Homeosis of the angiosperm flower:
Studies on three candidate cases of saltational evolution[1]

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Abstract

Gradualism, as outlined in DARWIN's influential book on the origin of species, maintains that evolution proceeds only by infinitesimally small inherited modifications. There is quite some evidence that gradual changes represent a frequent mode of evolution, but whether it is the only one remains highly disputed. Several lines of evidence, including cladistic analyses of character states as well as the fossil record, suggest that also profound sudden (saltational) changes may have occurred, and may have been crucial for the establishment of evolutionary novelties. In our group we use homeotic transitions in the angiosperm flower as a model for saltational changes during evolution. Three case studies on tulips, orchids and shepherd's purse are used to exemplify ancient as well as very recent homeotic transitions during evolution. The work on tulips suggests that changes in the spatial expression of class B floral homeotic genes represent an important molecular mechanism underlying the transition between sepaloid and petaloid organs during evolution. The work on orchids reveals how novel organs, such as the "lip" (labellum), may originate during evolution, and how then homeosis may contribute to the diversification of a novel flower *bauplan*. The studies on shepherd's purse provide an example for a natural floral homeotic mutant that has a fitness very similar to that of wild-type plants and thus has established stable populations in the wild. The importance of our findings for a better understanding of discontinuity in the fossil record (including the phenomenon of "punctuated equilibria") is discussed.

K e y w o r d s : Gradualism, macroevolution, Modern Synthesis, saltational evolution, punctuated equilibria, MADS-box gene, flower, homeosis.

Zusammenfassung

Der Gradualismus, der in DARWINS einflussreichem Buch über den Ursprung der Arten dargestellt wurde, behauptet, dass Evolution nur durch nahezu unendlich kleine erbliche Veränderungen voranschreitet. Es liegt tatsächlich eine ganze Reihe von Evidenzen dafür vor, dass graduelle Veränderungen eine häufige Art der Evolution darstellen, aber ob es die einzige Art ist, ist heftig umstritten. Es gibt eine ganze Reihe von Hinweisen darauf, unter anderem durch kladistische Analysen von Merkmalszuständen sowie durch die Analyse von Fossilmaterial, dass auch profunde (saltatorische) Veränderungen stattgefunden haben, und dass diese für die Etablierung evolutionärer Neuheiten wichtig gewesen sind. In unserer Arbeitsgruppe verwenden wir homöotische Transitionen in der Blüte der Angiospermen als ein Modell für saltatorische Veränderungen in der Evolution. Drei Fallstudien an Tulpen, Orchideen und Hirtentäschel werden benutzt, um ancestrale bzw. rezente homöotische Veränderungen in der Evolution exemplarisch darzustellen. Die Studie an Tulpen zeigt, dass Veränderungen in der räumlichen Expression von floralen homöotischen Genen der Klasse B einen wichtigen molekularen Mechanismus darstellen, der der Transition zwischen petaloiden und sepaloiden Organen zugrunde liegt. Die Untersuchungen an Orchideen lassen erkennen, wie Organe mit neuer Identität, wie z. B. die Lippe (Labellum), während der Evolution entstehen können, und wie Hömöosis dann zur Diversifizierung eines neuen Blütenbauplans beitragen kann. Die Studien am Hirtentäschel bieten ein Beispiel für eine natürliche florale homöotische Mutante, die eine Fitness ähnlich der von Wildtyp-Pflanzen hat und die daher stabile wilde Populationen ausgebildet hat. Die Bedeutung unserer Erkenntnisse für ein besseres Verständnis der Diskontinuitäten im Fossilmaterial (einschließlich des Phänomens der "punctuated equilibria") wird diskutiert.

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1. Introduction: The mystery of mysteries

Many human beings, including most biologists, have a deep and genuine interest in understanding as to how the complexity and diversity of life originated. Historically, living beings appeared so complex that it was taken for granted that someone much more powerful than humans must have created them. To Charles Darwin (1809–1882) we owe the extremely fruitful hypotheses, that living organisms originated by descent with modification, that is by biological evolution, and that evolution results from natural selection acting on heritable variation. However, while Darwin had quite something to say about the power of natural selection, he did not know much about heritable variation, and his speculations about the mechanism of inheritance did not stand the test of time (Stoltzfus 2006; Howard 2009).

In line with the title of his most influential book, "On the origin of species by means of natural selection", Darwin (1859) considered the origin of species as "the mystery of mysteries". To some it may thus come as a surprise that neither in this book, nor elsewhere, Darwin did explicitly tell us what species are, nor could he propose a mechanism by which new species originate. Rather, Darwin's book mainly deals with the change of existing species under natural selection, i. e. adaptation. (Up to now, the confusion of speciation with other kinds of evolutionary change, such as adaptation or the origin of novelties, is one of the most frequent misconceptions, severely impairing fruitful discussions about evolution (Theissen 2006).)

In his long intellectual journey towards writing up "On the origin of species …" Darwin originally started with some kind of saltationist's view (Eldredge 2005). Over the years, however, he developed a very extreme position by maintaining that evolution proceeds always in a countless number of unrecognizably small steps (Eldredge 2005), a view I'll call ultra-gradualism here. Darwin became absolutely convinced that "natural selection can act only by the preservation and accumulation of infinitesimally small inherited modifications", that is "without any great and sudden modifications" – in essence, *Natura non facit saltum* (nature never takes a leap) (e. g. Darwin 1859: 194). As has been recently argued by Howard (2009) this view may have been an important reason why Darwin, in contrast to Mendel, did not discover the basic principles of inheritance, and later ignored them as irrelevant for his interest in evolution, even though he had very similar evidence at hand.

In this respect it is interesting to know that his friend and supporter Thomas Henry Huxley warned Darwin that natural selection may also act on non-gradual changes, and that it is therefore unnecessary – and potentially dangerous for the credibility of his groundbreaking insights on natural selection – to link the powerful concept of nat-

ural selection to strict gradualism (Gould 1977a; Theissen 2009). It appears that Darwin did not have any compelling evidence that would have implied that evolution proceeds only by infinitesimally small steps; maybe it was just a prejudice.

Up to now, about 150 years later, strict gradualism has not found strong empirical support. Neither do the abrupt transitions in the fossil record immediately corroborate the strictly gradual evolution of new forms (Gould & Eldredge 1993; Schwartz 1999; Stoltzfus 2006; Theissen 2006), nor do cladistic analyses of traits in higher taxa such as animals and plants support the view that the major features of body plans and their constituent parts always originated in a gradual way (Vergara-Silva 2003).

The apparently sudden origin of new forms of plants (the "abominable mystery" of angiosperm origin) and animals ("Cambrian explosion" of animal body plans) in the fossil record worried already Darwin. Since true sudden origins of new forms and species were obviously out of discussion for Darwin, he preferred to explain the lack of gradual transformation series by a dramatic incompleteness of the fossil record. In case of the angiosperms, for example, he speculated that they had slowly evolved on a remote and no longer present continent in the southern hemisphere and thus left no traces in the fossil record during that time (Friedman 2009).

From both a scientific and epistemological point of view, such kinds of ad hoc hypotheses are unsatisfactory, and it is no wonder, therefore, that alternatives to Darwin's ultra-gradualism have been considered many times since the publication of "On the origin of species …". Given the way in which Darwin and the architects of the Modern Synthesis of evolutionary biology neglected the mechanisms of heritable variation and the intricate mechanisms of gene action during development it may also not come as a big surprise that especially geneticists, among them the most eminent experts of their times (such as William Bateson, Hugo De Vries, Carl Correns), had quite some problems to accept ultra-gradualism, or even fully rejected it (reviewed e. g. by Schwartz 1999; Stoltzfus 2006; Levit et al. 2008; Theissen 2009). Even though many of these scientists did not deny that gradual changes occur, for example during adaptation, they considered them unable to explain the origin of fundamental phenotypic novelties, or species and higher order taxa. Rather, sudden and discontinuous changes appeared necessary to explain the origin of profound evolutionary innovations or new species.

Diverse varieties of "saltationism" and "mutationism" denying the comprehensive explanatory power of gradual changes have been developed over the years (reviewed by Stoltzfus 2006; Levit et al. 2008). Most widely known are probably the saltational views of Richard Goldschmidt (1878–1958), who was convinced that true species are separated by "bridgeless gaps" that could not be overcome

by slow and gradual changes. GOLDSCHMIDT (1933, 1940) raised no objection to gradual changes within species, but he was convinced that the origin of new species required saltational events based on discontinuous variation (reviewed by DIETRICH 2000, 2003; THEISSEN 2006). GOLDSCHMIDT was well aware that the vast majority of discontinuous variants have disastrous effects on the fitness of organisms – hence he called them "monsters". But every once in a while, GOLDSCHMIDT argued, a "hopeful monster" is generated which is adapted to a new mode of life. According to GOLDSCHMIDT, macroevolution does not proceed by an accumulation of small changes within populations, but only by the rare success of hopeful monsters.

GOLDSCHMIDT (1940) presented two mechanisms for how saltational evolution might work. One mechanism, involving "systemic mutations", rejected the classical gene concept and thus contributed to the damage of GOLDSCHMIDT's scientific reputation (DIETRICH 2003). The second mechanism, however, involved "developmental macromutations" in "rate genes" or "controlling genes" that change early development and thus cause large effects in the adult phenotype. These kinds of mutations are quite similar to the ones considered in contemporary evolutionary developmental biology (see below).

While many aspects of the diverse saltational and mutational theories that have previously been suggested are now mainly of historical interest, the fact that ultra-gradualism became almost an obsession of DARWIN and the Modern Synthesis, and the Modern Synthesis the prevailing evolutionary theory, does not imply that gradualism is true, if only because the narrow conceptual framework of the Modern Synthesis, which is largely based on population genetics, may not provide a comprehensive explanation of evolution (reviewed e. g. by STOLTZFUS 2006; THEISSEN 2006, 2009).

In order to complement the Modern Synthesis with developmental genetics the nascent field of evolutionary developmental biology (evo-devo) is currently trying to figure out as to how changes at some key developmental control genes affect both the development and evolution of the body plans of plants and animals (GOULD 1997b; HAAG & TRUE 2001; THEISSEN et al. 2000, 2002; ARTHUR 2002; VERGARA-SILVA 2003; THEISSEN & MELZER 2007). Following this rationale, modern variants of mutationism and saltationism have been developed (BATEMAN & DI MICHELE 1994, 2002; SCHWARTZ 1999; STOLTZFUS 2006; THEISSEN 2006, 2009). For example, STOLTZFUS (2006) pointed out that mutations changing developmental processes are possible causes of discontinuity, directionality and dynamics in evolution. This is in remarkable contrast to the gradualistic doctrine of the Modern Synthesis that evolutionary change is always based on infinitesimal small modifications and that natural selection is the only source of directionality, creativity and dynamics in evolution.

At least among biologists it is widely accepted now that whenever there is natural selection acting on heritable variation, evolutionary change is an inevitable consequence. There is little doubt that this kind of "microevolution" contributes to the adaptation of living beings to their environment (including other living beings). Whether evolution exclusively proceeds in unrecognizably small steps, and whether these provide sufficient heritable variation to bring about fundamental novelties during evolution is less clear, however.

Speciation may often result from trivial events such as genetic drift following geographic separation or minor genomic or phenotypic changes leading to reproductive isolation. In my view, therefore, the tempo, mode and molecular mechanisms of evolution during the establishment of fundamental novelties do much more deserve to be considered the "mystery of mysteries" of life. I consider it an important scientific goal, therefore, to document and clarify putative cases of saltational evolution and to study their underlying molecular and ecological mechanisms. In our group we use homeotic changes of the angiosperm flower as promising model systems.

2. Homeosis and floral organ identity

Homeotic transitions represent dramatic yet coordinate changes in the body plans of organisms. The term "homeosis" had been introduced in 1884 by WILLIAM BATESON to describe a type of variation in which "something has been changed into the likeness of something else" (LEWIS 1994). Meanwhile several different definitions of homeosis exist, some broader, some more narrow, but they all boil down to structural changes in which organs or segments of an organism are replaced by organs or segments of another identity (SATTLER 1988). A well-known example is provided by

the *Antennapedia* mutant of the fruit fly *Drosophila melanogaster*, which has antennae replaced by leg-like organs. Since homeotically transformed organs lead to conflicts in homology assignments they can be considered being profound evolutionary novelties (THEISSEN 2005).

Homeotic mutants are quite frequent in plants (SATTLER 1988; MEYEROWITZ et al. 1989). Especially well-known are mutant plants with flowers that have more or less normal floral organs in places where organs of another type are typically found. These floral homeotic mutants have taught us a great deal about as to how the identity of the different organs is specified during flower development.

Many flowers of eudicotyledonous plants consist of four different types of organs, termed sepals, petals, stamens and carpels, which are often arranged in four whorls. In the model plant thale cress *(Arabidopsis thaliana)* many homeotic mutants fall into one of three classes: A, B or C. Ideal class A mutants have carpels rather than sepals in the first whorl, and stamens instead of petals in the second whorl. Class B mutants have sepals rather than petals in the second whorl and carpels instead of stamens in the third whorl. Finally, class C mutants have flowers in which reproductive organs (stamens and carpels) are replaced by perianth organs (petals and sepals, respectively), and in which the whorled arrangement of organs and determinacy of floral growth is lost (MEYEROWITZ et al. 1989; THEISSEN 2001). Many wild and ornamental plants have generated mutants with such a "filled flower" phenotype.

The defined classes of homeotic mutants have been explained by simple genetic models such as the ABC model of flower development (COEN & MEYEROWITZ 1991). In line with the three classes of homeotic mutants it proposes three different floral homeotic functions, termed A, B and C, to explain how the different floral organs adopt their unique identities during development. Specifically, the model maintains that class A genes specify sepals in the first floral whorl, class A+B genes petals in the second whorl, class B+C genes stamens in the third whorl and a class C gene carpels in the fourth whorl. Cloning of the floral homeotic genes in several model plants such as *Antirrhinum majus* (snapdragon), *Arabidopsis thaliana* and *Petunia hybrida* (petunia), during the 1980s and 1990s revealed that they all encode transcription factors, i. e. proteins that recognize specific DNA motifs of other genes and influence their transcription. In contrast to the homeotic genes of animals, however, they do not encode homeodomain proteins, but, with few exceptions, MIKC-type MADS-domain proteins (reviewed by BECKER & THEISSEN 2003; THEISSEN 2001). The combinatorial interaction of floral homeotic genes is probably based on the formation of multimeric transcription factor complexes that also include class E (or SEPALLATA-like) proteins, as outlined by the "floral quartet model" (THEISSEN 2001; THEISSEN & SAEDLER 2001).

3. Homeosis in the evolution of the flower

The simple genetic basis and suddenness of floral homeotic transitions raises the question as to whether similar changes also occurred during evolution. Some of the mutant phenotypes (e. g., petaloid rather than sepaloid first whorl floral organs, actinomorphic rather than zygomorphic flowers) resemble differences in character states between major organismic lineages. It is tempting to assume that the genes underlying such "phylomimicking mutants" define loci that play an important role in character changes during macroevolution, but this cannot be taken for granted (HAAG & TRUE 2001).

These considerations raise two questions. Did the angiosperm flower diversify during evolution via homeosis? If so, were these changes caused by mutations in orthologues of those genes that were defined as floral homeotic genes by developmental genetics? Several botanists have answered the first question already with a clear "yes". For example, there is evidence that in the flowers of several Zingiberales, some stamens have been replaced by a lip, some others by petaloid staminodes (KIRCHOFF 1991); likewise, the petals of Rosaceae (including roses) probably originated from stamens (RONSE DE CRAENE 2003).

In principle, homeotic changes in organ identity could occur in infinitely small steps, i. e. the only mode of evolution DARWIN could envision. The ease with which floral homeotic mutants are often generated by a mutation at a single gene locus makes it appears unlikely, however, that gradualistic scenarios always apply. Nevertheless it remains important to identify clear-cut cases of saltational homeosis, and to uncover its underlying molecular mechanisms and ecological and population genetics conditions. On the following I am going to briefly describe three ongoing case studies from our lab on ancient and more recent homeosis that, I hope, are on a good way along these lines.

4. MADS about saltational evolution:
three case studies

4.1. Tulips about tepal types

Tulips (genus *Tulipa*) and many other petaloid monocots such as lilies and orchids, have flowers comprising organs with identities quite similar to the ones of higher eudicotyledonous plants. However, first whorl organs are typically petaloid like second whorl organs rather than sepaloid. This finding suggests that a homeotic transition in the first floral whorl from sepaloid to petaloid organ identity, or vice versa, occurred during the evolution of flowering plants. (Actually, phylogenetic analyses suggest that such changes occured many times during the evolution of the angiosperms). According to the ABC model described

above, the development of petaloid organ identity requires the function of class A and class B floral homeotic genes, while sepaloid organs are specified by class A genes alone. Accordingly, a modified ABC model has been suggested for tulips, with class A+B genes specifying petaloid organs (tepals) in the first two floral whorls (KANNO et al. 2003).

Transgenic experiments in *Arabidopsis thaliana* revealed that the ectopic expression of the two class B genes *APETALA3* (*AP3*, a *DEF*-like gene) and *PISTILLATA* (*PI*, a *GLO*-like gene) is sufficient to homeotically transform sepals in the first floral whorl into petaloid organs (KRIZEK & MEYEROWITZ 1996). Such mutant flowers thus have two whorls of petaloid organs just like tulips (differences in organ number, shape etc. notwithstanding). Findings like this corroborate the view that heterotopic expression of class B genes in the first floral whorl may underlay petaloid organ identity in the first (outer) floral whorls of tulips and other petaloid monocots.

Three putative class B genes, two *DEF*-like and one *GLO*-like gene, could be molecularly cloned from tulip. In line with the modified ABC model, they were indeed found to be expressed, besides in stamens, not only in the petaloid tepals of the second floral whorl, but also in the organs of similar identity in the first whorl (KANNO et al. 2003). In a Viridiflora-type tulip (garden variety "Spring Green"), which has greenish, sepaloid stripes on the tepals, a reduction in the expression of the *DEF*-like genes, i. e. putative class B floral organ identity genes, has been observed, supporting the view that *DEF*-like MIKC-type MADS-box genes are involved in the development of petaloid organs in tulip and thus again corroborating the modified ABC model (KANNO et al. 2007). However, no reduction in the expression of the other type of putative class B gene, the tulip *GLO*-like gene, could be detected. This indicates that the regulatory interactions between class B genes in tulip are different from those in the eudicotyledonous plants *Arabidopsis thaliana* and *Antirrhinum majus*, where heterodimers of DEF-like and GLO-like proteins are required for the upregulation of the expression of the genes which encode them, so that a reduction in *DEF*-like gene expression leads also to a reduction in *GLO*-like gene expression, and vice versa (KANNO et al. 2007).

A non-synonymous single nucleotide polymorphism (SNP) in the MADS-box of one of the two *DEF*-like genes of tulip distinguishes all Viridiflora varieties from all the many other tulip varieties and species tested (HANNELORE SIMON and GT, unpublished data), further supporting the view that a conserved class B gene function involving *DEF*-like genes is required for the specification of petaloid tepals in tulips. The molecular mechanism by which the SNP affects sepaloid vs. petaloid organ identity is currently being investigated.

Taken together, our studies on tulips support the view that sudden and simple mutations at a single locus can bring about changes of perianth organs from a sepaloid to a petaloid identity, and vice versa, also in an evolutionary framework. Indeed, it seems that homeosis can happen in evolution by simple changes in floral homeotic genes.

4.2. MADS about orchids: the making of 0–3 lips

In contrast to tulips and many other petaloid monocots, the 30.000 or so different orchid species seem to have flowers of overwhelming morphological diversity. A closer look reveals, however, that this diversity is largely brought about by variation on a quite simple scheme. Just like the flowers of tulips, orchid flowers have two whorls of petaloid tepals surrounding the reproductive organs. But while the six tepals of a typical tulip flower are (almost) identical, three different kinds of tepals can be distinguished in the perianth of orchids: in the first floral whorl there are three outer tepals (often also termed "sepals"), while in the second whorl there are two lateral inner tepals ("petals") and a median inner tepal called lip or labellum (MONDRAGON-PALOMINO & THEISSEN 2008). While the outer tepals are often similar to the lateral inner tepals, the lip is usually quite different from the rest of the perianth organs, e. g. with respect to shape and colour. In case of slipper orchids, for example, the lip is the organ that forms the slipper.

Recently, we proposed a hypothesis, termed the "orchid code", explaining the developmental specification and evolutionary origin of organ identity in the orchid perianth (MONDRAGON-PALOMINO & THEISSEN 2008). It maintains that the identity of the different petaloid tepals in the perianth of orchid flowers is specified by the combinatorial interaction of four paralogous *DEF*-like genes with one *GLO*-like class B floral homeotic gene. Phylogeny reconstructions indicate that the different types of *DEF*-like genes fall into four distinct clades (clade 1–4) that originated very early during orchid evolution (MONDRAGON-PALOMINO & THEISSEN 2008; MONDRAGON-PALOMINO et al. 2009). Based on the conserved, clade-specific differential expression patterns of the genes the orchid code hypothesis maintains that differential expression of clade 3 genes distinguishes between inner and outer tepals, while differential expression of clade 4 genes distinguishes between the identities of the lip and that of lateral inner tepals.

Phylogeny reconstructions enabled to establish a relationship between the molecular evolution of clade 1–4 *DEF*-like genes, involving gene or even whole genome duplications, and the morphological differentiation of the orchid perianth (MONDRAGON-PALOMINO & THEISSEN, 2008; MONDRAGON-PALOMINO et al. 2009). The most recent common ancestor of orchids probably had an actinomorphic perianth composed of six almost identical tepals quite similar to that of tulips, in which an ancestral *DEF*-like

gene was uniformly expressed. The first duplication of the *DEF*-like gene gave rise to the ancestor of clade 1 and clade 2 genes, and to the ancestor of clade 3 and clade 4 genes. Evolution of differential expression of the precursor of clade 3 and clade 4 genes may have led to the establishment of different organ identities for outer (gene expression "off") and inner (gene expression "on") tepals. Similarly, another gene duplication gave rise to clade 3 and clade 4 genes, and differential expression of clade 4 genes led to the distinction between the lateral inner tepals (gene expression "off") and the lip (gene expression "on"). It is reasonable to assume that different *cis*-regulatory elements evolved in the four clades of *DEF*-like genes that are now responsible for the differential expression of these genes. These different paralogous *DEF*-like genes may thus respond to positional cues in different ways to obtain their characteristic expression patterns, involving a basipetal – acropetal gradient in case of clade 3 genes and an adaxial – abaxial (dorsal – ventral) gradient in case of clade 4 genes (MONDRAGON-PALOMINO & THEISSEN 2009). For the latter gradient, the high dorsal expression of some *CYCLOIDEA- (CYC-)* like transcription factors makes them good candidates (MONDRAGON-PALOMINO & THEISSEN 2009).

The evolution of new organ identities within the perianth (sub-identities of petaloid tepal identity) probably had a dramatic impact on the evolution of orchid floral diversity. It may have eliminated constraints and thus may have modularized the floral perianth, so that the different types of tepals (outer, lateral inner, lip) could now evolve individually, and thus often in dramatically different ways, in response to selection, e. g. by pollinators. It thus may well be that floral diversity in orchids is not only based on special selection regimes, but also on an unprecedented developmental genetic predisposition, i. e. four paralogous *DEF*-like genes, that originated close to the base of orchid evolution (MONDRAGON-PALOMINO & THEISSEN 2009).

Moreover, the evolution of new organ identities provided the potential for diversification of the orchid flower by homeotic transitions within the perianth. Floral homeotic terata, such as flowers without a lip or with three lips occur quite frequently in both nature and during plant breeding. They could well be simple gain- or loss-of-function mutants, changing the expression of a *DEF*-like or of a *CYC*-like gene. In nature, most of these mutants do certainly not survive for long. Once in a while, however, they may establish new lineages. For example, there is a variety of *Cattleya intermedia* termed *aquinii* that has the lateral inner tepals transformed into lip-like organs, possibly due to ectopic expression of its clade 4 *DEF*-like gene. In contrast, *Phragmipedium lindenii* is a "slipper orchid without a slipper", because its lip has adopted the identity of a lateral inner tepal, possibly due to loss of clade 4 gene function (MONDRAGON-PALOMINO & THEISSEN 2008,

2009). Thus homeotic transitions within the perianth have contributed to the diversification of the orchid flower during evolution.

4.3. Shepherd's purse: catching a hopeful monster

As outlined here and elsewhere (see citations above) several lines of evidence suggest that homeotic changes played a considerable role during the evolution of the flower. On the other hand, homeotic mutants have often been dismissed as hopeless monsters by population genetics based evolutionary theories such as the Modern Synthesis, due to their assumed low fitness in the wild (reviewed by THEISSEN 2006). In reality, we know almost nothing about the performance of homeotic mutants in natural populations and thus the ultimate difference between hopeless and hopeful monsters. To better understand the modes and mechanisms of saltational evolution via homeosis it appears thus important to study the fitness of promising homeotic mutants in natural habitats (BATEMAN & DIMICHELE 2002; VERGARA-SILVA 2003; DIETRICH 2003; THEISSEN 2006, 2009). Towards that goal we have established a floral homeotic variety of shepherd's purse *(Capsella bursa-pastoris)* as a promising model system (NUTT et al. 2006; HINTZ et al. 2006). The mutant variety was termed *Stamenoid petals (Spe)*, because its petals are completely replaced by stamens, while all other floral organs are unchanged. In contrast to most other floral homeotic mutants known, *Spe* has existed in a number of wild habitats throughout Europe for several decades, if not centuries. At least in these habitats the fitness of *Spe* must be quite similar to that of the wild-type. In our project we investigate how that is possible despite the drastic change in floral architecture.

It turned out that wild-type plants are visited by potential pollinators, such as hoverflies, wild bees and thrips about twice as frequent as *Spe* plants (ZIERMANN et al. 2009). Important reasons are probably attractive volatiles such as monoterpenes and 3,4-dimethylbenzaldehyde and optical cues which are both only provided by petals (ZIERMANN et al. 2009). Nevertheless, in our comparative studies the number of seeds per fruits was about the same in wild-type and mutant plants. This suggests that flower structure and floral visitation are obviously only of minor importance for shepherd's purse, very likely because it is a mainly self-pollinating plant. However, wild-type plants surprisingly produced more flowers, fruits and seeds than *Spe* plants because of differences in plant habit (more extensive branching in the wild-type). In contrast, under our conditions the germination rate of *Spe* seeds was higher than that of the wild-type (ZIERMANN et al. 2009). It remains to be investigated whether the observed linkages between floral phenotype, plant architecture and seed germination rate also hold at the population genetic level in

natural habitats. The genetic components underlying these characters also have not been identified yet (ZIERMANN et al. 2009), but I consider it very unlikely that all the differences between wild-type and *Spe* plants mentioned above are caused by mutation of the *Spe* locus alone. Segregation analyses to clarify this issue are currently underway.

The data available so far suggest that the similar fitness of *Spe* and wild-type shepherd's purse in the field represents a kind of evolutionary "stalemate" in which differences in plant architecture and germination capacity compensate each other (ZIERMANN et al. 2009).

In addition to a high selfing rate, considerable differences in the trait "time to flowering" have very likely helped *Spe* to persist in sympatry with wild-type plants (HAMEISTER et al. 2009). A lower genetic variation of the *Spe* subpopulation compared to the wild-type may have been caused by a recent local origin or recent introduction in one habitat in vineyards in southwest Germany (HAMEISTER et al. 2009). It is tempting to speculate that *Spe* represents a case of sympatric speciation in *statu nascendi*.

The floral homeotic phenotype of *Spe* is caused by a co-dominant mutant allele at a single locus conferring stamen identity in one of the two disomically inherited genomes of tetraploid *Capsella bursa-pastoris* (NUTT et al. 2006). Development of the *Spe* phenotype is probably brought about by ectopic expression of a class C floral homeotic gene in the organs of the second floral whorl (NUTT et al. 2006). Cloning of the relevant gene and understanding of its molecular lesion in the mutant are well underway (JANINE ZIERMANN, PIA NUTT and GÜNTER THEISSEN, unpublished data).

Until now it is widely believed by evolutionary biologists, especially those raised in the spirit of the Modern Synthesis and DARWIN's ultra-gradualism, that any mutation of major effect is unlikely to be tolerated by natural selection and thus generates nothing but "hopeless monsters" (AKAM 1998). However, varieties such as *Spe* demonstrate that organisms with a profound mutant phenotype can stay in wild populations for quite a long time and thus may establish novel organismic lineages.

5. On punctuated equilibria

Writing for a journal devoted to palaeobiology, one is tempted to interpret the potential of saltational evolution via homeosis in the light of the well-known abrupt transitions in the fossil record of both animals and plants. In fact, the fossil record provides little evidence for a gradual evolution of new forms (GOULD & ELDREDGE 1993). The observation is so dramatic, that the incompleteness of the fossil record, which certainly contributes to the phenomenon, cannot fully account for it. In their theory of "punctuated equilibria" ELDREDGE & GOULD (1972) explained the

discontinuity in the fossil record by the assumption that speciation usually occurs not in the centre of populations, but in periphery isolated populations that are unlikely to have left their traces in the fossil record at most places where the species of interest existed. The breaks in the fossil record thus just mark the migration of the descendant from the peripherally isolated area where it originated into the place of observation. This hypothesis, though *expressis verbis* attacking "phyletic gradualism", was based on allopatric speciation rather than non-gradualistic evolutionary mechanisms, and thus was easy to reconcile with the views of the Modern Synthesis. In line with this GOULD & ELDREDGE have indeed pointed out that their theory on punctuated equilibria is not a theory on saltational evolution (GOULD & ELDREDGE 1993). In my view, however, assuming that evolutionary changes occasionally can be saltational rather than gradual may sometimes provide a more straight-forward, and potentially even correct explanation for the lack of gradual series in the fossil record.

6. Outlook

Textbook versions of evolutionary biology, as e. g. enshrined by the Modern Synthesis, have assumed that DARWIN is right in assuming that evolution proceeds exclusively in infinitesimally small step, thus leaving no room for GOLDSCHMIDT's ideas about saltation. While DARWIN was convinced that evolution proceeds exclusively by infinitesimally small modifications, GOLDSCHMIDT assumed that new species arise only by saltational changes involving hopeful monsters. Many discussions about evolution in the past have been burdened with such black and white scenarios and false alternatives. Trying to provide a simple take home message I maintain that rather than being 100 % Darwinian, evolution might be 99 % Darwinian and 1 % Goldschmidtian; or 99.9 % and 0.1 %, that doesn't really matter – the numbers are deliberately arbitrary. It is only important to realize that besides gradual changes, also saltational evolution is a real phenomenon – hopeful monsters are here to stay (THEISSEN 2009). Likewise, also in case of punctuated equilibria there is no need to assume either/or scenarios. It appears more likely that the apparently sudden transitions in the fossil record have several different reasons, possibly not only, but also involving true saltational events (if only in rare cases). The importance of this long neglected mode of evolution, e. g. for the establishment of novelties, we are only just beginning to understand.

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