Biodiversity and Dollo’s Law: To What Extent can the Phenotypic Differences between Misopates orontium and Antirrhinum majus be Bridged by Mutagenesis?

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ABSTRACT

According to Dollo’s law, evolution is irreversible. Yet, of the eight derived features essentially distinguishing Misopates orontium from its closely related Antirrhinum majus, five differences have phenotypically been clearly diminished or fully overcome by mutant genes, so that Misopates orontium outwardly approaches, meets or even overlaps the features of Antirrhinum majus or vice versa (aspects of the life cycle, leaf form, flower size, flower colour and mode of fertilization). However, to date the morphological key distinguishing feature between the two genera, the strongly elongated sepals in Misopates (itself a feature being at odds with Dollo’s law), could not be reduced to that of the length of Antirrhinum nor could the development of the short Antirrhinum sepals be extended to that of the length of Misopates, in spite of extensive mutagenesis programmes with both species (agreeing with Dollo’s law as to the stasis of this difference). Also, the long sepal character strongly dominated almost all homeotic Misopates mutants. After a general discussion of Dollo’s law, its relevance for our mutants (and vice versa) is examined according to different evolutionary viewpoints. Furthermore, two concerns are raised: (1) To what extent can the hypothesis be substantiated such that the long and short sepals could really constitute genuinely persistent (“immutable”) characters? (2) To what magnitude can the unexpected constancy of a feature distinguishing genera like the sepal difference be generalized for systematics and paleontology? Moreover, four basic genetic explanations (losses of gene functions, redundancy, the origin of new genes and chromosome rearrangements) are examined in this connection, and their relevance for some pivotal questions on the origin of species is investigated. As far as the authors are aware, this is the first thorough paper on Dollo’s law in botany.

Keywords: apomorphic, mutations, plesiomorphic, reversibility and irreversibilty in plants: phenotypic, genetic, functional

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INTRODUCTION

The celebrated Human Genome Project has been the basis for the Chimpanzee Genome Project, which was inaugurated to approach, inter alia, the question of the genetic basis for the fundamental anatomical and other differences between Homo sapiens and Pan troglodytes, especially in the face of the strong sequence similarities found so far at the DNA and protein levels (for a review and unexpected new research, see Britten 2002; Holmes 2004; Orwant 2004; Watanabe et al. 2004).

A similar, but more modest, project focusing on the main genetic differences between closely related genera in the plant world was started with our Antirrhinum-Misopates project at the Max Planck Institute for Plant Breeding Research—the genetically well-studied genus Antirrhinum being the counterpart to humans of the aforementioned programme in comparison to the human-chimp-project (as well as many others studying the genetics of conscious and/or sensitive creatures with a complex nervous system and the capability of feeling pain), one of the great advantages of approaching basic questions of biodiversity by studying closely related plant genera is, among other points, the possibility to directly apply mutagenesis to address and unravel essential problems of the genetic basis of their differences as well as the question of the phenotypic reversibility (Dollo’s law) of the features distinguishing the two genera.

As quoted above, according to Dollo “an organism cannot return, even partially, to a former state already realized in the series of its ancestors”. This statement may directly be confronted with the morphologically most distinguishing feature of Misopates, the leaflike sepals. From an evolutionary perspective leaflike sepals are assumed to be a plesiomorphic (original) character whereas clearly defined and well developed sepals standing out against the leaves are assumed to be an apomorphic (derived) trait (see viewpoints A, C1, and C2a in the discussion). Since even from the most inclusive evolutionary presuppositions Misopates cannot be directly derived from an original ancestral angiosperm stock with leaf-like sepals, this trait would have returned – at least phenotypically – “to a former state already realized in the series of its ancestors” (see the further discussion of the pros and cons below). Moreover, comparable cases have been found in several different angiosperm families, so that this character would even include a high number of convergent reversals. Now, if it is assumed that such reversals have happened many times independently by point (and other) mutations and selection – the question may also be raised whether such a reversal could itself be reversed by further mutations and/or other factors back again into the direction of the derived state, i.e. the differentiated sepal. This would constitute another “return to a former state already realized in the series of its ancestors”.

Mutations are viewed to be the ultimate basis of any biodiversity by all biologists adhering to the synthetic theory: “...pure chance, absolutely free but blind, at the very root of the stupendous edifice of evolution: this central concept of modern biology...is the only one that squares with observed and tested fact” (Monod 1971, similarly Dawkins 1997, 2003; for an extensive documentation, see Lönnig 2002). Yet, this may not be the last word and the possibilities and limits of the origin of biodiversity due to
mutations have to be further investigated (Lönnig and Saedler 2002b). So to a certain extent our project provides a test for both the potential of mutations to produce the phenomena observed as well as the validity of Dollo’s law (for possible objections against the method, see the discussion). Concerning reversions, we must, of course, clearly distinguish between the different levels of biodiversity: reversions challenging Dollo’s law on the phenotypic level may have nothing to do with highly improbable reversions at the DNA-level restoring perhaps the original sequences coding for, or being involved in, the generation of the original features (for possible exceptions due to transposable elements and methylation, see again the discussion below). Modifying effects of non-allelic gene mutations shifting the manifestation of a mutant gene toward the original phenotype are probably most often involved in phenotypic reversions. Several important exceptions from Dollo’s law have been reported (Marshall et al. 1994; Teotonio and Rose 2000, 2001; Collin and Cipriani 2003). So far, the most spectacular departure from the law appears to be the “loss and recovery of wings in stick insects” (Whiting et al. 2003).

As for the systematic position of Misopates, it is so closely related to Antirrhinum that for centuries it was treated as one of the species of the genus Antirrhinum (Besler 1613; Tournefort 1700; Linné 1753; Miller 1768; Chavannes 1833) until it was raised to the level of a genus in 1840 by Rafinesque. Yet even after that revision many authors went on speaking of Antirrhinum orontium (Linné’s original species name) instead of Misopates orontium well into the 20th century (see, for instance, Hegi 1928; Bonnier and Douin 1935; Harrison 1960, also Antirrhinum orontium is usually cited in present synonym lists). Besides, in modern gene trees the two species usually cluster closely together (Gübitz et al. 2003; Hileman and Baum 2003). As for the etymology of the names, the following points may be worth mentioning: Antirrhinum, derived from Greek, anti, here meaning ‘like’, and rhis, rhinos, ‘nose’, probably referring to the nose-like capsule in its mature state and majus, Latin, comparative of Magnus, the former meaning ‘larger’ pertaining to the large size of the flowers (see Hartl 1974; Schubert and Wagner 2000). Misopates: according to Corneliuson (1997) is derived from Greek misos, ‘to hate’, and patein, ‘to step on’ (the author of the name seems to hate to step on that beautiful little plant), and the meaning of orontium seems to be derived from Latin oro, ‘to speak’, ‘to plea’, ‘to beg’, which may have something to do with the mouth-like form of the flower.

Surveying the differences between the two genera, it is to be noted that M. orontium is distinguished from the Antirrhinum majus essentially by the following features:

<table>
<thead>
<tr>
<th>Feature</th>
<th>Misopates orontium</th>
<th>Antirrhinum majus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Life cycle</td>
<td>Annual</td>
<td>Perennial</td>
</tr>
<tr>
<td>2) Leaves</td>
<td>30-50 mm x 1.6-6 mm</td>
<td>36-70 mm x 10-26 mm</td>
</tr>
<tr>
<td>3) Sepals</td>
<td>As long as or up to twice as long as the corolla</td>
<td>1/5th of the length of corolla</td>
</tr>
<tr>
<td>4) Flower (corolla) size</td>
<td>11-15 mm</td>
<td>33-46 mm</td>
</tr>
<tr>
<td>5) Flower colour</td>
<td>Light violet or almost white</td>
<td>Red (“purplish pink”)</td>
</tr>
<tr>
<td>6) Fertilization</td>
<td>Tendency to autogamy</td>
<td>Allogamous</td>
</tr>
<tr>
<td>7) Seed form</td>
<td>Bowl/cuplike</td>
<td>Elliptical/roundish</td>
</tr>
<tr>
<td>8) Seed formation</td>
<td>Also due to apomixis</td>
<td>Apomictic seed formation unknown</td>
</tr>
</tbody>
</table>

Presumably, a further significant difference between the two species appears to consist of the absence (Misopates) and presence (Antirrhinum) of larger numbers of active transposable elements (see the details under flower variegation below).

When Linné coined the name “Antirrhinum orontium” in 1753 for our Misopates orontium, he positively characterized the species by its morphological key feature: “calycibus corolla longioribus” and “calycibus flores superanthis” (“with sepals longer than corolla’ and ‘with sepals towering above the flowers”). Later, the extremely elongated sepals were the reason for Lamarck even to rename the species as “Antirrhinum calycinum” (Linné 1753; Lamarck quoted according to Hartl 1974) (see Fig. 1A).

Fig. 1 Juxtaposing Antirrhinum and Misopates and Misopates wildtype with a mutant. (A) Comparison between Antirrhinum majus (above) and Misopates orontium (below). Note that even in absolute terms the sepals of Misopates are more than twice as long than those of Antirrhinum. (B) Wild-type (left) and mutant sister plant (plona, right) are of the same age. The mutant is still green and keeps on growing and flowering whilst the wild-type sister plant is already withered and dry. The similar relation was found for a femally fertile deficiens mutant even after seed set as well as for the gigas mutant L 2002/1843. Bars: The bars represent 1 cm except for 2 (C) (2 cm), 3 (B), 5 (E) 5 (F), 6 (D), 7 (B) (0.5 cm), and 3 j (1 mm).
Also, the annual life cycle and the cup-like seeds belong to the more conspicuous characters of Misopates distinguishing it physiologically and morphologically as a genus from Antirrhinum. Moreover, there is a strong fertility barrier between the two genera. Of the few tentative F₁ hybrids, it has been reported that they can be obtained only with Misopates as the mother, that the Misopates features proved to be dominant over those of Antirrhinum and that the progeny of the hybrids did not display Mendelian segregation and recombination of the many different character pairs of the two genera as normally expected (Harrison and Darby 1955; Harrison 1960). Since Misopates displays a strong tendency to autogamy, it is not clear how many of the tentative hybrids constituted actual F₁-plants, or, as we suspect, whether perhaps almost all were just contaminations with the exception of perhaps a few cases of seed production due to apomixis (agamospermy) (see also Ernst 1918), which we have detected in Misopates. However, the apomictically produced seeds proved to be sterile so far (see the details below).

In relation to Antirrhinum in particular and the tribes of the Antirrhineae in general all the eight features specifying Misopates are classified as apomorph (derived) characters, which should hardly be reversible according to Dollo’s law (dollo in the discussion). With special emphasis on this issue as well as a possible potential of Antirrhinum itself to generate features bridging the phenotypic gap to Misopates, the following questions will be addressed and discussed in the present paper:

1) To what extent can the differences between the two genera be bridged by phenotypes due to mutant genes in either direction? In detail:
   a) To what extent can the distinguishing features of the leaves, flower size, flower colour and mode of fertilization of the two genera phenotypically be bridged by mutant genes?
   b) Can the (annual) Misopates life cycle be extended to that of Antirrhinum (perennial) or vice versa: have annual Antirrhinum mutants been isolated?
   c) Regarding the most prominent morphological difference we focussed our attention on the following points: can the long Misopates sepals be reduced in perhaps one mutant step (or several small ones) to the length of Antirrhinum sepals or – vice versa – have Antirrhinum mutants with comparatively long sepals ever been obtained in our own experiments or those of any other researchers.

Also, for reasons given below, the subsequent questions have been investigated in the mutant phenotypes:

2) To what extent are the mutant features of leaves, bracts and sepals correlated in Misopates?

3) What do the corresponding homeotic flower- and inflorescence mutants of Misopates and Antirrhinum reveal about the genetic differences between the two closely allied genera?

4) Does Misopates display features of regressive evolution (genetical and morphological losses of functions) especially in comparison to Antirrhinum or the tribes Antirrhineae in general?

Concerning the origin of the essential differences between the two genera, Erwin Baur stated after pointing out that selection of “small factor-mutations” and recombination were responsible for adaptations within the genus Antirrhinum: "However, the origin of A. majus and A. orontium [now Misopates orontium], which belongs to another genus section [now another genus] from a common ancestral form, can hardly be envisioned by these factors. For the time being we can only confess our "ignoramus" (Baur 1930; see also Lönngig and Saedler 2002a).

In addition to a test of Dollo’s law and the question on the power of mutagenesis, the following report also provides an investigation for Baur’s statement.

MATERIALS AND METHODS

Two lines of Misopates orontium have been used for the present studies: the nearly white flowering M. orontium from the Botanical Garden of Coimbra, Portugal (obtained via IPK of Gatersleben, FRG) and a typically violet flowering line derived from one wild Misopates plant spontaneously growing in Köln Vogelsang (the present geographical distribution of M. orontium includes not only the large parts of Middle and Northern Europe, and it has become a recent invader of many further parts of the world; for the details, see Hartl 1974; Sutton 1988; Haeppler et al. 2003). Since there are no cultivated lines known in Misopates (Hartl 1974), both these lines appear to represent different varieties of the same species in the wild. Also, there was no fertility barrier between the two lines (crosses were made in both directions). Nevertheless, it should perhaps be mentioned that the Misopates line from Coimbra is viewed to be a species of its own by some systematists, classifying it as Misopates calcynium (Franco 1971). For the genetic reasons just mentioned and further points (Lönning 2002), we do not follow this practice here. Moreover, in contrast to the description of M. calcynium presented by Sutton (1988), the sepals of the Coimbra line are slightly longer than those of the Vogelsang line of M. orontium. Although according to Stace (1997) M. orontium can have 2n=14 or 16 chromosomes, all Misopates 'species' so far investigated appear to have 2n=16 chromosomes (Sutton 1988) like the species of Antirrhinum (see, however, Pseudomisopates below).

Mutations in dry seeds were induced by fast neutrons and gamma rays in Siebendorf (IAEA), Austria, and Forschungszentrum Jülich, FRG, respectively. Seeds of M. orontium from the Coimbra line were treated with fast neutrons (three packages with 3.5 Gv, 5.5 Gv, and 7 Gv respectively) in 1999 in Siebendorf, and a second group in 2001 with gamma rays (200 Gv, 240 Gv) in Jülich. In 2001 seeds of the Vogelsang line were treated with gamma rays (200 Gy, 240 Gy), also in Jülich. Apart from the fact that the mutation frequency increases with the concentration of a mutagenic agent applied and that there can differences concerning the various kinds of lesions on the DNA-level (especially in chemical mutagenesis), no correlation of the types and strengths of the treatments with the types of mutants could be detected so far. In general this seems to be in agreement with the laws of probability for a non-directed process and the results of most other mutagenesis investigations. For instance, the almost infinite variation of the different kinds and doses of applied mutagens has not resulted in more of the better mutants in mutation breeding in spite of many earlier hopes and expectations (for further points and reviews on mutation research see Auerbach 1976; Lönning 1993, 2006).

Altogether 335,000 plants of Misopates have been investigated including ca. 10,800 M₂-families. Moreover, during the last 22 years 1.5 million Antirrhinum plants including some 30,000 M₂-families have been investigated by W-E L (mutagenesis by transposons, EMS, fast neutrons, gamma rays, and X rays as well as combinations of the mutagenic agencies). As for the logistic details, the same principles were followed as described in detail by Lönning and Huijser (1994), and Kunze et al. (1997). To solve the question, as to what extent the main phenotypic differences between the two species can be overcome by mutagenesis encompassing a test of Dollo’s law, to the best of our knowledge the present paper also reports the first (and at the same time extraordinarily large) mutagenesis programme with the wild species M. orontium.

Identification of mutants

Since locus identity test crosses between Misopates and Antirrhinum are not possible, the genes so far identified were recognized by sequence analyses of the homologous Antirrhineae/Misopates wild-type genes and their mutant deviations. Of the many mutants only those relevant for the questions of our topic have been considered in the present paper (a general overview of all the mutants isolated – being a topic of its own – is beyond the scope of the present paper). The numbers below (like L 2002/1283) refer first to the year in which an M₂ was evaluated, and second to the number of the segregating family; the letter L stands for the first author’s surname.)
For measuring the genetic distance between the two taxa, a series of additional genes have been sequenced (also) by PCR. If primers from one organism are also homologous to the sequence of another organism they can be used to sequence DNA from related species as has been done here for *A. majus* and *M. orontium* (the extensive tables concerning the oligos used and the positions of the oligo primers can be directly obtained from the authors). For further points on method, see Saikia et al. (1988).

The *Misopates* sequences have been submitted to the EMBL Nucleotide Sequence Database and can be retrieved by the accession numbers AM162204 to AM162213 and AM396483 to AM396489.

**RESULTS**

In the ensuing paragraphs, the results of the investigations are presented in accordance with the enumeration of the characteristics distinguishing the two species and genera as given in Table 1, followed by the topics of flower variegation and transposon activities, homeotic mutants, and genetic distance between the two species.

**Life cycle**

Annuality in plants is usually viewed to be an apomorphic (derived) feature. Three mutants of the normally annual *Misopates* have displayed a clearly elongated life cycle: *A. deficiens*, *A. plena*, and *A. floricaula*-like (*L. 2002/1283* mutant). Normally the average life span found in our field trials with the wild-type *M. orontium* lasted three to four months for the Vogelsang line, from which all three mutants have been derived (and up to four months in the Coimbra line). These mutants, however, now habitually live some 12 months and can be further propagated (probably indefinitely) by cuttings (until now for four years). Interestingly all three cases belong to the group of homeotic mutants (Figs. 1B, 4A, 4B, 4D-K). Moreover the female organs of the *deficiens*-mutant are fully fertile (instead of the stamina, compound styles and stigmas are produced). Yet even after seed-set the longevity does not seem to be reduced (extended propagation by cuttings has also been tried, but not achieved, for the wild-type).

Apart from these homeotic mutants, plants of the third generation of the leaf mutant L 2002/1843 (Figs. 2A-C), also derived from the Vogelsang line and further described below, reveal *gigas* growth at 15-17°C – they grow more than 230 cm long (the initial line grows up to only about 120 cm under the same conditions) and display an elongated life cycle (up to 10 months) (as for details on the term *gigas*, see Schubert and Wagner 2000). They can also be further propagated by cuttings, yet their life span appears to be limited to a few more months. Additionally, its seed production is strongly raised under these special environmental conditions. In contrast to these results at 15°C, the mutant can hardly be distinguished from the wild-type at 25°C (aside from its broad leaves).

As mentioned above, the unusual sepals of *Misopates* constitute the most prominent apomorphic feature morphologically distinguishing the two genera and species from each other, even to the point of inspiring Lamarck to coin the species name ‘*calcium*’. The sepals are as long as or up to twice as long as the corolla in *M. orontium* (developmental stages and modifications playing an important role in their variation), but the sepals of *Antirrhinum* are only ca. 1/5 the length of its corolla. Also, in absolute measurements, the *Misopates* sepals are still twice to three times as long as those of *Antirrhinum* (Fig. 1).

**Sepal- and leaf form correlations**

Coming back to the second question of the introduction, mutant deviations in leaf form appeared to be strongly correlated in *M. orontium* for all leaf organs, i.e. leaves, bracts and sepals. So when the mutant leaves were more slender, those of the bracts and sepals displayed the same phenomenon. When the leaves proved to be broader, this was also true for the rest of the leaf organs (until now we have detected only one clear exception from this rule: in comparison to the wild-type, mutant L 2004/495 displays shorter and broader leaves, yet the sepals are not correspondingly changed). Conversely, in *A. majus* the widths of the sepals seem to be relatively independent of the other leaf organs: the sepals proved to be as broad as usual in several slender leaf mutants (for an extreme example, see the mutant *phantastica*, see Baur 1926; Waite and Hudson 1995, 2001; Waite et al. 1998) as well as in the subspecies *tortuosum*, the latter case displaying very slender (linear) leaves, but broad sepals (Fig. 21). However, there are also *Antirrhinum* mutants where all three leaf organ types are affected by one and the same mutant gene (for example *abbreviata*, *acuminata*, *buxifolia*, *cincinnata*, *compacta*, to name but a few, for further mutants, details and references, see Stubbe 1966).

The usually strong correlation-results of leaf-mutation experiments in *Misopates* appear to be in agreement with the unanimous verdict of all morphologists commenting on the nature of the *Misopates* sepals as being essentially leaf-like (except, perhaps, the venation pattern with more parallel main veins, which corresponds to that of the sepals of *Antirrhinum*). In genetical terms this could mean that regulatory and target genes, which are expressed in *Antirrhinum* only in the leaves, but not in the bracts and/or...
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sepals, are now ectopically expressed in the *Misopates* sepals as well.

Even so, the exception of mutant 2004/495 from the correlation rule as mentioned above could be interpreted as a first hint at additional autonomous gene functions, which might be involved in the unusual calyx formation of *Misopates*.

**Mutagenesis of sepal length and numbers**

Two *Misopates* mutants displayed shorter sepals compared to those of the wild-type species (Figs. 2D-G, 3A, 3B). However, in the mutant L 2000/1554, which was derived from the nearly white flowering Coimbra line from Portugal, all the leaf organs proved to be shorter than normal in harmony with the rule just mentioned. Additionally, flower form was also slightly affected, yet fertility was almost normal. In the second mutant (L 2003/1191), derived from the Vogelsang line of *Misopates*, not only all the leaf organs were reduced in length, yet increased in width, but also the petals and male and female organs proved to be all extremely shortened, and so were the internodes, but the

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**Fig. 2 Leaf mutants of *Misopates* and *Antirrhinum*.** (A) (larger photograph). On the left: wild-type *Misopates orontium* with linear leaves; right (second tray): *Misopates* mutant L 2002/1843 with broad leaves. (B) (small figure within figure). From left to right: leaf of *A. majus*, normal leaf of *M. orontium*, *M. orontium* mutant L 2002/1843. (C) below the white string, except upper right: *Misopates* mutant L 2002/1843, above string, different culture varieties of *A. majus* some with moderately broader and others with more slender leaves than those of the *Misopates* mutant. (D) Broad-leafed *Misopates* mutant L 2003/1191 segregating in M2 population. (E), (F), (G): Mutant L 2003/1191 (enlarged). (E) compressed flowers of the mutants; (F) calyx consisting of broad sepals; (G) longitudinal section through ovary displaying normal looking ovules. For further details see the text. (H) *phantastica* mutant of *A. majus*. (I) Left, above and below: flower and leaf of *A. majus*, ssp. *majus*; right above and below: flower and leaf of *A. majus*, ssp. *tortuosum*: note that the width of the sepals is not correlated with the width of the leaves. (D-F) reveal that in *Misopates* there appears to be a stricter correlation for these features than in *Antirrhinum* (see also Fig. 4).
enlarged stem diameter (up to three times) was reminiscent of that of succulent plants (Fig. 2D-G). The stem was twisted. Moreover, fertility was strongly reduced in the latter mutant and the life cycle appeared to be abbreviated so that the mutants obtained so far, (1) dried up significantly earlier than the wild-types and (2) died without seed set, i.e. although ovule development appears to be normal, to date neither selfings nor pollination with wild-type pollen led to any seed being formed. Although stigma-like tissue appears at the style in early phases of development, the style appears to be closed at later stages. At present we propagate the mutant by using up the M2-seeds and by heterozygote sister plants: In 2005 the segregating mutants derived from M2 sister plants again displayed their typical characters (as expected), yet grew larger and lived longer than those of the M2 family.

Besides, sepal length can vary considerably in the totally sterile *plena* mutant of *Misopates*: flowers near the top of the inflorescences of older plants can show relatively short sepals (Fig. 3C): Yet this phenomenon hardly presents a clue to the questions raised.

The overall results concerning the problem formulated at the beginning – whether the enormously elongated *Misopates* sepals could be reduced to the length of the *Antirrhinum* sepals in perhaps one large mutant step or several smaller ones – has to be answered in the negative so far. The two mutant exceptions described above (not to mention the homeotic *plena* mutant) are hardly more than freaks, the pleiotropic effects of the mutant genes being recessive and thus due to losses of functions disturbing normal development and affecting the length and width of *all leaf organs* indiscriminately. Hence, the long sepal feature has proved to be extraordinarily stable until now.

As for *Antirrhinum*, no mutants with comparably long sepals have ever been detected in any of the mutagenesis experiments over the last 100 years.

**Sepal number**

In contrast to the length of the sepals of *Misopates*, their number can decidedly vary: (1) In mutant L 2002/5 the first flowers displayed only 3 to 4 more or less asymmetrically arranged sepals but the further upstream the flowers were positioned in the inflorescence, the more normal were their sepal numbers and symmetries; (2) one plant of L 2002/1323 also revealed special individual and ontogenetic asymmetries: the first flower had 7 sepals, but the following ones only 5.

No corresponding phenotypes have been described in *Antirrhinum*.

Homeotic sepal mutants with up to 7 sepals will be treated below under the section of homeotic mutants.

**Flower (corolla) size**

As hinted at in the introduction, at first sight one of the more impressive differences between the two species appears to lie in the size of the flowers (see Fig. 1). In relative terms the size of the flowers of *A. majus* is about twice the length and width of that of the *M. orontium* flowers (for absolute measurements see Table 1).

Concerning the basic questions addressed in the title of the present paper, it is to be noted that in 22 cases independently arisen *Misopates* mutant candidates (seven tested, all heritable) display enlarged flowers (Fig. 3D). On the other hand, some *Antirrhinum* mutants show reduced flower size. Thus flower mutants of both species appear to approach each other in this feature. Interestingly, the sepals in the enlarged *Misopates* flowers were correspondingly longer (also to be seen in Fig. 3D). The flower size mutants of both species include fertile lines showing no or only weak pleiotropic defects in other plant organs. However, it should also be noted that mutants in both species have been detected, which swing in the opposite direction: *Misopates* mutants with even smaller flowers than those of the wild-type and *Antirrhinum* mutants with larger flowers have been detected as well. An open question is whether the enlarged flower mutants of *Misopates* have already reached their selection limit for that character (for details on selection limits, see Lönnig 2001, 2002, 2004, 2006), or whether – by continued mutagenesis – further enlargements would be possible.

**Flower colour**

Flower colour differences of isolated but otherwise similar populations of a species have often been deemed to be sufficient to suggest new species names in morphological systematics (for documentation, see Lönnig 2002). The typical *M. orontium* flower colour as given in almost all field guides is light violet with a clearly darker violet venation pattern in the upper lip. Flower colour of *A. majus* ssp. *majus* is several degrees darker (purple). So far, none of the many flower mutants of the Vogelsang line of *Misopates* has really approached this deep red of *Antirrhinum* (anthocyanin and cell shape are significant in the latter, see Noda et al. 1993; Schwinn et al. 2006), but virtually all mutant colour deviations moved in the opposite direction: a series of *Antirrhinum* mutants revealed nearly all shades of brightening until the pure white of the *nivea* lines of *Antirrhinum* was reached. So the mutants of the two species find a common level in loss of function mutations diminishing or abolishing anthocyanin synthesis.

In addition to the lack of potential to produce steps in the direction of the deep red flower colours so far in *Misopates*, another difference has been noted: the *Antirrhinum* colour mutants and recombinants displaying a whole series of yellow and bronze colour shades seem to be absent in *Misopates* (the *Sulphurea* gene?), pointing, if corroborated, to a poorer equipment in the anthocyanin pathway in *Misopates* as compared to that of *Antirrhinum*.

In the nearly white flowering Coimbra line of *Misopates* there appeared one mutant which proved to be violet-flowering in the field (Fig. 3H), so much so that at first sight we were not sure whether perhaps a contamination of the seed materials with the Vogelsang line had occurred. However, that could definitely be excluded by further investigations: upon closer inspection anthocyanin synthesis appeared to be UV light dependent in this mutant, i.e. when the UV component was missing, the flowers of the mutant remained nearly white – in contrast to the violet *M. orontium* wild-type lines (Fig. 3F-I). So in the field, the colour of the almost white flowering wild Coimbra line had phenotypically reverted to the more common violet of its putative ancestors.

**Fertilization**

In contrast to the wild *A. majus*, there is a clear tendency to autogamy in the *M. orontium*. Although the flowers of wild *Misopates* plants are diligently visited by different species of bees in the experimental field as well as in the wild, if cross-fertilization is prevented, they invariably set seeds by autogamy, self-fertilization beginning rather early in anthesis, roughly one to two days before normal petal development is finished and when the flower is still closed but the pollen sacs are already opening.

Hence, in *Misopates* the series of multiple alleles for ensuring self-sterility so characteristic of almost all wild *Antirrhinum* species appears to be absent or non-functional in the former (see also Kusaba et al. 2001, and Nasrallah et al. 2002 on Arabidopsis thaliana as a case of loss of self-incompatibility in the Cruciferae). However, most *A. majus* culture varieties and the (further) mutants derived from them, are self-fertile as well. Yet in *Antirrhinum* seed set due to autogamy is decidedly weaker than in *Misopates* and to guarantee full seed production in the former, *A. majus* culture varieties and mutants are usually selfed. Nevertheless, on the loss of function level the two species
appear to approach each other again. (Loss of functional self-sterility alleles appears to have also occurred in the wild species Antirrhinum siculum). As for seed development without fertilization, see below the points under “seed formation due to apomixis”.

**Seed form**

The bowl- or cuplike seed form of *M. orontium* shown in Fig. 3J (in contrast to the elliptical-roundish seeds of *A. majus*) belongs to the more important and rather constant features distinguishing the two species. So the seeds of several *Misopates* mutants have been studied, yet to date no clear-cut deviations in mutant seed morphology have been detected. Several mutants, such as L 2000/1554 (leaves including the sepals reduced and somewhat twisted), L 2000/2064 (strong cycloidea allele), L 2002/691 (pure white flowers), L 2002/1843 (broad leaves; see above), L 2003/1191 (also broader leaves, but probably sterile, see above, M2-seeds investigated) and L 2202/2661 (larger...
flowers) all manifested the seed form of the wild-type. In sterile mutants there is the difficulty that seeds of heterozygote sister plants segregating the homozygous mutant seeds or – if available – M$_2$ seeds have to be examined. In both cases distinct, sharp and well-defined differences should be found among the seeds segregating 3 wild-type seeds to 1 mutant seed or – in cases with a deficit of recessives – correspondingly lower percentages of homozygous to seeds mutants would have to be discovered. In spite of the general stability of differences in the seed form between the two species, seed development is not always uniform, and it appears that reliable conclusions can be drawn only after repeated and extended investigations of larger seed materials, which remains to be done.

**Seed formation due to apomixis**

As far as we are aware, apomixis has never been reported for *Antirrhinum* – or for *Misopates* either. Even so, one of us (HS) had expected it in *Misopates* because of some alleged F$_1$-hybrid plants between *Misopates* and *Antirrhinum* displaying almost exclusively the *Misopates* phenotype (Harrison and Darby 1955; Harrison 1960). However, in a potentially autogamous plant species self-fertilizing itself early in anthesis, the production or transfer of at least some pollen grains can hardly be absolutely excluded (even in cases of early castrations). The case is, of course, different in species with massive or even regular apomictic seed production as found, for instance, in many Asteraceae. Yet, in crosses between *Misopates* (as mother plants) and *Antirrhinum*, normally there was not any seed set at all (some 100 pollinated flowers).

Nevertheless, the problem has – at least in principle – been solved by work with our *deficiens* mutant L 2002/2851 of *Misopates* (see text below and Fig 3J). This mutant displays a strong *deficiens* phenotype and is unable to produce any pollen at all. Yet surprisingly it can produce high numbers of seed capsules filled with rather cranky looking seeds (Fig 3J). Although none of these seeds has germinated as yet (59 capsules sown, ca. 40 seeds each), looking seeds (Fig. 3J) have also appeared in *Misopates* sequenced so far (see Table 2 below). The results obtained to date appear to point to reduced transposon activities in today’s *M. orontium* as compared to *A. majus*.

**Homoeotic mutants**

Because homoeotic mutants can disclose similarities of and differences between different taxa usually hidden in their normal development, it was assumed that they might help elucidate these questions also for *Misopates* and *Antirrhinum* as well as be relevant for Dollo’s law – the extent of reversibility of certain derived features of *Misopates* (see examples and discussion below).

Most of the homoeotic mutant phenotypes described for *Antirrhinum majus* have also appeared in *Misopates orontium: cycloidea, hemiradialis, floricaula, squamosa, plena, deficiens, fimbriata* and others. Wherever possible so far, the identity of the mutants was clarified by DNA sequence analyses (Table 2).

**Table 2 The homoeotic *Misopates* mutants.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutant</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLEXA</td>
<td>2002-745</td>
<td>small deletion*</td>
</tr>
<tr>
<td>SQUAMOSA</td>
<td>2003-765</td>
<td>large deletion*</td>
</tr>
<tr>
<td>FLORICAULA</td>
<td>2003-369 nu**</td>
<td>1 sub 12 del</td>
</tr>
<tr>
<td></td>
<td>aa***</td>
<td>1 change 4 del</td>
</tr>
<tr>
<td>FIMBRIATA</td>
<td>2002-1574 nu</td>
<td>frame shift &amp; stop</td>
</tr>
<tr>
<td>DEFICIENS A</td>
<td>2002-823 nu</td>
<td>21 del</td>
</tr>
<tr>
<td></td>
<td>aa</td>
<td>7 del</td>
</tr>
<tr>
<td></td>
<td>2002-1249 na</td>
<td>frame shift &amp; stop</td>
</tr>
<tr>
<td>CYCLOIDEA</td>
<td>2000-2064 nu</td>
<td>6 del</td>
</tr>
<tr>
<td></td>
<td>aa</td>
<td>2 del</td>
</tr>
</tbody>
</table>

* **the exact boundaries of the deletions were not identified**

**Floricaula-like mutants**

(Fig. 4A-C) (as for the detailed work on the corresponding *Antirrhinum* mutants, see Coen et al. 1990, Huijser et al. 1992): Because the morphological differentiation into leaves, bracts and sepals is absent in *Misopates* as compared to *Antirrhinum* and to most other members of the tribe *Antirrhineae*, in all our *floricaula*-like phenotypes (4 independently arisen cases, two of the mutant genes, *squa* and *flo*, have been identified so far – see Table 2) a repetition (or ‘star’ appearance) of homoeological regions (instead of bract repetition as in *Antirrhinum*). This may seem trivial (a lost differentiation feature cannot, of course, be repeated), yet in cuttings made from three nonflowering lines (L 2000/1089, L 2000/3582, both Combrande- derived, and L 2003/369, Vogelsang-derived), the leaves (repetitive bracts) appearing anew in the regenerated inflorescence were much smaller than those of the original mutant (Fig 4B), somewhat reminiscent of the small *Antirrhinum* bracts (in extreme cases they were even shorter
than in Antirrhinum). However, line 2003/765 (Vogelsang-derived; Fig. 4C), which regularly produces some malformed flowers (unexpectedly developing normal seeds regularly without artificial pollination) the repetitive leaves appear to be larger in the cuttings made so far as compared to the non-flowering phenotypes, nevertheless smaller than in the wild-type control.

Plena

(as for Antirrhinum, see Bradley et al. 1993; Lönnig and Saedler 1994): In agreement with the most distinguishing bauplan feature of the species, the plena mutant of M. orontium (L 2002/745, derived from the Vogelsang line) is clearly different from the plena mutant of Antirrhinum by the modified reiteration of the longer sepal feature within the flower from the otherwise corresponding Antirrhinum plena mutants. The character reappears in the fourth whorl of the mutant flowers (Fig. 4D, 4E). However, the inner sepals do not constitute a simple repeat of the outer wild-type Misopates sepal whorl, but are modified: they are clearly shorter (on average nearly intermediate between the sepal lengths of Antirrhinum and Misopates thus approaching the situation in the Antirrhinum mutant), and more tender and lighter green than those of the outer whorl, the pale green being possibly due to strong light protection in the innermost part of the flower. Moreover, the upper sepal appears to be generally longer than the lower one (some 2-3 mm). In contrast, in the plena mutants of Antirrhinum the inner sepals are about the size of the outer ones, sometimes even slightly longer (1-2 mm), but also lighter green. Besides, the plena mutant of Misopates can produce an additional shoot with 2 sepal-like leaves between the main axis and the pedicel so that at first sight it looks as if the flower had 7 sepals - a phenomenon not reported for Antirrhinum.

Moreover, the otherwise phenotypically closely corresponding plena mutant of A. majus obtained from the IPK (Gatersleben) develops – like our Misopates mutant – anthers at the upper parts of the second (inner) petal whorl. In Antirrhinum these anthers definitely produce some fertile pollen grains, which have successfully been used for the generation of hybrids, but in Misopates the anthers seem to be sterile. Yet at present it is not known whether similar sequences of the corresponding genes of the two species are mutated in comparable ways. As already mentioned above, in older inflorescences the length of the sepals of the upper flowers appears to be reduced (Fig. 3C).
Deficiens

The Deficiens gene of A. majus was the first homeotic plant gene to be cloned and characterized (Somer et al. 1990). As for Misopates, two deficiens (def) mutants have been derived from the Vogelsang line of M. orontium (L. 2002/823 and L. 2002/2851; Fig. 4F, 4G, 4I). In L. 2002/823 the style is short and strong, the petals are not completely transformed into sepal but display residual petaloid tissue (usually the sepal of the second whorl are about half as long as the normal ones but broader and often show a light violet rim) (Fig. 4F). Yet the mutant is slightly temperature sensitive: Under 25°C the petaloid features are strongly reduced, under 15°C they appear as shown in Fig. 4F (see also the double mutants in Fig. 7). At the DNA level this def mutant is characterized by a deletion of 21 bp starting in the K-box after downstream position 255 (in the corresponding protein 7 amino acids are missing: downstream residues nos. 86 to 92 inclusively; see also Table 2). Interestingly, the strongly temperature sensitive mutant def-101 of Antirrhinum is due to a deletion of three base pairs of the K-box only one step downstream: amino acid in position no. 93 is missing, a deletion of a lysine residue (Schwarz-Sommer et al. 1992).

The sequences of the wild-type (wt) and temperature sensitive (ts) def mutants of A. majus and M. orontium starting at position 253 of the coding sequences of the gene ending at position 300 (the entire coding sequences is 681 bp long for a putative protein of 227 aa in A. majus and 684 bp/228 aa in M. orontium).

\[
\begin{align*}
\text{A.m. wt:} & \quad \text{GAGAAAATGCAAGACACCTTGAAGAGCTGAATGAGGT} \\
\text{def-101:} & \quad \text{GAGAAAATGCAAGACACCTTGAAG---CTGAATGAGGT} \\
\text{M.o. wt:} & \quad \text{GAGAAAATGCAAGACACCTTGAAGAGCTGAATGAGGT} \\
\text{def: (ts):} & \quad \text{GAG-------------AAGCTGAATGAGGT} \\
\text{CAACAAGAAC} & \quad \text{CAACAAGAAC} \\
\end{align*}
\]

The 3 dashes above mark the 3 deleted base pairs in Antirrhinum (A.m. def-101 standing for Antirrhinum majus, mutant deficiens 101), and the 21 dashes denote the deleted bp in the corresponding Misopates mutant.

The second mutant – most probably a null mutant due to a deletion of seven bp, frameshift and stop – displays a strongly pronounced deficiens phenotype (Table 2; Fig. 4G, 4I): its second whorl is entirely sepal-like. Yet again – as was the case in the fourth whorl of the plena mutant – it is now the second whorl, which does not simply reiterate the sepal of the outer whorl. Instead, the inner sepal is shorter and more tender than the outer ones. Although in Antirrhinum the sepal of the second whorl are hardly distinguishable from the normal outer ones, in absolute terms the second whorl of the Misopates mutant again approaches the length of the corresponding whorl of the Antirrhinum mutant.

In contrast to the phenotype of the strong alleles of deficiens in Antirrhinum, the compound style appears to be decidedly more slender in Misopates (Fig. 4I).

Moreover, cross sections of the pistils of Antirrhinum and Misopates appear to point to a further basic difference between the two species: the Antirrhinum mutants manifest 5 loculi (Schwarz-Sommer et al. 1992), but the two Misopates mutants only 4 (Fig. 4J, 4K). Although a tiny stomainoid is present in the normal Misopates flower, pointing to normal Cycloidea gene function, the involvement of that gene in the differences between the two species cannot be excluded to date masmuch as the Cyc gene of M. orontium is 15 bp shorter than that of A. majus (nor can the presence of perhaps a very rudimentary fifth locus in the pistil’s early development of those Misopates mutants).

Cycloidea

The two cycloidea phenotypes derived from the Coimbra line of Misopates revealed a weak (herniradialis) and a strong phenotype respectively (Fig. 5A, 5B) (as for a detailed description and molecular characterization of similar mutants in Antirrhinum, see Luo et al. 1996, 1999; Galego and Almeida 2005; concerning Linaria, see some comments by Lönnig and Saedler 1997, but especially Cubas et al. 1999; Theßen et al. 2000). The strong phenotype often displays 6 sepals, 6 (fused) petals, and 6 stamens, but varies and the number 5 for all three organs can also be also found (often the radical phenotype appears to be very regular – much more so than in the corresponding Antirrhinum mutants). Moreover, the seed capsule commonly exhibits 4 pores instead of the normal number 3 in the wild-type. In contrast, the weak herniradialis allele manifests the wild-type number of 5 sepals, 5 petals, 4 stamens, and 3 pores (Fig. 5D-F). On the other hand, the strong cycloidea phenotypes of Antirrhinum disclose 5 sepals, 5 petals and 5 stamens, the weak ones 5, 5 and 4 or 5, respectively.

However, the Antirrhinum double mutant cycloidea/ dichotoma also shows 6 sepals, 6 petals and 6 stamens, the style being significantly shorter than the stamens and self-fertilisation rate is considerably lower than normal. Pore number appears to be 3 as in the wild-type. Although the weak herniradialis-like phenotype of Misopates manifests also only 3 pores, at first sight they look as if there were 4, yet a thin slit connects the seemingly two upper ones. The mutant produces an average seed set solely due to autogamy. However, the strong cycloidea allele, in which the style is also somewhat shorter than the stamens, should better be selfed to obtain sufficient seed numbers. To date, it is known that one of the two independently arisen Misopates cycloidea phenotypes (the strong one) is, indeed, due to a mutant cycloidea gene (see Table 2), and test crosses have been carried out to identify the basis of the second mutant phenotype: the F1 is wild-type and the F2 segregates both phenotypes – so the weak allele is assumed to be due to a mutant radialis gene.

Fistulata-like phenotypes

Also, three phenotypes were registered revealing fistulata-like flower aberrations (Fig. 5G; as for data on the fistulata phenotype in Antirrhinum, see McSteen et al. 1998; Motte et al. 1998). In contrast to Antirrhinum, the corolla is often closed in Misopates, but seed set is guaranteed by autogamy. Yet, nothing is known at present of the molecular basis of the Misopates mutants.

Fimbriata

(As for the investigations on the corresponding Antirrhinum mutants, see Simon et al. 1994; Ingram et al. 1997; Schultz et al. 2001, and for a comparison between Antirrhinum and Arabidopsis, Ingram et al. 1995): The Vogelsang-line derived Misopates fimbriata mutant L 2002/1574 represents one of the most striking phenotypes detected so far in Misopates (see Fig. 6A-G). A substitution and the deletion of two base pairs in an open reading frame of the gene resulting in a frame shift and a stop codon have most probably generated a null-allele (see Table 2). The F1 of this mutant displays the ensuing characteristics: a normal looking flower pedicel is followed first by 5 sepals and further by a bunch of sepal-like leaves. The sepal-like leaves are arranged around a series of nodes with strongly compressed internodes in between (see Fig. 6A). Whilst the plant keeps on growing and maturing, the internodes also elongate so that short branches develop. Additionally the “flowers” produce several fairly small styles and locule-like organs generating conventionally looking ovules. Although
the overall impression is that the development of these organs is strongly abnormal, rather unexpectedly these tiny organs are fertile and produce some seeds upon pollen transfer to the stigmas of the styles. The *Antirrhinum fimbriata* null mutant can produce a basically similar phenotype, and this is even true for the mutants of the corresponding *UFO*-gene in the distantly related *A. thaliana* (Ingram et al. 1995).

In contrast to *Antirrhinum* the *fimbriata* null mutant of *Misopates* has never produced petaloid tissue sectors as is regularly the case in *Antirrhinum* (Fig. 6E, 6G) or the weak *deficiens* allele of *Misopates* described above. Segregation: *fimbriata* segregation in the M₂ strongly deviated from the normal ratio: wild-type plants 511:63 *fimbriata* (8.1:1). This was probably due to sectorial mutant tissue in the M₁ plant (Gottschalk 1994). Segregation in the sister plants was normal: 394:124 (3.17:1).

**Mutants with petaloid sepals**

Mutant L 2003/971, derived from the Vogelsang line of *M. orontium*, displayed varying numbers of sepals (5-7) partially transformed into petaloid tissue sometimes fused with the lower lateral sepals (Fig. 6I, 6J). Phenotypically it is somehow intermediate between the *fimbriata*- and *cycloidea* mutants of *Antirrhinum*. Furthermore, mutant 2003/977 (petaloidy to be confirmed) manifests up to 7 sepals, of which the 2 adaxial ones can be fused with the lower lateral petals. Interestingly, the additional adaxial (median) sepal is often forked (Fig. 6K, 6L), thus representing another feature not reported for *Antirrhinum*. Both mutants display lowered fertility.

Further investigations appear to point to strong environmental influences on the expression and penetrance of these mutant features: M₃ sepal number deviations were severely reduced under mild greenhouse conditions in contrast to the M₂-phenotypes raised under strongly varying field parameters.

**Unidentified Misopates mutants**

In *Misopates* there appeared several mutants for which no corresponding phenotypes have (yet!) been detected in *Antirrhinum* and there seem to be potent reasons for the hypothesis that – as in several cases described above – further homologous mutant genes and perhaps even some non-homologous ones of *Misopates* and *Antirrhinum* might be involved in the development of phenotypes differing in

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Fig. 5 *Cycloidea* and putative *fistulata* alleles in *Misopates*. (A) *M. orontium* wild-type of the Coimbra line in comparison with its weak *cycloidea* allele. (B) Strong *cycloidea* allele of the same line. (C) The *cycloidea* phenotype of *Antirrhinum*. (D) (left): normal *orontium* capsule with 3 pores, right: *cycloidea* normally displaying 4 pores, but as shown in (E), can also develop 5 pores, yet in any case the capsule displays only 2 loculi as in the normal initial line (F). (G) *fistulata*-like phenotype of *M. orontium* in comparison to the *fistulata* mutant of *A. majus* (H). In contrast to the latter, the *M. orontium* mutant’s petals are closed.
Biodiversity and Dollo’s Law. Lönnig et al.

Fig. 6 (A-F) The *fimbriata* mutant of *Mispates orontium*. (A) Mutant phenotype of a flower with elongating internodes. (B) Flower with several styles; (C) Style of younger flower enlarged displaying ovules at its base of a style. (D) Capsule with seed set upon artificial pollination in that mutant (seed set upon pollination of such styles was regular). (E) Comparison of *fimbriata* null mutants of *A. majus* and *Mispates*; (F) *M. orontium fimbriata* flower internodes elongate to produce further “flowers”; (G) Similar situation in the *fimbriata* null mutant of *A. majus* (yet the *A. majus* mutant regularly displays some petaloid tissue whereas this has not been detected in *M. orontium*). (H) *Mispates M2* field. (I) *M. orontium* phenotype of L 2003/971 with the two middle sepals fused to petals. (J) The same plant displaying flower with 7 “free” sepals. (K) Plant of L 2003/977 with varying numbers of sepals (again 7 in the flower shown, the upper 2 are largely fused) and often show cycloidea-like petal deformations. (L) Same as (K), but flower removed. As far as the authors know, there are no *Antirrhinum* mutants, whose flowers produce 7 sepals.

some basic features of these closely related species.

The description and discussion of such further phenotypes, as the *bizarre* mutant (reduced petals, sterile) and several others of this category of unidentified mutants, will be given in another paper.

**Double mutants**

The double mutant between the temperature-sensitive weak allele of *deficiens* and the *plena* mutant consisted either of weakly petaloid organs of partly violet colour (15°C) or solely sepaloid structure (25-30°C) – see Fig. 7. Further double mutants are currently being generated.

**Dollo’s law and the ABC(DE) flower developmental models**

Several authors have argued with the German poet Johann Wolfgang von Goethe (1790) for a fundamental equivalence of the flower and the stem in the angiosperms (Coen and Carpenter 1993, there further references), implying that the different parts of the flower – sepals, petals, stamens, carpels – are essentially equivalent to the leaves of a shoot. This equivalence theory has had a long tradition especially in the German speaking world and has been known under the slogan “alles ist Blatt” (everything is leaf). Although determinacy, internode length, organ identity and phyllotaxy have all been changed in the flowering plants, these novelties have often been assumed to be “simply different modifications of a common growth plan”, produced by “different permutations of a few key features of plant
growth" (Coen and Carpenter 1993, p. 1175).

In the well-known but simplified ABC model of the master regulatory genes specifying flower development, the A-function is thought to designate sepal identity, the A- and B-functions cooperate for petal formation and the solitary C-function determines carpel identity — the A- and C-functions being antagonistic (for reviews see for example Coen and Meyerowitz 1991; Theißen and Saedler 1999; Theißen et al. 2000; Keck et al. 2003; Efremova et al. 2006).

Although recent research has refined the model (see for instance Theißen and Saedler 2001; Zahn et al. 2006) — for nature is often much more complicated than our paradigms predict or even can predict — let us assume for a moment that the homeotic mutants deficiens (B-mutant) and plena (C-mutant) discussed above are steps to the assumed ground state of angiosperm evolution, i.e. the leafy shoot. In that case the deficiens phenotype would represent "a former state already realized in the series of its ancestors" and, in contrast to Dollo’s law, a morphological reversal would, indeed, have occurred: the petals constitute an apomorphic feature, but a double whorl of sepals a more plesiomorphic state — as is known sepals are usually more leaf-like than petals. A similar statement could be made for the plena mutants: the petals, being much less differentiated than the stamens and carpels, could perhaps be viewed to constitute a step in the approach to the ground state just mentioned. Finally, the double mutant plena/deficiens consisting of sepaloid leaves only, could be interpreted to almost have reached that ground state in defiance of Dollo’s law, in which a shoot only displays leaves.

However, such an interpretation can be criticized for several basic reasons (Lönnig 1994):

1) In this scenario the assumed ground state of the angiosperms would have no sexual reproduction, which is in contradiction to any theory on the origin of species known to the authors.

2) In the homeotic mutants the flower organs are not transformed into simpler or more original ones, but the complex genetic programmes necessary for the petals, stamens and carpels are switched off by transposons or loss-of-function-mutations and are often substituted by other less differentiated ones. To illustrate the point in the words of Sattler (1988): "If a botanist in a biology department is replaced by a zoologist (as too often happens), the latter is not a transformed botanist simply because his predecessor was a botanist; he only occupies the same position. From the sameness of position, it does not follow that members occupying it are also essentially the same; they may be similar or very different."

3) There is no equivalence of the flower and shoot concerning sexual reproductive functions. Already at the beginnings of the 1980s, Kamalay and Goldberg (1980, 1984) had detected that "both the anther and the ovary contain approximately 10,000 diverse mRNAs that are not detectable in heterologous organ system mRNA or nuclear RNA populations" (Drews and Goldberg 1989). Since that time many further genes have been cloned and sequenced that are expressed only in the flower and hardly anywhere else (for an exhaustive literature survey see the annual bibliography of Georges Bernier, for example of the year 2001, or the following editions up to now).

The independent results of many research groups that large numbers of genes are expressed almost exclusively in the flower distinctly disproves the simple equivalence of flowers and shoots required by Goethe and his adherents.

4) As is now generally known to biologists, the MADS-box sequences are conserved from yeast to humans. Losses of functions in MADS-box genes perturb the various organisms quite differently, and — most important for the question of the ABC(DE) flower developmental models with regard to Dollo’s law — there is no trend to categorize the deviations as atavisms (for a further review on the developmental models, see Davies and Schwarz-Sommer 1994 and the more recent literature just mentioned above). To take again the first molecularly investigated homeotic flower mutant: hardly anyone would claim that the different (promoter-, MADS-box and K-box) alleles of the DEFICIENS gene of A. majus effectuating various degrees
of greenish petals (not to speak of the temperature-sensitive allele def4-101) would be the atavistic gene sequences of the angiosperms and thus be relevant for Dollo’s law. The same can doubtlessly be maintained for the alleles of the corresponding APETALA3 gene in Arabidopsis (Jack et al. 1992). The substitution of a complex developmental programme necessary for the formation of special flower organs by an earlier and simpler one does not indisputably confirm the equivalence of both (for further points see Lönnig 1994). Thus, clear evidence for the relevance of the ABC(DE) models of flower development for Dollo’s law appears to be missing at present.

**Genetic distance between Misopates and Antirrhinum**

The genetic distance (base substitutions) for protein coding sequences between humans and chimpanzees has been calculated to be about 1.23%, including the indels in noncoding sequences now roughly 5%, but up to about 80% of the proteins are different (Britten 2002; Watanabe et al. 2004; Weissbach 2004; Glazko et al. 2005). In Table 3 we have presented the divergence data for 18 nuclear genes for *M. orontium* and *A. majus* (comparison of altogether 14,140 bp). Although the distances vary strongly from gene to gene (see the details in Table 3), a putative medium overall divergence of 5.64 bp substitutions per 100 can be projected for the genomes of the two taxa, that is slightly more than 4.5 times the distance between the coding sequences of humans and chimpanzees.

<table>
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<tr>
<th>Gene</th>
<th>Sd/100</th>
<th>Sn/100</th>
<th>Sum</th>
<th>Sd</th>
<th>Sn</th>
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<th>Reading frame</th>
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<td>6.84</td>
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<td>96.84</td>
<td>797</td>
<td>246</td>
<td>549</td>
<td>164</td>
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</table>

Table 3 Genetic distances between *Antirrhinum majus* (Am) and *Misopates orontium* (Mo).

A. majus

B. domino

C. morifolia

D. hybrida

E. hyperborea

F. incana

G. integrifolia

H. heterophylla

I. hastata

J. hybrida

K. juniperina

L. koraii

M. macrosperma

N. media

O. melanoleuca

P. major

Q. montana

R. montana

S. morifolia

T. morifolia

U. macrocarpa

V. maximowiczii

W. maximowiczii

X. macrosperma

Y. media

Z. major

Average difference

Bases/100 5.64

*In the scientific literature only the abbreviations for the Deficient homologues are given: DEF1, DEF21, etc. The comparisons include only the reading frame regions including the stop codon, with the exception of INCOmposita, where the stop codon has not been sequenced. The introns and intergenic regions show high divergence and where not aligned. The column “reading frame” displays the total number of nucleotides.

On several phylogenetic presuppositions (and after several revisions during the last decades) not be discussed in this paper, a divergence time of 4.6 to 6.2 million years has been estimated for the two latter taxa (Chen and Li 2001), and according to a more recent review about 8 million years (Junker and Scherer 2006). A tentative extrapolation from these hypotheses to the pair *Misopates/Antirrhinum* would mean a divergence time between 21 to 36 million years.

**DISCUSSION**

**General discussion of Dollo’s law**

Before we discuss the relation of our empirical results to biodiversity and to Dollo’s law we should make some introductory remarks on Dollo’s law of irreversibility.

As far as the authors are aware, perhaps the most thorough discussion of Dollo’s law (including the translation of original French papers into the English language) has been presented by Gould (1970) and the basic points later reinforced by Gould and Robinson (1994). In the first paper the author contrasts Dollo’s law against several other largely discredited evolutionary laws, among them orthogenesis, recapitulation, Cope’s and Williston’s laws arguing that irreversibility on the one hand “is a phenomenon that quite different from the standard set of such ‘laws’”, because, in Dollo’s sense, “this would require that the organism retraces, exactly and in the same order, an extremely large number of steps” (Gould pp. 189, 198). Muller explained the point by “the sheer statistical improbability, amounting to an impossibility, of evolution ever arriving at the same complex genetic end-result twice” (Muller 1939). Reasons pointing in the same direction but from a thermodynamic vantage point have been presented by Blum 1962, see also Gould (1970).

Dollo himself explicited his law as follows (1913): “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 add 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.”

From the geneticist’s point of view, however, there are now several themes, which should be briefly considered to replenish the discussion (the first point could have been contemplated by Dollo himself to a certain extent, at least after the so-called rediscovery of Mendel’s laws in 1900).

1. The basic difference between genotype and phenotype in classical (Mendelian) genetics. In the text above we have repeatedly referred to differences between *Antirrhinum* and *Misopates*, which have phenotypically been clearly diminished or fully overcome by mutant genes, so that Misopates outwardly approaches, meets or even overlaps features of *Antirrhinum majus*. In polygenic traits many individual mutant genes can result in similar phenotypic aberrations: so totally different sequence deviations can lead to identical phenotypes. Double mutants (mutations in combination with suppressor mutations) can result in the reversal to near wild-type phenotypes although at the DNA level the original sequences may be entirely lost.

2. Transposable elements (TEs). Could a Dachshund or a Chihuahua ever revert to the phenotype of his ancestor, the wolf? Certainly not – if several losses of gene functions due to deletions or other complex sequences deviations are involved. However, if the key regulators were simply blocked by non-autonomous TEs which could be reactivated by some transposases in trans of active transposons (due to recombination or transposition), a reversion to the original wild-type gene function and corresponding phenotypes should be possible – astounding as it may appear especially in this case. Yet at least partial reversions to the wild-type phenotypes have been reported for several crop plants both under human care and in the wild. Strongly deviating phenotypes have, indeed, reverted to wild-type in the controlled experiments of many TE investigations: the *DEFiciens* mutant of *A. majus* regularly reverts to the wild-type phenotype when active TEs are involved (see Figs. 1, 4H, Sommer et al. 1990). The same is true for active TEs blocking genes of the anthocyanin pathway or other...
pathways or functions (for further examples, see Nevers et al. 1986; Lönnig and Saedler 1994; Kunze et al. 1997).

3. Epigenetics. The original DNA sequences of genes can even be entirely conserved, yet due to methylation the gene functions remain unexpressed. Reversal to the original function due to demethylation has also been repeatedly reported (for recent reviews, see Kalisz and Purugganan 2004; Tarig and Paszkowski 2004). So, even in the absence of any mutant DNA sequence variations strong phenotypic aberrations can be produced, be ‘inherited’ and revert to the wild-type after many generations (applying this to our Dachshund or Chihuahua examples, the ‘inhibited wolf’ could reappear in almost one step due to demethylation of the key regulatory genes).

4. Reversibility by recombination within species and genera. When an original species splits up into a series of subspecies by differential losses of gene functions, the original phenotype and even genotype may be retrieved in one or several steps of recombination as far as free recombination is possible (for details, see Lönnig 2002).

5. The law of the homologous series in hereditary variation (Vavilov 1922). This law describes, emphasizes, and predicts recurrent morphological and physiological phenotypes in related and non-related taxa. Von Sengbusch (2003) has summed up the law as follows:

(a) “Genetically closely related species and genera are characterized by similar series of hereditary variation with such a regularity that, knowing the series of forms within one species, one can predict the existence of parallel forms in other species and genera. The genetically closer the genera and Linnaeons within the general system are, the greater is the similarity in the series of their variation.”

(b) “Whole plant families are generally characterized by a definite pattern of variation penetrating all the genera and species comprising a family.” The author provides the ensuing example: “Brittle ears’ of gramineae is regarded as a primitive trait that occurs only exceptionally in cultivated varieties. The cross of certain varieties with stable ears would accentuate the cyclic and finite nature of functional reversals and variation per se within microevolution (Lönnig 1995, 2002, 2003b, 2005, 2006).

6. Gene mutations. Concerning point mutations (partly overlapping with points 5 and 7), reversals have been described for several plant and animal species (for reviews, see Gottschalk 1971; Lönnig 2002; Lewin 2003). Among many other topics, Gottschalk (1971) has reviewed all the changes of the sepal whorl known until then and believed to be due to gene mutations. Strongly elongated sepal mutants were found in *Lycopersicon* (mutants macrocalyx and torosa) and *Papaver* (leafy calyx). Later Vrebalov et al. (2002) sequenced the *Rin* locus of tomato (in the *rin* mutant fruits fail to ripen and the calyx is enlarged). It was detected that two mutant genes were involved in this phenotype, LeMADS-RIN in ripening and LeMADS-MC in sepal development and inflorescence determinacy, LeMADS-MC being the homologous gene of *Squamosa of Antirrhinum* and AP1 of Arabidopsis, respectively.

The phenomenon of calycanthemy, the transformation of sepal into petals so that the flower displays two petal whorls in extreme cases, has been detected in many angiosperm species and it has been classified as “back differentiation” (“Rückdifferenzierung”, i. e. reversals to a less differentiated state – Gottschalk 1971). As to fertility, which is thought to be a key factor for determining selection values, so far only the mutant *calyciflora* of *Nicotiana tabacum* (“subject to splitting of corolla tube and other morphological irregularities...three or four of the sepals are broad, roseate colored, and may form a whole or in part – White (1916) was deemed to be “fully fertile”, yet – even apart from its morphological irregularities – one may doubt whether it would have any chance of survival in the wild, inasmuch as even *N. tabacum* has never been found there. Most of the calycanthemy mutants display further hefty anomalies; many disclose reduced fertility or are sterile.

Many additional examples of calycanthemy are found in less recent works on plant teratology (Hessers 1869; Penzig 1922). Although hardly any of these cases were genetically analyzed so that a part of the instances might be simply due to modifications, the potential wealth of the information intrinsic in that older material becomes immediately apparent when one applies Stubbe’s rule to it, derived from his *Antirrhinum* studies (1966): “All alterations due to different environmental factors (modifications) have also been detected as mutants, but not all phenotypes due to mutations can be replicated by environmentally elicited modifications”. Concerning calycanthemy He et al. (2004) have raised the question whether nature has taken advantage of the possibility to ectopically express B-function genes, like *Def* and *Glo* in the sepal whorl thus producing two whorls of petals as found in the Liliaceae (further points on calycanthemy, see below).

7. Constancy of developmental pathways. Several authors have argued that the developmental programmes and pathways underlying (even) complex morphological features lost in the history of a genus or family can nevertheless be maintained on the genetic level for millions of years and have, indeed, been reactivated in several cases (Marshall et al. 1994; Collin and Cipriani 2003; Whitting et al. 2003).

On an experimental basis (probably in part due to point mutations, too) phenotypic reversal has been reported for *Drosophila* (Hall 1995; Teotónio and Rose 2000; and reviewed by the latter authors in 2001). Reversals from antibiotic resistance to sensitivity – predominantly due to losses of plasmids carrying resistance factors – has been detected in many lines of different species of micro-organisms (see, for example, Madigan et al. 2000). Bull (2000) also mentions reversibility in attenuated viruses of Sabin poliovirus vaccine, in domesticated organisms when returned to the wild, and of traits when artificial selection in lab experiments is relaxed. Last, but not least the famous reversals in the Axolotl (*Ambystoma mexicanum*), a larval salamander of mountain lakes of Mexico that usually lives without metamorphosing, should be mentioned in this paragraph (for details see, for example, Armstrong and Malacinski 1989).

8. Reversals from polyploidy to diploidy. A range of examples has been presented and discussed by Gottschalk (1976). Although Grant (1981) assumes that reversals from polyploidy to diploidy will not overturn polyploid trends, he has raised the question whether nature has taken advantage of the possibility to ectopically express B-function genes, like *Def* and *Glo* in the sepal whorl thus producing two whorls of petals as found in the Liliaceae (further points on calycanthemy, see below).

9. Homeomorphy. Additionally, a series of phenotypic reversals have been implied and described in paleontology under the term “homeomorphy” for more than 50 years now (homeomorphy being the result of a convergence which
Relevance of mutagenesis with Misopates and Antirrhinum for biodiversity and Dollo’s law

The assumption of the significance of mutants in general and homeotic mutants in particular mentioned for our topic in the introduction and later, inter alia that the homeotic mutants may disclose further similarities of and differences between various taxa usually hidden in the wild-type and non-homeotic mutant developments as well as be relevant for Dollo’s law – appears to be fully vindicated by the results obtained:

1) The (expected) similarities found at the level of the mutants, especially the homeotic deviations, reinforce the inference drawn from the ontogenies of the wild-type phenotypes of the two taxa to their common basic developmental patterns. Nevertheless, the temperature sensitivity in the weak deficiens allele due to a deletion in 21 bp as described above was, in fact, more than we had anticipated.

2) Conversely, most of the following differences and reversions in the corresponding mutants and phenotypes of the two species could hardly have been predicted for Misopates from the mere inspection of the wild-type on the basis of contemporary phylogenetic hypotheses:

(a) reversion into the direction of the putative original perennial life cycle (as mentioned above, normal life cycle of 3 to 4 months of Misopates extended to about 10 months in a non-homeotic mutant and to more than 4 years now in some (repeated) cuttings of homeotic mutants; this process can probably be extended indefinitely).

(b) supposed reversion to broad leaves,

(c) reversion of the almost white flowering Coimbra line to a violet flower colour,

(d) putative reversion to larger flowers,

(e) the strong reduction of sepal length in the cuttings of the floricaula-like mutants,

(f) the intermediate length of the inner ‘sepalas’ as well as the reduction of the outer sepals at the top of older inflorescences in the plena mutant (perhaps also its sterile anthers),

(g) the intermediate length of the second sepal whorl in the deficiens mutants and the short and broad style in the weak allele and the extraordinary slender and long style in the strong one,

(h) the absence of the fifth loculus in the deficiens mutants,

(i) the closed corolla in fistulata-like phenotypes,

(j) the absence of petaloid sectors in the fimbriata mutant comparable to those in the corresponding Antirrhinum phenotype,

(k) sepal number variation in one and the same mutant (up to 7 sepals),

(l) (at present) some hardly or not identifiable mutants.

(m) the absence of yellow flowering mutants (so far?),

(n) seed formation due to apomixis,

(o) four pores in the seed capsule of the cycloidea mutant of Misopates,

(p) until now, no hints for a reactivation of TEs by mutagenic agencies.

3) The differences between Misopates and Antirrhinum in combination with the fact that the most conspicuous morphological distinction between the two taxa, the enormously elongated sepals of Misopates, could not be reduced as an isolated morphological feature to that of the length of those of Antirrhinum (or vice versa), appears to point to a deeply rooted genetic and developmental disparity between them. This seems to be in accord with the overall distance that can be inferred from the sequence comparisons reported above.

Objections against the relevance of the experimental mutants for Dollo’s law

We will begin the discussion of this part by several objections that could be raised against the applicability of the presumed reversals reported above to falsify Dollo’s law and subsequently present the counter arguments:

First, nobody can be sure what the original features of the stem group of the family or tribus really consisted of. Second, the putative reversals reported here all belong to micro-evolutionary events, mostly due to losses of gene functions; however, Dollo’s law is essentially describing morphological macroevolution generally thought to be the result of gains of gene functions (often including new promoter- and gene sequences). Third, Dollo’s law was meant only for complex not for simple morphological structures.

The complexity criterion of Dollo’s law

Continuing the discussion with the third more general point (to return to the two others subsequently), one could, indeed, argue that at the genotypic level Dollo’s law is correct for any complex DNA rearrangements whose reversal would demand 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.” Applying this scenario to our Dachshund and Chihuahua examples (see general discussion of Dollo’s law above), the original wolf DNA sequences and corresponding phenotypes would never reappear even in millions of generations (except for phenotypes perhaps in the unlikely case that for any mutant gene a corresponding suppressor allele generating the original wild-type phenotype could be devised).

Or, to directly address some genetic differences between Misopates and Antirrhinum, – the divergence between the taxa given in Table 3 will never revert to a common sequence by random mutations, although individual bp can.

There is, however, no question that – when formulating his law – Dollo did not (and could not) think in terms of genetics at all (inasmuch as there was no discipline of genetics in any modern sense before 1900). He rather followed the “immortal Charles Darwin” (Dollo 1893), whose basic ideas on evolution Dollo had unreservedly accepted (“Louis Dollo was the most Darwinian of paleontologists in his time” – Gould and Robinson 1994). Now Darwin had formulated his Lamarckian pangeneses hypothesis according to which the causes, “which gave rise to the individual variations” etc. (see above) were the imprints of the environment on the organism becoming hereditary by the “gemmules” produced by the entire body continuously travelling to the germ cells indelibly preparing them for the inheritance of the acquired characters for the following generations (for further details and references, see Lönnig 2003a; – “indelibly” refers to Dollo’s “virtue of the
inductibility of the past’). In conjunction with Dollo’s strictly deterministic view of the natural world (he was educated in the mechanistic tradition that dominated late nineteenth-century science” – Gould 1970), irreversibility was an unavoidable inference for any complex constellation in evolution – and complex is what the constellations have always been.

Gould and others have tried to defend Dollo’s law arguing that “he applied irreversibility only to complicated morphologies”, but Dollo himself had definitely stated that “an organism cannot return, even partially, to a former state already realized in the series of its ancestors”. Moreover, Gould admitted that if the qualifying term “complex” is used to exclude any possible counter-instance, the statement becomes unfalsifiable” (Gould 1970).

To sum up the complexity objection, Dollo derived his law of irreversibility of evolution from the doubtful (some authors, especially of viewpoints C1 and C2, see below – would prefer to use the adjective “false”) mechanistic and deterministic perspective dominating the end of the 19th century in combination with a basically imperfect view of heredity. Apart from the missing distinction between phenotype and genotype and the frequent oversimplification above (mostly simply unknown to him), he himself did not clearly define “complex” (impossible) versus “simple” (possible) reversals. Since, sense latu, even both kinds of reversals have been described (see again Marshall et al. 1994; Bull 2000; Teotónio and Rose 2000, 2001; Collin and Cipriani 2003; Whitting et al. 2003), experimentally induced mutants could obviously also be relevant for Dollo’s postulate of general irreversibility – including the mutants of Misopates and Antirrhinum.

Micro- and macroevolution

The second objection mentioned above (the ‘putative reversals reported here all belong to micro-evolutionary events due to losses of gene functions; however, Dollo’s law is essentially describing morphological macroevolution mostly due to gains of gene functions (often including new promoter- and gene sequences)’) has in part already been answered in the last paragraphs. However, if the objection were correct, also the micro-evolutionary events due to temporary losses of gene functions by TEs and methylfication mentioned in the general discussion above might be classified as irrelevant for Dollo’s law.

Yet, Dollo did not distinguish between micro- and macroevolution as, nor, as mentioned above, did he delineate simple from complex reversions. Additionally, the absence of the difference between genotypes and phenotypes (whose distinction would have been possible for Dollo after 1900) appears to be a proton pseudos of Dollo’s law. As is well known today, losses of gene function can be compensated by suppressor- and modifier mutations to restore the original phenotype’s morphological structures (for some suppressor mutations with morphological effects see, for example, Hong and Spreitzer 1998; Hsieh 2000; Resnick et al. 2006), so phenotypic reversibility appears to be possible in both directions, losses and gains of functions – at least to a certain extent. The phenotype of Misopates mutant L. 2002/1843, for example, displaying the broad leaves normally found in A. majus is certainly due to a loss of function mutation: all its features are fully recessive when crossed with the wild-type recessiveness being a rather sure indicator of a loss of gene function. Yet there will hardly be any risk in predicting that by further mutation a mutant phenotype with slender leaves will reappear - being very similar to the original character of the Misopates wild-type (Vogelsang) line.

Conversely, the violet coloured mutant of the Misopates Coimbra line is certainly a gain of function, which in turn could be lost again in further mutagenesis experiments. The basis of this gain of function is presently unknown; demethylation of a regulator of the anthocyanin pathway could be among the possible causes. In any case, it is taken for granted that the necessary DNA sequences are still present in the Coimbra line.

From the neo-Darwinian standpoint of the origin of species and higher systematic categories by a putative process called “additive typogenesis” (see, for example, Heberer 1971; Cizhak et al. 1996; Sauer and Rehfeld 1999) macroevolution should be at least for the large extent not reversible by what may be called “subtractive typolysis”, i.e. by simple losses of the additionally acquired morphological features. However, since most new characters arise, not by simple additions but by integration of complex networks of gene functions rendering many systems to be irreducibly complex (Behe 1996, 2004; for a review, see Lönnig 2004), such systems cannot – in agreement with Dollo’s law – simply revert to the original state without destroying the entire integration pattern guaranteeing the survival of a species.

Nevertheless, extensive research on “regressive evolution” (for several review points, see Kunze et al. 1997; Lönnig 2002) has described a range of different species, genera and families, in which morphological features have, in fact, been lost due to mutations (yet within certain limits), in some cases perhaps reverting to some earlier and simpler morphological state. In many cases of regressive evolution, the life forms were given the status of species and genera of their own – the phenomenon thus partly belongs to “transspecific evolution”, the latter being the identification mark of macroevolution according to Mayr’s definition (Mayr 2001).

Yet, nobody will deny that the origin of higher systematic categories and Bauplans demands more than losses of gene functions (after all, ‘it is hard to think of oneself as an inactivated amoeba’; Crow 1981), and for the rise of these taxa as well as for the inception of irreducible complex systems, the debate continues whether mutations and selection alone will be sufficient to produce all the new genetic functions and innovations necessary for the cytoplasm, membranes, and cell walls (see, for instance, Behe 1996; Lönnig and Saederl 2002; Swift 2002; Campbell and Meyer 2003; Densbki 2004; Lönnig 2004, 2005).

The status of the original features of the tribes Antirrhineae

According to the third objection ‘nobody can be sure what the original features of the stem group of the family or tribes really consisted of”. This is certainly correct: most inferences concerning the reconstruction of the history of any higher taxa must remain chiefly hypothetical. In spite of some strong overall assertions, the uncertainty of the deductions as to the details is true for any evolutionary theory including the modern synthetic theory of evolution. Moreover, several researchers fully committed to naturalism prefer theories that question whether there has ever been something like a stem group of the tribes Antirrhineae at all (Teotonio 1953; Schwabe and Büllesbach 1998; Schwabe 1986; Schwabe and Büllesbach 1998; Schwabe 2001, 2002, 2004; Schwabe, personal communication 2003, 2004).

On the other hand it should be noted that even most critics of the modern synthesis accept direct phylogenetic connections within almost all of the lower systematic taxa at least up to the tribe or family level of the plant and animal kingdoms (for further points, see Lönnig and Saederl 2002; Meyer 2004). Yet concerning the evolutionary origin of the tribes Antirrhineae – i.e. usually the origin of the tribes or families and/or higher taxa themselves (for discussions of the details, see Lönnig 2002; Campbell and Meyer 2003). For our purposes we will refer to these three groups of researchers as representing viewpoints A, B, and C in the following text: A: modern synthesis, B: genomic potential hypothesis, and C: tendency to postulate a discontinuous origin of higher taxa either in combination
with purely naturalistic interpretations (C; Margulis and Sagan 1997; Schwarz 1999: Erwin 2000, 2004; Jablonski et al. 2000; Gould 2002; Müller and Newman 2003; Valentine and Jablonski 2003; Valentine 2004; Theissen 2005; and last but not least, Dollo himself) or, from a more or less typological vantage point, often including ID (C2), the hypothesis that, for example, irreducible complexity in certain organs and physiological processes (not to be equated to an interpretation of developmental probability and not to be explained by intelligent design). The latter, in turn, can be grouped into researchers postulating phylogenetic connections to putatively preceding taxa (C; Behe 1996; Denton 1998; Berlinski 2003a, 2003b; Conway-Morris 2003a, 2003b), and those doubting additional evolutionary links on a macro-evolutionary scale (C; Dembski 1998, 2002, 2003, 2004; Junker and Scherer 2001; Junker 2002; Swift 2002; and many further authors, in part already quoted above – see Campbell and Meyer 2003; Meyer 2004).

Apart from the fact that we do not aim to present a complete literature survey on the different positions here, but refer mainly to some relevant recent contributions, it should perhaps also be added that the following discussion is not meant to scientifically recount and evaluate the different views mentioned above. Rather, it is essentially dedicated to an interpretation of the described probability of Dollo’s law within the different frameworks and hypotheses, which are (also) presented in contemporary peer-reviewed scientific journals and/or peer-reviewed books. Only where a topic demands direct comparison of different ideas (like natural selection, see below), a few points will be referred to. (As for the different viewpoints, it need hardly be mentioned that the minority view of yesterday has become the standard vantage point of today and there is no convincing reason to believe that this will be any different in the future.)

Assuming with the majority of contemporary biologists (A, C1, C2a, and C2b) that there was, indeed, a common ancestral species of all the members of the Antirrhineae, – which characteristic features could have distinguished it? In the abstract and introduction of this paper we have spoken of “the eight derived features essentially distinguishing Misopates orontium from its closely related Antirrhinum majus”: (1) annuality, (2) linear leaves, (3) long sepals, (4) small flowers, (5) light violet flower colour, (6) autogamy, (7) cup-like seed form and (8) apomorphic seed production (for details, see Table 1). Concerning the sepals we have remarked that “since even from the most inclusive evolutionary presuppositions Misopates cannot be directly derived from an original ancestral angiosperm stock with leaves, in accordance to viewpoint B, multiple of these traits would have returned – at least phenotypically – (quoting Dollo) ‘to a former state already realized in the series of its ancestors’. According to the frame of reference C2b, the realization of Misopates would, in the absence of putative ancestors at the root of the angiosperms, signify a return to a more generalized (original) ground-plan of the flowering plants, i.e. to one without an advanced differentiation of leaves into bracts, calyx, corolla, etc. – as isolated organs – neither the long leaf-like sepals of Antirrhinum nor the short differentiated sepals of Antirrhinum be extended to the length of the corolla, yet in Sutton only one species out of the exactly 150 counted at present exhibits that feature, but six species out of seven in Misopates and the two Holzneria species. The exception in Misopates appears to be M. chrysothales from Morocco developing a corolla size of 11.5-14 mm and the length of adaxial sepal being 6-7.5 mm according to Sutton 1988. However, the data for the latter species should be checked.)

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Concerning Dollo’s law, the few isolated cases of leaf-like sepals within the tribus Antirrhineae, including the insulated case of Linaria chalepensis of the overall 150 Linaria species (Sutton 1988), seem to point to reversions to a more basic and undifferentiated state on the premises and data so far considered and obtained (viewpoints A, C1, C2). Nevertheless, in the tribus Antirrhineae there are also several further delicate sepal differentiations along with some more or less slight elongations of the sepals in relation to the corolla that appear to be more than just reversions to an original state (see Sutton 1988, for the sepal distinctions in several species of Schwenfirtia, where the adaxial calyx lobe is exceeding and overlapping the four lateral lobes and is also exceeding the corolla-tube, Linaria para- doxa with three fused adaxial lobes, Kicksia membranacea with broad sepals at base but slim elongations at the tips (“obspathulate to linear-lanceolate, acuminate”), Howellia ovata with sepals similar to Schwenfirtia, Holmgrenanthe petrophila with “conspicuously spinulose-dentate” sepals etc.).

Also, the unusual sepal development in several genera of the Solanaceae, the inflated-calyx-syndrome in Physalis, Nicandra, Przewalskia and others, is phenotypically, genetically and developmentally distinctly different from

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<td>Misopates orontium</td>
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<td>Misopates nanthe petrophila</td>
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<td>Misopates chrysothales</td>
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just a reversal to a “former state already realized in the series of its ancestors” (for a recently discovered example of a distinct case of independently arisen, but astonishingly similar structures in plants, see Glover et al. 2004, with further instances in the discussion; see also Theißen 2004); as to Physalis, see He and Saedler (2005).

Calycanthemy
As mentioned above, the phenomenon of calycanthemy has been reported in many plant families and been interpreted to be reversals into the direction of a more original morphological state of the flower by some authors. Besides, the question has been raised by He et al. (2004) whether nature has taken advantage of this possibility in the case of the monocot family Liliaceae, like lilies and tulips, displaying two whorls of petals (tepals) in their perianth. Thus, according to viewpoint C 1, the discovery of B-leaf form mutants with extended life cycles (see the details under point 1) seem to hint at a certain capacity to revert into the direction of the original state. Another interpretation could be the involvement of TEs (Lönnig and Saedler 2002), or epigenetic regulation (above), both in the generation of alternative life cycles, including a large potential of reversibility, i.e. “to return to a former state already realized in the series of its ancestors”. Referring to a not unproblematic molecular interpretation of the history of the genus Medicago – based on the most parsimonious reconstruction, the ancestral population is hypothesized to have consisted of autogamous annual individuals succeeded by recurrent evolution into the direction of perenniality and outcrossing. Nevertheless, “assuming an outcrossing, perennial ancestral state (partly supported by morphological features) does not fundamentally change the reconstruction” (Bena et al. 1998) – Thomas et al. (2000) comment: “However, a general conclusion that can be drawn from this and other studies of life-history is that annuality and perenniality are traits that recur time and again across the taxonomic range and that, with the right selection pressure, the propensity to generate either form of phenotype can be realized without the need for large-scale genetic innovation.”

So whatever happened in the cases of Misopates and Antirrhinum, Dollo’s law would have been violated either totally or in part by any of these scenarios. This appears to be equally true for the viewpoints A, C1, and C2a and C2b mentioned and briefly defined above. However, since the further vantage point B does not postulate any different ancestral species, all these annual and perennial forms would have been generated independently of each other and a “return to a former state already realized in the series of its ancestors” would be impossible.

Leaf form
Variation of leaf form from linear to almost orbicular occurs regularly within many genera of the Antirrhineae, including Antirrhinum itself (see Hartl 1974; Sutton 1988). Phenotypic reversibility into both directions appears to be possible. However, (almost) orbicular leaves have not been determined for any of the 7 species of the genus Misopates. Yet, the width of leaves of Misopates mutant L 2002/1843 (Fig. 2A-C) surpasses those of all the wild species of the genus and may be viewed as a reversal to an originally more inclusive spectrum of phenotypes in its polyvalent ancestor. It has to be investigated whether a loss of function mutation of perhaps a suppressor in mutant 2002/1843 may have released a more basic developmental state for a larger photosynthetic area (interestingly, the mutant is also as late in flowering as Antirrhinum majus and displays slightly enlarged flowers).

Also, the regular-repetitive range of leaf form variation within so many angiosperm genera, tribes, and families (see any work on plant taxonomy comparing several taxa) suggests polyvalent ancestors with a stark genetic potential to differentiate into lines with distinct leaf types (simple vs. compound leaves, different venation patterns, margins etc.), including an established capability to revert to earlier phenotypes.

As for the Antirrhineae, leaves vary from filiform, linear and lanceolate to cordate, sagittate, and reniform to ovate-cordate (Asarina). In accord with viewpoints A, C1, and C2b the case of the Misopates leaf mutant 2002/1843 suggests that Dollo’s verdict on the irreversibility to a preceding morphological state might be revised as follows regarding leaf form at the phenotypic level: “an organism can return, at least partially, to a former state already realized in the series of its ancestors”. As for viewpoint B, see especially the last paragraph on annuality vs. perenniality above.
Flower form

Historically the *cycloidea* and/or *radials* mutants of *Antirrhinum* and many other plants species with zygomorphic flowers have frequently been interpreted to be perfect textbook examples of reversals “to a former state...” – the assumed original radial flower form (viewpoints A, C1, and C2a, see, for example, Gustafsson 1979; Strasburger 2002). And since in *Misopates* the ‘reversal’ of the strong cycloidea (Fig. 5B) seems to be even more pronounced than in *Antirrhinum*, this example could now be added to the list of putative atavisms so far known in the plant kingdom. However, several authors have emphatically rejected the interpretation of an atavism here for the following reasons: (1) Zygomorph plant species can give rise to distinct kinds of radial mutants differing strongly from each another in their morphology. A species cannot have had differently looking ancestors as the basic starting point. (2) The radial flower form is often due to the 5 to 6 times repetition of a flower part already highly differentiated and synorganized for a special function within the zygomorphic flower form (i.e. usually reiteration of the middle part of the lower lip with lobe, gibba or spur and further unique structures of the ‘palate’, and the abaxial part of the flower tube like the hairs lining the abaxial folds, which “may serve to guide the long nectar at the base of the tube”; for references, see Sutton 1988) as well as total or almost total suppression of the upper lip. (3) Normal flower function is often strongly hampered by severe constriction of the entrance and other malformations and malfunctions (pollinators excluded, autogamy reduced, lower seed production and seed sterility, also, owing to nearly upright position, early fouling of all flower parts due to rain water in the flower under field conditions in *Antirrhinum*). For further information, see Linnaeus 1749; Penzig 1922; Goebel 1928; Arber 1970; Hartl 1974; Endress 1992; Luo et al. 1996, 1999; Lönnig and Saedler 1997; Cubas et al. 1999; Theilen 2000.

The *cycloidea* phenotypes of *Misopates*, *Antirrhinum* and others could, perhaps, rather be quoted as examples for Dollo’s and Gould’s conviction that certain forms – more or less superficially – approach a previous phyletic state but never fully reattain it. However, due to the many special structures and functional aberrations just enumerated, the *cycloidea* and *radialis* cases might not even qualify to be “so near but not quite” (Gould and Robinson 1994) to the assumed original state according to viewpoints A, C1 and C2a. So B and C2b could perhaps classify the *cycloidea* and similar mutants solely as teratological phenomena. Yet interestingly, these aberrant radial mutants in the plant kingdom can phenotypically revert to the original zygomorphic symmetry – either with no change on the DNA-level at all (in cases of demethylation) or, if transposons are involved, with complete restoration of the original DNA sequence in the 1% to 6% of transposon excisions without footprints and almost complete restoration in the functional rest with footprints (for the details on transposon excision in plants, see, for example, Scott et al. 1996).

For several further difficulties concerning the *cycloidea* gene family, including DNA sequence diversity for species of *Antirrhinum* and *Misopates*, see Vieira et al. (1999), and the reviews by Gübitz et al. (2003), and Hileman and Baum (2003); for facts and interpretations on some legume *cyc* genes according to different evolutionary models, see Ree et al. (2003). *cyc* flower form and Dollo’s law, the case of *Pseudomisopates rivas martinezii*, originally described as *Misopates rivas martinezii* (Sánchez-Mata 1988; Güemes 1997) may be mentioned here. In contrast to *Misopates*, *Pseudomisopates* is a self-sterile, spinous perennial displaying sepals shorter than corolla-tube, having only two pores in an ovoid capsule, generating seeds different in surface structure and overall form as compared to those of *Misopates*. Additionally, this species has a horizontal rhizome, and n = 7 chromosomes (*Misopates* n = 8).

Pseudomisopates may perhaps be interesting for Dollo’s law concerning corolla form and function: although being relatively removed from *Misopates* morphologically and physiologically, corolla morphology of *Pseudomisopates* is very similar to that of *Misopates* (Fig. 7C, 7D). Yet, whether this similarity is due to common ancestry, convergence, or a reversal in a history of several more or less slightly different corolla forms and colours cannot be answered at present.

Flower size

The supposition that the 22 independently arisen *Misopates* mutant candidates with larger flowers (Fig. 3D) be phenotypic revertirants into the direction of an earlier ancestral state rests mainly on the fact that most of the 20 European species of *Antirrhinum* – being the closest relatives of *Misopates* – have larger flowers than the latter (for *A. majus*, see Table 1), and that the one case of really small flowers within *Antirrhinum* (*Antirrhinum valentinum*: corolla 12-15 mm), being “a narrow endemic of south-eastern Spain” (Sutton 1988), appears to be the apomorphic state within this genus. Besides, the regular appearance of *Misopates* mutants with larger flowers suggests a relatively easy genetic (or epigenetic?) step in this direction. Also, flower size to guide the *Misopates* may be evaluated in connection with its annual life cycle and its tendency to autogamy.

Charnov and Schaffer (1973) had come to the conclusion that annuality would be preferred in hostile environments (for a further development of the argument, see Thompson et al. 2000). Interestingly, all annuals are selfers in the above mentioned genus *Medicago* guaranteeing seed set in the absence of any pollinators, but there are selfers and outcrossers in the perennials (for further points, see Bena et al. 1998). An apomorphic (derived) short life cycle with relatively small autogamous flowers could help coping with adverse conditions.

Moreover, rather intense variation of flower size has been reported for nearly any genus with several species in the angiosperms. Like annuality and perenniality as well as several distinct leaf forms, differences in flower size “recur time and again across the taxonomic range”. Thus, for viewpoints A, C1, C2a, and C2b the assumption that phenotypes with larger flowers had also appeared in the series of polyanvolent ancestors of *Misopates* does not appear to be far-fetched.

Flower colour

Because from viewpoints A, C1, and C2a, the absence of the anthocyanin pathway describes a more basal or “primitive” condition in any plant kingdom. Destroying this pathway may be viewed to be a phenotypic (in case of complete deletions even a genotypic) reversal “to a former state already realized in the series of its ancestors”, which would apply to virtually all the 19 colour mutants of the Vogelsang line of *M. orontium* (see the details under “flower colour” of the results and Fig. 3E). For viewpoint B and C2b these mutants would perhaps represent only more basic physiological states of the genomic configurations (B) or design (C2b) of flower colours.

In agreement with the inferences of Marshall et al. (1994), Collin and Cipriani (2003), and Whiting et al. (2003) referred to above, the reversion to the violet flower colour in the almost white flowering Coimbra line of *Misopates* requires that the developmental pathway underlying this complex physiological feature phenotypically lost in the history of this subspecies has nevertheless been maintained at the genetic level for all the time this subspecies has existed and has now, if only to a certain degree, been reactivated in the mutant representing another candidate for the thesis that “an organism can return, at least partially, to a former state already realized in the series of its ancestors”. One could, however, perhaps object that flower colour is not a morphological feature and thus lies outside the boundaries of Dollo’s law of irreversibility (see further points below).
Fertilization
In a tribus where cross fertilization is the rule, a tendency to autogamy and the absence of functional self-sterility alleles usually describes a derived (apomorph) state of fertilization. We predict that – as in Arabidopsis (Kusaba et al. 2001; Nasralla et al. 2002) – the inactivated self-sterility alleles will be found by further molecular investigations. Yet from the evolutionary vantage points of A, C1, C2a, and C2b these functions may again be viewed to be a return to “a former state already realized in the series of its ancestors”, for the apomorph autogamy can be interpreted to be the original state before the invention of the self-sterility alleles. Whether this putative pathway itself can be reactivated depends on the complexity of its deactivation mechanism: in case of several deletions and other complex mutations the probability will almost be zero and a reversal to functional self-sterility alleles would be due to ‘a circumstance so complex that we cannot imagine that it will ever occur’, to reformulate another passage of Dollo quoted above. If, however, the deactivation is due to reversible cut and paste mechanisms of TEs or epigenetic factors, its reactivation should be possible.

From viewpoint C2b the absence of functioning self-sterility alleles could simply be interpreted as a reversal to a more basic physiological state in the design of the fertilization mechanisms in angiosperms. However, viewpoint B (“Life is polyphyletic from its inception, there is no major branching” – Schwabe 2001) may perhaps have some basic problems with a rudimentary system of self-sterility alleles or would have to include this phenomenon in minor branching.

Again, as in the case of flower colour, it could be objected that differences and reversions in fertilization systems do not represent morphological features and hence have nothing to do with Dollo’s law of irreversibility. Rather it seems to be in agreement with his assertion that “functional or physiological reversals occur”. However, a differentiation of reversibility for structural and morphological characters vs. functional and physiological features is itself problematic and cannot be taken for granted without a further genetic and developmental foundation. For the underlying genetic and developmental basis of both, reversions in flower colour and self-sterility appear to be no less complex than the basis for many purely morphological features. If correct, this argument would constitute another valid point questioning Dollo’s law.

Seed form
The apomictic seed form in Misopates (Fig. 3J) is clearly a complex apomorphic feature characterizing all species of this genus. To the authors’ knowledge, this character does not occur anywhere else in the tribus Antirrhineae. To date, seed form reversions are unknown in Misopates.

Apomixis
Apomixis is viewed to be an apomorph character having been generated convergently multiple times in the history of a large number of angiosperm genera. Thus, from viewpoints A, C1, C2a, and C2b it cannot be excluded that this feature had already been realized in the series of its ancestors, subsequently been lost and then been reactivated again. Hardly anything is known at present about the occurrence and distribution of this feature in 27 genera of the Antirrhineae.

Photosynthetic apparatus
Dozens of mutants of the photosynthetic apparatus have been detected in our experiments – such mutants are, in fact, the most common ones in any mutagenesis trials with plants. The results indicate that there is an irreducibly complex core system as well as a variable part, the latter being especially esteemed in breeding colour varieties (including chimeras) in ornamentals. In the former any losses-of-function-mutations abolish the activity of the entire photosynthetic apparatus, and have to be all classified as lethal mutations, at least if the entire photosynthetic area of a plant is affected (usually white seedlings are still formed which soon die after the nutrients of the seeds are used up).

Although the origin of the irreducibly complex core system will not easily be explained by selection of successive point mutations in duplicated genes, – from evolutionary standpoint A, especially that of “additive typelessness”, maintenance of these losses of functions in the variable part could perhaps be interpreted as steps returning to “a former state already realized in the series of its ancestors”, i.e. steps on the way to an optimally functioning photosynthetic apparatus. Phylogenetically, C1 appears to be largely in accord with A (although not entirely with the causes generating the systems). From the vantage points of C2a and B, however, most of these still functioning mutants represent secondary deviations most probably never representing any of their ancestors. Yet C2b could perhaps argue that a less differentiated photosynthetic apparatus may be viewed as a reversal to a more basic or possibly to an imperfect physiological state in the design of photosynthesis.

As for the possible objection that photosynthesis does not belong to morphology proper, see comment under the topic of fertilization.

Significance of sepal variation and sepal constancy in Misopates orontium
Since several phenotypic differences distinguishing M. orontium from its closely related species A. majus, like properties of the life cycle, leaf form, flower size, flower colour, and fertilization, have phenotypically been diminished or fully overcome by mutant genes in otherwise normal phenotypes, – what possibilities of variation could exist in the rest of the differences, especially the long sepal feature being the morphologically most conspicuous trait so that Lamarck even suggested to coin the name “calycium” for the species?

As mentioned above, reduction of the length of sepals has been found so far only as a by-product of syndromes more or less strongly disturbing normal development producing “monster plants” in Misopates. Conversely, mutants with long sepals have never been detected in A. majus in more than a hundred years of research with mutants and recombinants of this species in several institutes nor in any culture varieties.

Before addressing the basic question whether this constancy may be simply due to limited materials or could hint at a deeper meaning in relation to systematics in general we will first discuss some basic findings of systematics and paleontology relevant for this problem.

The question of the general constancy of taxonomically relevant features
In contrast to Dollo, Darwin had proposed a theory of continuous evolution for the origin of new species and higher taxa by selection of innumerable “small 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).

In accordance with Darwin, the essence of the synthetic theory of evolution (modern synthesis, neo-Darwinism) is a slow, steady, and gradual origin of new species by stepwise selection of mutations, which “have only slight or even invisible effects on the phenotype” (Mayr 1970). Against this setting of continuous evolution it is easily comprehensible why Mayr, the doyen of the modern synthesis, has just recently called the phenomenon of morphological stasis (constancy) – so persistently characterizing the fossil record (for a review see Lönnig and Saedler 2002) – to be one of the basic unresolved problems of evolutionary biology. He specified the problem in a
In evolutionary biology we have species like horseshoe crabs. The horseshoe crab goes back in the fossil record over two hundred million years without any major changes. So obviously they have a very invariant genome type, right? Wrong, they don't. Study the genotype of a series of horseshoe crabs and you'll find there's a great deal of genetic variation. How come, in spite of all this genetic variation, they haven't changed at all in over two hundred million years while other members of their species in which they were living two hundred million years ago are either extinct or have developed into something totally different? Why did the horseshoe crabs not change? That's the kind of question that completely stumps us at the present time.

Beginning with the founder of embryology, Karl Ernst von Baer, many biologists have raised basic objections against the idea of gradual evolution (although Huxley had already expressed some dissatisfaction with gradualism as the almost exclusive mode of evolution before). Von Baer inferred that – “if small steps would have led to essential changes, these continuous alterations could only have been caused by continuous effects and that the accumulation of small changes would have generated a chaos of indeterminable forms”, thus disorder and confusion would be specifying the objects of taxonomy (von Baer 1886, see also Lamprecht 1966, 1974). However, von Baer submitted many arguments that “most species are very clearly delimited” and “provocatively constant”. Moreover, von Baer contested the idea that the initial “insensibly fine steps” could already have clear selective advantages. Like Dollo, von Baer postulated discontinuous evolution, but from a combination of viewpoints C1, C2a and C2b (see above).

Two of the great pioneers of general and systematic botany, Augustin Pyrame De Candolle, and Christian Konrad Sprengel made the following comments on the cardinal characters distinguishing species and genera from each other (1819/1821/1978, excerpts from pp. 95-97, – note the authors’ emphasis on the terms “invariable” (invariably), “invariableness” and “constant” in the ensuing paragraph):

By Species (species), we understand a number of plants, which agree with one another in invariable marks. In this matter every thing depends upon the idea of invariableness. When an organ, or property of it is changed neither by difference of soil, of climate, or of treatment, nor by continued breeding, this organ or property is said to be invariable. When, for instance, we have remarked for centuries, that Centifolia has always unarmed leaf-stalks, we say correctly, that this property of the Centifolia is invariable...What we know is, that from as early a time as the human race has left memorials of its existence upon the earth, the separate species of plants have maintained the same properties invariably...All properties of plants which are subject to change, form either a Subspecies (subspecies), or a variety (varietas)...By a Genus we understand the sum of the species which agree in certain constant properties of the essential parts. (p. 152) The generic character (character genericus) is the expression of the peculiar and invariable marks by which a genus of plants is distinguished from all others...every generic character must state shortly and distinctly the common marks which belong invariably to all species of the same genus. (p. 153) The generic character of the higher plants is borrowed solely from the organs of fructification. [Italics by the authors.]

Before proceeding to a discussion of the basic question, to what extent our work on Misopates and Antirrhinum is relevant for these statements on “invariableness” and vice versa, – expositions, which are roughly 200 years old and were first published 40 years before Darwin’s Origin in 1859, let us directly turn to some comments of modern systematics on the same questions (the arguments on the following points can also be found in Lönnig 2004).

Stace comments in agreement with almost all contemporary authors (1989):

Although flowers are no longer regarded as ‘essential’ and therefore taxonomically particularly important, they still provide the bulk of information contained in the diagnosis of angiosperm taxa. This is because in general the flowers appear to be more conservative than do most other organs.

Before he had already remarked that “This reliance on the flower is remarkable when one considers that most of the time the majority of angiosperms lack any flowers at all”. This appears to be also true for seed- and fruit-structures. Concerning the conservative key systematic characters he further points out that “endomorphic vegetative characters are more conservative than exomorphous ones” and continues:

Conservative characters are...most useful in delineating the higher taxa, where the emphasis is on the recognition of similarity between the members of a taxon.

Yet, for species and systematic categories below the species, he insists that the non-conservative characters seem to dominate.

And he is most probably correct in his analysis: considering the general shift in systematics during the last 250 years (Haeckel’s verdict for zoology that “related species which had been united within a genus by Linné and within a family by Cuvier, now constitute an inclusive order with several families and many genera” – implying that many of Linné’s species have been elevated to the position of genera during the last centuries – is also valid for botany: the history of the systematic status of Antirrhinum in relation to Misopates, not to mention the genera and species of the tribus Antirrhineae in general, may be used as a paradigm to illustrate this fact (see introduction above; for further details, see Lönnig 2002). Hence, one may conclude that the essentials have hardly changed in morphological systematics: The invariable characters delineating species and genera according to Linné, Cuvier, De Candolle, Sprengel and many others are now the conservative characters delineating higher taxa including the morphologically defined genera, tribus and families of today.

Stasis of systematic categories in time: some examples

Taking the descriptions of Linné for Antirrhinum and Misopates given some 250 years ago in his Species Plantarum, there is no difficulty in identifying the different species of today on the basis of his characterizations (see Linné 1753) – only the names have evolved. The same is true for the drawings and descriptions of plant species by Leonhard Fuchs (1543), Tabernaemontanus (1588) on maize and many other plants, even the portrayals by Dioskurides A.D. 78 and 288. Also, George Cuvier had absolutely no problem in identifying the mummified animals of old Egypt being several thousand years old (Cuvier 1830).

Yet, usually concerning such examples it is objected that they are simply nothing on the evolutionary time table. So let us have a look at the last 2.3 million years of European life history: Intriguingly this is characterized by “conservatively slow rates of evolution” (Lang 1994), and the author continues: “At the end of the tertiary the organisms consisted of species, almost all of which can be assigned to present genera, a large section even to living species. This applies not only for the European flora but also for its fauna.” The actualistic inferences drawn from present ecological indicator values to solve elementary questions of quaternary paleoecology are based on “this unusually far-reaching constancy of life forms down to the species” (Lang).

Additionally, about half of the genera of flowering plants found in geological formations dated to be 37 million years old have been assigned to present genera (Stanley 1986), and many well-known present plant families and genera have even been identified in cretaceous formations (taxa sometimes dated to be older than 100 million years before present).

Or, to take a glimpse at another well-known plant group,
the bryophytes. Agashe reports (1995):

"Members of both the major groups of bryophytes, i.e. Hepaticopsida (liverworts) and Bryopsida (mosses), are well represented in the known fossils. However, a detailed comparative study with modern bryophytes indicated that the group has remained almost unchanged since the Paleozoic time. Hence the fossil bryophytes do not help us much in understanding evolution except for the fact that they formed a prominent part of the vegetation from the Paleozoic onwards."

Hence, the widespread bryophytes have survived in their present *gestalt*, i.e. "almost unchanged", some 400 million years on earth.

A comprehensive survey about the phenomenon of the constancy of morphological characters and of entire taxa in the fossil record is beyond the scope of the present paper (for further Details, see Benton 1993; and for a review, see Lönnig and Saedler 2002b). The theory of punctuated equilibrium (Eldredge and Gould 1972; Gould and Eldredge 1993; Gould 2002), was developed to come to grips with the general phenomenon of abrupt appearance and stasis (constancy of the *gestalt* of organisms usually documented for millions of years) in the fossil record. The well-known "living fossils" are only a relatively small part of that general phenomenon.

For some recently discovered examples of the enormous stasis of morphological features, see Gao and Shubin (2003), Mayr (2004), and Gandolfo et al. (2004).

**Molecular stasis**

In the following paragraphs we will briefly examine the question whether molecular biology has contributed basic facts in accord with the findings on stasis found in systematics, morphology, and paleontology just mentioned.

As noted above, "several authors have argued that the developmental programmes and pathways underlying (even) complex morphological features lost in the history of a genus or family can nevertheless be maintained on the genetic level for millions of years and have, indeed, been reactivated in several cases (Marshall et al. 1994; Collin and Cipriani 2003; Whiting et al. 2003). This inference is in agreement with Ohno’s earlier hypothesis (1985) that even dispensable DNA-sequences “would not readily disappear from the genome of mammals (in particular) and vertebrates (in general). Instead they will linger for millions of years.”

Perhaps the peak of molecular stasis has been described by Lazcano and Miller (1996):

"After the explosive metabolic evolution that took place soon after the beginning of life, the basic genetic processes and major molecular traits have persisted essentially unchanged for more than three-and-a-half billion years, perhaps owing to the linkages of the genes involved and the complex interactions between different metabolic routes. At a macroevolutionary level, this represents a case of conservation that is even more striking than the maintenance of the major body plans that appeared at the base of the Cambrian, and which have remained basically unchanged for more than three-and-a-half billion years”, and the molecular mechanisms of animal ontogeny more than a billion years. On the background of the then prevailing idea of the synthetic theory, that ‘each particular gene is going to accumulate many changes over long periods of time and that this was how one organism turned into another’ (Shapiro), the discovery of molecular conservation was, indeed, ‘totally unexpected’ and ‘to a degree beyond anyone’s wildest expectations’ (De Robertis 1994).

Similar conservative sequences and functions dated to be at least some 300 million years old, are involved in the development of plants (Sommer et al. 1990; Schwarz-Sommer et al. 1990, 1992; Davies and Schwarz-Sommer 1994; Theißen and Saedler 1995, 1999; Theißen et al. 2000; Becker et al. 2001; Münster et al. 2002; Becker et al. 2003; De Bodt et al. 2003; Kirst et al. 2003; Kufuji et al. 2003; Zhang et al. 2004; and many others).

Now, since all these “old features”, anatomically as well as molecularly, are still with us, first the basic genetic question for viewpoints A, C1, C2a, and C2b should be addressed, how it is possible to derive stable features in any given plant or animal species by mutations in their genomes and to what extent could the answers be relevant for our results obtained so far for the main differences between *A. majus* and *M. orontium*.

Could this molecular stasis be the basis for the morphological constancy as found in paleontology – as well as of reversibility? But if so, how to generate stable characters by mutagenesis or otherwise at all?

**The origin of stable characters**

1) **Stable features due to losses of gene functions.** The simplest possibility to generate constant characters by gene mutations is to inactivate gene functions, which are redundant under special ecological conditions (see Lönnig and Saedler 1997). To give an easily comprehensible example: In thousands of angiosperma genera, species with coloured flowers exist side by side with species displaying white flowers, - “white” usually signifying a loss of function in the anthocyanin pathway or limited expression of the respective genes within such closely related species or subspecies. Provided that a sufficient loss of genetic information has taken place (for instance by deletions of the key gene functions), so that reversions to the original state can safely be excluded, the new feature (in this case “white flowers”) will be a stable character for aeons of time and over limitless geographical distribution.

Applying this inference to the origin of the features distinguishing *Misopates* from *Antirrhinum*, if the leaf-like bracts and sepal were due to the irreversible losses of functions of a repressor and/or its target genes, the feature would largely be constant. The sepal structure could then only be modified by an indirect route, i.e. the genes affecting the basic leaf programme now ectopically expressed. So in this case we should expect an essentially consistent correlation of mutant leaf deviations with corresponding aberrations in the bracts and sepal in *Misopates*. Apart from one exception, this was the rule we have found so far: Whenever there was a change in the sepal, a corresponding difference was also detected in the leaves and *vice versa* (see the leaf and sepal mutants mentioned above).

2) **Redundancy by gene duplications.** In a more or less simplistic model, generation of genetic redundancy by gene duplications could be another method to create rather constant features: the more extensive the redundancy the more stable the character (for the intricacies often found for real gene duplications, see Kunze et al. 1997; Lönnig 2002). Although this model does not guarantee absolute stability, functional gene duplications are thought to buffer the features of any anatomical or other system against mutant deviations of many kinds. Yet in contrast to the first possibility (constancy of features generated by losses of
gene functions) redundancy could – at least in theory – gradually be reduced until only the original functional gene is left, which, upon further mutation, would then also affect the constant character. To avoid this effect, partial redundancy tightly connected with the overall functions of a species system could be a solution (see below). Mutations in partially redundant genes being disadvantageous for the survival of the plant would quickly be lost by natural selection.

Although the generation of such redundancy can easily be imagined and given genomes correspondingly be interpreted, as far as the authors are aware, as yet there are no reports for any experimental generation of a comparable case of a mutable systematic feature becoming stable by individual gene duplications in a diploid organism. The situation appears to be somewhat different in polyploid organisms: experimentally induced polyploidy can have, indeed, a certain buffer function against the effects of gene mutations (Stebbins 1950, 1971; for a review see Lönnig 2002). For it appears to be clear that a recessive mutation, which normally has some effects on the phenotype in the homozygous state in the following generation in autogamous organisms, will not or hardly be detected in – for example – an octoploid plant line: the majority of dominant alleles buffers the potential effects of the recessive ones.

3) New genes with double functions: Another possibility to produce characters stable enough to be relevant for systematics could be the generation of genes with double functions, one for a key morphological feature and concomitantly a second function for a basic role in an important physiological process, so that, again, any loss of function mutations would be selected against whenever appearing in the wild and in extreme cases also in cultivated populations. In several plant species the gene coding for chalcon synthase (CHS) appears to have such a double function being (1) at the basis of the anthocyanin pathway and simultaneously displaying (2) basic functions for resistances of several kinds (frost, disease, UV), so that no wild populations without Nivea functions (the gene coding for CHS) are known in Antirrhinum or Misopates so far. Moreover, some plant species are endowed with more than one copy of the Nivea gene. However the basic problem remains: how to derive a new non-redundant gene with double functions from an old one by duplications and substitutions? Viewpoints B to C might find the problem especially interesting. (The problems how to derive entirely new gene functions and gene reaction chains from old ones have been described by Kunze et al. 1997, Axe 2000, 2008, Lönnig 2002, Behe and Sanders 2004.)

A network of interdependent functions – substantially irredicibly complex – is hinted at in the comment of Lazcano and Miller quoted above trying to explain the basic genetic processes and major molecular traits that have persisted essentially unchanged for more than three-and-a-half billion years “perhaps owing to the linkages of the genes involved and the complex interactions between different metabolic routes” (italics added).

4) Chromosome rearrangements. “Normal” chromosome rearrangements as well as those due to transposons could be involved in some of the possibilities mentioned above. Due to rapidly growing evidences for chromosomal hot spots of transposable element visits in combination with the data of the theory of the chromosomal field (Lina de Faria 1986, 1999), we have discussed the possibility of partly predetermined chromosome rearrangements, which could also be relevant for the origin of new chromosome races and species in the plant- and animal kingdoms (Lönnig and Saedler 2002b). According to the investigations of Harrison (1956, 1960) the chromosomes of Antirrhinum and Misopates display so many differences so that pairing is strongly disturbed, leading to sterility in his putative F1 plants. However, to date no chromosome map of Misopates is available to compare it with the data known from Antirrhinum (for the latter, see Schwarz-Sommer et al. 2003). Granted the findings reported by Harrison are correct, gene position effects could also be involved in the generation of the differences between A. majus and M. orontium. Yet, because gene positions should in part be reversible by further chromosome rearrangements, character reversion to the original “wild-type” should be considered for this possibility (except, of course, for chromosome rearrangements implying the deletion of the genes in question).

Data pointing to regressive evolution (losses of gene functions) in Misopates in comparison with Antirrhinum

Among several other authors, it was especially Gould who has emphasized the point that there is no general trend for improvement in evolution (Gould 1998, 2002). Moreover, regressive evolution due to losses of gene functions has been widely documented for the animal and plant world alike (for a brief review, see Lönnig 2002). As for Misopates, the data found so far mostly appear to point to the absence of gene functions possibly by regressive evolution in Misopates as compared to Antirrhinum, yet the following hypotheses of our discussion have to be further checked and investigated:

1) Loss of function of a repressor and/or its target genes otherwise involved in the differentiation of leaves, bracts and sepals present in almost all the other 328 species of the tribus Antirrhineae. In Misopates the main leaf programme runs directly up into the sepals. In this connection it is relevant to note that within the tribus the Misopates bracts and sepals definitely represent apomorphic, not plesiomorphic, features.

2) Perhaps loss of function of a gene necessary to produce functional pollen in the plena mutant of Misopates with anthers (the corresponding phenotype of Antirrhinum mutant is fertile). However, since none of the mutants have been sequenced so far, at present it could be argued that the situation may be different at the DNA level of the corresponding loci.

3) In the deficiens mutants of Misopates the fifth locale appears to be absent, probably pointing to another absence or loss or reduction of gene function.

Moreover, the phenotype of the double mutant cyc/dich of A. majus has already been realized by the monoallelic strong cyc/oid mutant of Misopates also described above. Thus, some monogenic mutants of Misopates appear to display the phenotypes of double mutants of Antirrhinum.

4) As already noted above, the almost white flower colour in the wild Coimbra Misopates orontium line is probably due to a “loss” or suppression of function and can phenotypically revert to the violet of its assumed ancestors.

Natural selection

If Misopates has evolved its dramatically long sepals as a shelter for the inner flower whorls – the hypothesis most often advanced to explain the origin of this feature – the question might immediately be raised, how all the other 99% of the Antirrhineae (or the angiosperms in general) have managed to survive and successfully flourish without correspondingly elongated sepals.

As already emphasized, Darwin had suggested a theory of gradual evolution for the origin of new species and higher systematic categories by selection of – to repeat this vital point – 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). And among other topics it was mentioned that von Baer contested the idea that the initial “insensibly fine steps” could already have had clear selective advantages and that both, Dollo (viewpoint C1) and von Baer (C2) in combination with C2a and C2b had postulated a discontinuous origin of species and higher taxa (followed by a long line of biologists up to the present day).

Briefly extending this controversy to the origin of the enormously elongated sepals of *Misopates orontium*, the difficulties of both positions (entirely or in part) may immediately become translucent for this case, too.

Assuming with viewpoints A, C1, C2a and C2b, that the common ancestor of the tribus Antirrhineae displayed relatively short sepals in comparison to the corolla as one of its original characteristics – 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 mutations to “stretch” them in an extended series of insensibly fine steps to their present magnitude in cooperation with (2) an enormous selection pressure again concentrating particularly on this morphological character? Also, similar questions might be raised for the less conspicuous, but also derived morphological features, especially the cuplike seeds, alone or in combination (perhaps by effects of pleiotropic genes)?

For some physiological characters the problems seem to be easier, although insensibly fine steps seem to reach their limits here, too. Sometimes it is argued that the morphological deviations might be just by-products of physiological changes. Moreover, 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 1997; Müller and Theißen 2003).

Also, the saltatorial view of C1 will meet enormous problems regarding the origin of the long sepal feature if the latter is part of an irreducibly complex system. Yet, in that case B and C2 (both) will see corroborating evidence of their views.

If, however, the long sepals of *M. orontium* were just due to some neutral losses of gene functions (perhaps best classified under C1), these losses should be detectable in comparison with related species displaying normal sepals.

**CONCLUSION**

Dollo’s statement that “an organism cannot return, even partially, to a former state already realized in the series of its ancestors” and that “structural or morphological reversal does not occur” etc. (see above) has to be qualified for several apomorph features of *Misopates* (longevity, leaf form, flower size and colour and mode of fertilization), at least phenotypically. However, as the most important morphological difference between *Misopates* and *Antirrhinum*, i.e. the length of the sepals, so far no convergence due to mutations has been achieved and this agreement with Dollo’s law raised the possibility of a reversal, an irreversibly constacy of this key feature may be due to an irreversible genetic constitution in its present state (see the details above). Future investigations will solve this question.

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