scientists regard themselves as free-minded individuals but often spurn diversity." And "...too much conformism means that we are missing important opportunities to gain new knowledge. ... After all, many 'weird' ideas – from quantum physics to prions – turned out to be true and provided enormously enriching insights" (Gannon 2007, p 885). However, following this important advice here and thus to present an in-depth-discussion of the mutagenesis results found in *Physalis* with the two laws mentioned above in mind (including a historical perspective and non-conformist approaches to evolution), may also require a correspondingly unusual length of the *Discussion*-section, as is the case in the present article.

Physalis mutants and Dollo's law

In the paper *Biodiversity and Dollo's Law: To What Extent can the Phenotypic Differences between Misopates orontium and Antirrhinum majus be Bridged by Mutagenesis* (Lönnig *et al.* 2007), the authors have extensively discussed the meaning and applications of Dollo's law (to which I may refer my readers for additional information) as well as several putative exceptions to this law (Marshall *et al.* 1994; Bull 2000; Teotonio and Rose 2000, 2001; Collin and Cipriani 2003; Whiting *et al.* 2003; to be added are now are Pagel 2004; Zander 2006; Domes *et al.* 2007), so that it will suffice here to focus our attention on the ensuing points relevant for our topic instead of repeating the entire account already presented earlier.

In medias res: Among other things, Louis Dollo asserts that according to his law "[A]n organism cannot return, even partially, to a former state already realized in the series of its ancestors" (Dollo 1893, as also noted above) and "Functional or physiological reversal occurs; structural or morphological reversal does not occur" (Dollo 1903; see also Gould 1970; Gould and Robinson 1994).

Before inspecting the lantern mutants with these dicta in mind, we have to briefly consider another item of Dollo's evolutionary viewpoint in particular and some hypotheses of the contemporary theories of evolution in general. Dollo also supposed that "evolution is discontinuous, irreversible and limited" (Dollo 1893). (To avoid endless repetitions of the verbal description of the different standpoints, it is henceforth called **viewpoint A**, and the alphabetic method is also used for the further vantage points discussed below.) Most evolutionists today believe, however, that evolution is continuous, reversible (at least in special cases or to a certain degree), and unlimited (**viewpoint B**). Yet, whatever hypothesis a phylogeneticist prefers at the present state of the art concerning lantern formation in *Physalis* and further genera of the Solanaceae – i. e. whether he believes that the ICS evolved several times independently of each other in a continuous process by random mutations and selection (thus 'convergently' according to the dominant evolutionary or neo-Darwinian theory) in the plant family of Solanaceae (**viewpoint B₁**) or possibly constituted a plesiomorphic

(original) feature for this family, so that the character either *devolved* smoothly or was lost more or less abruptly several times independently of each other in many of the family's genera (favoured by Hu and Saedler 2007; Khan *et al.* 2009) (**viewpoint B₂**) – according to any of the past and present evolutionary hypotheses and further theories on the origin of species (see below), the ICS sooner or later constitutes an apomorphic (derived) feature for the following reasons:

(1) If one discards the extremely improbable hypothesis that all the independently arisen lantern reduction (and other) mutants in the mutagenesis experiments were due to sequence deviations in one and the same gene, the data of some 100 ICS mutations vigorously suggest that, in addition to a few master regulatory genes (He *et al.* 2004; He and Saedler 2005, 2007a, 2007b), also a rather large number of particular target genes of a gene network or a cascade of genes will be involved in the formation of the ICS (and this appears to be one of the most important inferences from my mutagenesis research in *Physalis*) – several of the genes most probably being pleiotropic. This inference may extend earlier studies of gene expression in male and female flower organs (Kamalay and Goldberg 1980, 1984; Drews and Goldberg 1989) now to the development of the ICS, at least to a certain degree. (2) The Chinese lantern is an extraordinarily specified feature not only from a genetical viewpoint, but also developmentally and anatomically (He *et al.* 2004; He and Saedler, 2005, 2007a, 2007b; Hu and Saedler 2007). (3) To my knowledge, no evolutionist hypothesizes that the entire class of angiosperms represented by some 56 orders, 457 families, 13,208 genera and between 250,000 to 400,000 species is derived from a common ancestor displaying such a special sepal feature like the Chinese lantern or any other comparably specialized character (the very term "heterobathmy" usually applied here, in German "Spezialisationskreuzungen" a translation of Dollo's "chevauchement [overlappings] de specialisation", in English also "specialization-crossings" and "cross-specializations" – for the detailed history of the term see Nelson 2004, p. 131 – implies the irreversibility of complex special traits as a basic criterion to exclude species displaying them from being ancestors to others without these characters). Also, none of the intelligent design proponents (see again Behe 1996/2006; Dembski 1998; Dembski and Wells 2007; Lönnig 2004, 2009b; Meyer 2009; and many of the further authors critical to Darwinism mentioned in the introduction above, not to mention the time-honoured school of German botanists of the so-called *idealistic morphology* going back to Goethe 1790; cf. Troll 1984) – which is henceforth called **viewpoint C** – would hypothesize that the morphological potential of the basic *bauplan* of the angiosperms already involved the realization of a specific feature like the ICS from its very beginning. Thus, according to all known scientific theories on the origin of species, the ICS constitutes an apomorphic character.

So, (a) *when*, (b) *where* and (c) (functionally) *why* did this apomorphic feature first appear? The following discussion will show that these questions are tightly interconnected with each other so that often no clear dividing lines can be drawn between them.

(a) According to several data of the paleontological record, the ICS appears to be at least 28,4 million years old (*Physalis pliocenica*, Szafer 1961; May 1997, 2001; see also *The Paleobiology Database* 2009), thus *Physalis* belongs to the "living fossils" – a term created by Darwin for life forms that haven't changed much since their first appearance in earth history. However, the plant family of Solanaceae may be about twice as old (*Solanites*, a genus without lanterns from the Lower Eocene, London clay, dated to be 56-49 Ma old, Berry 1914; Martinez-Millan 2007) and the earliest angiosperms including *Archaeofructus*, unexpectedly a genus without sepals and petals, some 125-135 million years (Sun *et al.* 2002; Ji *et al.* 2004; Gandolfo *et al.* 2004; Kutzelnigg 2008; Miller 2009; Pennisi 2009; Stockey *et al.* 2009; Rothwell *et al.* 2009; Taylor *et al.*

2009).

If correct – I use the conditional clause because the plant fossil record is, of course, still imperfect, not least for the Solanaceae – **B2** would have been disproved and the origin of the ICS by multiple convergences would be strengthened for viewpoints **A** (irreversible) and **B1** (at least partially reversible), whereas **C** could live with both options, although it tends by its very nature to favor multiple independent origins of the Chinese lantern. However, if **B2** were definitely disproved, **A** and **B1** would nevertheless have the problem to explain the improbable feat of multiple independent origins of a rather complex trait like the ICS by the ateleological and thus purely naturalistic process of random mutations and selection – one of the reasons why Hu and Saedler (2007) tend to favour multiple losses of the ICS (see below), for separate losses of complex form and function appear to be more probable than independent gains, even granting the best genetical starting points realistically possible for the latter.

As to the present question *when* the ICS first appeared, let us briefly add another challenge for **B2** here: The hypothesis of a plesiomorphic ICS for the Solanaceae implies the presence of the Chinese lantern at the very base of a family, for which 96 genera and about 2,297 species have been described (D'Arcy 1991), some 75 species belonging to *Physalis* and almost 1,000 species being affiliated to the closely related genus *Solanum*. Since of these 96 genera only species within the genera *Cuatrecasas*, *Exodeconus*, *Margaranthus*, *Nicandra*, *Physalis*, *Physaliastrum*, *Physochlaina*, *Przewalskia*, *Withania*, and possibly *Anisodus*, feature an ICS, which completely masks the developing and mature fruit (He *et al.* 2004; Hu and Saedler 2007), all the other 86 genera of the family with some 2,000 species would have either lost the ICS, or were descendants of species that have lost it. If correct, one would perhaps tend to assume that the losses of function of the ICS would have happened rather early in the history of the family, otherwise a late scenario with perhaps a thousand and more independent losses of Chinese lanterns without at least very plausible – or better still – *plainly recognizable* selective advantages at certain points in their history, appears to be almost as improbable and hence as unreal as a series of independent gains. However, if the assumed losses occurred early in the history of the Solanaceae, it might not be easy to explain the occurrence of the ICS in the range of the 10 different (not always closely related) genera mentioned above. For some more detailed points on **B2**, see the ensuing discussion (b) "where" the ICS arose.

(b) So concerning the question *where* the ICS arose, He *et al.* (2004, p. 150) mentioned the possibility of polyphyly even within the genus *Physalis* observing that "the large majority" of its species "grow in Mexico, 21 in Guatemala, 10 in Nicaragua and 9 in Panama". However, "another genus, *Przewalskia* featuring the ICS, exclusively grows in the alpine regions of China" and *Physalis alkekengi* with a fossil record of some 12 million years (Geissert *et al.* 1990) is also assumed to have arisen in the Old World (Eurasia). The authors then raise the question: "Does this suggest polyphyly of the ICS? Studies based on morphology and on chloroplast DNA suggest even polyphyly of *Physalis*, but this has to be verified molecularly using trait determining genes."

Hu and Saedler have continued to address the question of monophyly *vs.* polyphyly in their paper of 2007. They estimated that the frequency of 9 (including *Anisodus* possibly 10) genera displaying the ICS of the altogether 96 genera of the Solanaceae would be compatible *either* with a multiple origin of this character, *or* "alternatively, the trait could represent a plesiomorphic character that was lost in most lineages during the evolution of the Solanaceae." And they emphasized the different contradictory possibilities of interpretation presented by the molecular data from their evolutionary point of view as follows: "Whereas phylogeny reconstructions suggest independent multiple origins of ICS, expression of the trait-determining *MPF2* gene in floral

tissues, however, seems to be plesiomorphic. Therefore, ICS might have arisen multiple times or multiple losses might have occurred in the evolution of non-ICS featuring species." Moreover, the authors state that "...the phylogeny based on cDNA sequences of *MPF2*, the ICS-determining gene, matches the topology of the species tree as inferred from the cp data, indicating not only that the subtribes of the Solanaceae are polyphyletic but also that the ICS has arisen multiple times. Even within the Physaleae, ICS seems to have evolved several times independently." ... "However, the *MPF2*-like gene expression studies do not support this hypothesis but rather suggest an alternative: plesiomorphic nature of *MPF2*-like gene expression and secondary mutations in the ICS pathway."

Additionally, the authors ascertained that most of the 114 species belonging to 35 genera they had investigated in the Solanaceae also displayed constitutive expression of *MPF2* and they conclude: "This raises the intriguing possibility that ICS might actually be a plesiomorphic basal character in the family" (italics added). – As already mentioned above, this vantage point means that the feature arose once and very early in the assumed phylogenetic history of the Solanaceae at an unknown location but was subsequently lost for the great majority of the genera and species of the Solanaceae. Thus, according to **B2**, the question *where* the feature arose would be identical with the currently unresolved questions exactly where and when the first representatives of the Solanaceae originated (the fossil record of the Solanaceae is sketchy and the molecular trees are often inconsistent and sometimes even strongly contradictory). However, concerning losses of gene functions resulting in growth inhibition of the ICS, one of the possibilities of **B2** is that ICS losses could have happened autonomously and recurrently on most continents where the family was represented, at least if one does not assume very early losses (see, however, the problem mentioned for late losses under (a)).

In contrast to **B2**, polyphyly is the answer according to **A**, **B1** and **C**, but I have to admit anew that unfortunately no scientific answers are presently possible to the question exactly *where* the multiple beginnings and formations of the ICS occurred. Nevertheless, one might at least speculate that it happened independently of each other in Eurasia and the Americas.

(c) Last but not least, let us briefly address the question, *why* (functionally) this apomorphic feature has appeared either once (**B2**) or several times independently of each other in the Solanaceae (possibilities within viewpoints **A**, **B1**, and **C**). Concerning viewpoint **C**, it may be mentioned that losses of functions are a distinct additional option in the sense of a purely naturalistic *devolution* process and such losses may also be considered, at least to a certain extend, by **B1**. In other words, what could have been the biological/mutational/genetic and selective advantages for the genesis of the ICS – once or several times independently of each other and (especially for **B2**) its assumed multiple losses?

Now, the reader may feel my dissatisfaction when I first have to point out that here the conundrum for any viewpoint except theory **C** (see below) is aggravated by the almost complete lack of evidence for ostensibly clear anatomical and/or physiological functions of the Chinese lanterns. Nevertheless, He *et al.* (2004, p. 150) mentioned one possible purpose: "The function of the inflated calyx is not entirely clear, but in certain species like *Przewalskia tangutica* it seems to reduce the specific weight and thus might facilitate wind dispersal of the fruits."

Let us be sure that in the plant kingdom there is an enormous wealth of different and distinctively effective methods of fruit and seed dispersal from the parachutes in dandelions (*Taraxacum officinale*) to the catapults of the squirting cucumber (*Echballium elaterium*) (Levey *et al.* 2002; Fenner and Thompson 2005; Stuppy and Kessler 2008; Kessler and Stuppy 2009). Moreover, it appears that the great majority, in fact >90% of the 2,297 species of the Solanaceae – from sea coasts (where hypothetically modi-

fied watertight lanterns could be imagined to help transport them all around the globe) to high mountain ranges like the Andes (up to the vegetation limit) – can live and reproduce quite happily dispersing their seeds without first putting the fruit into lantern-like coverings. Thus, if lanterns were a generally effective method of seed dispersal or perhaps even more effective than the other methods in the large majority of the species and genera of this and other plant families *under the same ecological conditions* – why, then, is it such an extremely rare phenomenon in the plant kingdom in general and still uncommon in the Solanaceae in particular? (Focussing on the latter, the question may at least be relevant for all the ecological conditions where *Physalis* and further lantern developing species of the 10 genera mentioned above are growing side by side with non-ICS Solanaceae.) Seed dispersal by animals – another functional option sometimes mentioned for the ICS – is also at least as effective (and perhaps even more so) in many other plant genera without lantern formation.

Even so, there is of course no doubt that the lanterns *could* provide a target for wind dispersal at least due to their relatively large surface area. Yet, in the experimental fields the lanterns were usually found near or in the vicinity of their mother plants, even after strong wind invasions (except when the immediate surroundings were rather flat and smooth, for instance due to tarpaulin covering without further 'obstacles', like plants, pots and trays) – possibly the still considerable weight of the mature fruit being the main factor hindering immediate large distance dispersal by winds, but further environmental conditions may be relevant for seed dispersal in *Physalis*. A study of fruit dispersal in the wild could perhaps be helpful to definitely solve this question.

At present, the above quoted (under-)statement that "the function of the inflated calyx is not entirely clear" is not only correct but the problem seems to become even more delicate for any selection theory when we consider the question *why* (functionally) from the perspective of the past (Darwin) and also of the most dominant current evolutionary theory (neo-Darwinism or modern synthesis):

According to Darwin and the present modern synthesis a gradual evolution from small normal sepals to full lantern formation is thought to have happened in a multistep process by mutations with "small or even invisible effects on the phenotype" (Mayr), or, to put it in Darwin's prose with added emphasis, by "innumerable slight variations" or "extremely slight variations" as well as "infinitesimally small inherited variations", and again, to adequately emphasize one of Darwin's central ideas on evolution, by "infinitesimally small changes", "infinitesimally slight variations" and "slow degrees", i.e. "steps not greater than those separating fine varieties", "insensibly fine steps" and "insensibly fine gradations", "for natural selection can act only by taking advantage of slight successive variations; she can never take a leap, but must advance by the shortest and slowest steps" and "the transition [between species] could, according to my theory, be effected only by *numberless small gradations*" (Darwin 1859, 1868, 1877/1896; check also van Wyhe, 2002-2009: The Complete Works of Charles Darwin Online).

This theorizing past and present leads us to a problem, which I have already briefly touched above in conjunction with seed dispersal and which I had also faced before in *Misopates* (Lönnig *et al.* 2007). To apply the essential questions and objections concerning the latter on the related case in *Physalis*, namely:

If *Physalis* has evolved its dramatically longer sepals forming the ICS as a shelter for the developing fruit – the hypothesis often advanced to explain the origin of this feature – the question might immediately be raised, how all the other >90% of the Solanaceae (or >99% of the angiosperms in general) have managed to survive and successfully flourish without correspondingly elongated sepals forming an ICS.

Also, especially for viewpoint **B2**, the question may be

raised (analyzing the above scenario), which distinctive selective advantages would necessitate an ample series of independent *losses* of such a functionally very important and vital feature the ICS must inescapably be according to any selection theory? Do such theories not constitute a *contradictio in adjecto*?

And applying the additional queries for the origin of the long *Misopates* sepals directly to the sepals of *Physalis* eventually forming the ICS:

Assuming with the modern synthesis that the postulated common ancestor of the Solanaceae first displayed relatively short sepals in comparison to the corolla as one of its original characters – which could have been (1) the genetic factors focussing especially on the length of the sepals (of the many morphological characters) by rounds of repetitive 'small' mutations to "stretch" and mould them *in an extended series of insensibly fine steps* (see above) to their present size and form in cooperation with (2) an enormous selection pressure again concentrating particularly on this morphological character to eventually result in the specific structure of the ICS? Moreover, it would have to be postulated that this highly unlikely process would have happened about 10 times independently of each other. The speculations of Khan *et al.* (2009) on positive Darwinian selection for the ICS of *Physalis* and *Withania* depend on several pre-suppositions and assumptions not to be discussed here, except perhaps mentioning the fact that many angiosperm genera survive and flourish successfully without any maintenance of a microclimate within a lantern, providing humidity for a developing berry in a drier environment (see also discussion above) – apart from the fact that a threshold would have to be postulated for such a microclimate to be effective.

At present, at least, there appears to be no convincing evidence in agreement with the modern synthesis or neo-Darwinian theory of evolution, even after some 150 years of theorizing.

However, to ward off such objection against his favoured theory, Mayr (1963) claimed that "one can never assert with confidence that a given structure does not have selective significance." And Simpson (1953) argued that "the fallibility of personal judgements as to the adaptive value of particular characters...is notorious", – referring especially to features of animals quite unlike any now living. Dobzhansky (1975) asserted that "not even a biologist of Grassé's experience can judge reliably which characters are useful, neutral, or harmful in a given species." These statements may illustrate the frequency and depth of the problem. Yet the perceptive reader may wonder whether such statements can ever be falsified (Grassé 1977; Brady 1982; ReMine 1993; Wesson 1991; Müller and Newman 2003).

Just to emphasize the key message of the last paragraph: If there is no way to falsify such expositions, they are outside science (Popper), as appears to be the fact for large parts of the selection hypothesis generally (for further points, see Lönnig 2001). Although it might as well be possible to almost infinitely invent various new environmental scenarios to explain a phenomenon by selection, this infinity of mostly non-testable explanations (often just-so-stories) itself may put the theory outside science.

In contrast to any selection theory like modern neo-Darwinism, it is sometimes argued that some morphological deviations might just be neutral by-products of different (selectionally unlinked) genetic and physiological changes in a species, in the case of *Physalis* perhaps the recruitment of *MPF2* into the floral quartet (He and Saedler 2005, 2007a, 2007b; for the floral quartet see Theißen and Saedler 2001). However, in 2007 Hu and Saedler had already analyzed 7 steps in the development of the ICS that could be mutational targets interrupting lantern formation (see below). Also, as noted above, the results of some 100 lantern mutants, which appeared independently of each other in my mutagenesis experiments, further imply the existence of many fine tuned and coordinately expressed target genes. Hence, it seems to be obvious that by just one or a few *ran-*

dom mutational steps the Chinese lantern cannot be obtained.

This inference can also be applied to Dollo's verdict (as noted above) that "evolution is discontinuous, irreversible and limited" and implicitly to the hopeful monster theory (Goldschmidt) and its modern versions according to Gould (2002/2007) and Theißen (2005, 2009, there further references up to the present). Thus, the hypothesis that comparably complex new structures could be generated in just one discontinuous step appears to be (1) genetically very improbable, (2) there are (still) hardly any distinct selective advantages that could be argued for the maintenance of such a new structure, and (3) there would be basic problems to verify or falsify Dollo's or Goldschmidt's approach.

Now, the question may also be raised, whether vantage point C can ever be verified or be falsified. As will be noted below, clear criteria to identify arguments for C have been presented by Behe, Dembski, Dembski and Wells, Lönnig, Meyer and others. Moreover, it is to be observed that C can, of course, be falsified by proving (among other points) that the probability to form an ICS by purely natural processes is *high*, that specified complexity is *low*, and finally, by generating an ICS by random mutations in a species displaying none (this would add just another case to the assumed multiple convergently generated examples according to A and B1). However, in the latter case the question would also have to be painstakingly explored, whether this species belonged to the group of Solanaceae having lost its original ICS and would now have regained it in one or very few mutational steps. In that improbable case one would meet a *double reversion* to disprove Dollo's law. Yet other cases of convergence have also been postulated (Glover *et al.* 2004; Theißen 2004).

And still several additional relevant points on the question of reversibility

Even at the risk of testing some of the reader's patience, there are still several additional relevant points to be mentioned for a thorough discussion of the topic:

In the case of the long *Misopates* sepals, the very feature was already at odds with Dollo's law. For (1) leaflike sepals are assumed to be a plesiomorphic character and (2) because, even from the most inclusive evolutionary presuppositions, *Misopates* cannot be directly derived from an original ancestral angiosperm stock with leaf-like sepals (for the details, see Lönnig *et al.* 2007).

Now, what about *Physalis*? Although ICS formation may remind us somewhat of leaf development, the Chinese lantern is, as pointed out above, undoubtedly a very specialized and thus derived feature anatomically and genetically, i.e., it constitutes a developmental system being much more than just a reversion to leaflike sepals. Nevertheless, it may perhaps be argued from viewpoints B1 and B2 that at least resumption of sepal growth is obviously necessary in order to form larger leaf-like areas for the ICS (if only as one component of a very special overall new morphological trait), and this may be interpreted as a reversion "to a former state already realized in the series of its ancestors" (and in support the theorists could perhaps point out to mutants 240 Gy/962, 220 Gy/3922 and 280 Gy/46, see Fig. 3), whereas viewpoint C may explain growth resumption as just a necessary by-product to reach the goal, namely lantern formation by possibly a common molecular growth module expressed after fertilization in the WT's sepals again, a module which may also be detected elsewhere in flowering plants (roots, shoots, leaves and flowers). Anyway, there is no exception to the fact that the ICS is a derived feature.

Having established that the Chinese lantern is an apomorphic trait and focussing now on the entire feature, let us anew inspect Dollo's assertion that "[A]n organism cannot return, even partially, to a former state already realized in the series of its ancestors" (Dollo 1893). From the evolutionary points of view (A, B1, B2) the assumed ancestors

probably had rather normal sepals as also the present *Physalis* displays them until fertilization. Examining the mutants exhibiting lantern reductions (see above) *prima facie* it seems rather clear from these vantage points that – to re-write Dollo's dictum – "an organism can, indeed, return to a certain extent to a former state already realized in the series of its presumed ancestors". As for C, it could be argued that normal sepals belong to the characteristic structures of the basic angiosperm type, which would be reapproached or regained by such a reversion.

However, one may object that these mutants show nothing but a purely phenotypical reversion irrelevant for Dollo's law. For Dollo explicated his law as follows (1903, Gould 1970):

"The irreversibility of evolution is not simply an empirical law resting on facts of observation, as many have believed. It has deeper causes which lead it, in the last analysis, to a question of probabilities as with other natural laws. ... In order for it [evolution] to be reversible, we would have to admit the intervention of causes exactly inverse to those which gave rise to the individual variations which were the source of the first transformation and also to their fixation in an exactly inverse order – a circumstance so complex that we cannot imagine that it has ever occurred."

Yet, the problem with this objection is that Dollo never distinguished between genotype and phenotype as has been discussed in detail by Lönnig *et al.* 2007 (as for the molecular level, Dollo's law appears to have recently been supported by Bridgham *et al.* (2009), but the authors unfortunately do not discuss phenomena and possible molecular reasons for them like those described in the Pottiaceae by Zander 2006, and several further studies referred to by him). Thus, Hu and Saedler might insist that for their position it is sufficient that the ICS can morphologically be reduced (a point clearly illustrated by the mutants, see especially Fig. 1 to Fig. 11), and the authors could further surmise that a full reduction of the lanterns to normal (or almost normal) sepals will possibly happen in extended mutagenesis programmes with *Physalis* as well. Also, the authors could argue that it would *not* be necessary "to admit the intervention of causes exactly inverse to those which gave rise to the individual variations which were the source of the first transformation and also to their fixation in an exactly inverse order", because the loss of function of a trait having a polygenic basis can in many cases be caused by just one loss-of-function mutation in only one gene as, for example, the multiple independent losses of anthocyanin synthesis in many plant species illustrate, often involving only one dysfunctional gene of the entire pathway of about 10 genes. Moreover, as has been repeatedly pointed out that losses (also) of organ function have occurred many times independently in species in the wild by "regressive evolution": losses of pigments and eye function in cave animals, wing reduction in island insects and birds around the world, rattle snakes without rattles, losses of dispersion systems in island plants, etc. on oceanic islands and elsewhere (Diamond 1983, Schemmel *et al.* 1984, some points in Lönnig 2002, 2005, 2006, Becker and Lönnig 2004, in *Astyanax mexicanus* see, for example, Protas *et al.* 2007). Losses of organ functions have also been recorded for cultivated plants and many domestic animals (e.g. Galloways have lost their horns by mutations, a recessive character, Manx-cats and hairless dogs may also be mentioned in this connection).

However, one inference is sure: If, in agreement with B2, multiple losses of such a specific trait like the apomorphic ICS really took place in the past in different genera independently of each other, *reverting the ICS to normal sepals*, the process possibly being accompanied by further genetic alterations leading to the formation of new groups of the Solanaceae at the same time (like *Capsicum baccatum*, *Lycianthes biflora*, *Tubocapsicum anomalum*, *Withania solanacea*, and *Vassobia breviflora*), this would constitute one of mightiest blows against the validity of Dollo's law of irreversibility that the history of biology would have

witnessed so far.

Notwithstanding these arguments and counterarguments, there are also some acute points, which may be raised in favour of Dollo's law and against the idea of multiple losses of the ICS. The first of these arguments refers to the possibilities and limits of what random mutations can really achieve. This question has been carefully examined and recently published by Behe (2007) in his book *The Edge of Evolution*. One of his interim findings even on microorganisms and viruses, with their extremely short generation times and enormous numbers of individuals, reads as follows (2007, p 162):

"Indeed, the work on malaria and AIDS demonstrates that after all possible unintelligent processes in the cell – both ones we've discovered so far and ones we haven't – are at best of extremely limited benefit, since no such process was able to do much of anything. It's critical to notice that no artificial limitations were placed on the kinds of mutations or processes the microorganisms could undergo in nature. Nothing – neither point mutation, deletion, insertion, gene duplication, transposition, genome duplication, self-organization nor any other process yet undiscovered – was of much use."

So, judging from these results in microorganisms and viruses, how much can mutations really do (a) to generate and/or (b) later also reverse the formation of such structures in the angiosperms, here the Chinese lantern, back to more or less normal sepals *in the wild*? (c) Have we really obtained partial ICS reversions in the mutants, which could survive *in the wild*?

To make a long story short, (a) the development of the ICS by at least 7 larger steps according to Hu and Saedler (2007), mainly discovered by He and Saedler before (He *et al.* 2004; He and Saedler 2005, 2007a, 2007b) – (1) recruitment of the *MPF2* gene into the floral quartet, (2) "hormone transport from the ovaries in the innermost to the outer whorl, the calyx", (3) (4) (5) "various functions in the hormone cascade, MPF2-like protein structure and modification and its nuclear transport", (6) calyx cell division, and (7) calyx cell elongation – (without mentioning all the so far unidentified target genes, whose existence I infer from the many lantern mutants) appears to be in agreement with the results of Behe's studies (2007): it seems to be very improbable that the current evolutionary theories like the modern synthesis (continuous evolution) or the hopeful monster approach (in one or very few steps) can satisfactorily explain the origin of the ICS. Also, it has yet to be studied and tested in detail whether C can be applied in this case according to the scientific criteria discussed in detail elsewhere (Behe 1996; Dembski 2004; Behe 2006, 2007; Dembski and Wells 2009; Lönnig 2009b; Meyer 2009). Just to briefly repeat these criteria here to convey to the reader some items which could be tested for C, mostly following Dembski 2004 and for point (f) Behe 1996/2006 and 2007:

(a) High probabilistic complexity (e.g. a combination lock with ten billion possible combinations has less probability to be opened by just a few chance trials than one with only 64,000).

(b) Conditionally independent patterns (e.g. in coin tossing all the billions of the possible sequences of a series of say flipping a fair coin 100 times are equally unlikely (about 1 in 10^{30}). However, if a certain series is specified before (or independently of) the event and the event is found to be identical with the series, the inference to ID is already practiced in everyday life).

(c) The probabilistic resources have to be low compared to the probabilistic complexity (refers to the number of opportunities for an event to occur, e.g. with ten billion possibilities one will open a combination lock with 64,000 possible combinations about 156,250 times; vice versa, however, with 64,000 accidental combinations, the probability to open the combination lock with 10 billion possible combinations is only 1 in 156,250 serial trials).

(d) Low specificational complexity (not to be confused with specified complexity): although pure chaos has a high

probabilistic complexity, it displays no meaningful patterns and thus is uninteresting. "Rather, it's at the edge of chaos, neatly ensconced between order and chaos, that interesting things happen. That's where specified complexity sits" (Dembski 2004).

(e) Universal probability bound of 1 in 10^{150} – the most conservative of several others (Borel: 1 in 10^{50} , National Research Council: 1 in 10^{94} , Loyd: 1 in 10^{120} – for the details see again Dembski 2004).

"For something to exhibit specified complexity therefore means that it matches a conditionally independent pattern (i.e., specification) of low specificational complexity, but where the event corresponding to that pattern has a probability less than the universal probability bound and therefore high probabilistic complexity". For instance, regarding the origin of the bacterial flagellum, Dembski calculated a probability of 10^{-234} .

(f) Perhaps it will also be useful checking for irreducible complexity (IC) on the molecular/genetic level, although IC appears to be absent in the present case on the anatomical level ("By *irreducibly complex* I mean a single system composed of several well-matched, interacting parts that contribute to the basic function, wherein the removal of any one of the parts causes the system to effectively cease functioning" - Behe 1996, 2006).

A full elucidation of the entire network and cascades of gene functions involved in the development of the ICS would be helpful for a final scientific solution of the question.

(b) I entirely agree with the statement that "the complexity of the mechanism underlying development of an ICS should make the process vulnerable to secondary loss-of-function mutations" (Hu and Saedler 2007). Thus, loss-of-function mutations could at least interfere with normal lantern formation at the 7 different developmental steps enumerated above. Yet, there is perhaps one question still to be solved: In virtually all the cases of regressive evolution (degeneration), some typical rudimentary structures are still to be found. This is also true in the lantern mutants and modifications (see **Figs. 2, 3, 11**). The reason for this phenomenon most probably is that parts of the genetic network involved in the formation of complex structures like the ICS are pleiotropic and thus often also necessary for essential functions as leaf and/or flower development. Hence, loss of function in these genes would also mean loss of other vital organ functions, but "you cannot be dead and evolve" (Nelson 2007). However, in most of the Solanaceae species and genera without lantern formation no rudimentary structures can be detected, *Anisodus* perhaps being an exception. Its lantern appears to be imperfectly developed and somewhat deformed. But at the present state of knowledge it may also be argued that the normal sepals themselves constitute the "rudimentary" structures in most of the Solanaceae. If this were true, Dollo's law would have no chance to survive as a law (at least not in botany).

Additionally, in the section about leaf mutants I noted that several independently arisen leaf mutants of *Physalis* displayed entire margins. Entire leaf margins appear to be the original state of the angiosperm leaves. Moreover, several wild *Physalis* species also have leaves with entire margins. So these observations may be relevant for Dollo's law as well, showing again that partial (phenotypic) reversion is possible.

(c) He and Saedler detected that in the WT *Physalis* line fertilization was necessary for ICS development. Intriguingly, due to loss-of-function mutations fertilization was not necessary anymore in several cases of the (usually) smaller lantern mutants. Not only fertile mutants but even several sterile (male and/or female) ones with no fruit development at all were able to regularly develop an ICS. Provided that such a mutant could survive in the wild (a sterile one perhaps by vegetative propagation like the *cycloidea* mutant of *Linaria*), from viewpoints **B1** and **B2** this could be interpreted as a partial reversion "to a former state already realized in the series of its ancestors", and by C as a



Fig. 15 Lethal mutants due to losses of functions in the photosynthetic apparatus. The many independently segregating mutants regularly appearing in any mutagenesis program may point to an irreducibly complex core system of genes and functions to guarantee a minimal function of the photosynthetic system.

more basic or intermediary state of function in the design of the ICS.

Although many further reflections and reasonings could be devised on the possible origins and losses of the ICS, the present state of knowledge based on molecular evidence and mutagenesis research at least seems to clearly reveal a certain trend so often found in science in most of its branches over the last 150 years in general and evolutionary biology in particular, namely that the apparently so easily to be generated and hence perhaps also fully reversible ICS appears to be genetically and molecularly *much more complex than originally predicted*. This trend towards hitherto unexpected complexities worsens the problems for viewpoints **A**, **B1** and **B2**, but strengthens the position of **C**, because **C** welcomes any complex processes and structures (the more specified complexity involved in a feature the better and best is irreducible complexity). Besides, it is independent of the improbabilities inherent in the rest of the hypotheses and also independent of selective advantages; the new structures could just be neutral (but of course not be strongly disadvantageous).

The law of recurrent variation

The law of recurrent variation was first formulated by Lönnig 1986, 1995 (in German) and extended 2005, 2006 (in English). It complements Vavilov's "law of homologous series in hereditary variation" (Vavilov 1922, 1951; for the differences see Lönnig 2005). It states that (now supplementing the quotation from the introduction) "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 limits for the micro-quantitative part of variation" (Lönnig 2006).

To test this law thoroughly in *Physalis* to the point of checking the exact phenotypical limits of the individual organs in particular and of the species as a whole in general, at least several 100,000 M_2 -families, or, still better, as suggested above, 1,000,000 M_2 -families should be carefully investigated. And, additionally, the best thing would be that all the mutant genes be sequenced – a task widely beyond the scope of the present paper, and of course, of a magnitude, which could employ a number of international groups of geneticists for a lifetime even in our age of molecular genetics. However, reverse genetics could be of help here to actuate many of the potential of individual phenotypes necessary to be studied for this law by probably less ex-

penditure ("Reverse genetics is an important complement to forward genetics" – Ahringer 2006). Nevertheless, to isolate all the possible alleles with different effects on the phenotype would be an additional large task also by this method.

Notwithstanding the extent of the problem, as expected from and predicted by the law of recurrent variation and already shown in some detail above, even in the 8,600 M_2 -families certain phenotypes have appeared repeatedly and in some cases even regularly, among them the smaller lantern mutants shown above (**Fig. 8**). Besides – virtually also known from any other larger plant mutagenesis project – an almost endless array of mutants of the photosynthetic apparatus (not shown here, but see the supplementary material), i.e. white and yellow mutants and even some dark green ones segregated recurrently in my material. Here the many (usually white or almost white) lethal mutants seem to point to an irreducibly complex core system of genes and corresponding functions necessary to guarantee at least a minimal function of the photosynthetic system (**Fig. 15**) for irreducibly complex systems, see again Behe 1996/2006, 2007; Dembski and Wells 2009; Lönnig 2004, 2009b; Meyer 2009).

Also, all the loss-of-function mutations interrupting the 7 developmental steps as envisaged by Hu and Saedler to reverse the ICS to a small calyx would, of course, be in full agreement with the law of recurrent variation (with or without rudimentary structures in the original sense of the term).

I have quoted Mayr above that there are no laws in biology except in functional biology. Although there are some differences of opinion (Snaydon 1987), reproduction biology belongs to *Functional Plant Biology* (see, for example, the Journal with the same title, R. Munns, ed. in chief, 2009). Since reproduction biology without genes and genetics is unimaginable, both may be looked at as part of functional biology of an organism *sensu lato*. Nevertheless, Mayr is in most cases certainly correct when he writes (to repeat):

"Biologists often use the word law, but for something to be a law, it has to have no exceptions. A law must be beyond space and time, and therefore it cannot be specific. Every general truth in biology though is specific. Biological "laws" are restricted to certain parts of the living world, or certain localized situations, and they are restricted in time. So we can say that there are no laws in biology, except in functional biology which, as I claim, is much closer to the physical sciences, than the historical science of evolution."

The "biogenetic law" (Haeckel, virtually totally wrong, as evaluated from its original definition, cf. Theißen and Saedler 1995; Junker and Scherer 2006), von Baer's law, orthogenesis, Cope's and Williston's laws, Allen's rule, Bergmann's rule, and others belong to the list of examples for laws that appear to be restricted to certain parts of the

living world (or were even found to be nearly entirely wrong by further research). As to the Mendelian laws, every geneticist knows, of course, that there are exceptions due to tight chromosomal linkage, cytoplasmic inheritance and horizontal gene transfer. Even so, any geneticist empirically working with eucaryotes like plants and animals also knows that *the overwhelming majority* of his investigations on the inheritance of the traits he studies follows these basic laws and that they are definitely *not* "restricted to certain parts of the living world, or certain localized situations", and that they are generally also *not* "restricted in time" (for further discussions on the concepts of "laws" and related topics in biology, see Van der Steen and Kamminga 1991; Elgin 2003; Bock 2004; Waters 2007).

As is usually known only to historians of science, the disciples of Darwin had enormous problems to accept the laws of inheritance following the decades of 1866 (Mendel's publication) and especially again at the turn of the 19th to the 20th century (for the details, see Lönnig 2003). In fact, Darwinists had done almost everything possibly for (anti-)science to suppress the basic discoveries on inheritance, even to the point of defaming and/or "forbidding" and misinterpreting further research on them as far as was possible. Historian of science Conway Zirkle commented (1964, p.68): "The controversy became so bitter that in 1903 the British periodical *Nature* closed its columns to the Mendelians. The columns of *Biometrika* had already been closed to them". In spite of all this opposition, the Mendelian laws survived (accompanied by a decline of Darwinism in the first decades of the 20th century) simply because they proved to be true in more than 95% of the analyses and both, the theoretical and practical biologists – not least in plant breeding research – were able to successfully work with these laws, which, in fact, constituted one of the most fertile revolutions that ever happened in biology. Just to very briefly illustrate some new empirical results: I recently found segregations like the following ones in *Misopates* for a *deficiens* mutant: 341:108 (3.16:1), 369:124 (2.98:1), and 523:177 (2.95:1) (altogether 1233:409 = 3.01:1) and for a *plena* mutant 196:66 (2.96:1). Hence, the Darwinian antagonism, hostility and resistance were misdirected (to put it mildly). This and other examples show that there is hope for true scientific laws or rules to eventually be accepted even in the face of powerful irrational opposition.

I anticipate that the same will be true for the law of recurrent variation. There can be no doubt that the law of recurrent variation is a genuine law of nature, which has already been tested in many species by generating literally billions upon billions of mutations. John C. Sanford, inventor of "the bolistic gun" ("gene gun") process, pathogen-derived resistance, and genetic immunization" (Sanford 2005), has come independently of me to virtually the same conclusion concerning mutation breeding and its repercussions for the synthetic theory:

"... millions and millions of plants were mutagenized and screened for possible improvements. ... Vast numbers of mutants were produced and screened, collectively representing many billions of mutation events. ... However, from all this effort, almost no meaningful crop improvement resulted. The effort was for the most part an enormous failure, and was almost entirely abandoned."

Regarding an apparent exception to this rule, the low phytate corn, Sanford comments (pp. 25/26):

"The low phytate corn was created by mutagenizing corn, and then selecting for strains wherein the genetic machinery which directs phytic acid production had been damaged. Although the resulting mutant may be desired for a specific agricultural purpose, it was accomplished through net loss of information (...) and the loss of biological function." "If essentially no truly beneficial mutations (i.e. resulting in more net information) could be recovered from this vast science guided process, why do we think the identical process, in the absence of any guiding intelligence, would be more fruitful in nature?"

According to Sanford, the strongly anticipated but

failed revolution by mutation breeding shows, to put it succinctly, that neo-Darwinism or the modern synthesis/synthetic theory "is wrong" and thus "our current understanding of the history of life is also wrong" (p 5) (as for the topics of rounds microevolution by cumulative selection vs. Goldschmidt's hopeful-monster-hypothesis, see the introduction above).

This and further points as well as the generalization of the findings in the law of recurrent variation can be understood and in principle also be tested by any rational mind on earth. And it remains to be added that in any other branch of science such clear and so often reproduced findings would have long been formulated and accorded the status of a general and genuine law of nature and thus of science.

Applying the law to mutation genetics in *Physalis*, the following predictions and retrodictions can be made: (1) Mutagenesis, even on the scale of 1,000,000 M₂ families, will not produce a genuine new species or "primary species" (no entirely new genes, no novel gene reaction chains and matching cytoplasm and correspondingly no new positive features) able to survive in the wild under similar or identical ecological conditions (only on thinly populated islands without adequate competitors, there could, perhaps, be a chance of some mutants to survive in the wild, possibly like the "monster" *cycloidea* line of *Linaria* on the Swedish island Scarpascaer near Stockholm (Linné 1749; Gustafsson 1979; Luo *et al.* 1996; Lönnig and Saedler 1997; Cubas *et al.* 1999; Luo *et al.* 1999; Theißen 2000; for secondary or systematic species in contrast to primary species, see Lönnig 2002 with all the details including the important role of the cytoplasm on 624 pp.). (2) *Physalis pubescens* ssp. *floridana* may have had a richer gene pool in the past and could have lost a part of its original genetic potential during its history, i.e., some of the potential not necessary to survive at its respective habitats over longer periods of time thus losing some of its primary flexibility and adaptability (for details see Ohno 1985; Lönnig and Saedler 1997; Lönnig 2002; Alonso-Blanco *et al.* 2005). This may constitute a depauperation which – due to a loss of genetic buffering possibilities – could also restrict reductions of organ development to a limit still to be exactly determined (including the ICS?).

(3) Most probably all our mutants have already appeared many times independently of each other in the history of the species and they will continue to do so to time indefinite, as a simple calculation reveals: the average (spontaneous) mutation rate of 1:10⁵ per gene per generation means that every gene mutates a thousand times in any generation in a population of say some 100 million plants. Multiply this with the number of possible generations according to the data given for the fossil record of the genus *Physalis* and the species *P. alkekengi* above and any residual doubts may be eventually diminished to zero.

As for further biological predictions and retrodictions on the basis of the law of recurrent variation as well as details about the possible repercussions for the present theories of evolution, see Lönnig (2005, 2006). Moreover, Meyer (2009) has recently carefully evaluated and critiqued the genetic algorithms presently *en vogue* to rescue evolution from its limits (Dawkins, Küppers, Schneider's *Ev*, and Adami and Brown's *Avida*). His conclusion: "Invariably, evolutionary algorithms succeed in producing specified information (or its functional equivalent) as the result of preexisting information, or the programming skill of computer scientists, or both." "...the probabilistic resources of the entire universe equal 10¹³⁹ trials, which, in turn, corresponds to an informational measure of less than 500 bits," which may be subsumed on the far right of the saturation curve illustrating decrease of the number of new mutant phenotypes in increased number of experiments until saturation limits are asymptotically approached (Lönnig 2006). However, in contrast to the entire universe, a genus like *Physalis* would, of course, have only a minimal part of this already rather limited potential of probabilistic potential of 500 bits at its disposal whereas at the same time it could

loose much more than gain.

Less than 500 bits "represents the maximum information increase that could be reasonably expected to occur by chance from the big-bang singularity to the present – without assistance from an intelligent agent. Systems that exist over a shorter period of time will have correspondingly smaller envelopes of maximal information increase and will, in any case, usually experience informational loss or degradation without input from an agent" (Meyer 2009, p. 294).

Provided these calculations are fully applicable to the basic questions on the origin of species, – so far all the known facts of mutation genetics are in full agreement with them – it appears to be more than unlikely to generate the whole world of living organisms by the neo-Darwinian method. For the many details on the evolutionary algorithms I would like to refer the interested reader to Meyer's book and all the references therein as well as Dembski and Marks II (2009a, 2009b), and Ewert *et al.* (2009).

Last but not least, it may not be inappropriate to say a word on the length of the present *Discussion* within the framework of two biologic laws: Theoretical consideration may be just as important in science as are new empirical results. Concerning this point Meyer (2009, p 139) draws attention to "Copernicus's *De Revolutionibus orbium coelestium*, Newton's *Principia*, and the papers Einstein produced in his *annus mirabilis*, his miracle year of 1905", and one may, of course, add the work of Max Planck, Werner Heisenberg and Darwin's *On the Origin of Species* and others (for further information, see Meyer 2009; Wiker 2009). On the basis of empirical and theoretical concerns, here I have tried to consider to some extent both the historical perspective and the scope of the most important present paradigms concerning the origin of life forms. Although some of these paradigms still represent minority positions, this may change in the near future.

ACKNOWLEDGEMENTS

I am obliged to Maret Kalda who made the photographs of **Fig. 12A, B, and C** and Prof. Heinz Saedler, MPIZ, for the discussions and his generous financial support to facilitate the field trials. Prof. Chaoying He, State Key laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Fragrant Hill, Nanxincun 20, 100093 Beijing, China, participated in the field work after a bicycle accident of W.-E. L. and molecularly identified the *Globosa* gene for the mutant shown in **Fig. 12**. He continues to work on some of the mutants. Also, I have appreciated the excellent work of our gardeners Sybille Richter and Manfred Pohé as well as the careful assistance of Lydia Kurinjaja (now Lydia Jedig), Franz Heister, Nail Okutucu and several further temporary assistants involved in the field and greenhouse work (see the list in the supplementary information). Kurt Stüber, MPIZ, helped to manage the photographs with different computer programmes.

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