Wolf-Ekkehard Lönnig:

Max-Planck-Institut für Züchtungsforschung, Köln, 12 April 2007 and 20 March 2008

Physalis: Field and Greenhouse Observations and Experiments

PART 2 (MAIN PART): MUTAGENESIS in *Physalis* and a glimpse at the law of recurrent variation 2005/2008

Max Planck Institute for Plant Breeding Research

MAX-PLANCK-GESELLSCHAFT

Hinweis 31. 3. 2009: Durch "Verkleinern" des Dokuments von 453 MB auf 2,64 MB haben einige Fotos nicht mehr die ursprüngliche Schärfe.

PART 2: MAIN PART

Mutagenesis

in *Physalis* and a glimpse at the law of recurrent variation

Gamma irradiation was performed in

Forschungszentrum Jülich

by

Dipl.-Ing. Manfred Thomé

The law of recurrent variation

(Lönnig 1986, 1995, 2005, 2006)

“Science is the endeavor to deduce from single observations something of general validity, to extrapolate from the known to the unknown and to examine critical arguments regarding such deductions.”

REINHOLD VON SENGBUSCH

“The continually improved knowledge of mutants in *Antirrhinum* has provided some essential experience [or results]. During the years each new large mutation trial [mutagenesis experiment] showed that the number of really new mutants recognized for the first time, was steadily diminishing, so that the majority of the genetic changes was already known.”

HANS STUBBE

“The larger the mutant collections are, the more difficult it is to extend them by new mutation types. Mutants preferentially arise that already exist."

WERNER GOTTSCHALK

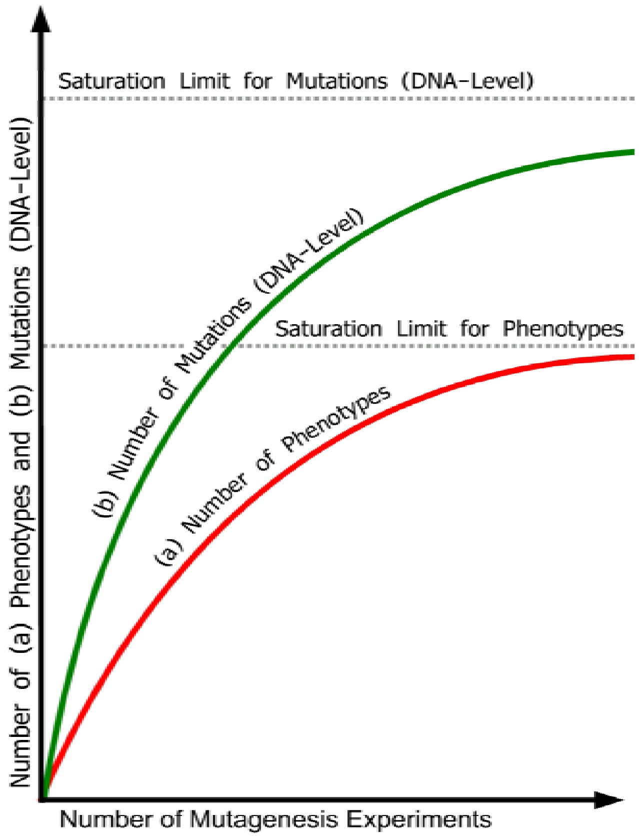


Figure on the left (from Lönnig 2006): Idealized saturation curves illustrating decrease of the number of new mutant phenotypes and new mutant genes in increased number of experiments until saturation limits are reached. Abscissa: increasing number of mutation experiments. Ordinate: (a) number of mutant phenotypes, and (b) number of mutations (DNA-level) with effects on the phenotype. Because mutations at different loci can cause similar or identical phenotypes (see Table 1 above), the curve for the number of mutant genes is distinct from that of the number of new phenotypes. The redundancy problem – for example, some phenotypes appear only when 2 or more genes have been mutated (see further points in the text) – widens the distance between the two curves. The real curves will be different for different organisms, depending, among other things, on the genetic complexity of the species investigated as well as on the scale and specificity of the experiments realized (different kinds and quantities of radiation, chemical mutagens, transposons, t-DNA). The common ground of all curves is the finite number of mutant phenotypes and mutant genes with effects on the phenotype (apart from a micro-quantitative rest of variations due to, for example, environmental and epigenetic factors, position effects, and ‘junk DNA’, which, however, does not change the basic situation).

Photo by Manfred Thomé, Forschungszentrum Jülich: The seedbags are put into this fixed radiation tube and brought to the fuel elements 6 m below the surface of the water.

M_1: Different retardation of seed germination under 200 to 500 Gy (2 June 2005)

Even after gamma treatment with 400 and 500 Gy many seeds germinate, but later most of the seedlings die (for percentages see next slide) and only very few adult plants are obtained.

Physalis pubescens ssp. *floridana*

Seeds sown	Plants obtained	Percent
Control: 352 seeds	188 plants	53.40 %
200 Gy* 994 seeds	715 plants	71.93 %**
300 Gy 965 seeds	118 plants	12.23 %
400 Gy 842 seeds	13 plants	1.54 %
500 Gy 648 seeds	4 plants	0.62 %

***"Gy=**Das *Gray* ist nach dem britischen Physiker und Vater der Radiobiologie, Louis Harold **Gray**, benannt.

Die frühere (vor dem 31. Dezember 1985) Einheit war das *rad.* Die Einheit ist der Quotient aus der aufgenommenen Energie und der Masse des Körpers. 1 Gy = 1J/kg = 100 rad (Das **Internationale Einheitensystem**, auch einfach **SI** (Abk. für frz.: **Systéme international d'unitè**) genannt, verkörpert das moderne metrische System und ist das am weitesten verbreitete Einheitensystem für *physikalische* Einheiten" (Wikipedia). ("The derived unit for absorbed dose, specific energy (imparted), kerma (Gy). **One gray is equal to the dose of one joule of energy absorbed per one kilogram of matter**. It is named after the British physician L. H. Gray (1905-1965)".) (http://jscience.org/api/javax/units/SI.html#GRAY) (Or: "One *gray* is the absorption of one joule of radiation energy by one kilogram of matter.") "**Kerma** ist ein Begriff aus der Strahlenphysik. Der Begriff ist eine Abkürzung für die Wörter *Kinetic Energy released per unit mass* oder auch *Kinetic Energy released in matter*. Die Kerma ist die auf Sekundärteilchen der ersten Generation übertragene Bewegungsenergie dividiert durch die bestrahlte Masse."

**It has been found several times independently that relatively "small" dosages for plant seeds can have a stimulating effect on germination.

Mutants segregating in M_2-families

M_2-families. Upper left: *Misopates*. Upper left below (L 2005/251): *Antirrhinum.* Two examples of *Physalis,* enlarged below.

Dozens of such mutants have arisen independently of each other in different M_2-families (law of recurrent variation, many different genes involved) and also in different plant species (law of homologous series in variation, Vavilov).

Mutants of *Physalis pubescens*, ssp. *floridana*

Left: *Physalis acutifolia.*

Right: Mutant(s) of *Physalis pubescens*

A: 240 Gy/2412

B: 240 Gy/1017

C: 240 Gy/2499

Left: **240 Gy/2810**

Below: **240 Gy/3298**

240 Gy/2710

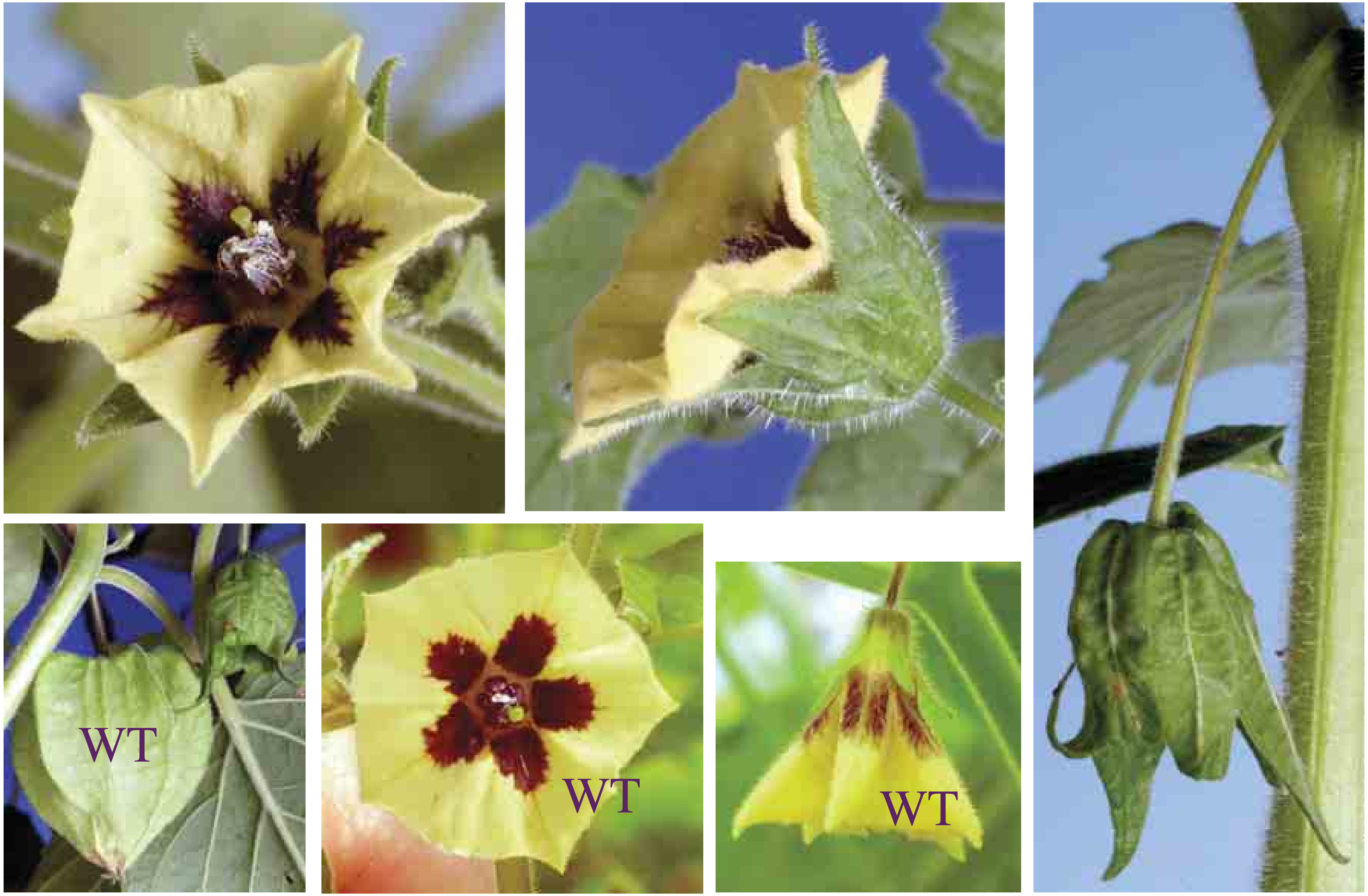
Right

260Gy/389

220 Gy/849

Our first lantern mutant: 240 Gy/962

Detected in the experimental field on Friday 7 July 2006 at 18.03 h (first thought to be possibly due to a mutant *MPF3* gene corresponding to *AP1 (APETALA1)* in *Arabidopsis* and *SQUA* (*SQUAMOSA*) in *Antirrhinum majus*). However, not confirmed so far (question open). In 2007 two very similarly looking mutants were detected: 220Gy/3922 and 280Gy/46.



Further photos of mutant 240 Gy/962; left below: comparison to wildtype lantern and flower

And later two further very similar mutants:

220 Gy/3922

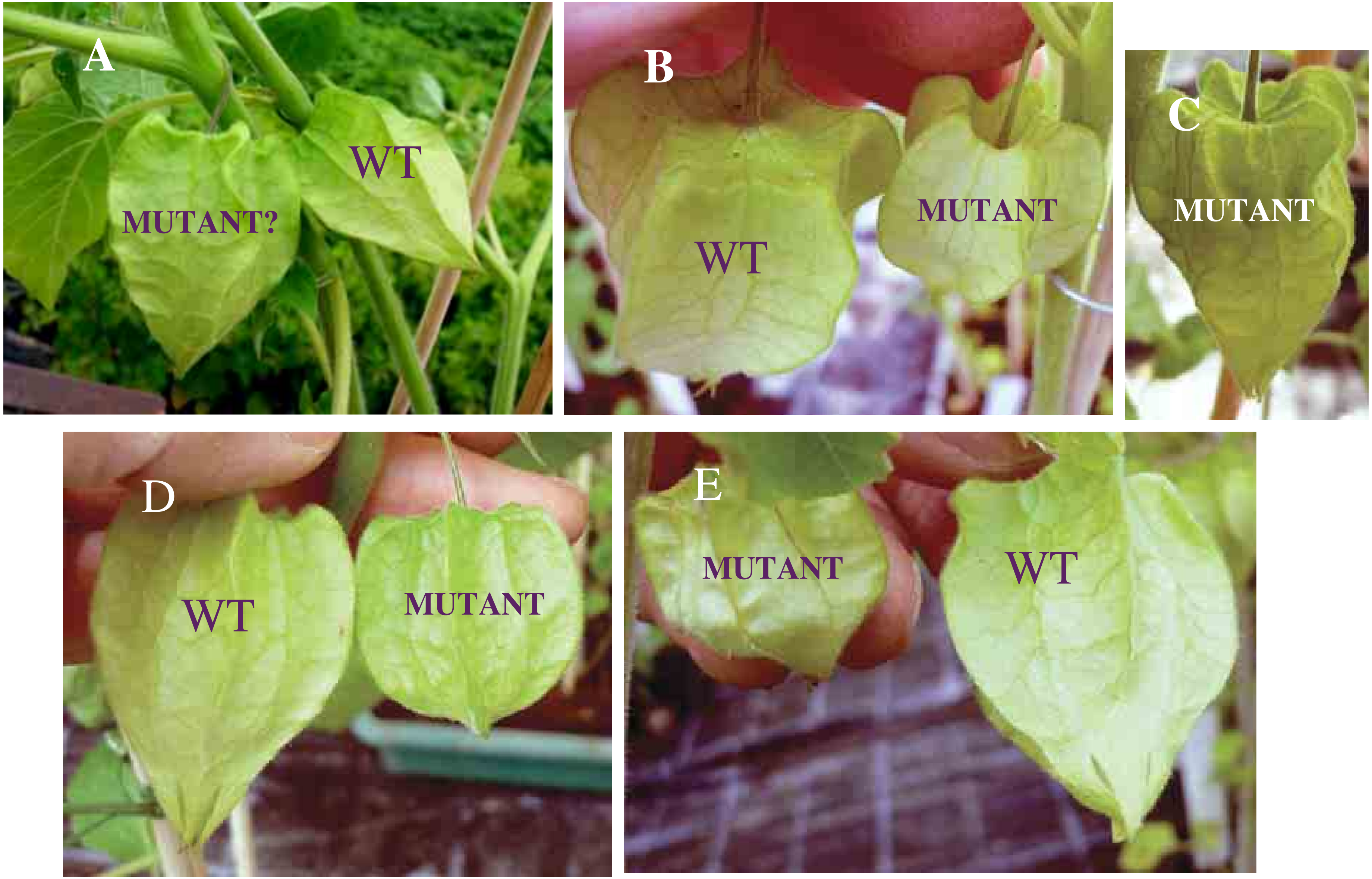
Phenotypically very similar is also mutant 280 Gy/46

on the right below ----->

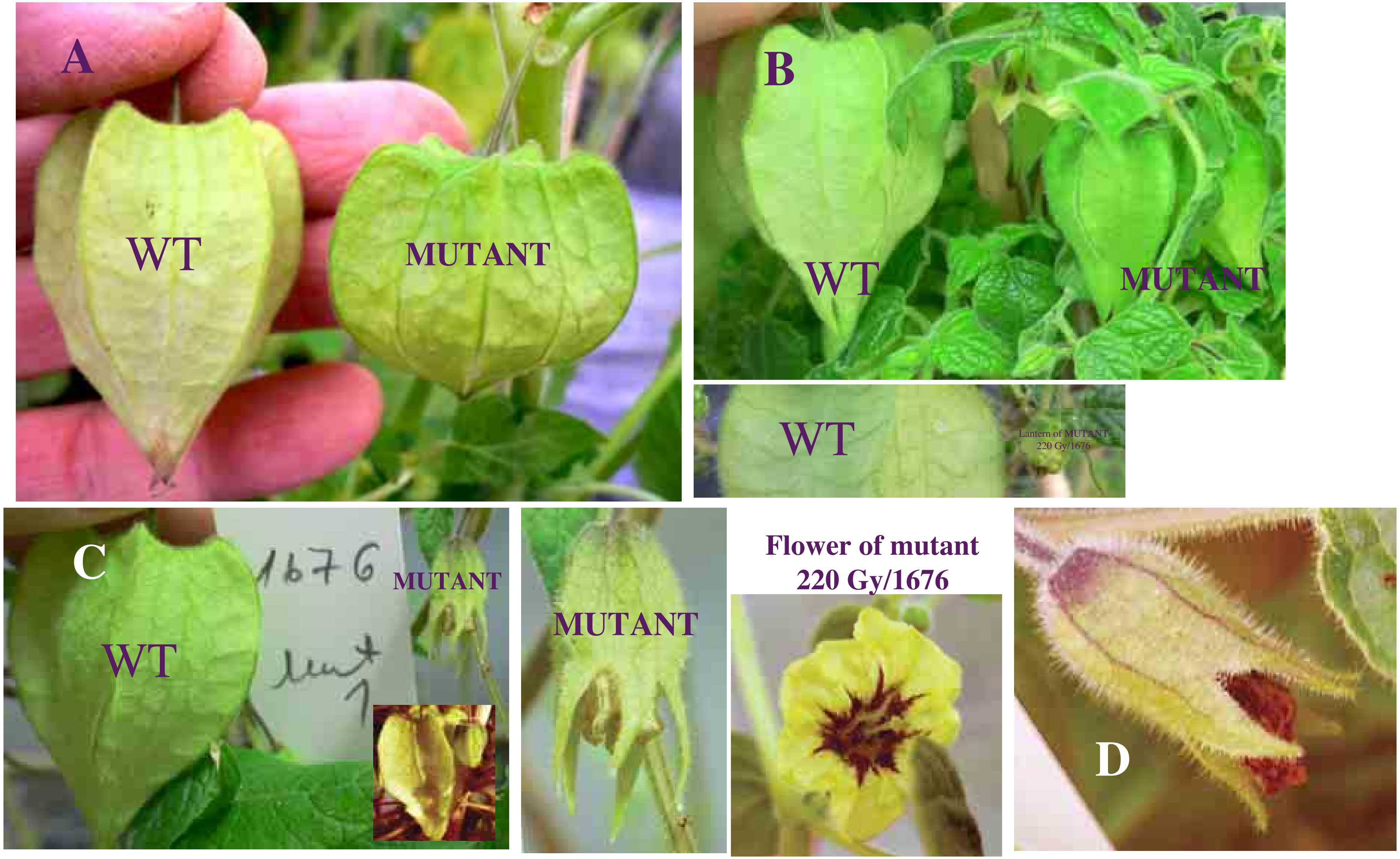
The <u>three</u> mutants have arisen independently of each other in different M_2-families: law of recurrent variation.

Further Lantern Mutants

(1) 240 Gy/985: Lantern as long as WT, but slimmer, (2) 240 Gy/941: Lantern smaller, (2a) 240 Gy/1543: rather small whitish lantern, and 2b: another similar one above: 240 Gy/1880, (3) 240 Gy/975: Lanterns again narrower than WT and rims undulating, (4) 240 Gy/985 (cf. also (1)): Lanterns similar to (3).



(A) 240 Gy/2506: Lantern larger? (B) 240 Gy/2782: Lantern overall smaller,
(C) 240 Gy/1559: Lantern deformed, (D) 240 Gy/938 and (E) 240 Gy/1460(?): Lantern shorter.



(A) 240 Gy/2455 (2544): Lantern shorter and broader, (B) 220Gy/1900: Lantern shorter and narrower/slimmer, (C) 220 Gy/1676: Lantern starts growing without fertilization but often soon falls off, yet some produce small lanterns, (D) and a second independently arisen similar mutant: 220 Gy/801 (also sterile).

And similar phenotype in mutant

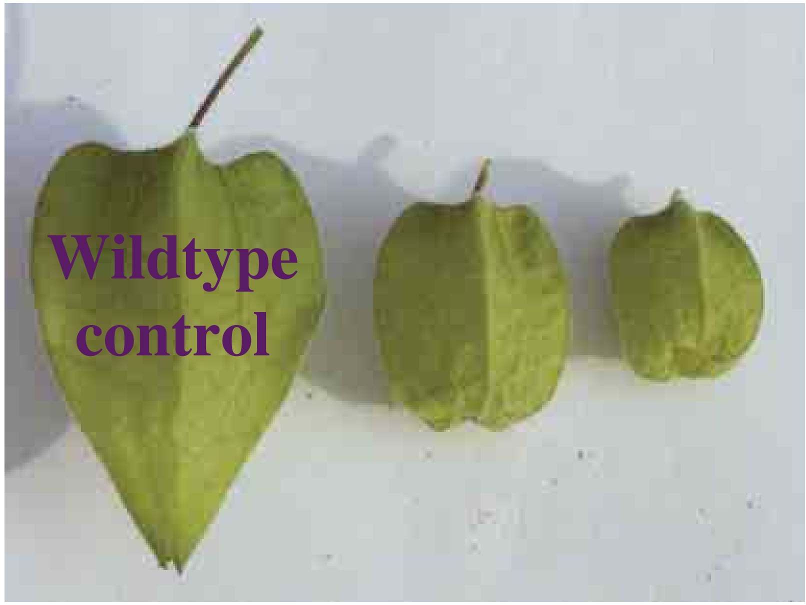
220 Gy/801



However, in contrast to mutant 220 Gy/1676 (again below), it can produce some berries with seeds due to autogamy.

The two phenotypically very similar mutants have arisen independently of each other in different M_2-families (law of recurrent variation). Locus identity testcrosses will reveal - as in the many other new cases of different but also repetitively appearing similar phenotypes shown in the slides above and below - whether different loci are involved or only different alleles.

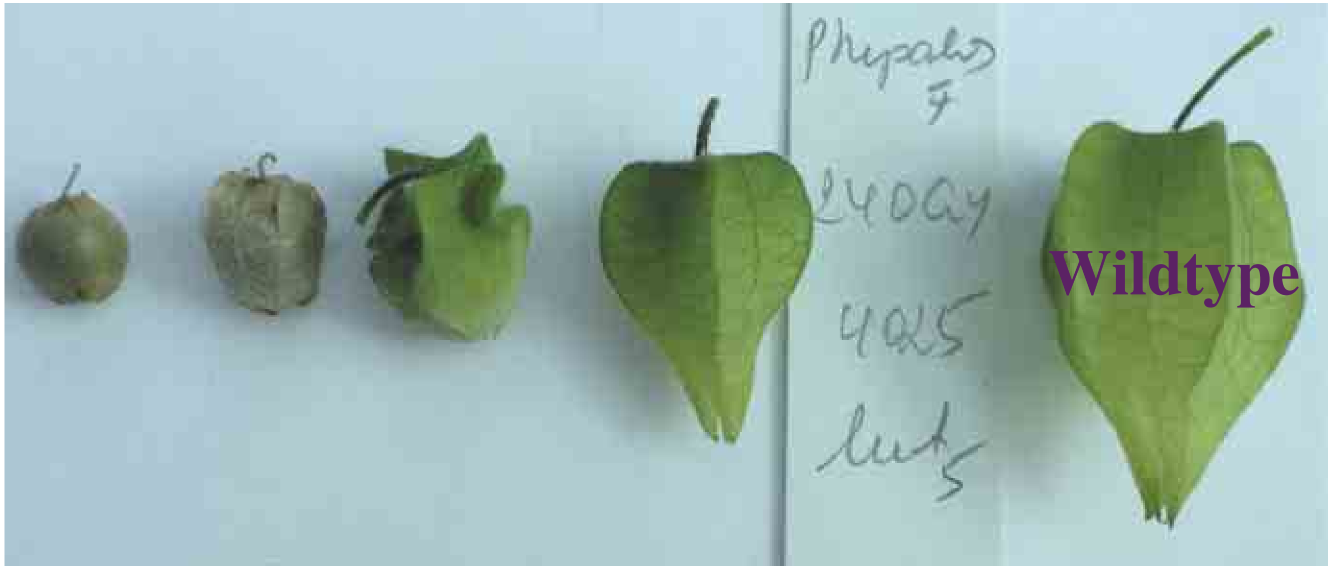
220 Gy/2666



Mutant 220 Gy/2666: Smaller flowers, honey guide enlarged, sepals already smaller in the flower, all lanterns open, size reduced, more roundish than wildtype (tips missing), fertility strongly reduced (male and female), yet occasionally can produce a fruit. Mutant 220Gy/166 (below) looks very similar:

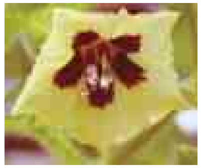
The mutants have arisen independently of each other in different M_2-families (law of recurrent variation).

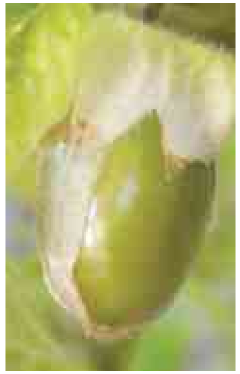
240 Gy/4025



Different types of lanterns (size and form) in one and the same mutant. *In the field* the lanterns seem to have been *much more strongly reduced* than under the mild greenhouse condition (strong modifications).

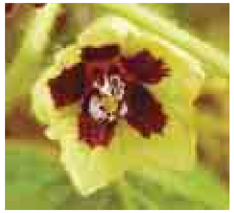
Lantern reduction and variation in 220 Gy/3911





Lantern reduction in mutant 240 Gy/938 (M_3[!])

280 Gy/246



Mutant 280 Gy/246 develops individual fruits with a strongly reduced lantern. However, it also produces normally looking lanterns, which are smaller than those of the wildtype (see below on the right).

260 Gy/1820

Leaves smaller, reminding a bit of the leaves of birch trees. Petals often deformed, not fused. Male and female parts usually sterile; only after many repeated trials berry development. In this case the petals stay at the growing calyx. In comparison to the wildtype, the calyx remains rather small.

Multiplication of ovaries and often reduction of petals in mutant 240 Gy/3812 (below)

260 Gy/1521 Mut 1

(Small flowers, reduction of petals)

Below: 220 Gy/4184

(dwarf/cripple mutant: growth of sepals and petals also reduced)

Mutant 260Gy/386 solely produces numerous ***lanterns without any seeds at all***

Same in the dwarf mutant 260Gy/1521: leaves and lanterns much smaller than in the wildtype (mutant not shown)

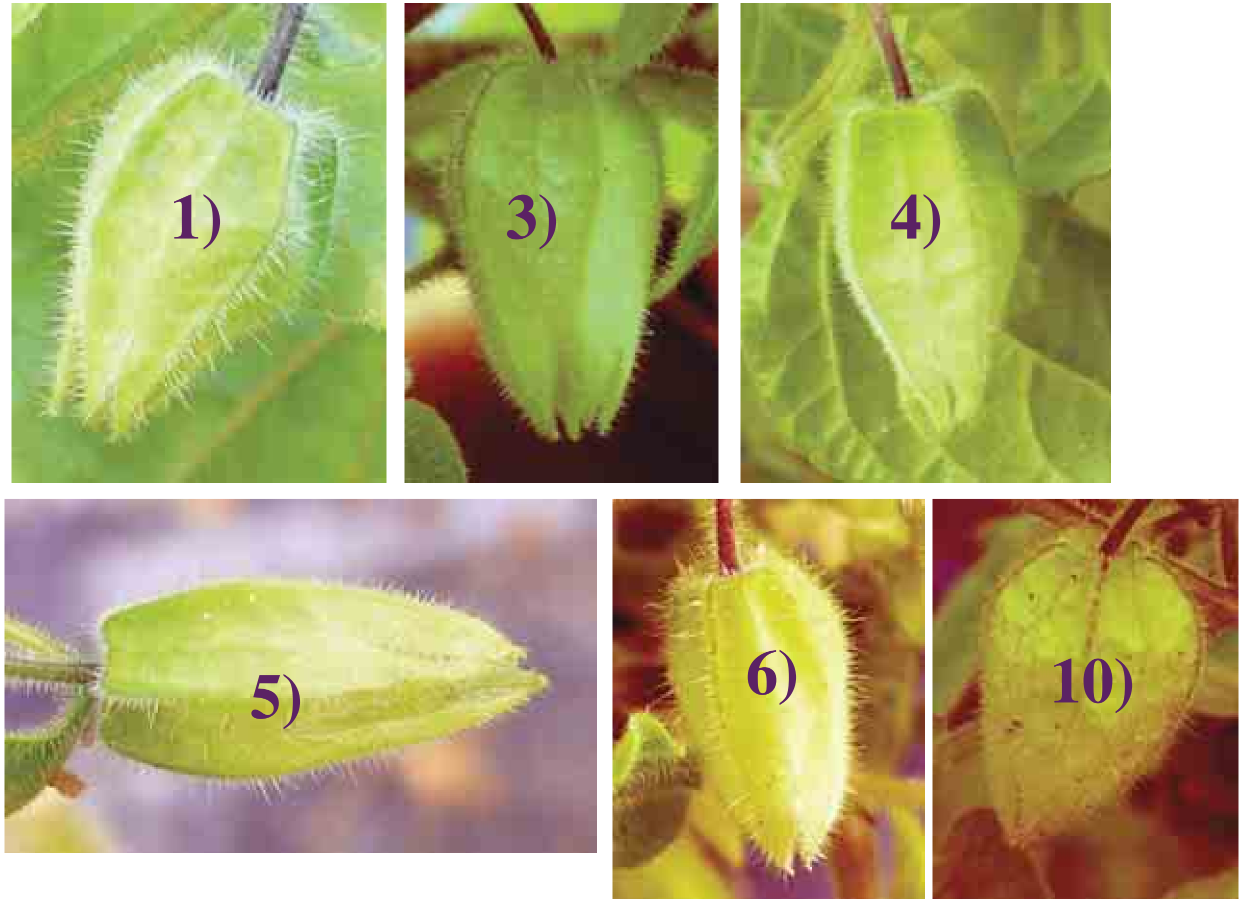
(Left) 240Gy/938: in the mutant the berry is often larger than the calyx

Below: 260Gy/537 (dwarf) with smaller lanterns

All the mutants (so far) developing lanterns without fertilization (law of recurrent variation)

Important Lesson: lantern formation can be decoupled from fertilization!

1) 220Gy/ 711
2) 220Gy/ 740
3) 220Gy/1543
4) 220Gy/1577
5) 220Gy/1869
6) 220Gy/1899
7) 240Gy/ 941
8) 240Gy/1236
9) 240Gy/1958
10) 240Gy/2701
11) 260Gy/ 386
12) 260Gy/ 527
13) 260Gy/ 946
14) 260Gy/1521



Again: all these similar mutants (and many more, see next slide) have arisen independently of each other in different families.

Law of recurrent variation

260 Gy/1405(Mut1)

260 Gy/1698(Mut 1)

240 Gy/4082(Mut 2)

220 Gy/395(Mut 3)

260 Gy/1277

240 Gy/3857

260 Gy/1521

220 Gy/2855(Mut1)

220 Gy/1985(Mut 1)

Altogether almost 50 such mutants. And again: the mutants have arisen independently of each other in different M_2-families (law of recurrent variation).

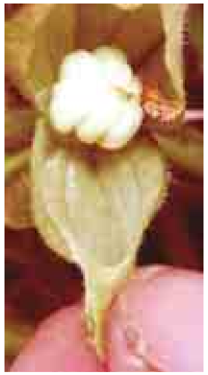
220 Gy/3764

220 Gy/3582

220 Gy/2390

240 Gy/3645

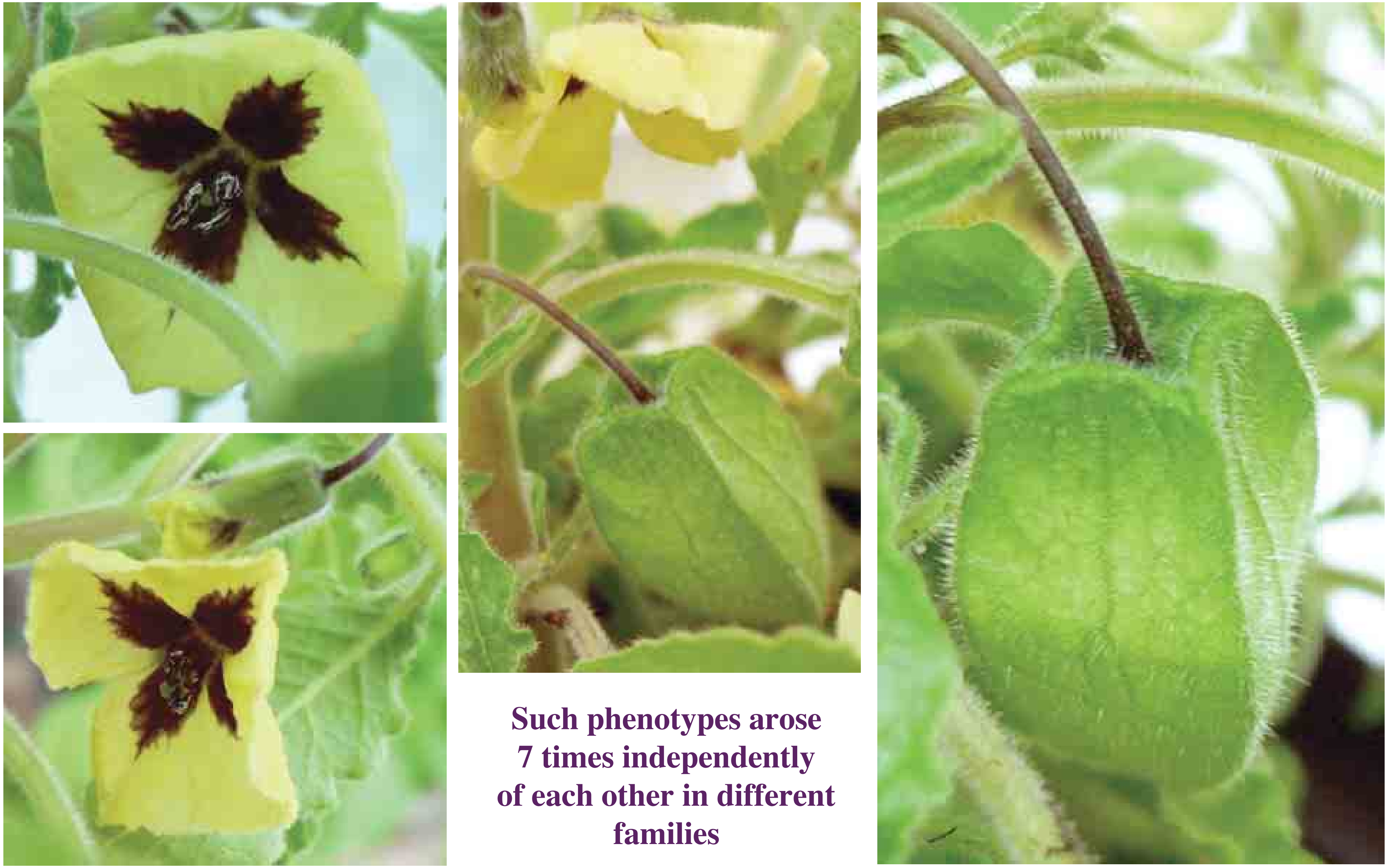
260 Gy/1305



260 Gy/1729 rims strongly undulating; similarly in 260 Gy/1222 and 220 Gy/409 (but in latter some also with wildtype rims)

Also 240 Gy/3979: vierzählig

(similar: mutants 220 Gy/468, 220 Gy/3008, 220 Gy/3205, 220 Gy/3902, 220 Gy/2503 and 240 Gy/3645)



Such phenotypes arose 7 times independently of each other in different families


Mutant 260Gy/1750: the first *deficiens*-like (homeotic) mutant in *Physalis*: two whorls of sepals, yet the inner whorl often with residual petal-like tips. Upper row: 5 different flowers. Below: in addition to the second whorl of sepals, the flowers clearly display several overies (with ovules) and styles and stigmata. Seed production is found upon pollination.

Mutant 260Gy/1750: the first *deficiens*-like (homeotic) mutant in *Physalis:* enlarged

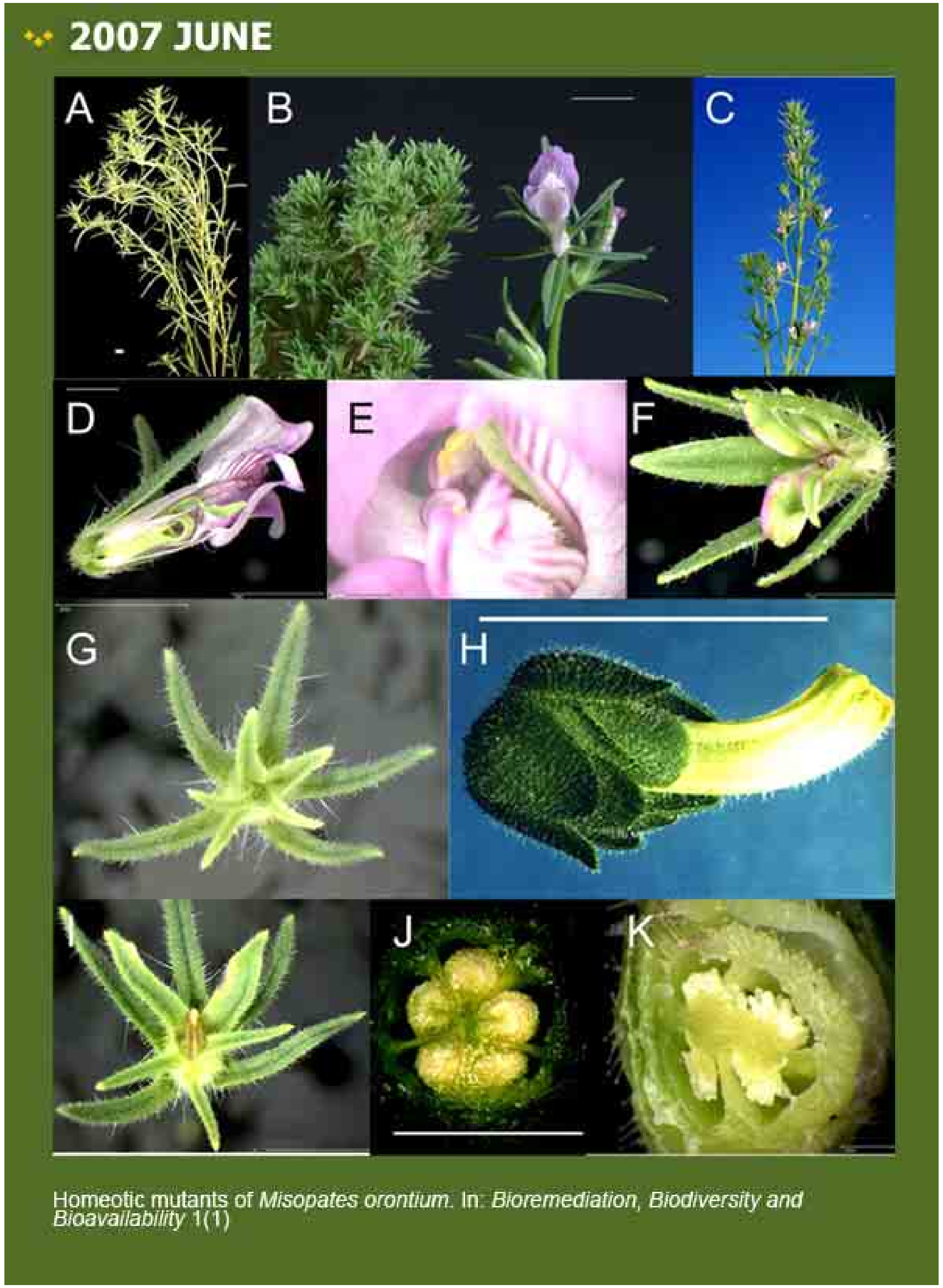
Mutant 260Gy/1750: the first *deficiens*-like (homeotic) mutant in *Physalis*: further enlarged

Mutant 260Gy/1750: the first *deficiens*-like (homeotic) mutant in *Physalis:* lantern within lantern.

Mutant 260Gy/1750: the first *deficiens*-like (homeotic) mutant in *Physalis*: outer lantern opened.

Reminds of Vavilov: The law of homologous series in variation (I have obtained similar homeotic mutants - i.e. with two whorls of sepals - in *Antirrhinum* and *Misopates* and they have also been decribed in *Arabidopsis* and many other plant species).

Photo of the month 6/2007 of Biorem. Biodiv. & Bioavail. 1:1-30
(Lönnig et. al. 2007)

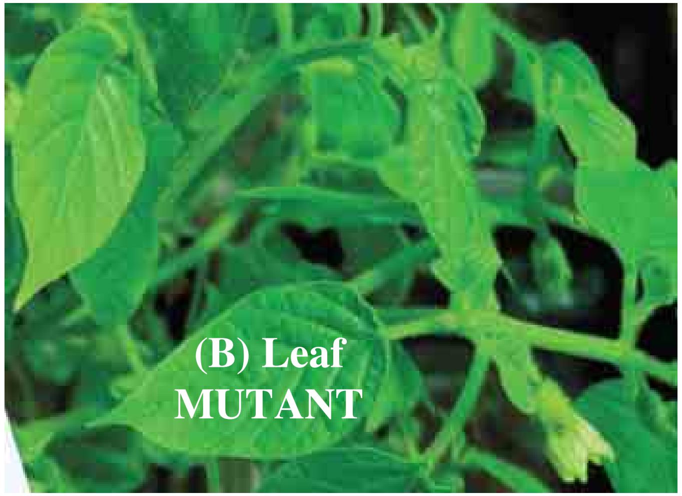


Homeotic mutants of *Misopates orontium*. In: *Bioremediation, Biodiversity and Bioavailability* 1(1)

Left (F-K) *deficiens* of *Misopates* and *Antirrhinum*.
And in comparison: *Physalis* below.

The stark difference: Several separate ovaries, styles, and stigmas in *Physalis*.

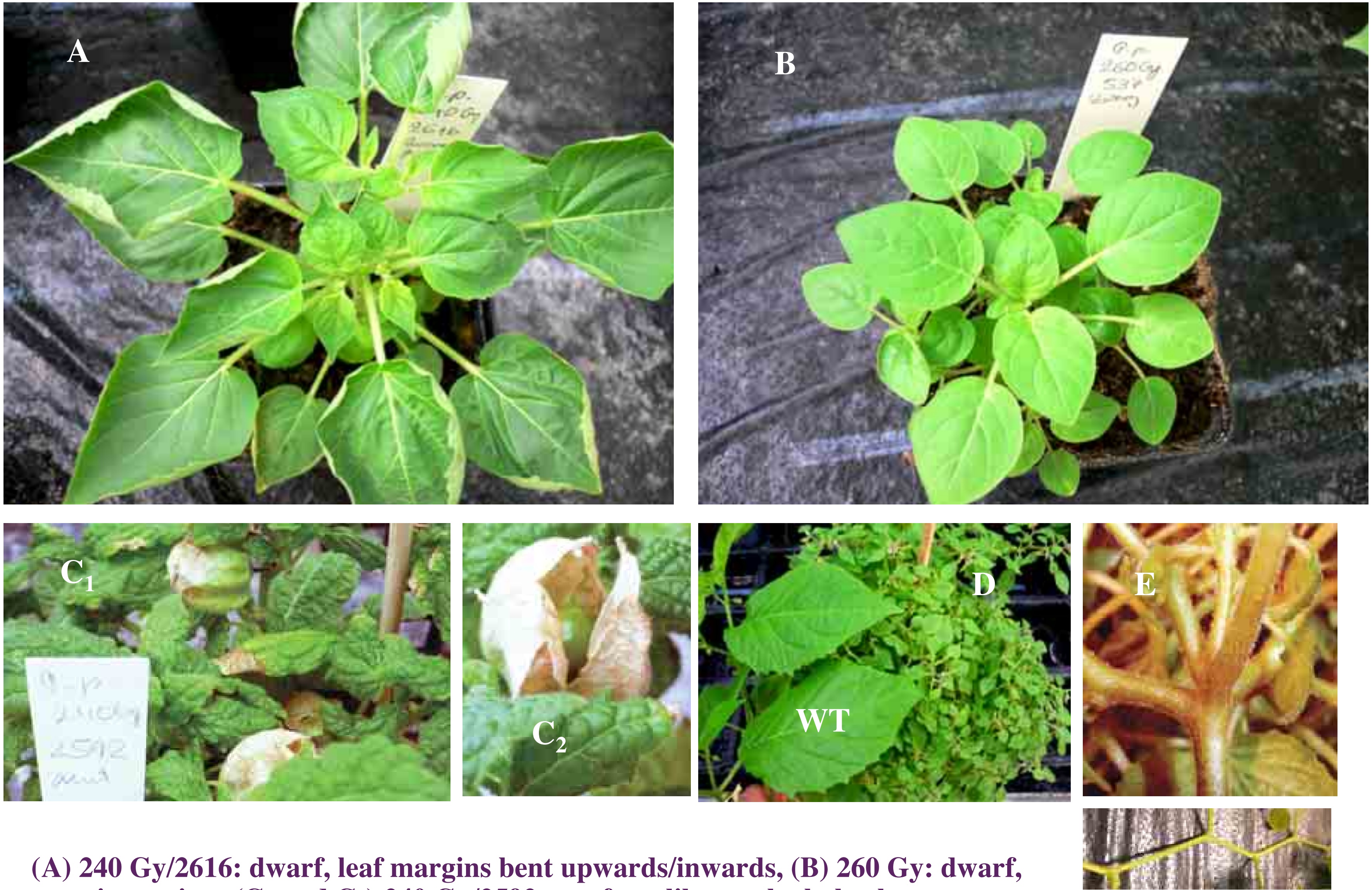
Leaf Mutants (loss-of-function macromutations)



(C)

(A) 220 Gy/1092/3: slim leaves, lanceolate, often twisted, (B) 240 Gy/1236: margins entire (also in 260 Gy/497), (C) 240 Gy/2435, (D) 240 Gy/1409 (phenotype appeared 6 times independently in different M_2-familes).

(Again: Many further often almost identical leaf form mutants appeared independently of each other in additional M_2-families: law of recurrent variation.)



(A) 240 Gy/2616: dwarf, leaf margins bent upwards/inwards, (B) 260 Gy: dwarf, margins entire, (C_1 and C_2) 240 Gy/2592: surface like curly kale, lanterns smaller, often deformed, (D) 220 Gy/1503: leaves very small, margins almost entire (WT: left), (E) same mutant line: branching pattern seems to be richer than in wildtype. **(Many further often very similar leaf mutants appeared independently in additional M_2-families: law of recurrent variation.)**

And more on the:

„Curly kale“ mutant 260 Gy/1498

which reminds also a bit of brussels sprouts (Rosenkohl) **and which is extraordinarily fertile**

Possibly 3 times independently arisen (has to be checked).

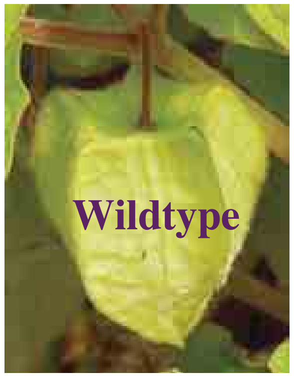
Mutant 240 Gy/4082 and similar one on the right: 260 Gy/1948

And also on the right picture within picture mutant 280 Gy/216: short pedicel, many flowers and lanterns upright

280 Gy/123 Mut 3

Chlorophyll reduction in lanterns, anthocyanin and chlorophyll reduction in upper parts of the shoots and chlorophyll reduction to a certain extent also in the upper leaves.

249 Gy/3911 Mut 5 (lantern with 9 “corners“)



Honey guides reduced or enlarged in size, colour varying

(Again: all the mutants have arisen independently of each other in different M_2-families: law of recurrent variation)

Wildtype

220 Gy/149

220 Gy/436

220 Gy/1747

220 Gy/2233Mut 2
variable

220 Gy/3121

220 Gy/2834

220 Gy/3911: Almost WT (see folio above) and others

240 Gy/4082

260 Gy/1950Mut 2

220 Gy/2442

240 Gy/4045

220 Gy/1676

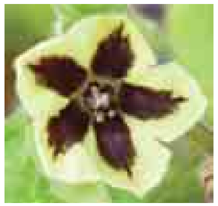
220 Gy/3068

220 Gy/281Mut 2

Wildtype and 220 Gy/2085

220 Gy/3100

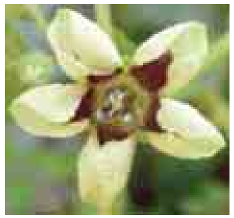
240 Gy/2344



220 Gy/2031 Mut3

220 Gy/60 and WT

240 Gy/3812



...and further numbers: 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 etc.

220 Gy/2930

(similar phenotype in mutant 240 Gy/4116)

200 Gy/93

(veins darker green than surrounding tissue)

260 Gy/1215

Totally sterile,
all dying off later

220 Gy/2728

220 Gy/3678

240 Gy/4016

260 Gy/1811

Phenotype arose 4 times independently of each other in different M_2-families.

240 Gy/3482

(veins lighter green than surrounding tissue, perhaps mutation of a microRNA involved as in a similar case in Arabidopsis?)

Similarities between individual mutant features of *Physalis pubescens, ssp. floridana,* and individual features of other wild *Physalis* species

(Similarities according figures and text of O.Vargas Ponce et al. (2003): La familia Solanaceae en Jalisco, Mexico)

Physalis aggregata "Inflorescencia con flores solitarias o casi [beinahe] agregadas, de 2-4 flores por la cercanía [Nähe] de los entrenudos" p. 22 **(260 Gy/1773, 220 Gy/1978?)**

Physalis ampla "Herbacea anual, erecta, ramificada, hasta de 60 cm de largo" p. 25 **(anual: probably several of our dwarf mutants)**

Physalis angulata "Cáliz 10-costada o globoso en el fruto, las costillas [Rippen]inconspicuas" p. 29 **(globoso: mutant 240Gy/2455)**

Physalis angustiphysa "Cáliz 5-angulado en el fruto, con diez.. líneas conspicuas de 2.6-4.2 cm de largo, 1.8-2 cm de ancho" p. 32 **(220 Gy/2739, 260 Gy/1821)**

Physalis hastatula "Hojas... En ocasiones asimétrico... Margen entero o con 1-2 dientes en ambos lados [beidseitig]" p. 48 **(240 Gy/3645)**

Physalis hederifolia p. 51 **(240 Gy/3572, 260 Gy/1820)**

Physalis leptophylla "Hojas alternas... margen entero" p. 58 **(margen entero: 220 Gy/3764, 240 Gy/1236, 260 Gy/537, 220Gy/2764)**

Physalis lignescens "lignificado en la base" p. 61 **(200 Gy/250, 240 Gy/2265, 260 Gy/1689)**

Physalis longipedicellata "Flor solitaria... pedicelo en flor de 1.7-4.5 cm de largo... Cáliz... Muy grande para la baya" p. 67 **(pedicelo: 220 Gy/166, 220 Gy/2503, 240 Gy/962)**

Physalis mcvaughii "...máculas compuestas, de color café, ca. de 7 mm de largo..." p. 71 **(large honey guides, smaller flowers: mutants (220 Gy/2666, 220 Gy/2344, 220 Gy/2085)**

Physalis microcarpa "Flor ...ca. de 2 mm de largo... Fruto una baya ca. de 7 mm de diámetro" p. 74 **(220 Gy/2390: very small flowers, small berries; similar: 220 Gy/3106)**

Physalis minuta "Hojas margen entero;.. Corola... 4-9 mm de diámetro... Fruto 8 mm e diámetro" p. 79 **(240 Gy/204, 240 Gy/2317: margen entero)**

Physalis subrepens "erecta hasta 30 cm después procumbente" p. 107 **(procumbens: 220 Gy/750, 240 Gy/4027)**

Lantern Constancy through Time

"When I see that species even in a state of nature do vary little and seeing how much they vary when domesticated, I look with astonishment at a *species which has existed since one of the earlier Tertiary periods*...This fixity of character is marvellous." Charles Darwin 1852.

***Physalis alkekengii*: several discoveries of seeds and fruit: dated to be up to**

11.61 million years old (Late/Upper Miocene)

F.Geissert,, H. J. Gregor, D.H. Mai, W. Boenigk, and T. Guenther. 1990. Die "Saugbaggerflora", eine Frucht- und Samenflora aus dem Grenzbereich Miozän-Pliozän von Sessenheim im Elsass (Frankreich). *Documenta Naturae* **57**: 1-207.

***Physalis pliocenica*: several discoveries of seeds and fruit: dated to be up to**

28.4 million years old (Upper Oligocene, Chattian)

D.H.Mai. 1997. Die oberoligozänen Floren am Nordrand der Sächsischen Lausitz. *Palaeontographica Abteilung B* **244**:1-124.

Hence, *Physalis* belongs to the "living fossils".

Darwin proposed a theory of continuous evolution for the origin of new species and higher taxa by selection of innumerable "small steps", "steps not greater than those separating fine varieties", "insensibly fine steps", "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" (Darwin 1859, 1896).

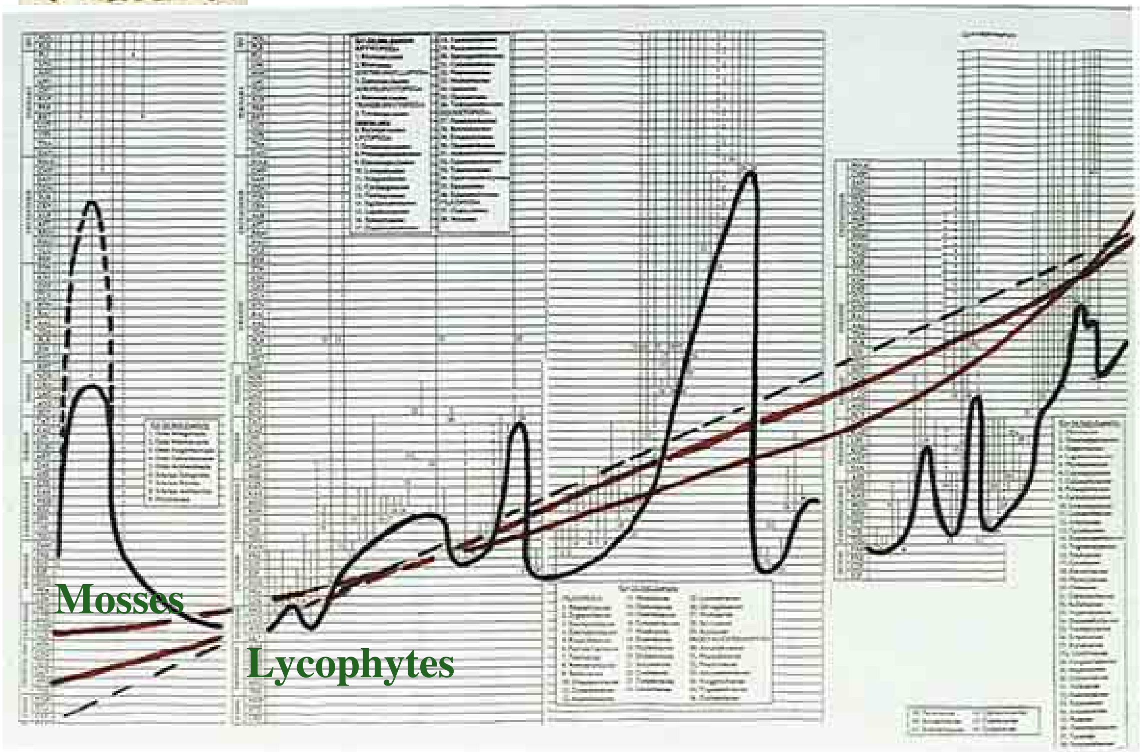
Heracleitus of Ephesus (about 544 BC to ca. 475 BC), describing the essence of nature by his famous verdict: panta rhei, ouden menei (all things flow, nothing abides). For almost everything in the plant and animal genomes seems to be in a permanent process of flux so that in the long run one should hardly expect any constant genomic (and corresponding morphological) characters at all.

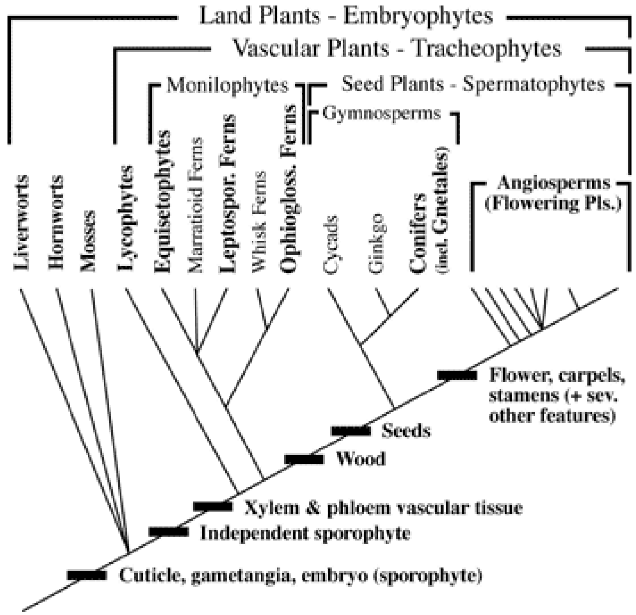
From Haeckel 1904

“The worst use of theory is to make men insensible to fact.” Lord Acton

Liverworts: Upper Devonian to recent (385.3 million years).

But Lycophytes were earlier: Upper Silurian to recent: *Cooksonia* and *Baragwanathia longifolia*, dated to be 424 million years old.





From San Diego Natural History Museum

Composed according to M.J.Benton (Ed.): The Fossil Record 2 (mosses - on the far left - and others checked for the latest results).

Phylogenetic expectations in contrast to the present state of the art of the fossil record - the only real historic facts available.

Incidentally, this present state of the art of the fossil record is almost 100 years old. I think this means something extraordinarily (the present phylogenetic schemes are basically wrong).

And what's the Giraffe got to do with Physalis?

Remember please that Darwin proposed a theory of continuous evolution for the origin of new species and higher taxa by selection of innumerable "small steps", "steps not greater than those separating fine varieties", "insensibly fine steps", "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" (Darwin 1859, 1896).

1. *Giraffa* is a living fossils (at least 12 million years old) appearing abruptly in the fossil record.

2. According to the latest research, the long-necked giraffe displays a duplication of one of its neck-vertebrae and a loss of a thoracic vertebra. - *How was this possible when natural selection "can never take a leap"?* The same question may be asked for the new joint in boid snakes* and many other discontinuous biological phenomena.

3. Moreover, without an exception, all selection theories advanced so far have been disproved (or are at least shown to be doubtful) for the origin of the long-necked giraffes even according to some of the most enthusiastic Darwinians.

4. The hypothesis of Gould and Vrba about exaptations is being discussed among giraffe researchers now.

5. Additionally, synorganization has posed several basic problems even for a macromutation in the case of the giraffe (for the details and the ID-question, see Lönnig 2006 and 2007).

Some of the points may also be relevant for *Physalis*: 1. abrupt appearance in the fossil record, 2. constancy as a living fossil, 3. testability of selection theories: question for the advantage of every one of the "innumerable small steps", 4. exaptation/neutral theory, 5. question of the probability of one or more macromutation(s). However, a basic difference is: *no strict functional synorganization* known in the *Physalis* lantern.**

*"On the isolated island of Mauritius, former home of the dodo, two genera of boid snakes.....share a feature present in no other terrestrial vertebrate: the maxilliary bone of the upper jaw is split into front and rear halves, connected by a movable joint. In 1970, my friend Tom Frazzetta published a paper entitled "From hopeful Monsters to Bolyerine Snakes". He considered every preadaptive possibility he could imagine and rejected them in favor of discontinuous transition. How can a jawbone be half broken?" Stephen Jay Gould: The Return of Hopeful Monsters. Natural History, **86**: June-July 1977, pp. 22-30.

Giraffa photograph from Grzimeks Tierleben, Bd. 13.

****You can get mutants forming lanterns without fertilization and mutants with rather small sepals (small open lanterns instead of the large wildtype ones) producing large berries with many seeds.**

Possible future projects:
Looking for some homeotic mutants, which are still missing

A) First missing but later found: In April 2007 a *deficiens-like* homeotic mutant was still missing in *Physalis*. However such a mutant was detected on 3 September 2007. In April of that year I had asked the following questions:

What will it look like before and after fertilization? Will it produce a double lantern after pollen transfer? See the answers above.

- **B) A (real) *plena* mutant** (mutant 220 Gy/2031 only with petaloid stamina)
- **C) The double mutant *plena/deficiens***

Will it produce an endless repetition of lanterns within lanterns?

Work to be done: another 100,000 plants (more than 4,000 M_2-families in the field - perhaps in China by Prof. Chaoying He)

Appendix:

Illustrating Functional Synorganization by the Example *Giraffa camelopardalis*

(besides the fact that the Giraffe cows are about 1m smaller than the bulls refuting the generally accepted feeding-competition-hypothesis of natural selection)

1. Hight up to 5.80 m: blood pressure must be extremely high to force blood up its long neck: systole 340, diastole 230.
2. Requires a very strong heart, which weighs more than 10kg, walls: diameter about 8 cm.
3. "...when the giraffe lowers its head to eat or drink, the blood rushes down and could produce such high pressure in the head that the blood vessels would burst. To counter this effect, the giraffe is equipped with a coordinated system of blood pressure controls." (Davis & Canyon) Special valves necessary.
4. Arterial walls much thicker than normal, special rete mirabile with extraordinary elastic vessels.
5. "...the fluid that bathes the cells of the body is maintained at a high pressure; this is largely achieved by the thick skin, which is tightly stretched over the body and which functions like the anti-gravity suit worn by pilots of fast aircraft." (McGowan)
6. Large volume of air in the trachea. This air is unavailable for respiration and the space it occupies is the dead space. The dead space has a volume of about 2,5 l, the rate of ventilation has to be increased. A resting giraffe takes about 20 breaths per minute, man 12, elefant 10. (McGowan)
7. Many muscles, tendons, and bones had to be modified accordingly.
8. „...to prevent profuse bleeding... all arteries and veins in the giraffe's legs are very internal. The capillaries that reach the surface are extremely small, and the red blood cells are about one-third the size of their human counterparts, making capillary passage possible. " (Hofland)
9. Ontogeny and birth modified.
10. „A miracle is an event that should appear impossible to a Darwinian in view of its ultra-cosmological improbability within the framework of his own theory. Now speaking of macromutations, let me observe that to generate a proper elephant [or giraffe], it will not suffice suddenly to endow it with a full-grown trunk [or neck respectively]. As the trunk [neck] is being organized, a different but complementary system - the cerebellum - must be modified in order to establish a place for the ensemble of wiring that the elephant [giraffe] will require to use his trunk [or neck]. These macromutations must be coordinated by a system of genes in embryogenesis." P. M. Schützenberger.

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(Some 10,000 M_1-families and 8,500 M_2-families so far - altogether about 235,000 plants - investigated so far and ca. 2000 mutants found.)

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10 (11) peer-reviewed publications* and 4 peer-edited** ones

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