Friedrich-Schiller-Universität Jena

seit 1558

# Tracking the molecular background of phenotypic change in fruit evolution and plant domestication

## Dissertation

Teresa Lenser

# Tracking the molecular background of phenotypic change in fruit evolution and plant domestication

**Dissertation**

Zur Erlangung des akademischen Grades

„doctor rerum naturalium“ (Dr. rer. nat.)

Vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät

der Friedrich Schiller Universität Jena

von Diplom-Biologin Teresa Lenser

geboren am 06. Juli 1981 in Bottrop

**Gutachter**

1. Prof. Dr. Günter Theißen (Jena)

2. Prof. Dr. Klaus Mummenhoff (Osnabrück)

3. Prof. Dr. Cristina Ferrandiz (Valencia)

**Tag der öffentlichen Verteidigung**

Dienstag, 15.April 2014

# Table of contents

**1. Introduction** ........ 3

**1.1 The molecular background of phenotypic change** ........ 3

**1.2 Convergent versus parallel evolution and the role of molecular convergence** ........ 5

**1.3 Fruits as an adaptive trait** ........ 7

**1.4 Fruits of the Brassicaceae** ........ 9

**1.5 Aims of this work** ........ 11

**2. Manuscripts** ........ 13

**2.1 Overview of the manuscripts** ........ 13

**2.2 Manuscript I** ........ 16

Teresa Lenser and Günter Theißen (2013) Conservation of fruit dehiscence pathways between *Lepidium campestre* and *Arabidopsis thaliana* sheds light on the regulation of *INDEHISCENT*. *The Plant Journal*, 76, 545-556

**2.3 Manuscript II** ........ 34

Andreas Mühlhausen[†], Teresa Lenser[†], Klaus Mummenhoff, and Günter Theißen (2013) Evidence that an evolutionary transition from dehiscent to indehiscent fruits in *Lepidium* (Brassicaceae) was caused by a change in the control of valve margin identity genes. *The Plant Journal*, 73, 824-835.

([†]These authors contributed equally to this work)

**2.4 Manuscript III** ........ 56

Teresa Lenser and Günter Theißen (2013) Molecular mechanisms involved in convergent crop domestication. *Trends in Plant Science*, DOI: 10.1016/j.tplants.2013.08.007, *in press*

**3. Discussion** ........ 69

**3.1 Lepidium species as models for fruit evolution – the ups and downs** ........ 69

**3.2 Conservation of fruit development – Brassicaceae and beyond** ........ 71

**3.3 The role of molecular convergence during plant evolution** ........ 74

**3.4 Beyond the ivory tower – practical contributions of this thesis** ........ 76

**3.5 A fruity taste of things to come** ........ 78

**4. Summary** ........ 82

**Zusammenfassung** ........ 83

**5. Bibliography** ........ 84

**6. Danksagung** ........ 97

**7. Ehrenwörtliche Erklärung** ........ 98

**8. *Curriculum vitae*** ........ 99

**9. Konferenzbeiträge und Publikationen** ........ 100

# 1. Introduction

Evolutionary biologists and breeders alike are intrigued by the question how the molecular networks of a given organism translate into a defined phenotypic appearance. The former because they seek to explain how modifications of these networks could give rise to the immense diversity of life that populates the earth. The latter because they wish to manipulate these networks in order to improve relevant traits of domesticated species in a concerted manner.

This question can be approached at two different levels. In detailed case studies, researchers focus on a particular trait in a very limited number of species to search for mutations or alterations in molecular pathways which cause phenotypic differences (see for example Bharathan *et al.*, 2002; Yoon and Baum, 2004; Gompel *et al.*, 2005; Fourquin *et al.*, 2013; Zhang *et al.*, 2013). A considerable number of such in-depth surveys is a prerequisite for higher level approaches which aim at combining such information in order to gain more general insights on how phenotypic changes are molecularly achieved (see for example Hoekstra and Coyne, 2007; Stern and Orgogozo, 2008; Gompel and Prud'homme, 2009; Martin and Orgogozo, 2013).

In this thesis, character evolution in angiosperm plants is studied at both levels focusing on fruit evolution in the plant family of Brassicaceae in detail and surveying data on the molecular background of convergent plant domestication.

## 1.1 The molecular background of phenotypic change

Understanding the molecular mechanisms of phenotypic change has been a long-standing goal in evolutionary biology (King and Wilson, 1975; Jacob, 1977). Topics that have been under particular debate in this context include the relative roles of protein-coding versus *cis*-regulatory mutations (Hoekstra and Coyne, 2007; Wray, 2007; Stern and Orgogozo, 2008), the relative contribution of mutations in single genes of large phenotypic effect versus mutations in many genes of small phenotypic effect (Orr, 2005; Theißen, 2009; Pritchard and Di Rienzo, 2010), the importance of gene and genome duplications (Flagel and Wendel, 2009; Van de Peer *et al.*, 2009; Innan and Kondrashov, 2010; Kaessmann, 2010), and the level of

predictability of the molecular changes associated with phenotypic evolution (Stern and Orgogozo, 2008; Stern and Orgogozo, 2009; Papp *et al.*, 2011). Obtaining integrative scientific opinions concerning these matters has proven to be difficult because individual case studies may point in opposite directions. For example, mutations in protein-coding as well as in *cis*-regulatory regions have been demonstrated to play a role during domestication of rice (*Oryza sativa*): a single missense mutation within the *sh4* locus causes reduced seed shattering in all known rice cultivars (Li *et al.*, 2006), whereas a single nucleotide polymorphism (SNP) in a *cis*-regulatory region of the *qSH1* locus has led to the strong non-shattering behavior of *japonica* rice (Konishi *et al.*, 2006). In the same line, hypermuscularity in sheep (*Ovis aries*) was found to rely on a *cis*-regulatory mutation that creates a microRNA (miRNA) target site in the 3' untranslated region (3' UTR) of the myostatin gene (Clop *et al.*, 2006), whereas changes in coding regions of respective orthologous genes cause similar phenotypic alterations in cattle (*Bos taurus*) and dog (*Canis lupus familiaris*) (Grobet *et al.*, 1997; Mosher *et al.*, 2007).

During recent years, the amount of case studies identifying molecular changes that drive phenotypic evolution has increased rapidly, thus allowing for central evolutionary questions to be approached in a more comprehensive way. Systematic surveys of published data have led to some popular working hypotheses about the molecular principles of phenotypic change, as for example a preferential fixation of mutations with limited pleiotropic effects (Gompel and Prud'homme, 2009; Kopp, 2009), or an important role of gene duplications for the evolution of phenotypic novelties (Flagel and Wendel, 2009; Kaessmann, 2010). Nevertheless, such surveys remain merely descriptive and conclusions may depend to a significant amount on personal weighting and interpretation of the available data. For instance, when reviewing studies on the number of genetic changes involved in the alteration of individual traits, some authors emphasize that adaptive events are highly polygenic (Pritchard and Di Rienzo, 2010), while others concentrate more on the importance of single mutations with large phenotypic effect (Theißen, 2009; Nadeau and Jiggins, 2010). A by far more unbiased way to approach superordinate evolutionary questions is to do the data-analysis in a strictly quantitative way. In their pioneering work, David Stern and Virginie Orgogozo quantitatively analyzed a large dataset of evolutionary relevant mutations and could demonstrate that the relative contribution of *cis*-regulatory versus protein-coding mutations strikingly differs between short-term and long-term evolutionary events (Stern and Orgogozo, 2008). The dataset was later expanded and is publically available for further quantitative analysis (Martin and Orgogozo, 2013).

Regardless of whether molecular changes of adaptation are analyzed in a surveying or strictly quantitative way, certain biases and constraints due to inherent characteristics of the process of data collection have to be considered. The preferential investigation of known candidate genes might lead to a disproportionally high amount of mutations becoming identified in orthologous loci (Stern and Orgogozo, 2008). Also the relative number of coding versus *cis*-regulatory mutations underlying phenotypic evolution was proposed to be biased because potentially important coding changes, especially nonsense mutations, are easier to identify by plain sequence analysis than potentially important changes in *cis*-regulatory regions (Stern and Orgogozo, 2008). Finally, there is also a bias toward the detection of mutations with large phenotypic effects, simply because more subtle effects may not be discernible with the techniques which are available to date (Rockman, 2012; Martin and Orgogozo, 2013). Some authors argue that despite these biases, general and profound hypotheses about the molecular mechanisms of phenotypic variation can be obtained by comprehensively analyzing data about the currently known 'loci of evolution' (Martin and Orgogozo, 2013). This point of view is supported by a vast body of studies drawing their conclusions based on exactly this line of thinking (see for example Hoekstra and Coyne, 2007; Wray, 2007; Stern and Orgogozo, 2008; Gompel and Prud'homme, 2009; Stern and Orgogozo, 2009; Nadeau and Jiggins, 2010; Conte *et al.*, 2012). Nevertheless, the above mentioned constraints cause other researchers to question the informative value of such approaches in general (Rockman, 2012).

## 1.2 Convergent versus parallel evolution and the role of molecular convergence

One of the hot topics in molecular evolution, its predictability, is often addressed by studying and comparing causative mutations underlying cases of repeated and independent evolution of the same phenotypic trait, a phenomenon called parallel or convergent evolution (Wood *et al.*, 2005; Arendt and Reznick, 2008; Gompel and Prud'homme, 2009; Stern and Orgogozo, 2009; Manceau *et al.*, 2010; Elmer and Meyer, 2011; Lobkovsky and Koonin, 2012; Martin and Orgogozo, 2013).

In a classical sense, these terms have often been applied to distinguish between cases of independent evolution of the same phenotypic trait in closely related (parallelism) or distantly related (convergence) species (Arendt and Reznick, 2008; Elmer and Meyer, 2011). This

terminology is already rather superficial because it lacks a clear discrimination between what is considered closely versus distantly related (Elmer and Meyer, 2011). Nevertheless, matters have gotten even worse since some of the genetic loci underlying the respective phenotypic alterations have been discovered and authors have started to integrate this additional level of molecular information into the same terminology concept (Elmer and Meyer, 2011). From this point on, parallel evolution was defined by some as similar phenotypic changes caused by similar molecular mechanisms while convergent evolution was considered to be similar phenotypic changes caused by distinct molecular mechanisms (Elmer and Meyer, 2011). This definition is again ambiguous because molecular mechanisms may be considered as similar on the level of genetic pathways, discrete genes, or even individual nucleotide mutations (Yoon and Baum, 2004; Elmer and Meyer, 2011). On top of these vague definitions, a literature survey reveals that both terms are used in a highly arbitrary way in recent publications (Arendt and Reznick, 2008).

This lack of a consistent terminology stimulated different authors to suggest new concepts on how best to describe the phenomenon of repeated phenotypic evolution. Some suggested that only one term, 'convergent evolution', should be used to describe the independent evolution of phenotypic similarity (Arendt and Reznick, 2008). Others argue that the distinction of different evolutionary timescales is essential to acknowledge differences in underlying principles and thus, both terms should be kept (Leander, 2008). In both cases, the molecular background of phenotypic change is not directly included in the suggested terminology but needs to be specified as accessory information. Scotland (2011), on the other hand, combines both concepts by restricting the term convergence to describe repeated change of the same character at the phenotypic level, and the term parallelism to indicate identical molecular changes underlying this phenomenon. Which of these definitions will reach overall acceptance within the scientific community remains to be seen. In this thesis, convergence is used as the sole term to indicate the synchronous change of phenotypic characteristics during evolution, thus basically following Arendt and Reznick (2008). If phenotypic convergence is caused by changes at orthologous loci, the term 'molecular convergence' is applied.

Although no consensus on a universal terminology has been reached so far, many recent studies report in unison that molecular convergence plays an important role during evolutionary processes (see for example Colosimo *et al.*, 2005; Protas *et al.*, 2006; Liu *et al.*, 2010b; Feldman *et al.*, 2012). This statement has been discussed extensively based on data derived from natural adaptation in animal systems (Arendt and Reznick, 2008; Gompel and

Prud'homme, 2009; Nadeau and Jiggins, 2010; Elmer and Meyer, 2011), an especially prominent example being the repeated involvement of mutations within the melanocortin-1-receptor coding gene in coat-color changes of species as distantly related as for example lizards, mammoths and mice (Majerus and Mundy, 2003; Mundy, 2005; Hoekstra *et al.*, 2006; Römpler *et al.*, 2006; Gross *et al.*, 2009; Rosenblum *et al.*, 2010). Results from laboratory-controlled evolutionary studies likewise back the idea that molecular convergence is a common phenomenon during evolution (Lobkovsky and Koonin, 2012). Although some cases of molecular convergence are also known from research on plant systems (Yoon and Baum, 2004; Christin *et al.*, 2007; Kivimäki *et al.*, 2007), the amount of data is still limited and little effort has been put into summarizing these data in order to extract possible general trends. This thesis contributes to filling this gap by reviewing data on molecular convergence during crop domestication and by investigating the molecular background of a specific case of phenotypic evolution: the switch from dehiscent to indehiscent fruits in two species of the angiosperm plant family of Brassicaceae.

## 1.3 Fruits as an adaptive trait

The angiosperms (flowering plants) are named after their possession of fruits, structures that develop from mature ovaries after fertilization and enclose and protect the plant’s seeds (Figure 1) (Seymour *et al.*, 2008; Ferrandiz, 2011). Fruits also ensure seed dispersal by very different and highly specialized means (Ferrandiz, 2011). Fleshy fruits provide nutritious parts in order to attract animals as dispersal agents. Dry fruits may develop floating devices for water dispersal, feather or wing-like structures to facilitate dispersal by wind or a sticky texture for attaching to animal vectors. Others open in a controlled process called dehiscence in order to release their seeds for individual dispersal. Because successful dispersal of seeds is an important determinant of plant fitness, fruits are often considered as a key innovation of the angiosperm lineage and a major factor determining its evolutionary success (Scutt *et al.*, 2006; Lorts *et al.*, 2008; Ferrandiz, 2011).

From an evolutionary perspective, fruit morphology is considered to be an important adaptive trait for optimizing a plant’s seed dispersal strategy in response to different environmental conditions and life-history traits (Knapp, 2002; Seymour *et al.*, 2013). For example closed forest habitats were found to be correlated with the occurrence of fleshy fruits while dry fruits were found to be more common in open deserts and plains (Bolmgren and Eriksson, 2005;

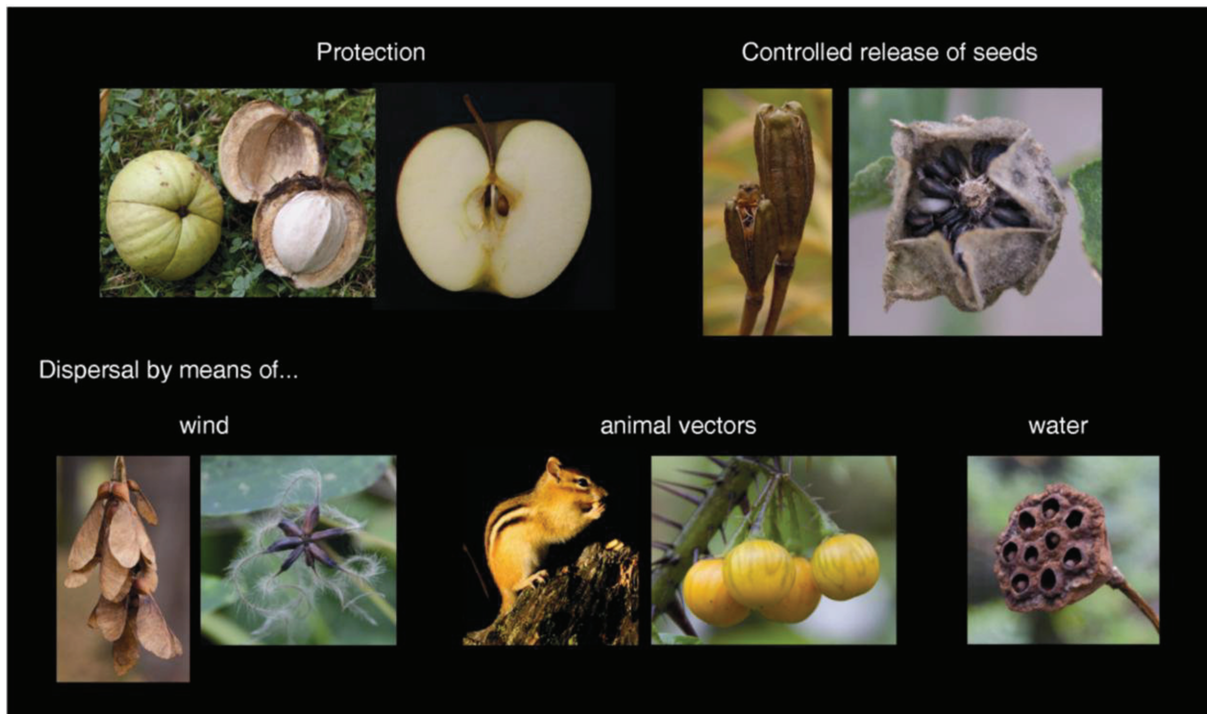


**Figure 1: Multifaceted roles of angiosperm fruits.** Fruits are considered as a key innovation of the angiosperm lineage. They offer **protection** for the developing seeds (left: *Carya laciniosa*; right: *Malus domestica*), may allow for the **controlled release of seeds** (left: *Lilium regale*; right: *Sida rhombifolia*) or enable seed dispersal by means of **wind** (left: *Acer* spec., right: *Clematis recta*), **animal vectors** (left: chipmunk (*Tamias* spec.) acting as a dispersal agent, right: *Solanum atropurpureum*), or **water** (*Nelumbo nucifera*). Chipmunk picture courtesy of Martina Börner (University of Bielefeld).

Lorts *et al.*, 2008). In the same line, within the family of Rubiaceae the development of fleshy fruits was found to be correlated with a shrubby growth habit in contrast to herbaceous plants where dry fruits were more frequently found (Bremer and Eriksson, 1992). In accordance with its adaptive value, fruit morphology was reported to be evolutionary highly plastic when surveying major angiosperm lineages (Lorts *et al.*, 2008) as well as when concentrating on certain plant families in particular (Bremer and Eriksson, 1992; Morgan *et al.*, 1994; Wagstaff and Olmstead, 1997; Clausing *et al.*, 2000; Bradford and Barnes, 2001; Davis *et al.*, 2001; Hall *et al.*, 2002; Patterson and Givnish, 2002; Zjhra *et al.*, 2004). This high plasticity indicates that there are no major phylogenetic constraints acting on fruit evolution in general which makes it a good model for studying the molecular background of character evolution (Lorts *et al.*, 2008; Seymour *et al.*, 2013).

Besides their great importance for plant fitness under natural conditions, fruits and their products are also an indispensable part of human civilization. We not only consume them in huge amounts and feed them to our livestock but also dress in them, apply them to our skin, use them in various industrial applications and, recently, let them even fuel our cars. This

versatile and copious usage is only possible due to the development of modern high-performance crops producing fruits which are specially adapted to human demands by a process called domestication (Doebley *et al.*, 2006; Miller, 2007; Purugganan and Fuller, 2009; Gross and Olsen, 2010; Olsen and Wendel, 2013). Fruit characters that have been repeatedly the target of domestication-related changes include size and number (to increase yield), shape, color, taste, and fragrance (to meet human preferences), and dehiscence behavior (to reduce seed-loss). Therefore, fruits have been extensively modified due to natural adaptation as well as artificial selection processes, thus offering the chance to compare the molecular changes imposed by both processes on the plants' genomes and metabolic circuits.

## 1.4 Fruits of the Brassicaceae

In this thesis, two manuscripts focus in particular on fruits of the Brassicaceae. This plant family comprises approximately 3500 species of mostly perennial herbs that typically form two-valved capsular fruits which are called siliques (if they are more than three times as long as broad) or silicles (if they are less than three times as long as broad) and open longitudinally upon maturity (Al-Shehbaz, 2001). However, aberrations from this typical form are common and the resulting diversity in fruit morphology has traditionally been used to assign phylogenetic relationships within the family (Al-Shehbaz, 2001). Only recently, extensive molecular studies have revealed that many fruit characters have been subject to substantial convergence and are thus not phylogenetically meaningful (Al-Shehbaz, 2001; Bailey *et al.*, 2006; Beilstein *et al.*, 2006; Franzke *et al.*, 2011). One of these characters is the formation of indehiscent (non-opening) fruits which can be observed in more than 50 Brassicaceae genera distributed over the whole phylogeny (Appel and Al-Shehbaz, 2003; Franzke *et al.*, 2011). From an ecological point of view, the significance of indehiscent fruits is not yet clear although it has been speculated that specialized morphological structures of the fruit may assist certain forms of seed dispersal or that the gradual release of seeds through slow decomposition of fruit valves may increase dormancy and thus allow the formation of seed banks (Imbert, 2002; Mühlhausen *et al.*, 2008). A few of the evolutionary switches from dehiscent to indehiscent fruits happened within the genus of Lepidium, one of the largest genera of the Brassicaceae consisting of approximately 250 species typically forming two-seeded silicles (Al-Shehbaz, 2001; Mummenhoff *et al.*, 2001; Mummenhoff *et al.*, 2009; Al-Shehbaz and Mummenhoff, 2011).

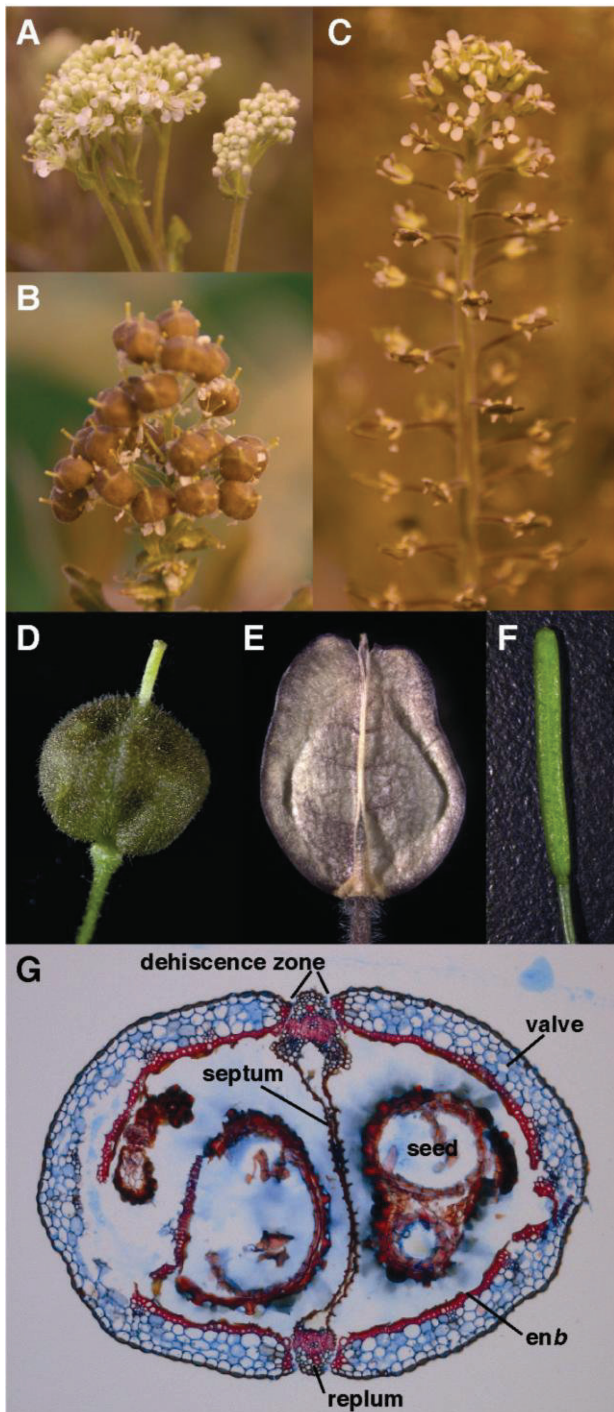


**Figure 2: Brassicaceae model species of this thesis**. Typical inflorescences of *L. appelianum* (**A**, **B**) and *L. campestre* (**C**). Both species produce silicles (**D**, *L. appelianum*; **E**, *L. campestre*), which are less than three times as long as broad. *A. thaliana*, on the other hand, produces typical siliques (**F**), which are more than three times as long as broad. A cross section of an *A. thaliana* fruit (**G**) depicts typical elements of a dehiscent Brassicaceae fruit. Lignified cells are stained in red while non-lignified cells are counterstained in blue. en*b* = endocarp layer *b*.

Although the developmental pathway leading to fruit dehiscence has been studied extensively in *Arabidopsis thaliana* - the major plant model system and a member of the Brassicaceae family - the conservation of this pathway within the family was largely unknown at the onset of my work (for reviews on fruit development in Arabidopsis see for example Dinneny and Yanofsky, 2005; Girin *et al.*, 2009; Ostergaard, 2009; Grieneisen *et al.*, 2013; Seymour *et al.*, 2013). Likewise, nothing was known about the molecular changes that had caused the switch from dehiscent to indehiscent fruits within any species of Brassicaceae, thus making it impossible to assess the relative role of molecular convergence in this special case of convergent character evolution. The two closely related species *Lepidium campestre* (field pepperweed; dehiscent fruits) and *Lepidium appelianum* (globe-podded hoary cress; indehiscent fruits) were chosen in this work as study systems to address these questions further (Figure 2). Both plants are diploid (2n = 2x = 16), which makes them well accessible for genetic analyses, and closely related to *A. thaliana*, which may facilitate the adaptation of molecular techniques (Figure 3).

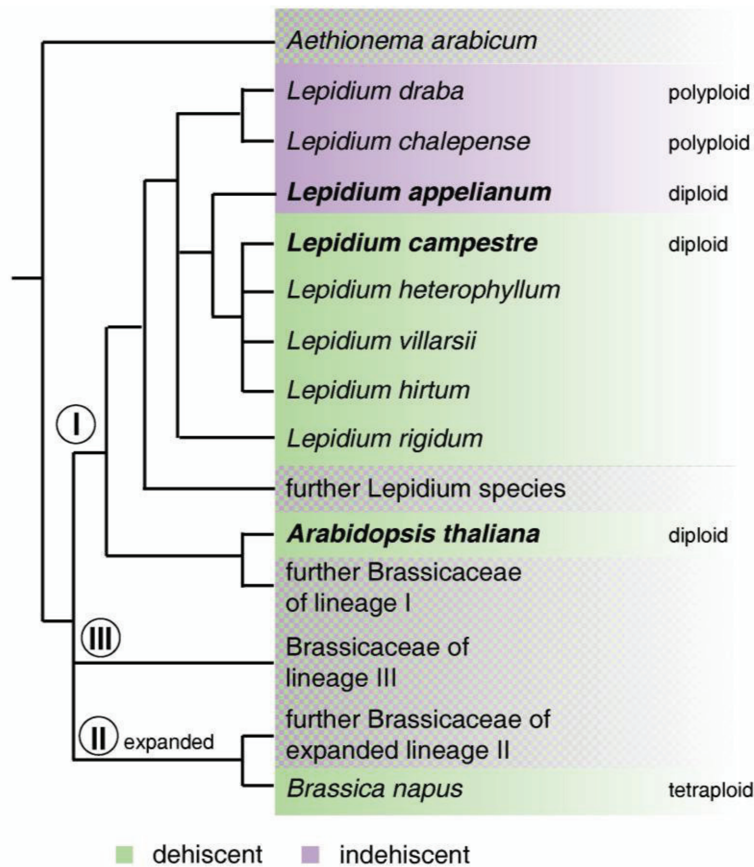


**Figure 3: Phylogenetic relationship of Brassicaceae model species.** Shown is a simplified phylogeny of Brassicaceae including the three major lineages (I, II, and III). Colors are used to distinguish between species with dehiscent (green) and indehiscent (violet) fruits. A checked pattern of both colors is applied if both, dehiscent and indehiscent fruits, can be found. The three main model species used in this thesis (marked in bold) all belong to lineage I. For relevant species, the ploidy level is given.

## 1.5 Aims of this work

As stated above, assessing the relative role of molecular convergence during evolution and characterizing the factors promoting this phenomenon is one aspect on the way to understanding the molecular mechanisms of character evolution, which is one of the central goals in evolutionary developmental biology. Because these questions have not been extensively examined with respect to plant adaptation, this thesis is centered on the molecular background of convergent plant evolution in both, natural and domestic environments.

As an example for convergence in natural adaptation, the repeated switch from dehiscent to indehiscent fruits within the Brassicaceae was chosen as a study system. As a prerequisite for finding molecular differences causing changes in dehiscence behavior, it was first essential to answer the question how well the developmental pathway leading to fruit dehiscence is

generally conserved within the Brassicaceae. I approached this first aim by establishing a transformation system for the dehiscent plant *L. campestre* and using it to manipulate the expression of genes orthologous to known fruit developmental genes of *A. thaliana*. By phenotypic analyses of fruits and gene expression studies, gene functions and pathway connectivity could be compared between *L. campestre* and *A. thaliana* (manuscript I).

The subsequent aim was to elucidate the molecular changes responsible for one of the adaptive changes from dehiscent to indehiscent fruits within the Brassicaceae. Thus, gene expression data were compared between dehiscent *L. campestre* and indehiscent *L. appelianum*, and resulting candidate genes were further analyzed via sequence analysis and heterologous transformation (manuscript II). Assessing the role of molecular convergence during Brassicaceae fruit evolution was another aim addressed by comparing and combining my own molecular data with that of other studies.

Transfer of knowledge on molecular principles from evolution to domestication research holds great potential for future crop improvement. Thus, the final aim was to estimate the role of molecular convergence for crop domestication on the basis of preexisting data and to evaluate the question whether the same determining factors known from research on natural adaptation also act on adaptation in domestic environments (manuscript III).

# 2. Manuscripts

## 2.1 Overview of the manuscripts

**I.** **Teresa Lenser and Günter Theißen** (2013) Conservation of fruit dehiscence pathways between *Lepidium campestre* and *Arabidopsis thaliana* sheds light on the regulation of *INDEHISCENT*. *The Plant Journal*, **76**, 545-556.

Here we compare the molecular fruit developmental pathways between *Arabidopsis thaliana* and *Lepidium campestre*. We report high conservation of gene function and pathway connectivity between both species and identify ALCATRAZ and SPATULA as repressors of *INDEHISCENT* expression.

All reported experiments were performed by me. I wrote the first draft and prepared the figures of the manuscript. Günter Theißen designed and supervised the project and corrected and improved the whole manuscript.

Overall own contribution: 90%

I hereby certify the accuracy of the statements on the contributions of the authors.

Günter Theißen

**II. Andreas Mühlhausen**[†], **Teresa Lenser**[†], **Klaus Mummenhoff, and Günter Theißen** (2013) Evidence that an evolutionary transition from dehiscent to indehiscent fruits in *Lepidium* (Brassicaceae) was caused by a change in the control of valve margin identity genes. *The Plant Journal*, **73**, 824-835.

([†]These authors contributed equally to this work)

By comparative expression analyses we show that a loss of expression of valve margin identity genes causes the formation of indehiscent fruits in *Lepidium appelianum*. By heterologous transformation studies and sequence analysis we further target the question which genetic changes cause the altered gene expression pattern.

I performed the quantitative RT-PCR analysis, the heterologous transformation analysis, cloned half of the candidate genes of the Lepidium species and, together with Andreas Mühlhausen, wrote the first draft of the manuscript and prepared the figures. Andreas Mühlhausen did the other half of the cloning, the sequence analyses, investigation of gene copy number, and expression analysis via *in situ* hybridization and northern blot. Klaus Mummenhoff and Günter Theißen designed and supervised the project. All authors participated in correcting and improving the manuscript.

Overall own contribution: 40%

I hereby certify the accuracy of the statements on the contributions of the authors.

Günter Theißen

**III. Teresa Lenser and Günter Theißen** (2013) Molecular mechanisms involved in convergent crop domestication. *Trends in Plant Science*, DOI: 10.1016/j.tplants.2013.08.007, *in press*

We review genes determining key traits of crop domestication highlighting the relative importance of molecular convergence. We further discuss factors that increase the probability for molecular convergence to occur and suggest that a transfer of knowledge from evolutionary biology to domestication research might assist developing crop improvement strategies.

I wrote the first draft of the manuscript and prepared the figures. Günter Theißen provided the initial idea, wrote the chapter "*From evolution back to domestication – on the origin of new crop species*" and corrected, completed and improved the whole manuscript.

Overall own contribution: 80%

I hereby certify the accuracy of the statements on the contributions of the authors.

Günter Theißen

## 2.2 Manuscript I

**Teresa Lenser and Günter Theißen** (2013) Conservation of fruit dehiscence pathways between *Lepidium campestre* and *Arabidopsis thaliana* sheds light on the regulation of *INDEHISCENT*. *The Plant Journal*, **76**, 545-556

the plant journal

SEB Society for Experimental Biology

*The Plant Journal* (2013) **76**, 545–556 doi: 10.1111/tpj.12321

FEATURED ARTICLE

# Conservation of fruit dehiscence pathways between *Lepidium campestre* and *Arabidopsis thaliana* sheds light on the regulation of *INDEHISCENT*

**Teresa Lenser and Günter Theißen***

*Department of Genetics, Friedrich Schiller University Jena, Philosophenweg 12, D-07743 Jena, Germany*

Received 20 June 2013; revised 22 August 2013; accepted 30 August 2013; published online 10 October 2013.
*For correspondence (e-mail guenter.theissen@uni-jena.de).

## SUMMARY

**The mode of fruit opening is an important agronomic and evolutionary trait that has been studied intensively in the major plant model system *Arabidopsis thaliana*. Because fruit morphology is highly variable between species, and is also often the target of artificial selection during breeding, it is interesting to investigate whether a change in fruit morphology may alter the developmental pathway leading to fruit opening. Here we have studied fruit development in *Lepidium campestre*, a Brassicaceae species that forms silicles instead of siliques. Transgenic *L. campestre* plants with altered expression levels of orthologs of *A. thaliana* fruit developmental genes (*ALCATRAZ, FRUITFULL, INDEHISCENT* and *SHATTERPROOF1,2*) were found to be defective in fruit dehiscence, and anatomical sections revealed similar changes in tissue patterning as found in respective *A. thaliana* mutants. Gene expression analyses demonstrated a high degree of conservation in gene regulatory circuits, indicating that, despite great differences in fruit morphology, the process of fruit opening remains basically unchanged between species. Interestingly, our data identify *ALCATRAZ* as a negative regulator of *INDEHISCENT* in *L. campestre*. By mutant analysis, we found the same regulatory relationship in *A. thaliana* also, thereby shedding new light on how *ALCATRAZ* drives separation layer formation.**

**Keywords: fruit opening, dehiscence, fruit development, regulatory network evolution, *Lepidium campestre, Arabidopsis thaliana*.**

## INTRODUCTION

Fruit opening in thale cress (*Arabidopsis thaliana*) mainly depends on correct formation of certain tissues within the fruit. Primarily important are two layers of cells that form directly at the valve/replum border and together constitute the dehiscence zone. The separation layer faces the replum and consists of small isodiametrically shaped parenchyma cells (Rajani and Sundaresan, 2001). Prior to fruit opening, these cells secrete cell wall-degrading enzymes to mediate breakdown of the middle lamella, resulting in cell separation (Meakin and Roberts, 1990; Petersen *et al*., 1996; Ogawa *et al*., 2009). The lignified layer faces the valve, and is connected to the lignified endocarp layer *b*, which is located on the inside of the fruit valves (Spence *et al*., 1996). These lignified stripes of cells form a rigid scaffold that builds up tension upon ripening, when the fruit dries and shrinks, forcing the valves apart from the replum at the weakest point, i.e. the separating cells of the separation layer.

The molecular basis of dehiscence zone formation is the expression of transcription factor-encoding 'dehiscence zone identity genes' specifically at the valve/replum border (Rajani and Sundaresan, 2001; Liljegren *et al*., 2004). These genes comprise the redundant MADS box genes *SHATTERPROOF1* (*SHP1*) and *SHP2*, and the basic helix-loop-helix (bHLH) genes *INDEHISCENT* (*IND*) and *ALCATRAZ* (*ALC*), which are positively regulated by the *SHP* genes (Liljegren *et al*., 2004). A functional *IND* gene appears to be important for formation of both the separation layer and the lignified layer, while *ALC* contributes to separation layer formation only (Rajani and Sundaresan, 2001; Liljegren *et al*., 2004). Another MADS box gene, *FRUITFULL* (*FUL*), and the homeobox gene *REPLUMLESS* (*RPL*) act as negative regulators in the fruit valves or the replum, respectively, to restrict expression of dehiscence zone identity genes to the thin stripe of the dehiscence zone

© 2013 The Authors
The Plant Journal © 2013 John Wiley & Sons Ltd

(Ferrandiz *et al.*, 2000; Roeder *et al.*, 2003; Liljegren *et al.*, 2004). By regulating several downstream targets, *IND* acts to establish an auxin minimum as well as a cytokinin and gibberellic acid maximum at the valve/replum border; these local hormone distributions are crucial for correct dehiscence zone formation and pod shatter (Sorefan *et al.*, 2009; Arnaud *et al.*, 2010; Marsch-Martinez *et al.*, 2012). One downstream effect of the presence of active gibberellic acid at the dehiscence zone is activation of ALC by degradation of DELLA repressor proteins, which makes IND an indirect activator of ALC (Arnaud *et al.*, 2010). IND additionally induces the expression of *SPATULA* (*SPT*), another bHLH gene that contributes to separation layer formation partially redundantly with *ALC* and interacts with *IND* to establish the auxin minimum at the dehiscence zone (Girin *et al.*, 2011; Groszmann *et al.*, 2011).

*Arabidopsis thaliana* is a member of the Brassicaceae family, which includes plants with a great variety of fruit shape and size (Al-Shehbaz, 2001; Bowman, 2006). As most members produce dehiscent fruits, an interesting question is whether the fruit developmental pathway leading to fruit dehiscence in *A. thaliana* is conserved in other Brassicaceae species despite great morphological differences. Only a few studies provide limited insights into the molecular basis of fruit development of Brassicaceae species other than *A. thaliana* (Petersen *et al.*, 1996; Chauvaux *et al.*, 1997; Ostergaard *et al.*, 2006; Ogawa *et al.*, 2009; Sorefan *et al.*, 2009; Girin *et al.*, 2010; Avino *et al.*, 2012; Mühlhausen *et al.*, 2013). They all suggest a high degree of conservation in the fruit developmental pathway of various Brassicaceae species with dehiscent fruits. However, no comprehensive study analyzing the function and regulatory relationships of several members of the pathway in one species has been reported so far.

Therefore, we have studied fruit development in field pepperweed (*Lepidium campestre*), another diploid member of the Brassicaceae family. Fruits of *L. campestre* are very distinct from fruits of *A. thaliana* in overall appearance. They are shorter but much wider (and in Brassicaceae nomenclature, are therefore called silicles rather than siliques), and only produce two seeds. Nevertheless, it was shown that the tissue patterning at the valve/replum border is very similar between the two species, with all components considered to be important for proper dehiscence in *A. thaliana* also present in *L. campestre* (Mummenhoff *et al.*, 2009). This may qualify *L. campestre* as a good complementary model system to study fruit development and fruit opening.

For functional analyses using reverse genetic approaches, the ability to generate transgenic organisms is a crucial requirement. The most widely used technique to transform *A. thaliana* is the floral-dip method (Clough and Bent, 1998; Bechtold *et al.*, 2000; Desfeux *et al.*, 2000). Success with this protocol (with slight variations) has also been demonstrated for several other Brassicaceae species (Qing *et al.*, 2000; Curtis and Nam, 2001; Tague, 2001; Wang *et al.*, 2003; Bartholmes *et al.*, 2008; Lu and Kang, 2008). Nevertheless, a previous attempt to transform *L. campestre* via floral dip proved unsuccessful (Eriksson, 2009).

In this study, we establish a floral dip-based transformation system for *L. campestre* and use it to generate transgenic plants with abnormal expression levels of genes orthologous to known *A. thaliana* fruit developmental genes. The effect on *L. campestre* fruit morphology and on the expression of other members within the pathway was determined, and compared to known phenotypes of respective *A. thaliana* mutant plants. Our data reveal a high degree of conservation between the fruit developmental pathways in both species. This conservation also applies to the role of *ALC* as a repressor of *IND* expression, a regulatory connection that was detected in *L. campestre* and was later on also found in *A. thaliana*.

## RESULTS

### *Lepidium campestre* may be transformed via floral dip

As it has been reported that *Agrobacterium* strains vary strongly in their ability to transform various plant species and even different ecotypes (Clough and Bent, 1998; Trieu *et al.*, 2000; Tague, 2001; Bartholmes *et al.*, 2008), we initially tested three different strains (GV3101, LBA4404 and AGL1) for their ability to infect and transform *L. campestre*. Selection of $T_1$ seeds using Basta solution resulted in six and three surviving offspring for plants treated with the GV3101 and LBA4404 strains, respectively, and no surviving offspring for AGL1-treated plants (Table 1). This corresponds to transformation effi-

**Table 1** Transformation efficiency dependent on *Agrobacterium* strain

| *Agrobacterium* strain | Number of treated plants | Absolute number of seeds | Number of transformants | Mean transformation efficiency (%) |
|---|---|---|---|---|
| *LBA4404* | 9 | 3851 | 3 | 0.1 |
| *AGL1* | 9 | 4418 | 0 | 0 |
| *GV3101* | 9 | 3384 | 6 | 0.19 |

Using three different *Agrobacterium tumefaciens* strains for floral-dip transformation of *L. campestre*, the transformation efficiency was determined as the number of positive transformants per number of seeds.

© 2013 The Authors

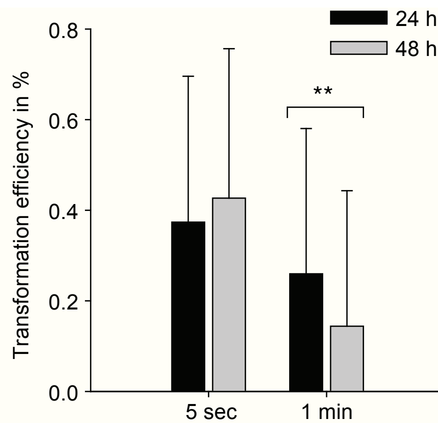


**Figure 1.** Transformation efficiency as a function of dipping time and age of the *Agrobacterium* culture.
The transformation efficiency resulting from floral dipping was determined for plants that were immersed for 5 sec or 1 min in infiltration solution containing *Agrobacteria* grown for 24 or 48 h. Values are the mean transformation efficiency and standard deviation for 19 plants per treatment. Dipping for 1 min compared to 5 sec results in significantly lower efficiency (**$P \leq 0.01$).

ciencies of 0.19% (GV3101), 0.1% (LBA4404) and 0% (AGL1). For further optimization, we concentrated on the GV3101 strain, because it produced the highest number of Basta-resistant plants.

Two further parameters – age of *Agrobacterium* culture and the exposure time of plants to the infiltration medium – were tested for their influence on transformation efficiency. The results are presented in Table S1 and Figure 1. They indicate that the age of the *Agrobacterium* culture does not influence transformation efficiency, but immersing inflorescences for only 5 sec compared to 1 min significantly increases it. Consequentially, the standard protocol used for further floral-dip transformation of *L. campestre* included use of the GV3101 *Agrobacterium* strain grown for 24 h with a dipping time of 5 sec, resulting in an approximate transformation efficiency of 0.4%.

To test whether the *bar* gene stably integrates into the *L. campestre* genome and is inherited into the next generation, $T_2$ seeds were tested for their Basta resistance. Based on the rules of Mendelian inheritance, we expected approximately 75% of seedlings to be resistant if one transgene integrated into the genome of the $T_1$ plant (3:1 segregation), approximately 93% resistant seedlings for independent integration of two transgenes (15:1 segregation), and more than 98% surviving offspring if three or more independent transgene integrations took place. Offspring seedlings from 29 randomly chosen primary transformants were selected using Basta solution, and the results were analyzed by a chi-square test to show whether they fit one of the expected distributions (see Figure 2a and Table S2). For 22 plants, the proportion of Basta-resistant offspring seedlings was above 65%, thereby matching at least one of the expected distributions, but for seven plants, the ratio was below 50%, suggesting the action of gene-silencing mechanisms. In line with this, Southern blot hybridization on genomic DNA of $T_1$ plants revealed that low levels of Basta-resistant offspring correlate with increasing numbers of transgene integrations (Figure 2b,c). These results indicate that transgenes stably integrate into the *L. campestre* genome and are inherited to the next generation, but are subject to transgene silencing as a consequence of multiple transgene integrations, a finding comparable to what is known for other plant species (Muskens *et al.*, 2000; Wang and Waterhouse, 2000; De Buck *et al.*, 2001).

#### Altering fruit developmental gene expression in *L. campestre* changes fruit dehiscence capability

To analyze to what extent the fruit developmental gene pathway known to exist in *A. thaliana* siliques is conserved in *L. campestre* silicles, the expression level of fruit developmental gene orthologs of *L. campestre* (henceforth referred to as e.g. *LcIND* versus *AtIND*) was manipulated. RNAi hairpin constructs targeting *LcIND*, *LcALC* and *LcSHP* and a cDNA fragment of the complete *LcFUL* coding region were placed under the control of the CaMV 35S promoter and transformed into *L. campestre*. In theory, this should lead to a reduction of the expression levels of *LcIND*, *LcALC* and *LcSHP1/2*, and to ectopic over-expression of *LcFUL*. Fruits of positively transformed plants were subjected to the random impact test to quantify their susceptibility to dehiscence. No difference in dehiscence behavior was detected between wild-type plants and those that were transformed with an empty vector construct (Figure 3). However, transformation with the RNAi constructs resulted in fruits with reduced dehiscence capability in five of 10 transformants (*LcIND*), one of 10 transformants (*LcSHP1*) and 14 of 17 transformants (*LcALC*; Figure 3). Of 10 plants carrying the 35S:*LcFUL* construct, three produced fruits with reduced dehiscence capability, while the fruits on one plant shattered more readily than wild-type fruits (Figure 3).

Overall gene expression levels of *LcIND*, *LcALC*, *LcFUL*, *LcSHP1* and *LcSHP2* were compared between dehiscent fruits of control plants and fruits of *L. campestre* transformants with alterations in dehiscence behavior via quantitative RT-PCR. Those genes that were targeted by RNAi all showed an approximately 10-fold decrease in expression level compared to control fruits (Figure 4a). In 35S:*LcFUL* plants, fruits with reduced dehiscence capability expressed *LcFUL* at least 20 times more strongly than control fruits, while fruits with increased dehiscence susceptibility showed a reduction of overall *LcFUL* expression level (Figure 4a). In addition to these changes, which were the direct result of the transformed constructs, secondary changes in other genes were noted that must be due to regulatory connections of these genes with the primary targeted

© 2013 The Authors

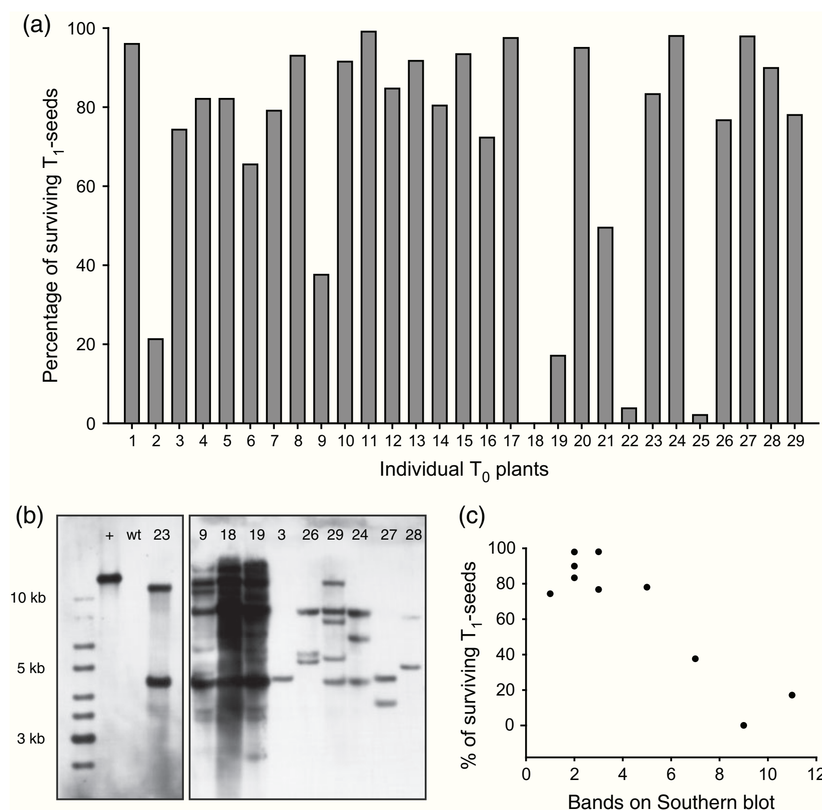


**Figure 2.** Transgene integration and inheritance.
(a) Percentage of Basta-resistant $T_2$ seeds of 29 primary-transformed *L. campestre* plants.
(b) Southern blot hybridization using a *bar* gene-specific probe on linearized pGPTV-Bar plasmid (+) and on genomic DNA from wild-type (wt) and primary-transformed *L. campestre*. Plant numbers are consistent with those shown in (a).
(c) For 10 primary-transformed *L. campestre* plants, the percentage of Basta-resistant $T_2$ seeds was plotted against the number of *bar*-specific bands on a Southern blot.

genes. In RNAi-*LcIND* fruits, the expression level of all other tested genes remained unchanged except for *LcSHP1*, which showed a slight but significant increase in expression (Figure 4a). Fruits over-expressing *LcFUL* showed a decrease in *LcIND* expression level, while *LcSHP2* expression was up-regulated (Figure 4a). RNAi-*LcALC* fruits expressed *LcSHP2* at a slightly higher level than control fruits, and showed an approximately sixfold increase in *LcIND* expression (Figure 4a). For both the RNAi-*LcSHP* plant and the 35S:*LcFUL* plant with decreased *LcFUL* expression, no statistical analysis of the gene expression data could be performed, because only one transformant was available in each case. However, analysis of individual plants indicated an overall decrease in expression level for *LcALC*, *LcIND* and *LcSHP2* in RNAi-*LcSHP* fruits, and an overall increase in expression level for all genes under study in fruits in which *LcFUL* was down-regulated (Figure 4a).

#### Exploring the regulation of *IND* in *A. thaliana*

*ALC* is not known to act as a negative regulator of *IND* during fruit development of *A. thaliana*. Therefore, the strong increase of *LcIND* expression in RNAi-*LcALC* fruits was unexpected. To investigate whether this regulatory connection is restricted to *L. campestre* or also applies to *A. thaliana*, relative expression levels of *IND* were compared between *A. thaliana* plants with reduced *ALC* expression (*alc* and RNAi-*ALC* plants) and respective controls (wild-type and empty vector control plants). In both cases, the decrease in *ALC* expression in the transgenic lines was accompanied by a two- to fourfold increase in *IND* expression in flowers as well as in fruits (Figure 4b). These data suggest that *ALC* works as a repressor of *IND* expression in both *A. thaliana* and *L. campestre*. Because constitutive *SPT* expression was shown to rescue the indehiscent *alc* fruit phenotype (Groszmann *et al.*, 2011), relative expression levels of *IND* were also investigated in flowers of two *A. thaliana spt* lines (*spt-1* and *spt-2*). An approximate twofold increase in *IND* expression compared to wild-type suggests that, like *ALC*, *SPT* represses *IND* in *A. thaliana* flowers, although probably to a lesser extent (Figure 4b). In a previous study, a 400 bp region in the promoters of *A. thaliana* and *Brassica rapa IND* genes was found to be highly conserved and sufficient for valve margin expression (Girin *et al.*, 2010). An alignment of homologous regions from several species of Brassicaceae reveals the presence of a conserved E-box motive in addition to the conserved CArG box identified previously (Figure S1; Girin *et al.*, 2010), thus identifying a potential binding site for ALC and SPT in the *IND* promoter.

#### Correlation between levels of fruit dehiscence and gene expression levels

By transforming two RNAi-*LcALC* constructs into *L. campestre*, a number of independent transformants were

© 2013 The Authors

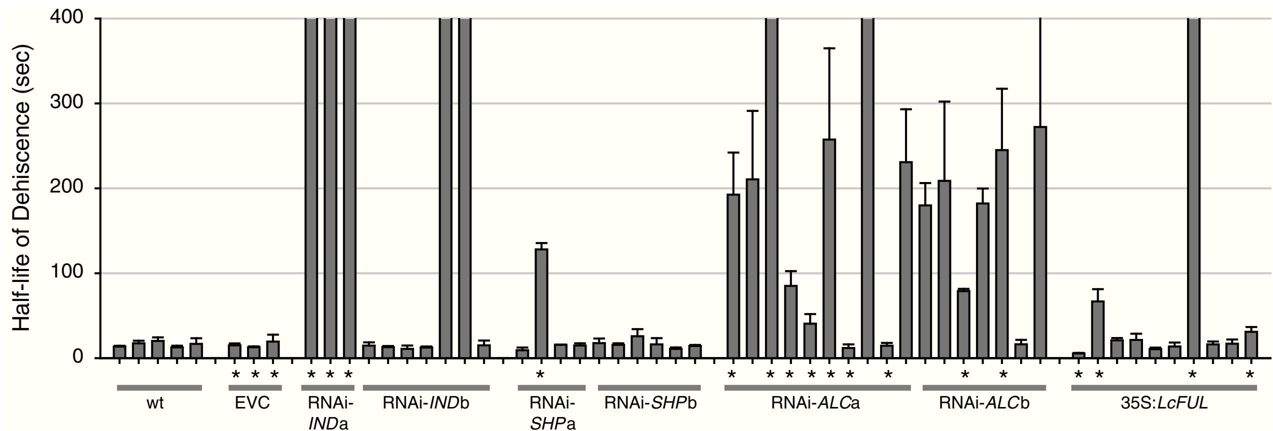


**Figure 3.** Dehiscence capability of various *L. campestre* transformants.
The half-life of dehiscence as determined by the random impact test is shown for *L. campestre* wild-type plants (wt) and plants that were transformed with an empty pFGC5941 vector (EVC), a 35S:*LcFUL* construct or various RNAi constructs targeting *LcIND*, *LcSHP* and *LcALC*, respectively. RNAi constructs contain sequence information upstream (construct a) or downstream (construct b) of the conserved transcription factor binding domain of each gene. Error bars represent standard deviations. Plants that were later subjected to quantitative RT-PCR gene expression analysis are indicated by asterisks.

obtained that differed strongly in their fruit dehiscence capability from fully dehiscent via tardily dehiscent to completely indehiscent (Figure 3). To analyze whether the level of fruit dehiscence correlated with gene expression level, dehiscence half-life was plotted against the relative expression level of *LcALC*, and, because of the newly discovered regulatory connection to *LcIND*, also against the relative expression level of *LcIND* (Figure 4c). This analysis demonstrates that an increase in dehiscence half-life correlates well with decreasing *LcALC* and increasing *LcIND* mRNA levels.

#### Mis-expression of fruit developmental genes changes fruit morphology in *L. campestre*

Fruit morphology was compared between control plants transformed with an empty vector and those transformants that showed altered dehiscence behavior in the random impact test. When directly inspecting mature fruits under high magnification, some fruits of RNAi-*LcIND* plants showed a complanate replum that almost merged with the surrounding valve tissue, while, in control fruits, the replum was always bulgingly raised (Figures 5a,b and S2a,b). Fruits of the 35S:*LcFUL* plant that shattered more readily compared to wild-type fruits were much smaller than control fruits and had papery thin valves and smaller seeds (Figures 5c and S2c). By analyzing lignin-stained cross-sections of fruits, it was found that all *L. campestre* plants with alterations in fruit dehiscence behavior also showed changes in tissue patterning at the valve/replum border. However, the exact nature of these changes was specific for every gene under study. RNAi-*LcIND* plants produced fruits that lacked a separation layer and showed a continuous degree of fusion between endocarp layer *b* (en*b*) and the lignified vascular bundle that is connected to the replum (Figure 5g–i). A lignified layer could not be distinguished, but it is not clear whether this was due to its complete fusion with the lignified replum tissue or because it was not formed at all. In some fruits, the replum was severely flattened compared to the control (Figure 5f,g). Fruits of RNAi-*LcALC* plants lacked a separation layer, and the connection between the replum and the vascular bundle was narrower, while the lignified layer was wider than in control fruits (Figure 5f,j). The fruits of the single RNAi-*LcSHP* plant with reduced fruit dehiscence showed reduced lignification of the lignified layer, while the separation layer appeared to be intact (Figure 5k). In the case of 35S:*LcFUL* plants, the fruits with reduced dehiscence lacked a discernable separation layer and lignified layer, and showed fusion of the en*b* with the replum (Figure 5l), thereby greatly resembling RNAi-*LcIND* fruits. The only difference to these fruits is that the en*b* fusion is restricted to the replum and does not include the vascular bundle, as it does in RNAi-*LcIND* fruits (Figure 5g,h,l). The single 35S:*LcFUL* plant with increased shattering susceptibility developed fruits that completely lacked a lignified layer, a separation layer and an en*b*. Additionally, the mesocarp was greatly reduced, and only the replum with its attached vascular bundle was morphologically comparable to control fruits (Figure 5e,m).

### DISCUSSION

#### Floral-dip transformation of *L. campestre*

The development of new model species is an important issue in the field of evolutionary biology (Abzhanov *et al.*, 2008). Here we describe a floral dip-based transformation protocol for *L. campestre*, with a transformation efficiency of approximately 0.4%. This may facilitate future studies on gene function in a Brassicaceae species of biological and agronomic interest (Eriksson, 2009). In a prior study by another group, it was reported that *L. campestre* could not

© 2013 The Authors

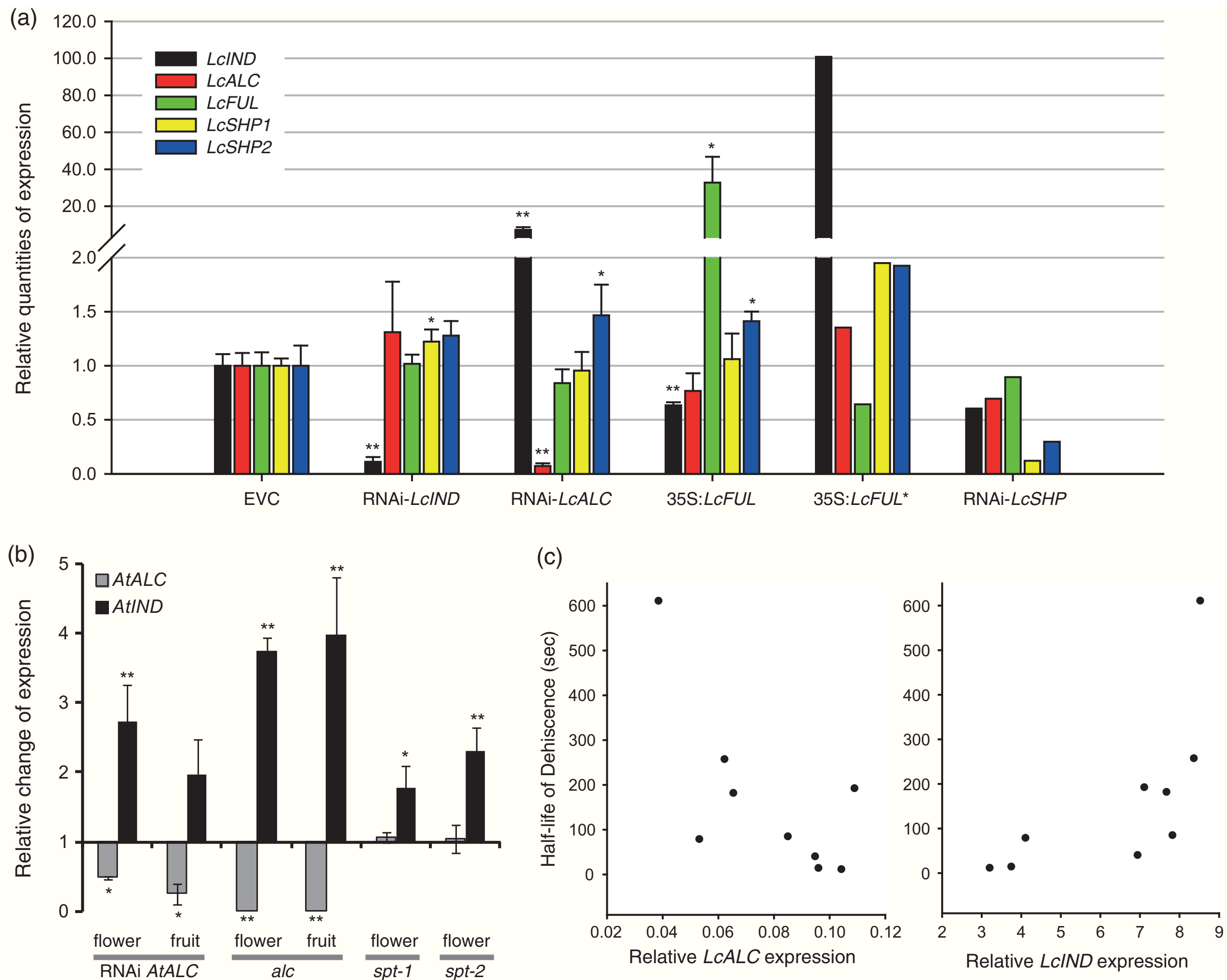


**Figure 4.** Expression analysis of fruit developmental genes.

(a) Relative expression levels of fruit developmental genes were determined by quantitative RT-PCR in fruits (stage 17) of transgenic *L. campestre* plants. Expression quantities are presented relative to expression level in the empty vector control (EVC), which was set to 1 for each gene. Error bars represent standard deviations and are given if at least three individual plants were tested. Columns without error bars were obtained by analyzing a single plant in each case. 35S:*LcFUL** represents the plant that produced small fruits with increased dehiscence susceptibility unlike the normally shaped fruits with normal or reduced dehiscence susceptibility of all other 35S:*LcFUL* transformants. Significant differences compared with control plants are indicated by asterisks (*$P \leq 0.05$, **$P \leq 0.01$).

(b) Relative *AtALC* and *AtIND* expression was analyzed in *A. thaliana* flowers (stage 13/14) and fruits (stage 17). Values shown are relative changes of expression compared to the EVC (in the case of RNAi-*AtALC*) or to *A. thaliana* wild-type (in the case of *alc*, *spt-1* and *spt-2*).

(c) Dehiscence half-lives as obtained by the random impact test of individual RNAi-*LcALC* transformants were plotted against the relative expression levels of *LcALC* and *LcIND*, respectively.

be transformed by simple floral dipping (Eriksson, 2009). This discrepancy may be due to the fact that different *Agrobacterium* strains were used in both studies. Additionally, only a few seeds were screened for transgene integration by Eriksson (2009), and therefore transformation events occurring with low efficiency may have been missed.

The analysis of transgene integration following floral dip of *L. campestre* showed that the majority of transformed plants carried more than one T-DNA insertion (Figure 2b), a phenomenon that is also observed for floral-dip transformation of *A. thaliana* (De Buck *et al.*, 2004). Multiple T-DNA integrations are generally undesirable because an observed phenotype may not be transferred to the next generation if it is influenced by several loci that segregate independently. Furthermore, the presence of multiple T-DNA copies has been associated with transgene expression variability and induction of transgene silencing (Muskens *et al.*, 2000; Wang and Waterhouse, 2000; De Buck *et al.*, 2001), while expression of single-copy transgenes is mostly uniform and stable (De Buck *et al.*, 2004; Schubert *et al.*, 2004). This is in line with our findings that the number of Basta-resistant $T_2$ offspring plants decreased with increasing T-DNA copy number, with first

© 2013 The Authors

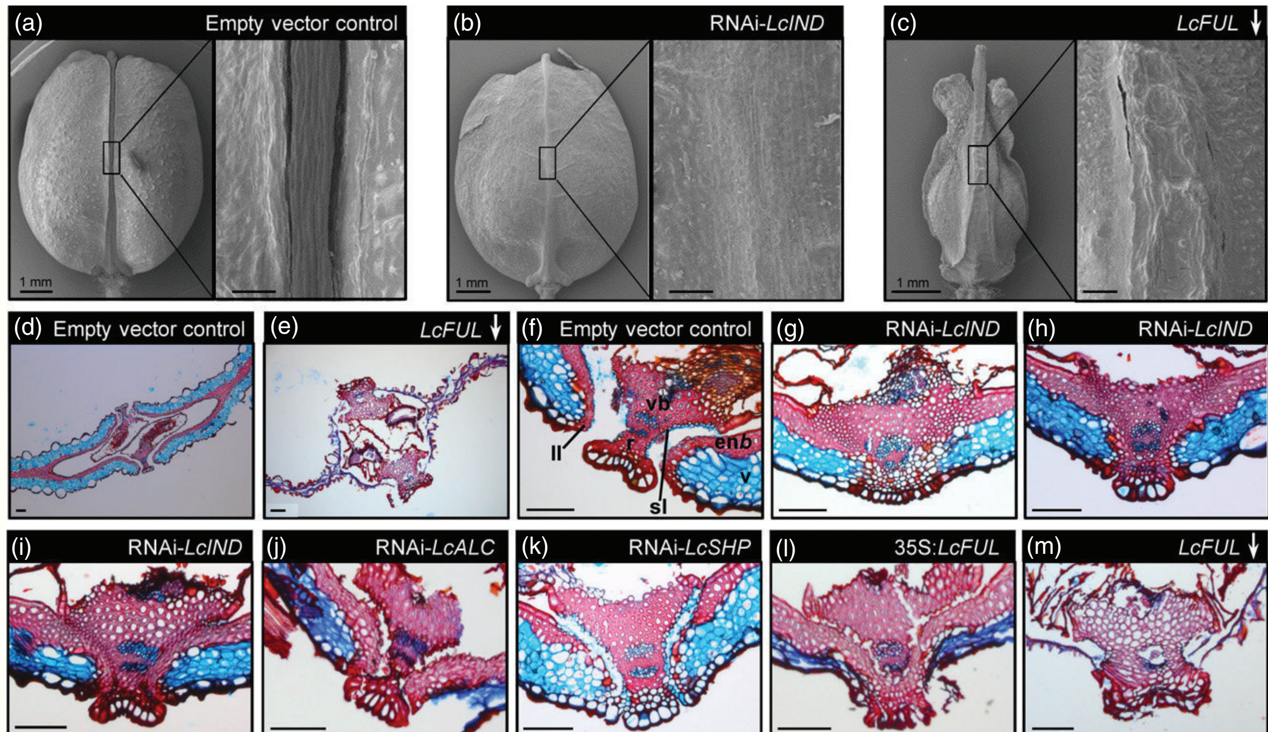


**Figure 5.** Fruit phenotypes of *L. campestre* transformants mis-expressing fruit developmental genes.
Scanning electron microscopy images of whole fruits of stage 19 with magnification of the valve/replum border (a–c) and 8 μm cross-sections of fruits with lignified cell-walls stained in red and non-lignified cell-walls stained in blue (d–m) are shown. Fruits of the empty vector control (a, d, f) show tissue patterning similar to fruits of *A. thaliana*, including fruit valves (v), replum (r), lignified layer (ll), separation layer (sl), endocarp layer *b* (en*b*) and the vascular bundle (vb). RNAi-*LcIND* (b, g–i), RNAi-*LcALC* (j), RNAi-*LcSHP* (k) and 35S:*LcFUL* (l) fruits show various degrees of loss of dehiscence zone tissue. Reduction of *LcFUL* expression level leads to the formation of small fruits without dehiscence zone, en*b* and mesocarp tissue (c, e, m). Scale bars = 100 μm, unless indicated otherwise.

signs of transgene silencing showing in plants with three copies (Figure 2c). Therefore, as in *A. thaliana*, for certain applications, it will be necessary to select *L. campestre* single-copy transformants by screening a pool of transformants or specifically enriching them, for example by using Cre-mediated site-specific recombination techniques (Srivastava *et al*., 1999; De Paepe *et al*., 2009) or treatment with niacinamide (De Block *et al*., 1997). Keeping these obstacles in mind, the floral-dip transformation technique introduced here may be successfully applied in *L. campestre*, thereby facilitating its use as a model system in evolutionary and developmental biology.

### High level of conservation between the fruit developmental pathways of *A. thaliana* and *L. campestre*

In *A. thaliana*, several mutant lines are known that produce indehiscent fruits. In this study, the phenotypes of some of these mutants (35S:*FUL*, *shp1/2*, *ind* and *alc*) were mimicked by RNAi and ectopic gene expression in *L. campestre*. The alteration in gene expression for all five *L. campestre* genes under study resulted in formation of indehiscent fruits, thereby indicating that their overall function in fruit development is conserved between both Brassicaceae species. Looking more closely at the particular morphological changes and alterations in gene expression within the fruits of transformed *L. campestre* plants, the high level of conservation was confirmed, although certain differences were detected as well. For example, the 35S:*LcFUL* fruits closely resembled the corresponding *A. thaliana* transgenic plants in lacking both layers of the dehiscence zone (Ferrandiz *et al*., 2000). Interestingly, the one plant with reduced levels of *LcFUL* expression (most probably due to transgene silencing) strongly resembles an *A. thaliana ful* mutant with a reduction in fruit and seed size, where only the replum develops normally (Gu *et al*., 1998). However, the fruits did not show ectopic lignification throughout the valves as reported for *A. thaliana ful* mutants (Ferrandiz *et al*., 2000) but instead completely lacked the en*b* and had greatly reduced mesocarp tissue. These findings suggest that the function of *FUL* orthologs in valve tissue development is more substantial in *L. campestre* than in *A. thaliana*, where it is mainly responsible for suppressing the expansion of expression of valve-margin identity genes (Liljegren *et al*., 2004). Nevertheless, this conclusion should be treated with caution as only fruits of one plant were analyzed, and the unintended transgene silencing may have had side-effects on expression of other MADS box genes due to high sequence similarities within

© 2013 The Authors

the DNA-binding domain. The regulatory function of *FUL* as a repressor of *SHP1/2*, *IND* and *ALC* appears to be conserved in the *Lepidium* ortholog because the expression levels of these downstream targets mostly change accordingly in response to *LcFUL* up- or down-regulation (Figure 4; Ferrandiz *et al.*, 2000; Liljegren *et al.*, 2004). The finding that *LcSHP1/2* does not show a decrease in overall expression level in *LcFUL* over-expressing fruits may be due to *FUL*-independent high transcript abundance in developing seeds (Ferrandiz *et al.*, 2000; Ostergaard *et al.*, 2006; Mühlhausen *et al.*, 2013).

*SHP1/2* are activators of *IND* and *ALC* in both *A. thaliana* and *L. campestre*, as indicated by a reduction in the expression level of both targets in RNAi-*LcSHP* fruits (Figure 4; Liljegren *et al.*, 2000, 2004). As in *A. thaliana*, down-regulation of *SHP1/2* led to reduced lignification within the dehiscence zone, but in contrast to *Atshp1/2* fruits, fruits of the RNAi-*LcSHP* plant formed an intact sepration layer (Figure 5k; Ferrandiz *et al.*, 2000). This is probably due to the fact that there was still considerable *LcSHP2* expression present, so the phenotype is not as strong as that of a complete *shp1/2* knockout.

Like its orthologs in *A. thaliana* and *B. rapa* (Liljegren *et al.*, 2004; Girin *et al.*, 2010), *LcIND* contributes to formation of both the separation layer and the lignified layer, as a reduction in expression level leads to elimination of both tissues (Figure 5g–i). Its involvement in replum formation also seems to be conserved, although, instead of an expansion of replum tissue as in Arabidopsis and *Brassica* (Girin *et al.*, 2010), RNAi-*LcIND* fruits showed a more complanate replum (Figures 5b,g and S2b). Whether this suggests opposing functions of the different *IND* orthologs in replum formation or merely represents morphological variability in character formation needs to be further analyzed. As expected from *A. thaliana*, *LcIND* does not severely influence the expression level of *LcFUL*, *LcSHP1/2* or *LcALC*.

In *A. thaliana*, *AtALC* is thought to contribute exclusively to separation layer formation, as *alc* mutant fruits possess a well-defined lignified layer but lack separation layer tissue (Rajani and Sundaresan, 2001). The same is true for RNAi-*LcALC* fruits, where no separation layer cells may be distinguished at the valve margin, but the lignified layer is wider compared to wild-type (Figure 5j). This corresponds well with reports from *alc* fruits in which ectopically lignified cells form a bridge between lignified layer and replum, thereby preventing regular pod dehiscence (Rajani and Sundaresan, 2001; Groszmann *et al.*, 2011). In addition to these effects, RNAi-*LcALC* fruits showed a strong reduction of tissue connecting replum and vascular bundle (Figure 5j), indicating that *LcALC* may contribute to lower replum formation. Surprisingly, expression analysis showed a sixfold increase in the overall *LcIND* expression level in RNAi-*LcALC* fruits compared to the empty vector control (Figure 4), implying that *LcALC* is a repressor of *LcIND*.

### *ALC* and *SPT* act as repressors of *IND*

Based on our *L. campestre* data suggesting that *LcALC* may have a negative regulatory effect on *LcIND* expression, the same regulatory connection was demonstrated for the respective *A. thaliana* orthologs in a subsequent experiment (Figure 4b). Thus we hypothesize it to be a common feature of the Brassicaceae fruit developmental pathway. Further expression analyses indicated that *SPT*, the closest paralog of *ALC* (Toledo-Ortiz *et al.*, 2003), also has repressing activity on *IND*. This functional redundancy between *ALC* and *SPT* probably explains why constitutive *SPT* expression rescues the *alc* mutant phenotype (Groszmann *et al.*, 2011). Our findings add further information to the understanding of Brassicaceae fruit dehiscence zone formation (Figure 6). As known previously, the directly adjacent formation of the lignified layer and the separation layer are crucial for correct fruit dehiscence to occur. *IND* contributes to lignification of the lignified layer and en*b* layer tissue (Liljegren *et al.*, 2004), and additionally acts in separation layer formation by mediating the release of ALC and SPT from DELLA repressor proteins (Arnaud *et al.*, 2010). Our data suggest that, following activation, a major function of ALC and SPT is down-regulation of the *IND* expression level within the separation layer, thus preventing IND-induced lignification of separation layer cells. This is also supported by the fact that reduced *ALC* expression leads to ectopic

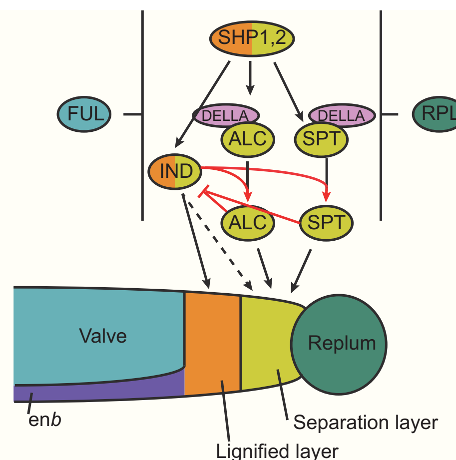


**Figure 6.** Model for ALCATRAZ (ALC) and SPATULA (SPT) function in fruit dehiscence zone formation.
Regulatory pathway leading to fruit dehiscence zone formation as recently described (Dinneny and Yanofsky, 2005; Arnaud *et al.*, 2010; Girin *et al.*, 2011; Groszmann *et al.*, 2011). The upstream regulators FRUITFULL (FUL) and REPLUMLESS (RPL) repress dehiscence zone formation, while SHATTERPROOF1 (SHP1) and SHP2 activate dehiscence zone formation, by regulating INDEHISCENT (IND), ALC and SPT. Only IND contributes to formation of the lignified layer, while IND, ALC and SPT promote separation layer formation. It is unclear whether IND solely acts through release of ALC and SPT from DELLA repressor proteins, or whether it has additional direct roles in separation layer formation. Our findings suggest a regulatory feedback loop between IND and ALC/SPT (highlighted in red) that may help to establish the separation layer by decreasing overall IND concentrations in this area. en*b*, endocarp layer *b*.

© 2013 The Authors

lignification of those cells that normally form the separation layer (Rajani and Sundaresan, 2001; Groszmann *et al.*, 2011; this study), probably due to unusually high *IND* activity. Due to their opposing reciprocal regulation, *ALC* and *SPT* appear to be connected with *IND* in a feedback loop that ensures particularly stable levels of the active protein products within the separation layer (Figure 6).

Recently, it has been found that, in addition to their function in dehiscence zone formation, *ALC*, *SPT* and *IND* are also involved in various aspects of gynoecium development and may play a role during pollen tube development and anther dehiscence (Cai and Lashbrook, 2008; Groszmann *et al.*, 2011; Kay *et al.*, 2013). It will be interesting to study how the *IND*-repressing activity of *ALC* and *SPT* contributes to these pathways. Another interesting aspect will be to test whether *IND* is a direct or indirect target of *ALC* and *SPT* repressor activity. As bHLH domain transcription factors, both proteins bind to DNA as homo- or heterodimers (Heim *et al.*, 2003), with possible interaction candidates from yeast two-hybrid analyses being IND, or SPT and ALC themselves (Liljegren *et al.*, 2004; Girin *et al.*, 2011; Groszmann *et al.*, 2011). An E-box within a 400 bp region of the *IND* promoter that is sufficient for valve margin expression (Girin *et al.*, 2010) was identified as a good candidate site for direct binding of ALC and SPT proteins, and the complete conservation of this sequence motif in all investigated sequences from various Brassicaceae species strongly suggests its functional importance (Figure S1). Nevertheless, further evidence is required to clarify this aspect.

### Connecting genes with fruit morphology

Our systematic approach to study the fruit developmental pathway in *L. campestre* reveals a high degree of conservation of gene function and pathway connectivity compared to *A. thaliana*, even though both fruit size and form are very different in the two species. Only minor differences in expression pattern or gene function were detected, and the functional relevance of these remains to be determined. These data confirm insights obtained in other Brassicaceae with dehiscent fruits (Ostergaard *et al.*, 2006; Girin *et al.*, 2010; Avino *et al.*, 2012). On the other hand, it was shown in two independent studies that the same pathway is severely disturbed in fruits of indehiscent Brassicaceae species (Avino *et al.*, 2012; Mühlhausen *et al.*, 2013), indicating that fruit dehiscence versus overall fruit size and form are controlled by independently operating modules of the overall genetic network patterning a Brassicaceae fruit. It will be interesting to study (i) whether changes within the fruit developmental pathway are involved in evolution of other fruit characteristics other than fruit dehiscence, and (ii) which genes are involved in the evolution of fruit characteristics such as size and shape. Some species within the Brassicaceae develop fruits with an additional abscission zone, the so-called joint, and involvement of genes from the fruit developmental pathway in joint formation has been discussed but not demonstrated so far (Hall *et al.*, 2006; Avino *et al.*, 2012). Genes known to influence fruit size or shape in *A. thaliana* include *ROTUNDIFOLIA4* (Narita *et al.*, 2004), *DEVIL* genes (Wen *et al.*, 2004), the cytochrome P450 gene *CYP78A9* (Ito and Meyerowitz, 2000), the genes encoding *A. thaliana* heterotrimeric G protein $\beta$ and $\gamma$ subunits (Lease *et al.*, 2001; Li *et al.*, 2012), and *KNUCKLES* (Payne *et al.*, 2004). These genes may serve as candidates for future evolutionary studies.

An important issue in Brassicaceae fruit developmental research is the hope of transferring knowledge from well-studied model species to agriculturally important crop plants. An often cited desire is to genetically design fruits with reduced dehiscence capability to increase seed yield (Spence *et al.*, 1996; Ostergaard *et al.*, 2006). The high level of conservation within the fruit developmental pathway of all dehiscent Brassicaceae species tested so far suggests that this strategy is indeed promising. We found that the severity of the indehiscence phenotype correlates well with the expression level of certain genes from the fruit developmental pathway, a fact that was also observed for the *SHATTERING ABORTION1* gene in rice (Zhou *et al.*, 2012). This indicates that fine-tuning gene expression levels may allow plant breeders to obtain fruits with optimal dehiscence capability for various climatic or harvest conditions.

## EXPERIMENTAL PROCEDURES

### Plant material and growth

Plants were grown in a greenhouse on seedling substrate (Klasmann-Deilmann GmbH, Geeste, Germany, http://www.klasmann-deilmann.com/), vermiculite (Klasmann-Deilmann GmbH) and sand (8:1:1), supplemented with 1 g $L^{-1}$ Osmocote mini (The Scotts Miracle-Gro Company, Marysville, OH, USA, http://www.scotts.com) and 1 g $L^{-1}$ Triabon (COMPO Expert GmbH, Münster, Germany, http://www.compo-expert.com) with a 16 h photoperiod at 20°C and 8 h without illumination at 15°C. Six weeks after germination, *Lepidium campestre* plants were vernalized at 4°C with 8 h illumination per day for 8 weeks, then moved back to the greenhouse to induce flowering. In the case of *Arabidopsis thaliana*, ecotype Columbia-0 was used for transformation with RNAi constructs, while the *alc*, *spt-1* and *spt-2* mutations were in the Landsberg *erecta* background (Alvarez and Smyth, 1999; Rajani and Sundaresan, 2001). Flower and fruit stages were assigned as described by Smyth *et al.* (1990).

### Floral-dip transformation

Several protocols for floral-dip transformation of various Brassicaceae species (Clough and Bent, 1998; Curtis and Nam, 2001; Tague, 2001; Martinez-Trujillo *et al.*, 2004; Bartholmes *et al.*, 2008) were used as a guideline to optimize this technique for *L. campestre*. The *Agrobacterium tumefaciens* strains GV3101/pMP90 (Van Larebeke *et al.*, 1974; Koncz and Schell, 1986), LBA4404 (Hoekema *et al.*, 1983) and AGL1 (Lazo *et al.*, 1991) containing binary plasmids pGPTV-Bar (http://biotech.unl.edu/pgptv-bar) or pFGC5941 (http://www.chromdb.org/rnai/pFGC5941.html) were grown at 28°C on YEB medium (5 g/l of each, beef extract, peptone, and sucrose, 1 g/l yeast extract, 0.5 g/l $MgSO_4$) complemented with

© 2013 The Authors

50 μg ml$^{-1}$ kanamycin and 50 μg ml$^{-1}$ rifampicin (all strains) and additionally with 25 μg ml$^{-1}$ gentamycin (GV3101). Overnight cultures (5 ml) were used to inoculate 500 ml of YEB medium, and *Agrobacterium* cultures were subsequently grown for 24 or 48 h under constant rotation at 200 rpm. Cells were pelleted by centrifugation at 16°C and 5500 ***g*** for 15 min, resuspended in infiltration medium (5% sucrose, 0.02% Silwet L-77, LEHLE SEEDS, Round Rock, TX, USA, http://www.arabidopsis.com) to a final $OD_{600}$ of 2.0, and kept at room temperature in the dark for approximately 2 h. Dipping of plants started before the first flower buds had opened, and was repeated weekly until no new flowers were produced, usually resulting in 3–5 runs of dipping. Infiltration medium was constantly agitated using a magnetic stirrer, and plant inflorescences were completely immersed for 5 sec or 1 min, aiming for complete wetting of all bud surfaces. Very dense inflorescences were gently spread apart prior to dipping. Afterwards, plants were covered in plastic bags to retain humidity and kept without direct illumination for 24 h. When dipping was completed, plants were cultivated until all fruits were dry and seeds could be collected. To select for positively transformed offspring, seeds were germinated and seedlings were repeatedly sprayed with 0.01% Basta solution (Aventis CropScience Deutschland GmbH, Hattersheim, Germany, www.sanofi.de) at 3–4 day intervals until all Basta-sensitive seedlings had died. Transformation efficiency was calculated for each plant as the number of surviving offspring divided by the approximate number of seeds, which was estimated by weighing. To analyze the segregation of Basta resistance in the next generation, approximately 100 $T_2$ seeds were sown, resulting seedlings were counted and selected via spraying with Basta solution. When selection was complete, the number of Basta-resistant offspring plants relative to the number of seedlings was determined. Frequency distribution was analyzed using the chi-square test implemented in SPSS software (IBM Corporation, Armonk, NY, USA, www.ibm.com). *A. thaliana* plants were transformed using standard procedures (Zhang *et al.*, 2006).

### Southern hybridization

In order to analyze the number of transgene copies in the genome of *L. campestre*, total DNA was isolated from leaf material using a DNeasy plant maxi kit (Qiagen N.V., Hilden, Germany, www.qiagen.com). Approximately 10 μg of DNA were digested with *Eco*RI (ThermoFisher Scientific Corporation, Waltham, MA, USA, http://www.thermoscientificbio.com/fermentas/) overnight, separated on 1% w/v agarose gels, blotted onto positively charged nylon membranes (Roti-Nylon plus; Carl Roth GmbH + Co. KG, Karlsruhe, Germany, www.carlroth.de), and hybridized with digoxigenin-labeled probes at 68°C. Probes were prepared by PCR onto pGPTV-Bar plasmid using DIG DNA labeling mix (Roche Applied Science, Penzberg, Germany, http://www.roche-applied-science.com) with primers 5′-CTGAAGTCCAGCTGCCAG-3′ and 5′-GAGA-CAAGCACGGTCAA CT-3′. PCR amplification involved 30 cycles of 95°C (45 sec), 62°C (45 sec) and 72°C (30 sec) in an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Deutschland, www.eppendorf.de). Probes were bound using anti-digoxigenin-AP, Fab fragments (Roche Applied Science), and signal detection was performed using CSPD (Disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2′-(5′-chloro)tricyclo [3.3.1.1 3,7]decan}-4-yl) phenyl phosphate) (Roche Applied Science).

### Cloning of RNAi and 35S constructs

To manipulate the expression level of specific fruit developmental genes in *L. campestre*, fragments of these genes were PCR-amplified from plasmid clones and inserted into multiple cloning sites of the pFGC5941 binary vector (http://www.chromdb.org/rnai/pFGC5941.html). All primers used contained restriction sites for site-directed cloning, and are listed in Table S3. In case of the RNAi constructs, two constructs were designed for each of the three transcription factor coding genes (*LcIND*, *LcALC* and *LcSHP1*) based on cDNA fragments located either upstream (construct a) or downstream (construct b) of the DNA sequence coding the conserved DNA-binding domain. These cDNA fragements, ranging in size from 188 bp (*LcIND*b) to 457 bp (*LcSHP*b), were inserted in reverse complementary orientation into the multiple cloning sites on both sites of the *CHSA* (petunia *CHALCONE SYNTHASE A*) intron that is part of the pFGC5941 plasmid, thus forming a hairpin RNA when transcribed. For the 35S construct, a cDNA fragment containing the whole coding region of *LcFUL* was inserted into the *Nco*I–*Sma*I restriction sites of pFGC5941, thereby removing the CHSA intron from the plasmid.

### Lignin staining and microscopic analysis

Fruits of stage 19 were fixed in FAA (2% formaldehyde, 5% glacial acetic acid, 60% EtOH, 0.1% Tween-20) for 24 h, embedded in Paraplast (Carl Roth GmbH + Co. KG) and sectioned. For lignin analysis, sections were de-waxed and stained for 2 min with safranin/astra blue (Sigma Aldrich Corporation, St. Louis, MO, USA, http://www.sigmaaldrich.com) (Gerlach, 1984), resulting in red staining of lignified cell-walls and blue staining of non-lignified cell-walls, followed by microscopic analysis using a Leica DM5500 B microscope (Leica Microsystems GmbH, Wetzlar, Germany, http://www.leica-microsystems.com). For scanning electron microscopy, dry fruits were coated with gold using an Emitech K500 sputter coater (Quorum Technologies Ltd., Laughton, UK, www.quorumtech.com), and examined using an FEI XL30 ESEM microscope (FEI, Hillsboro, OR, USA, www.fei.com).

### Gene expression analysis via quantitative RT-PCR

Total RNA from *L. campestre* fruits was extracted using an RNeasy plant mini kit (Qiagen N.V.). Total RNA from *A. thaliana* was extracted using QIAzol lysis reagent (Qiagen N.V.) for flowers and the protocol described by Onate-Sanchez and Vicente-Carbajosa (2008) for fruits. cDNA synthesis and quantitative RT-PCR were essentially performed as described previously (Mühlhausen *et al.*, 2013). Primers are listed in Table S4. For normalization, relative quantities of expression were divided by a normalization factor, the geometric mean of the relative quantities of expression of the normalizer genes *LcGAPDH*, *LcRAN2*, *LcUBQ10* and *LcTIP41-like* in the case of *L. campestre*, and by the relative quantities of expression of *AtTUB2* in the case of *A. thaliana*.

### Quantifying dehiscence using the random impact test

To be able to compare the susceptibility of fruits from different plants to dehiscence, previously described protocols for the random impact test (Morgan *et al.*, 1998; Bruce *et al.*, 2002; Arnaud *et al.*, 2010) were adapted for *L. campestre* fruits. Silicles of stage 19 (Smyth *et al.*, 1990) were harvested randomly from wild-type and transgenic plants and kept under constant environmental conditions at 25°C and 50% relative humidity for at least 3 days to achieve a consistent moisture content. Twenty silicles were placed together with six 5 mm steel balls in the grinding jar of an MM 400 mixer mill (Retsch GmbH, Haan, Germany, www.retsch.com) and agitated at 9 Hz for cumulative times of 5, 10, 20, 40, 80 and 160 sec or until all fruits had opened. After each interval, the number of open silicles was recorded. Fruits were counted as open when one or both valves were completely detached. Using the method

© 2013 The Authors

described by Bruce *et al.* (2002), the time variable was linearized by a log transformation, and the logit function was applied to the percentage of open fruits at each time point. Thus, the data were transformed into a near-linear relationship, for which the slope and intercept were derived by linear fitting. From this, the dehiscence half-life, i.e. the estimated point in time when half of the fruits had opened, was calculated. To increase reliability, the mean half-life of three replicate samples was determined per plant.

## ACKNOWLEDGEMENTS

We would like to thank Pia Nutt for support with plant transformation, Florian Rümpler for help with statistics, and Lydia Gramzow for bioinformatic support and for her green thumb. Many thanks also to Anne Karpinski und Jane Gräf for their assistance with cloning the RNAi constructs, and to Domenica Schnabelrauch (Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany) for her sequencing efforts. We are further grateful to Thorsten Lenser for help with random impact test data analysis, and to Susanne Schilling and Dajana Lobbes for their advice on Southern blot hybridization. Many thanks also to Dominik Schmidt (Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Jena, Germany) for providing us with the *Agrobacterium* strains, and to Venkatesan Sundaresan (Department of Plant Biology, University of California at Davis, CA) for seeds of the *alc* mutant. We further thank Elisabeth Hommel, Julia Kappes and Moira Walters for technical assistance with quantitative RT-PCR, and David Smyth (School of Biological Sciences, Monash University, Melbourne, Vic., Australia) and Fabian Vaistij (Department of Biology, University of York, York, UK) for kindly providing *spt* mutant seeds. Scanning electron microscope pictures were acquired with the kind help of Hans Pohl (Institute of Systematic Zoology and Evolutionary Biology with Phyletic Museum, University of Jena, Germany). We offer special thanks to Andreas Mühlhausen and Klaus Mummenhoff (Department of Botany, University of Osnabrück, Germany) for many crucial discussions about crucifers, and an anonymous reviewer for valuable comments on a previous version of the manuscript. This work was supported by a grant from the Deutsche Forschungsgemeinschaft to G.T. (TH 417/6-1).

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

**Table S1.** Transformation efficiencies.

**Table S2.** Basta resistance in $T_2$ offspring seeds.

**Table S3.** Primers for transformation constructs.

**Table S4.** Quantitative RT-PCR primers.

**Figure S1.** Alignment of *IND* promoters.

**Figure S2.** Stereomicroscopic images of whole fruits.

## REFERENCES

**Abzhanov, A., Extavour, C.G., Groover, A., Hodges, S.A., Hoekstra, H.E., Kramer, E.M. and Monteiro, A.** (2008) Are we there yet? Tracking the development of new model systems. *Trends Genet.* **24**, 353–360.

**Al-Shehbaz, I.A.** (2001) *Brassicaceae (Mustard Family). eLS.* Chichester, UK: John Wiley & Sons Ltd. DOI: 10.1038/npg.els.0003690.

**Alvarez, J. and Smyth, D.R.** (1999) *CRABS CLAW* and *SPATULA*, two Arabidopsis genes that control carpel development in parallel with *AGAMOUS*. *Development*, **126**, 2377–2386.

**Arnaud, N., Girin, T., Sorefan, K., Fuentes, S., Wood, T.A., Lawrenson, T., Sablowski, R. and Ostergaard, L.** (2010) Gibberellins control fruit patterning in *Arabidopsis thaliana*. *Genes Dev.* **24**, 2127–2132.

**Avino, M., Kramer, E., Donohue, K., Hammel, A. and Hall, J.** (2012) Understanding the basis of a novel fruit type in Brassicaceae: conservation and deviation in expression patterns of six genes. *Evodevo*, **3**, 20.

**Bartholmes, C., Nutt, P. and Theissen, G.** (2008) Germline transformation of Shepherd's purse (*Capsella bursa-pastoris*) by the 'floral dip' method as a tool for evolutionary and developmental biology. *Gene*, **409**, 11–19.

**Bechtold, N., Jaudeau, B., Jolivet, S., Maba, B., Vezon, D., Voisin, R. and Pelletier, G.** (2000) The maternal chromosome set is the target of the T-DNA in the *in planta* transformation of *Arabidopsis thaliana*. *Genetics*, **155**, 1875–1887.

**Bowman, J.L.** (2006) Molecules and morphology: comparative developmental genetics of the Brassicaceae. *Plant Syst. Evol.* **259**, 199–215.

**Bruce, D.M., Farrent, J.W., Morgan, C.L. and Child, R.D.** (2002) Determining the oilseed rape pod strength needed to reduce seed loss due to pod shatter. *Biosyst. Eng.* **81**, 179–184.

**Cai, S. and Lashbrook, C.C.** (2008) Stamen abscission zone transcriptome profiling reveals new candidates for abscission control: enhanced retention of floral organs in transgenic plants overexpressing Arabidopsis *ZINC FINGER PROTEIN2*. *Plant Physiol.* **146**, 1305–1321.

**Chauvaux, N., Child, R., John, K., Ulvskov, P., Borkhardt, B., Prinsen, E. and Van Onckelen, H.A.** (1997) The role of auxin in cell separation in the dehiscence zone of oilseed rape pods. *J. Exp. Bot.* **48**, 1423–1429.

**Clough, S.J. and Bent, A.F.** (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735–743.

**Curtis, I.S. and Nam, H.G.** (2001) Transgenic radish (*Raphanus sativus* L. *longipinnatus* Bailey) by floral-dip method – plant development and surfactant are important in optimizing transformation efficiency. *Transgenic Res.* **10**, 363–371.

**De Block, M., Debrouwer, D. and Moens, T.** (1997) The development of a nuclear male sterility system in wheat. Expression of the *barnase* gene under the control of tapetum specific promoters. *Theor. Appl. Genet.* **95**, 125–131.

**De Buck, S., Van Montagu, M. and Depicker, A.** (2001) Transgene silencing of invertedly repeated transgenes is released upon deletion of one of the transgenes involved. *Plant Mol. Biol.* **46**, 433–445.

**De Buck, S., Windels, P., De Loose, M. and Depicker, A.** (2004) Single-copy T-DNAs integrated at different positions in the Arabidopsis genome display uniform and comparable β-glucuronidase accumulation levels. *Cell. Mol. Life Sci.* **61**, 2632–2645.

**De Paepe, A., De Buck, S., Hoorelbeke, K., Nolf, J., Peck, I. and Depicker, A.** (2009) High frequency of single-copy T-DNA transformants produced by floral dip in *CRE*-expressing Arabidopsis plants. *Plant J.* **59**, 517–527.

**Desfeux, C., Clough, S.J. and Bent, A.F.** (2000) Female reproductive tissues are the primary target of *Agrobacterium*-mediated transformation by the Arabidopsis floral-dip method. *Plant Physiol.* **123**, 895–904.

**Dinneny, J.R. and Yanofsky, M.F.** (2005) Drawing lines and borders: how the dehiscent fruit of Arabidopsis is patterned. *BioEssays*, **27**, 42–49.

**Eriksson, D.** (2009) *Towards the domestication of* Lepidium campestre *as an undersown oilseed crop.* PhD Thesis. Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Alnarp, Sweden.

**Ferrandiz, C., Liljegren, S.J. and Yanofsky, M.F.** (2000) Negative regulation of the *SHATTERPROOF* genes by FRUITFULL during Arabidopsis fruit development. *Science*, **289**, 436–438.

**Gerlach, D.** (1984) *Botanische Mikrotechnik, eine Einführung, 2. Aufl.* Georg Thiema Verlag, Stuttgart, 1977.

**Girin, T., Stephenson, P., Goldsack, C.M.P., Kempin, S.A., Perez, A., Pires, N., Sparrow, P.A., Wood, T.A., Yanofsky, M.F. and Ostergaard, L.** (2010) Brassicaceae *INDEHISCENT* genes specify valve margin cell fate and repress replum formation. *Plant J.* **63**, 329–338.

**Girin, T., Paicu, T., Stephenson, P. *et al.*** (2011) INDEHISCENT and SPATULA interact to specify carpel and valve margin tissue and thus promote seed dispersal in Arabidopsis. *Plant Cell*, **23**, 3641–3653.

**Groszmann, M., Paicu, T., Alvarez, J.P., Swain, S.M. and Smyth, D.R.** (2011) *SPATULA* and *ALCATRAZ* are partially redundant, functionally diverging bHLH genes required for Arabidopsis gynoecium and fruit development. *Plant J.* **68**, 816–829.

© 2013 The Authors

**Gu, Q., Ferrandiz, C., Yanofsky, M.F. and Martienssen, R.** (1998) The *FRUITFULL* MADS-box gene mediates cell differentiation during Arabidopsis fruit development. *Development*, **125**, 1509–1517.

**Hall, J.C., Tisdale, T.E., Donohue, K. and Kramer, E.M.** (2006) Developmental basis of an anatomical novelty: heteroarthrocarpy in *Cakile lanceolata* and *Erucaria erucarioides* (Brassicaceae). *Int. J. Plant Sci.* **167**, 771–789.

**Heim, M.A., Jakoby, M., Werber, M., Martin, C., Weisshaar, B. and Bailey, P.C.** (2003) The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. *Mol. Biol. Evol.* **20**, 735–747.

**Hoekema, A., Hirsch, P.R., Hooykaas, P.J.J. and Schilperoort, R.A.** (1983) A binary plant vector strategy based on separation of *vir*- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. *Nature*, **303**, 179–180.

**Ito, T. and Meyerowitz, E.M.** (2000) Overexpression of a gene encoding a cytochrome p450, *CYP78A9*, induces large and seedless fruit in Arabidopsis. *Plant Cell*, **12**, 1541–1550.

**Kay, P., Groszmann, M., Ross, J.J., Parish, R.W. and Swain, S.M.** (2013) Modifications of a conserved regulatory network involving INDEHISCENT controls multiple aspects of reproductive tissue development in Arabidopsis. *New Phytol.* **197**, 73–87.

**Koncz, C. and Schell, J.** (1986) The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of *Agrobacterium* binary vector. *Mol. Gen. Genet.* **204**, 383–396.

**Lazo, G.R., Stein, P.A. and Ludwig, R.A.** (1991) A DNA transformation-competent Arabidopsis genomic library in *Agrobacterium*. *Nat. Biotechnol.* **9**, 963–967.

**Lease, K.A., Wen, J.Q., Li, J., Doke, J.T., Liscum, E. and Walker, J.C.** (2001) A mutant Arabidopsis heterotrimeric G-protein β subunit affects leaf, flower, and fruit development. *Plant Cell*, **13**, 2631–2641.

**Li, S.J., Liu, Y.J., Zheng, L.Y.** *et al.* (2012) The plant-specific G protein γ subunit AGG3 influences organ size and shape in *Arabidopsis thaliana*. *New Phytol.* **194**, 690–703.

**Liljegren, S.J., Ditta, G.S., Eshed, H.Y., Savidge, B., Bowman, J.L. and Yanofsky, M.F.** (2000) *SHATTERPROOF* MADS-box genes control seed dispersal in Arabidopsis. *Nature*, **404**, 766–770.

**Liljegren, S.J., Roeder, A.H.K., Kempin, S.A., Gremski, K., Ostergaard, L., Guimil, S., Reyes, D.K. and Yanofsky, M.F.** (2004) Control of fruit patterning in Arabidopsis by INDEHISCENT. *Cell*, **116**, 843–853.

**Lu, C.F. and Kang, J.L.** (2008) Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by *Agrobacterium*-mediated transformation. *Plant Cell Rep.* **27**, 273–278.

**Marsch-Martinez, N., Ramos-Cruz, D., Irepan Reyes-Olalde, J., Lozano-Sotomayor, P., Zuniga-Mayo, V.M. and de Folter, S.** (2012) The role of cytokinin during Arabidopsis gynoecia and fruit morphogenesis and patterning. *Plant J.* **72**, 222–234.

**Martinez-Trujillo, M., Limones-Briones, V., Cabrera-Ponce, J.L. and Herrera-Estrella, L.** (2004) Improving transformation efficiency of *Arabidopsis thaliana* by modifying the floral dip method. *Plant Mol. Biol. Rep.* **22**, 63–70.

**Meakin, P.J. and Roberts, J.A.** (1990) Dehiscence of fruit in oilseed rape (*Brassica napus* L.) 2. The role of cell-wall degrading enzymes and ethylene. *J. Exp. Bot.* **41**, 1003–1011.

**Morgan, C.L., Bruce, D.M., Child, R., Ladbrooke, Z.L. and Arthur, A.E.** (1998) Genetic variation for pod shatter resistance among lines of oilseed rape developed from synthetic *B. napus*. *Field Crops Res.* **58**, 153–165.

**Mühlhausen, A., Lenser, T., Mummenhoff, K. and Theißen, G.** (2013) Evidence that an evolutionary transition from dehiscent to indehiscent fruits in *Lepidium* (Brassicaceae) was caused by a change in the control of valve margin identity genes. *Plant J.* **73**, 824–835.

**Mummenhoff, K., Polster, A., Muhlhausen, A. and Theissen, G.** (2009) *Lepidium* as a model system for studying the evolution of fruit development in Brassicaceae. *J. Exp. Bot.* **60**, 1503–1513.

**Muskens, M.W.M., Vissers, A.P.A., Mol, J.N.M. and Kooter, J.M.** (2000) Role of inverted DNA repeats in transcriptional and post-transcriptional gene silencing. *Plant Mol. Biol.* **43**, 243–260.

**Narita, N.N., Moore, S., Horiguchi, G., Kubo, M., Demura, T., Fukuda, H., Goodrich, J. and Tsukaya, H.** (2004) Overexpression of a novel small peptide ROTUNDIFOLIA4 decreases cell proliferation and alters leaf shape in *Arabidopsis thaliana*. *Plant J.* **38**, 699–713.

**Ogawa, M., Kay, P., Wilson, S. and Swain, S.M.** (2009) ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1 (ADPG1), ADPG2, and QUARTET2 are polygalacturonases required for cell separation during reproductive development in Arabidopsis. *Plant Cell*, **21**, 216–233.

**Onate-Sanchez, L. and Vicente-Carbajosa, J.** (2008) DNA-free RNA isolation protocols for *Arabidopsis thaliana*, including seeds and siliques. *BMC Res. Notes*, **1**, 93.

**Ostergaard, L., Kempin, S.A., Bies, D., Klee, H.J. and Yanofsky, M.F.** (2006) Pod shatter-resistant *Brassica* fruit produced by ectopic expression of the *FRUITFULL* gene. *Plant Biotechnol. J.* **4**, 45–51.

**Payne, T., Johnson, S.D. and Koltunow, A.M.** (2004) *KNUCKLES* (*KNU*) encodes a C2H2 zinc-finger protein that regulates development of basal pattern elements of the Arabidopsis gynoecium. *Development*, **131**, 3737–3749.

**Petersen, M., Sander, L., Child, R., van Onckelen, H., Ulvskov, P. and Borkhardt, B.** (1996) Isolation and characterisation of a pod dehiscence zone-specific polygalacturonase from *Brassica napus*. *Plant Mol. Biol.* **31**, 517–527.

**Qing, C.M., Fan, L., Lei, Y., Bouchez, D., Tourneur, C., Yan, L. and Robaglia, C.** (2000) Transformation of Pakchoi (*Brassica rapa* L. ssp *chinensis*) by *Agrobacterium* infiltration. *Mol. Breed.* **6**, 67–72.

**Rajani, S. and Sundaresan, V.** (2001) The Arabidopsis myc/bHLH gene *ALCATRAZ* enables cell separation in fruit dehiscence. *Curr. Biol.* **11**, 1914–1922.

**Roeder, A.H.K., Ferrandiz, C. and Yanofsky, M.F.** (2003) The role of the REPLUMLESS homeodomain protein in patterning the Arabidopsis fruit. *Curr. Biol.* **13**, 1630–1635.

**Schubert, D., Lechtenberg, B., Forsbach, A., Gils, M., Bahadur, S. and Schmidt, R.** (2004) Silencing in Arabidopsis T-DNA transformants: the predominant role of a gene-specific RNA sensing mechanism versus position effects. *Plant Cell*, **16**, 2561–2572.

**Smyth, D.R., Bowman, J.L. and Meyerowitz, E.M.** (1990) Early flower development in Arabidopsis. *Plant Cell*, **2**, 755–767.

**Sorefan, K., Girin, T., Liljegren, S.J., Ljung, K., Robles, P., Galvan-Ampudia, C.S., Offringa, R., Friml, J., Yanofsky, M.F. and Ostergaard, L.** (2009) A regulated auxin minimum is required for seed dispersal in Arabidopsis. *Nature*, **459**, 583–586.

**Spence, J., Vercher, Y., Gates, P. and Harris, N.** (1996) 'Pod shatter' in *Arabidopsis thaliana*, *Brassica napus* and *B. juncea*. *J. Microsc.* **181**, 195–203.

**Srivastava, V., Anderson, O.D. and Ow, D.W.** (1999) Single-copy transgenic wheat generated through the resolution of complex integration patterns. *Proc. Natl Acad. Sci. USA*, **96**, 11117–11121.

**Tague, B.W.** (2001) Germ-line transformation of *Arabidopsis lasiocarpa*. *Transgenic Res.* **10**, 259–267.

**Toledo-Ortiz, G., Huq, E. and Quail, P.H.** (2003) The Arabidopsis basic/helix--loop-helix transcription factor family. *Plant Cell*, **15**, 1749–1770.

**Trieu, A.T., Burleigh, S.H., Kardailsky, I.V.** ***et al.*** (2000) Transformation of *Medicago truncatula* via infiltration of seedlings or flowering plants with *Agrobacterium*. *Plant J.* **22**, 531–541.

**Van Larebeke, N., Engler, G., Holsters, M., Van den Elsacker, S., Zaenen, I., Schilperoort, R.A. and Schell, J.** (1974) Large plasmid in *Agrobacterium tumefaciens* essential for crown gall-inducing ability. *Nature*, **252**, 169–170.

**Wang, M.B. and Waterhouse, P.M.** (2000) High-efficiency silencing of a β-glucuronidase gene in rice is correlated with repetitive transgene structure but is independent of DNA methylation. *Plant Mol. Biol.* **43**, 67–82.

**Wang, W.C., Menon, G. and Hansen, G.** (2003) Development of a novel *Agrobacterium*-mediated transformation method to recover transgenic *Brassica napus* plants. *Plant Cell Rep.* **22**, 274–281.

**Wen, J.Q., Lease, K.A. and Walker, J.C.** (2004) DVL, a novel class of small polypeptides: overexpression alters Arabidopsis development. *Plant J.* **37**, 668–677.

**Zhang, X.R., Henriques, R., Lin, S.S., Niu, Q.W. and Chua, N.H.** (2006) *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nat. Protoc.* **1**, 641–646.

**Zhou, Y., Lu, D.F., Li, C.Y.** ***et al.*** (2012) Genetic control of seed shattering in rice by the APETALA2 transcription factor *SHATTERING ABORTION1*. *Plant Cell*, **24**, 1034–1048.

© 2013 The Authors

**Figure S1 - Alignment of *IND* promoter regions.** Genomic regions homologous to the *IND* promoter element that was found to be highly conserved between *Arabidopsis thaliana* and *Brassica rapa* by Girin et al. (2010) were identified from the Brassicaceae species *Arabidopsis lyrata*, *Capsella rubella*, *Thellungiella halophila*, *Schrenkiella parvula*, *Sisymbrio irio*, *Leavenworthia alabamica*, and *Aethionema arabicum* using BLAST. Respective sequences were aligned using seaview (Gouy *et al.,* 2010). Conserved hypothetical CArG-box and E-box elements were identified and highlighted in blue and pink, respectively.

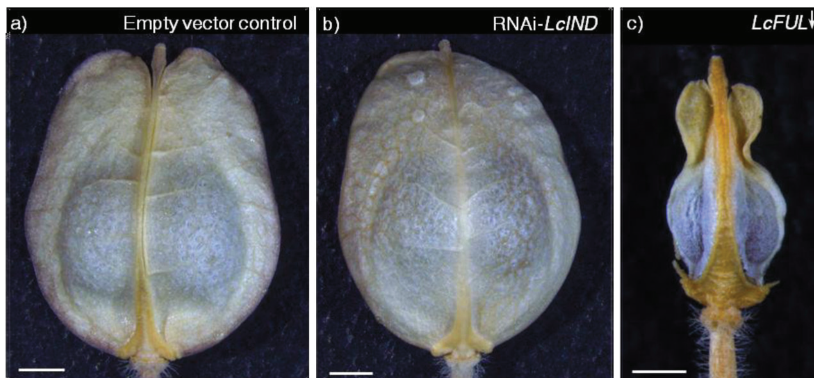


**Figure S2 – Stereomicroscopic images of whole fruits.** Whole fruits of stage 19 are shown. a) empty vector control; b) RNAi-*LcIND*; c) fruits with reduced expression level of *LcFUL*. Pictures were taken using a Leica M205 FA stereomicroscope (Leica, Germany) employing the Multifocus module of the Leica Application Suite software. Scale bars = 1 mm.

**Table S1 - Transformation efficiency dependent on age of *Agrobacterium* culture and dipping time.** Using two different growth conditions and dipping times for GV3101-mediated floral dip transformation of *L. campestre*, the transformation efficiency was determined for each plant individually as number of positive transformants per number of seeds. Individual transformation efficiencies were averaged to give the mean transformation efficiency per treatment.

| *Age of Agrobacterium culture* | *Dipping time* | *Number of treated plants* | *Absolute number of seeds* | *Number of transformants* | *Mean transformation efficiency* |
|---|---|---|---|---|---|
| *24h* | 5 sec | *19* | *7670* | 27 | 0.37% |
| *24h* | 1 min | *19* | *6360* | 17 | 0.26% |
| *48h* | 5 sec | *19* | *6760* | 29 | 0.43% |
| *48h* | 1 min | *19* | *3090* | 6 | 0.14% |

**Table S2 - Segregation of Basta-resistance in $T_1$-offspring seeds of primary transformants and frequency distribution by $\chi^2$-test.**

| *Plant No.* | *Number of Basta-sensitive seedlings* | *Number of Basta-resistant seedlings* | *Segregation ratio* | *$\chi^2$ value for 3:1 distribution/p-value* | *$\chi^2$ value for 15:1 distribution/p-value* | *$\chi^2$ value for 63:1 distribution/p-value* |
|---|---|---|---|---|---|---|
| *1* | 6 | *143* | *23.83:1* | - | 1.257/0.262 | 5.883/0.015 |
| *2* | 111 | *30* | *0.27:1* | - | - | - |
| *3* | 36 | *104* | *2.89:1* | 0.038/0.845 | - | - |
| *4* | 21 | *96* | *4.57:1* | 3.103/0.078 | - | - |
| *5* | 24 | *110* | *4.58:1* | 3.592/0.058 | - | - |
| *6* | 40 | *76* | *1.9:1* | 5.563/0.018 | - | - |
| *7* | 34 | *129* | *3.79:1* | 1.491/0.222 | - | - |
| *8* | 6 | *80* | *13.33:1* | - | 0.078/0.781 | - |
| *9* | 78 | *47* | *0.60:1* | - | - | - |
| *10* | 11 | *118* | *10.73:1* | - | 1.142/0.285 | - |
| *11* | 1 | *106* | *106:1* | - | 5.160/0.023 | 0.274/0.600 |
| *12* | 21 | *116* | *5.52:1* | 6.835/0.009 | - | - |
| *13* | 11 | *122* | *11.09:1* | - | 0.927/0.336 | - |
| *14* | 22 | *90* | *4.09:1* | 1.714/0.190 | - | - |
| *15* | 11 | *156* | *14.18:1* | - | 0.032/0.857 | - |
| *16* | 39 | *102* | *2.62:1* | 0.532/0.466 | - | - |
| *17* | 3 | *117* | *39.00:1* | - | 2.880/0.090 | 0.686/0.408 |
| *18* | 83 | *0* | *0:1* | - | - | - |
| *19* | 92 | *19* | *0.21:1* | - | - | - |
| *20* | 6 | *115* | *19.17:1* | - | 0.344/0.557 | 9.074/0.003 |
| *21* | 50 | *49* | *0.98:1* | - | - | - |
| *22* | 76 | *3* | *0.04:1* | - | - | - |
| *23* | 17 | *85* | *5.00:1* | 3.778/0.052 | - | - |
| *24* | 2 | *99* | *49.5:1* | - | 3.143/0.076 | 0.115/0.735 |
| *25* | 94 | *2* | *0.02:1* | - | - | - |
| *26* | 24 | *79* | *3.29:1* | 0.159/0.690 | - | - |
| *27* | 2 | *95* | *47.5:1* | - | 2.904/0.088 | 0.157/0.692 |
| *28* | 9 | *80* | *8.89:1* | 10.521/0.001 | 2.266/0.132 | - |
| *29* | 18 | *64* | *3.56:1* | 0.407/0.524 | - | - |

**Table S3 - Overview about primers used for generating transformation constructs.**

| Gene name | Accession no. | Primer sequence[a] | Name of construct | Length of PCR product in bp |
|---|---|---|---|---|
| *LcALC* | FR727240 | 5'- AACCATGGATGGGTGATTCCGACAACTGTG-3'<br>5'-AAGGCGCGCCGCATAGCCGGAGGGAGAAAC -3'<br>5'-AATCTAGAATGGGTGATTCCGACAACTGT G-3'<br>5'-AAGGATCCGCATAGCCGGAGGGAGAAAC-3' | RNAi *ALC*a | 189 |
| | | 5'-AACCATGGGGCCTAAACCCTATGCAATTACCAC-3'<br>5'-AAGGCGCGCCACATGTTTGACTCCTGTGTTTGGC-3'<br>5'-AATCTAGAGGCCTAAACCCTATGCAATTACCAC-3'<br>5'-AAGGATCCACATGTTTGACTCCTGTGTTTGGC-3' | RNAi *ALC*b | 247 |
| *LcIND* | FR727239 | 5'- AACCATGGCCAAAGAAGCATGATGAGCCTC-3'<br>5'-AAGGCGCGCCTTATTCTTACGTTACGCCGGTTAGG -3'<br>5'-AATCTAGACCAAAGAAGCATGATGAGCCTC-3'<br>5'-AAGGATCCTTATTCTTACGTTACGCCGGTTAGG-3' | RNAi *IND*a | 296 |
| | | 5'- AACCATGGCCTCACTCTCACCTTGGACCTC-3'<br>5'-AAGGCGCGCCGCGAGCAAGCTAGCAAGTGC -3'<br>5'-AATCTAGACCTCACTCTCACCTTGGACCTC-3'<br>5'-AAGGATCCGCGAGCAAGCTAGCAAGTGC-3' | RNAi *IND*b | 188 |
| *LcSHP1* | FR727235 | 5'- AACCATGGCAAATAAAAGAGAGAGAGAGAATC-3'<br>5'- AAGGCGCGCCTCTATTCTCTTTATCTCTATCTTCC-3'<br>5'-AATCTAGACAAATAAAAGAGAGAGAGAGAATC-3'<br>5'-AAGGATCCTCTATTCTCTTTATCTCTATCTTCC-3' | RNAi *SHP*a | 181 |
| | | 5'- AACCATGGTCCTCCTTCTGTCACTGAAGC -3'<br>5'-AAGGCGCGCCGTCTTGACCAGAGAATTGCTGATTTG -3'<br>5'-AATCTAGATCCTCCTTCTGTCACTGAAGC -3'<br>5'-AAGGATCCGTCTTGACCAGAGAATTGCTGATTTG-3' | RNAi *SHP*b | 457 |
| *LcFUL* | FR727237 | 5'- AACCATGGCAGGGTTGTCGTTTCTCTC -3'<br>5'- AACCCGGGATGAACTAAACATATATATAAGTTCAATGATGAG -3' | 35S::*LcFUL* | 913 |

a: Forward (upper line) and reverse (lower line) primer sequences; restriction sites are underlined

**Table S4 - Overview about primers used for quantitative RT-PCR analysis.**

| Gene name | Accession no. | Primer sequence[a] | Amplicon length (bp) | Mean PCR efficiency[b] |
|---|---|---|---|---|
| *LcGAPDH* | FR728677 | 5'-CTATCAAGGAGGAATCTGAAGGCAAAC-3'<br>5'-ACGAAGTCAGTTGAGACGACATCATC-3' | 78 | 0.953 |
| *LcRAN2* | FR728678 | 5'-CTGCTGGGATACTGCTGGAC-3'<br>5'-GTAACTTGCTTTGCCTTCACTTGC-3' | 224 | 0.940 |
| *LcUbq10* | FR733641 | 5'-ACCAGCAGCGTCTCATCTTC-3'<br>5'-TTCTGAATGTTGTAGTCGGCTAAGG-3' | 72 | 0.907 |
| *LcTip41 like* | FR728679 | 5'-GCTTATGAGATTGAGAGAGACGAGAA-3'<br>5'-GGATACCCTTTCGCAGATAGAGAC-3' | 122 | 0.919 |
| *AtTUB2* | NM_125664 | 5'-AAACTCACTACCCCCAGCTTTG-3'<br>5'-CACCAGACATAGTAGCAGAAATCAAGT-3' | 61 | 0.951 |
| *LcALC* | FR727240 | 5'-AACAAGAGAAATGGAGGTAGACAG-3'<br>5'-ATTCATAACGGCTAAAGTCTGC-3' | 227 | 0.912 |
| *AtALC* | NM_001085341 | 5'-TGAAAAGAAGAGGAGGAGCAAG-3'<br>5'-CATTCATAACGGCTAAAGTCTGG-3' | 158 | 0.895 |
| *LcFUL* | FR727237 | 5'-GATGGTTTGATGGAGAGAATCGGAC-3'<br>5'-CTACTCATTGGTGGTCGGACG-3' | 110 | 0.935 |
| *LcIND* | FR727239 | 5'-TAAGCGACGATCCTCAGACG -3'<br>5'-AAGAACTTGGTGTATCGGATGGC -3' | 147 | 0.963 |
| *AtIND* | NM_116229 | 5'-TAAGCGACGATCCTCAGACG-3'<br>5'-AATCCTCACCTGCCGTTTCAAG-3' | 167 | 0.948 |
| *LcSHP1* | FR727235 | 5'-AGAAGAGAGAGAAATGGAGTTGCAGC-3'<br>5'-ACACTTGATTCCTGTTGTACTTCTGG-3' | 99 | 0.942 |
| *LcSHP2* | FR727236 | 5'-GTGTGTCTTCTTCTCATCAGTCGG-3'<br>5'-GAGGTGGTTGCTCTTGGTCG-3' | 101 | 0.969 |
| *AtSPT* | NM_119857 | 5'-TATGACTGTGAAAGCGAGGAAG-3'<br>5'-GAAGGACCTGACTTGGAAGAG-3' | 71 | 0,937 |

a: Forward (upper line) and reverse (lower line) primer sequences
b: Average PCR efficiency for a certain amplicon group as calculated by LinRegPCR (11.0)

## 2.3 Manuscript II

**Andreas Mühlhausen[†], Teresa Lenser[†], Klaus Mummenhoff, and Günter Theißen** (2013)

Evidence that an evolutionary transition from dehiscent to indehiscent fruits in *Lepidium* (Brassicaceae) was caused by a change in the control of valve margin identity genes. *The Plant Journal*, **73**, 824-835.

([†]These authors contributed equally to this work)

the plant journal
SEB Society for Experimental Biology

*The Plant Journal* (2013) **73**, 824–835 doi: 10.1111/tpj.12079

# Evidence that an evolutionary transition from dehiscent to indehiscent fruits in *Lepidium* (Brassicaceae) was caused by a change in the control of valve margin identity genes

**Andreas Mühlhausen[1,†], Teresa Lenser[2,†], Klaus Mummenhoff[1,‡] and Günter Theißen[2,*,‡]**
[1]*Department of Biology, Botany, University of Osnabrück, Barbarastraße 11, D-49076 Osnabrück, Germany, and*
[2]*Department of Genetics, Friedrich Schiller University Jena, Philosophenweg 12, D-07743 Jena, Germany*

Received 5 March 2012; revised 5 November 2012; accepted 9 November 2012; published online 18 January 2013.
*For correspondence (e-mail guenter.theissen@uni-jena.de).
†These authors contributed equally to this work.
‡These authors contributed equally to this work.

## SUMMARY

**In the Brassicaceae, indehiscent fruits evolved from dehiscent fruits several times independently. Here we use closely related wild species of the genus *Lepidium* as a model system to analyse the underlying developmental genetic mechanisms in a candidate gene approach. *ALCATRAZ* (*ALC*), *INDEHISCENT* (*IND*), *SHATTERPROOF1* (*SHP1*) and *SHATTERPROOF2* (*SHP2*) are known fruit developmental genes of *Arabidopsis thaliana* that are expressed in the fruit valve margin governing dehiscence zone formation. Comparative expression analysis by quantitative RT-PCR, Northern blot and *in situ* hybridization show that their orthologues from *Lepidium campestre* (dehiscent fruits) are similarly expressed at valve margins. In sharp contrast, expression of the respective orthologues is abolished in the corresponding tissue of indehiscent *Lepidium appelianum* fruits, indicating that changes in the genetic pathway identified in *A. thaliana* caused the transition from dehiscent to indehiscent fruits in the investigated species. As parallel mutations in different genes are quite unlikely, we conclude that the changes in gene expression patterns are probably caused by changes in upstream regulators of *ALC*, *IND* and *SHP1/2*, possible candidates from *A. thaliana* being *FRUITFULL* (*FUL*), *REPLUMLESS* (*RPL*) and *APETALA2* (*AP2*). However, neither expression analyses nor functional tests in transgenic plants provided any evidence that the *FUL* or *RPL* orthologues of *Lepidium* were involved in evolution of fruit indehiscence in *Lepidium*. In contrast, stronger expression of *AP2* in indehiscent compared to dehiscent fruits identifies *AP2* as a candidate gene that deserves further investigation.**

**Keywords: fruit dehiscence, valve margin, evolutionary developmental biology, character evolution, Brassicaceae, *Lepidium campestre*, *Lepidium appelianum*.**

## INTRODUCTION

In the Brassicaceae, a family of angiosperms with 338 genera and 3700 species (Warwick *et al.*, 2010) that includes the major flowering plant model system *Arabidopsis thaliana*, molecular analyses have demonstrated that many morphological characteristics on which traditional systematic relationships are based are homoplasious rather than homologous. Fruit structures in particular have proven to be highly labile during evolution, and all molecular phylogenetic data consistently indicate that many species with similar fruits may be only distantly related, whereas species with dramatically different fruits may be very closely related (Mummenhoff *et al.*, 2005, 2009; Franzke *et al.*, 2011). This implies that developmental processes controlling fruit shape and structure are extremely plastic in evolution. The typical Brassicaceae fruit is dehiscent, and is considered to represent the ancestral fruit type in the family (Hall *et al.*, 2002). Nevertheless, species with various indehiscent fruits are found in 20 tribes distributed over the whole Brassicaceae phylogeny (Figure 1) (Appel and Al–Shehbaz, 2003), indicating that this character evolved many times independently. Here we investigate the evolutionary shift from dehiscent to indehiscent fruits by comparing two closely related Brassicaceae species, i.e. *Lepidium campestre* (L.) W.T. Aiton and *Lepidium appelianum* Al–Shehbaz.

With about 250 species, the genus *Lepidium* is one of the major genera of the Brassicaceae (Al–Shehbaz and Mummenhoff, 2011). Typically, *Lepidium* species produce



© 2012 The Authors
The Plant Journal © 2012 Blackwell Publishing Ltd

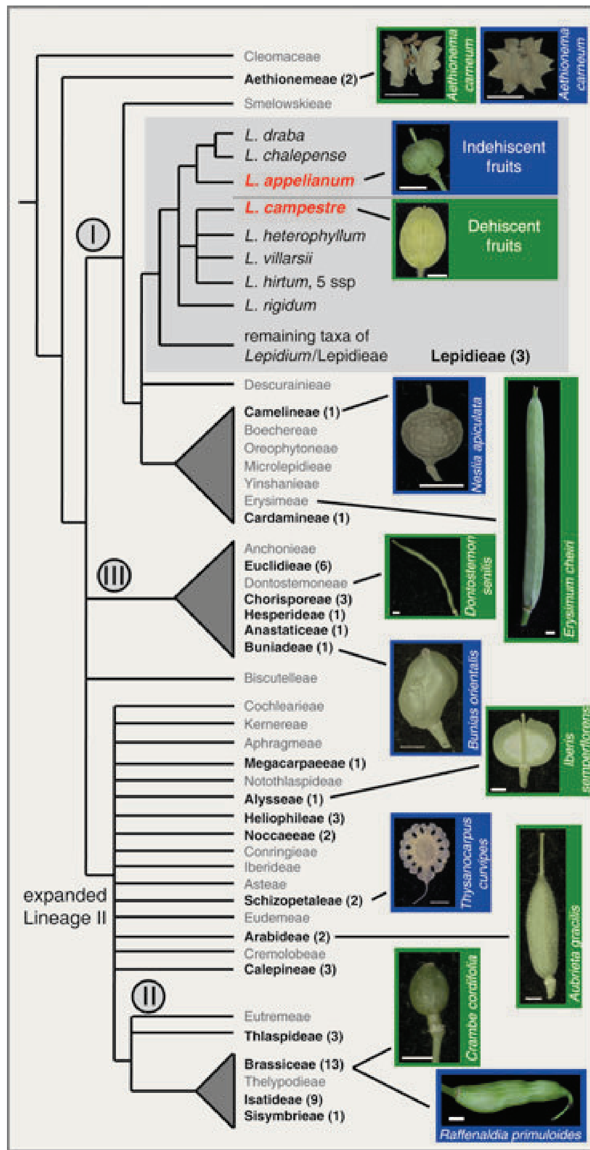


**Figure 1.** Distribution of indehiscent fruits within Brassicaceae.
The phylogeny including the three major lineages of Brassicaceae (I, II, and III) is based on a multi-gene analysis (Couvreur *et al*., 2010; Franzke *et al*., 2011), and illustrates that indehiscent fruit types evolved independently in 20 tribes (bold). The occurrence of indehiscence (number of genera per tribe) is shown in parentheses. The relationships of *L. campestre*, *L. appelianum* and their closest relatives are shown in detail. Sample images of dehiscent fruits (green margin) and indehiscent fruits (blue margin) are shown to demonstrate fruit diversity within the family. Scale bars = 2 mm, except for *Aethionema carneum* indehiscent fruits (1 mm). *Cleome* (Cleomaceae) as representative of the outgroup was used to root the phylogeny.

two-seeded dehiscent fruits, but the genus also comprises species with indehiscent fruits, such as *L. appelianum*, previously considered as a member of the genus *Cardaria* (*Cardaria pubescens*) (Mummenhoff *et al*., 2001, 2009; Al-Shehbaz *et al*., 2002). The two chosen species *L. campestre* (dehiscent fruits) and *L. appelianum* (indehiscent fruits) (Figure 1) are especially suited as models for our purpose because their close relatedness ensures that the shift of fruit type only happened quite recently during evolution. Additionally, both species are diploid ($2n = 2x = 16$), which simplifies genetic analyses, and their close relationship to Arabidopsis facilitates the adaptation of molecular techniques.

As has been demonstrated before (Mummenhoff *et al*., 2009), the anatomy of *L. campestre* wild-type fruits resembles *A. thaliana* wild-type fruits, in that they form a well-defined dehiscence zone (DZ) at the valve margin (Figure 2), resulting in a similar fruit opening mechanism. In contrast, *L. appelianum* fruits fail to open, only releasing the seeds during decomposition of the fruit valves. Anatomical studies show that they do not form a DZ but are surrounded by a continuous ring of lignified cells (Figure 2) comparable to indehiscent fruits of certain *A. thaliana* mutants, such as *35S::FUL*, *ful* or *ind ful* (Ferrandiz *et al*., 2000b; Liljegren *et al*., 2004). However, because the fruits of all these mutants lack a DZ, it is not possible to unequivocally relate the fruits of *L. appelianum* to one of them in particular.

*Arabidopsis thaliana* exhibits the typical Brassicaceae fruit, termed a silique, in which the process of fruit opening is quite well understood. At a morphological level, accurate patterning of relevant tissues within the fruit is crucial to allow fruit dehiscence (Figure 2). The dominant structures of the fruit are the two fruit valves that enclose the developing seeds. They are connected to the replum by the DZ (or valve margin), which consists of a stripe of lignified cells, the lignified layer, and an adjacent region of small thin-walled cells, the separation layer (Spence *et al*., 1996; Rajani and Sundaresan, 2001). The cells of the lignified layer are connected to the lignified endocarp layer *b* located on the inside of the valves. During fruit ripening, the whole fruit dries and shrinks, and only the lignified structures stay rigid, thereby creating a spring-like tension within the fruit. At the same time, the middle lamellae of the separation layer cells degenerate, acting as a predetermined breaking zone at which the pressure tears the valves apart from the replum, resulting in fruit opening (Meakin and Roberts, 1990, 1991; Spence *et al*., 1996).

At the molecular level, fruit dehiscence is controlled by several genes encoding transcription factors that are required for proper establishment of the DZ (Figure 2a). Two redundant MADS box genes, *SHATTERPROOF1* (*SHP1*) and *SHATTERPROOF2* (*SHP2*) are expressed in the DZ, where they activate the basic helix-loop-helix protein-encoding genes *INDEHISCENT* (*IND*) and *ALCATRAZ* (*ALC*). Additionally, *SHP1* and *SHP2* contribute to valve margin development autonomously (Liljegren *et al*., 2000, 2004). *IND* contributes to formation of both the lignified layer and the separation layer, especially mediating lignification of the valve margin cells, whereas *ALC* is essential for separation layer formation (Rajani and Sundaresan, 2001; Sorefan

© 2012 The Authors

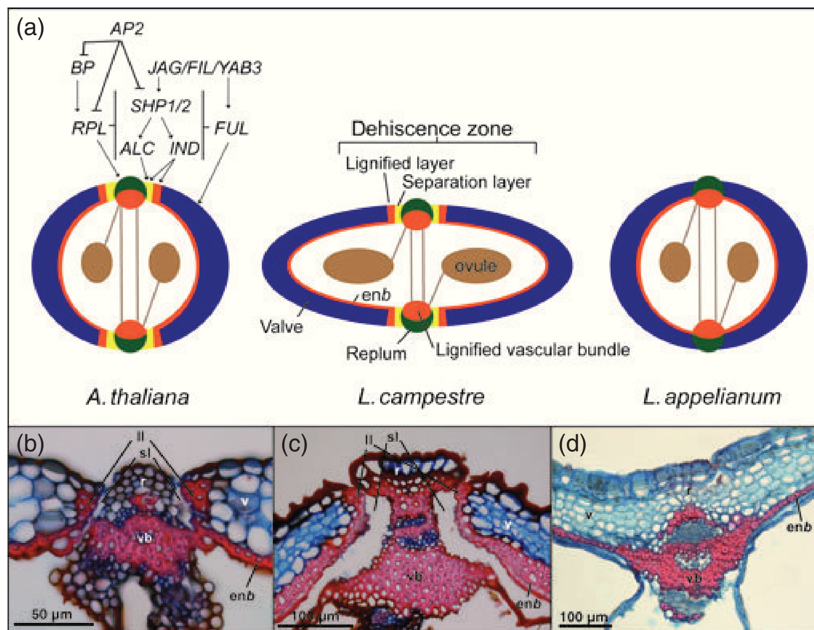


**Figure 2.** Comparison of fruit anatomy between *A. thaliana*, *L. campestre* and *L. appelianum.*

(a) Schematic cross-section of wild-type fruits of *A. thaliana* (left), *L. campestre* (middle) and *L. appelianum* (right), including the gene regulatory pathway responsible for tissue patterning in *A. thaliana* fruits.

(b–d) Lignin-stained (red) cross-sections of stage 19 wild-type fruits of *A. thaliana* (b), *L. campestre* (c) and *L. appelianum* (d). In dehiscent fruits of *A. thaliana* and *L. campestre*, a dehiscence zone (DZ) consisting of a separation layer (sl) and a lignified layer (ll) separates valves (v) and replum (r), while in indehiscent fruits of *L. appelianum*, the endocarp layer *b* (en*b*) is fused to the lignified vascular bundle (vb), and thus no DZ is visible.

*et al.*, 2009). It is crucial for correct fruit patterning that the expression of these four 'valve margin identity genes' is restricted to the DZ, a process that is mediated by negative regulators. One of these proteins is encoded by the MADS box gene *FRUITFULL* (*FUL*), which is expressed in the fruit valves and mainly contributes to valve cell differentiation and expansion (Gu *et al.*, 1998; Ferrandiz *et al.*, 2000b). Another such protein is encoded by the *BEL1*-like homeobox gene *REPLUMLESS* (*RPL*), which is expressed in the replum, but is not necessary for replum development *per se* (Gu *et al.*, 1998; Ferrandiz *et al.*, 2000b; Rajani and Sundaresan, 2001; Roeder *et al.*, 2003). Note that the *RPL* gene is also known as *PENNYWISE* (Smith and Hake, 2003), *BELLRINGER* (Byrne *et al.*, 2003), *VAAMANA* (Bhatt *et al.*, 2004), *LARSON* (Bao *et al.*, 2004) and *BLH9* (Cole *et al.*, 2006). Recently, the floral homeotic gene *APETALA2* (*AP2*) (Bowman *et al.*, 1989) has been identified as a negative regulator of *RPL* and the *SHP* genes (Ripoll *et al.*, 2011).

In *A. thaliana*, mutants for all seven genes described above have been characterized that show impaired dehiscence. Single mutants of *ind* or *alc* and *shp1/2* double mutants are all defective in DZ formation, leading to inability of the valves to detach from the replum (Liljegren *et al.*, 2000, 2004; Rajani and Sundaresan, 2001; Wu *et al.*, 2006). In *rpl* mutants, ectopic *SHP1/2*, *IND* and *ALC* expression induces the conversion of replum cells into valve margin-like cells, leading to partial indehiscence (Roeder *et al.*, 2003; Sorefan *et al.*, 2009). Moreover, both the *ful* knockout mutation as well as *35S::FUL* overexpression induce indehiscence in *A. thaliana*. In *ful* plants, valve cells are transformed into valve margin cells due to ectopic expression of valve margin identity genes (*SHP1/2*, *IND* and *ALC*), but in addition the overall architecture of the fruit is highly disturbed because the fruit valves fail to elongate (Gu *et al.*, 1998; Ferrandiz *et al.*, 2000b). On the other hand, fruits of *35S::FUL* mutants look normal except for the lack of a DZ caused by the suppression of valve margin identity genes by ectopic *FUL* expression (Ferrandiz *et al.*, 2000b; Ostergaard *et al.*, 2006). In *ap2* knockout plants, increased expression of *RPL*, *SHP2* and *IND* causes a delay in fruit opening due to expansion of the replum and the lignified layer (Ripoll *et al.*, 2011). Thus, it is tempting to speculate that such simple changes in gene expression are involved in independent evolution of indehiscent from dehiscent fruits within the Brassicaceae.

In the current study, we compare the expression patterns of *L. appelianum* and *L. campestre* orthologues of the *A. thaliana* genes (henceforce referred to as *AtALC*, *AtFUL*, *AtIND*, *AtRPL*, *AtSHP1*, *AtSHP2*, and *AtAP2* for clear discrimination from the *Lepidium* orthologues). Whereas the expression patterns in dehiscent *L. campestre* closely resemble those found in *A. thaliana*, we observed an absence of expression of valve margin identity genes (*LaALC*, *LaIND* and *LaSHP1/2*) in the valve/replum border in indehiscent *L. appelianum* fruits. We conclude that, at least in some wild *Lepidium* species, the switch from dehiscent to indehiscent fruits is indeed accompanied by changes within the regulatory pathway predicted from the *A. thaliana* mutant system, and that the causal genetic change has probably affected an upstream regulator of *LaSHP1* and *LaSHP2*. Therefore, our study exemplarily clarifies the molecular genetic basis of fruit dehiscence between two closely related wild species.

© 2012 The Authors
The Plant Journal © 2012 Blackwell Publishing Ltd, 

## RESULTS

### Sequence analysis of fruit developmental genes

One goal of our work was to uncover differences in DNA sequence and expression patterns of putative fruit developmental genes to identify candidate genes that may have contributed to the shift from dehiscent to indehiscent fruits in *Lepidium*. Based on the close relationship between Arabidopsis and *Lepidium*, we designed primers derived from *A. thaliana* fruit developmental gene sequences to isolate the respective orthologues from *L. campestre* and *L. appelianum*. We isolated cDNAs of *ALC*, *FUL*, *IND*, *RPL*, *SHP1* and *SHP2* from both *Lepidium* species, and initial BLAST (National Center for Biotechnology Information, Bethesda, MD, USA) searches revealed high degrees of DNA sequence similarity to the respective fruit developmental genes from *A. thaliana*. Phylogenetic analyses of the respective gene families were performed, indicating that all genes under study are putative orthologues of the corresponding *A. thaliana* fruit developmental genes (Figure S1). To exclude the existence of additional closely related paralogues, we performed Southern blot hybridization on genomic DNA from *L. campestre* and *L. appelianum* using various restriction enzymes. For *LcSHP1* and *LaSHP2*, we found two bands, most likely due to cross-hybridization (as revealed by the sizes of the obtained bands). In all other cases, only one band per lane was observed, suggesting that all genes tested are single-copy genes (Figure S2).

The amino acid sequences of all orthologues were infered from cDNA sequences to facilitate the search for structural differences between genes from indehiscent *L. appelianum* and dehiscent Brassicaceae species, including *L. campestre*. The amino acid sequence identity between the putative orthologues of *A. thaliana* and *Lepidium* ranges from 73.5% (AtALC versus LaALC) to 95.5% (AtFUL versus LaFUL). Amino acid sequence identities between orthologous *Lepidium* proteins are always higher than 93%. Amino acid alignments (Figure S3), also including sequences of other dehiscent Brassicaceae species, were performed in order to search for amino acid substitutions that appear only in proteins of the indehiscent *L. appelianum*. These are considered as candidate changes that may have contributed to the shift from dehiscent to indehiscent fruits during the evolution towards extant *L. appelianum*. We detected a total of 20 amino acid positions (marked by arrows in Figure S3) at which the sequence of *L. appelianum* differs from all other Brassicaceae species under study.

### Expression of valve margin identity genes is absent from the valve/replum border of *L. appelianum* fruits

In order to compare the expression patterns of putative fruit valve margin identity genes (i.e. *ALC*, *IND* and *SHP1/2*) between *L. appelianum* and *L. campestre*, their overall expression level was analysed in six tissues (root, stem, leaf, flower, fruit stage 15/16, and fruit stage 17) by quantitative RT-PCR and Northern blot hybridization (Figure 3a–d and Figure S4). Furthermore, *in situ* hybridization was performed on sections of flowers and fruits (stages 10–15, according to Smyth *et al.*, 1990) for a more detailed insight into the spatial expression patterns (Figure 4a–h). It was found that, in both species, all four valve margin identity genes are mainly expressed in flowers and fruits and only at lower levels, if at all, in roots, stems and leaves. The only exceptions are *LaALC* in roots and *LaSHP2* in stems, which show expression levels comparable to those in flowers and fruits. For *ALC*, the overall expression level in fruits of *L. appelianum* was significantly higher than in fruits of *L. campestre* (Figure 3d), accompanied by a clear change in spatial distribution. In *L. campestre*, *ALC* is strongly expressed in the DZ (Figure 4e), but was exclusively detected in the ovule in *L. appelianum* (Figure 4a). *IND* is expressed at the valve/replum border in *L. campestre* fruits, but is not detectable in fruits of *L. appelianum* (Figure 4b,f), consistent with a 2.5–7-fold decrease in the overall expression level in *L. appelianum* flowers and fruits compared to *L. campestre* (Figure 3a). *SHP1* and *SHP2* are both expressed in the DZ and ovules in *L. campestre* (Figure 4g,h) but only in ovules in *L. appelianum* (Figure 4c,d). Regarding the overall expression level, *LcSHP1* is significantly more highly expressed than *LaSHP1* in flowers and fruits (stage 15/16), while *LcSHP2* is significantly more highly expressed than *LaSHP2* in flowers whereas expression is significantly lower in fruits (stage 17) (Figure 3b,c). In summary, we show that, in *L. campestre* dehiscent fruits, all four valve margin identity genes are expressed in the DZ, but that such expression patterns at the valve/replum border are absent from *L. appelianum* indehiscent fruits.

### The spatio-temporal expression patterns of *FUL* and *RPL* in the two *Lepidium* species are similar

As *AtFUL* and *AtRPL* are known to be upstream regulators of valve margin identity genes in *A. thaliana* (Ferrandiz *et al.*, 2000b; Roeder *et al.*, 2003; Liljegren *et al.*, 2004), their expression patterns were also analysed and compared between the two *Lepidium* species. *FUL* was found to be expressed in all analysed tissues except for roots, and the overall expression level was significantly higher in *L. campestre* stems and fruits of stage 15/16 compared to *L. appelianum* (Figure 3e,f). In both species, *in situ* hybridization shows mRNA accumulation in the fruit valves, but not in the replum (Figure 4i–n). Therefore, the expression patterns look superficially similar between *L. appelianum* and *L. campestre*, but small differences at the valve/replum border cannot be excluded, because especially in this region fruit anatomy differs significantly between the two species. *RPL* is expressed in all tissues analysed, with significantly higher expression levels for *LcRPL* in leaves and flowers

© 2012 The Authors

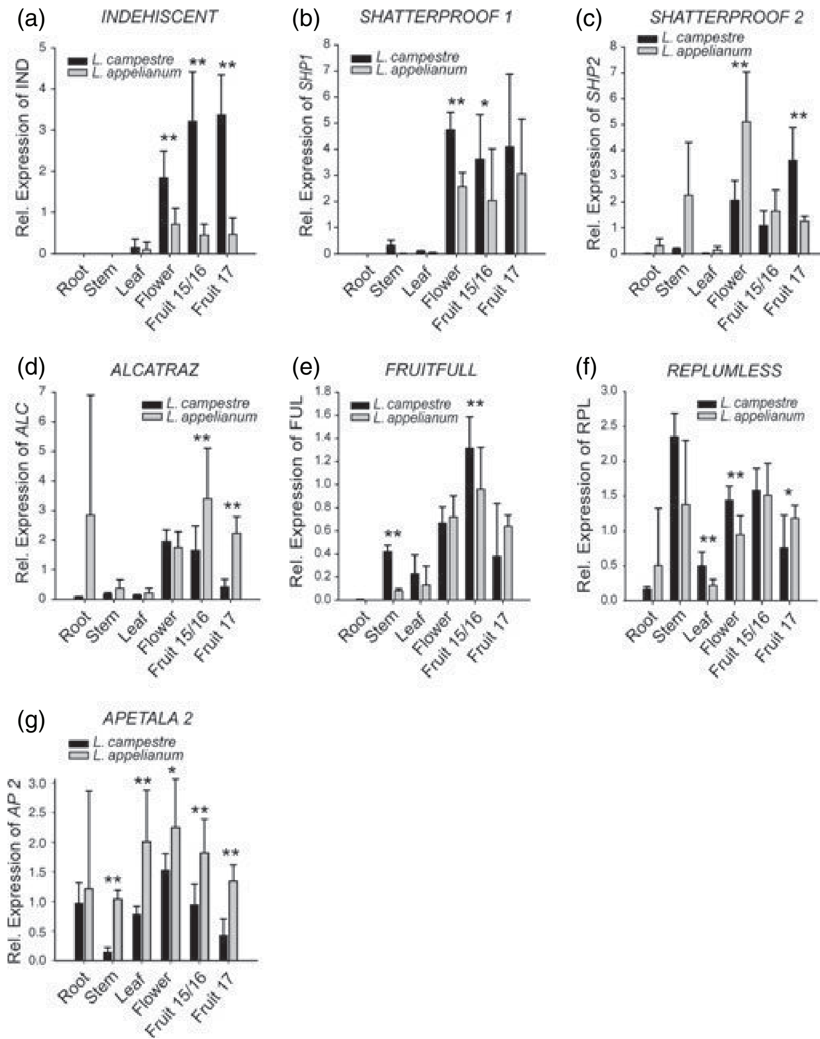


**Figure 3.** Gene expression analysis via quantitative RT-PCR.
Relative expression levels of *IND* (a), *SHP1* (b), *SHP2* (c), *ALC* (d), *FUL* (e), *RPL* (f) and *AP2* (g) in various tissues as indicated below the columns. Significant differences between *L. appelianum* and *L. campestre* are indicated by asterisks (*$P \leq 0.05$, **$P \leq 0.01$).

and for *LaRPL* in fruits of stage 17 (Figure 3f) when comparing the two *Lepidium* orthologues. Unfortunately, spatial expression patterns for *RPL* were only detected via *in situ* hybridization at the tip of the inflorescence meristem, and in all whorls of the developing flower (Figure 4k,n). Detection by *in situ* hybridization was not possible in mature flowers or fruits (Figure 4j,m) although the quantitative RT-PCR results clearly show such expression.

### *LaAP2* is up-regulated in indehiscent *L. appelianum*

During preparation of this paper, the floral homeotic gene *AtAP2* was identified as a negative regulator of genes controlling valve margin identity in *A. thaliana* (Ripoll *et al.*, 2011). As an initial step to investigate this new candidate gene, we isolated orthologous partial cDNAs from both *Lepidium* species (*LaAP2* and *LcAP2*) and analysed their expression by quantitative RT-PCR. We found a significant increase in *LaAP2* expression compared to *LcAP2* expression in all tissues except roots (Figure 3g).

### Ectopic expression of *FUL* and RPL orthologues from both *Lepidium* species does not reveal functional changes in coding regions

As no major difference in expression pattern were detected in *Lepidium* between the two orthologues of *FUL* and, likewise, between the two orthologues of *RPL*, we wished to analyse whether the amino acid differences found during our sequence analysis cause a change in protein function. Therefore, cDNAs of the coding regions were cloned into a binary plasmid under the control of a CaMV 35S promoter, and transformed into *A. thaliana* (*RPL* orthologues) or *L. campestre* (*FUL* orthologues). For the *RPL* orthologues, the most obvious effect for all constructs was formation of multiple cauline leaves at the base of second-order inflorescence shoots (Figure 5a–d). Furthermore, in some transformants of each construct, an alteration in phyllotaxy and irregular elongation of internodes was observed (Figure S5). These phenotypic changes are consistent with those

© 2012 The Authors

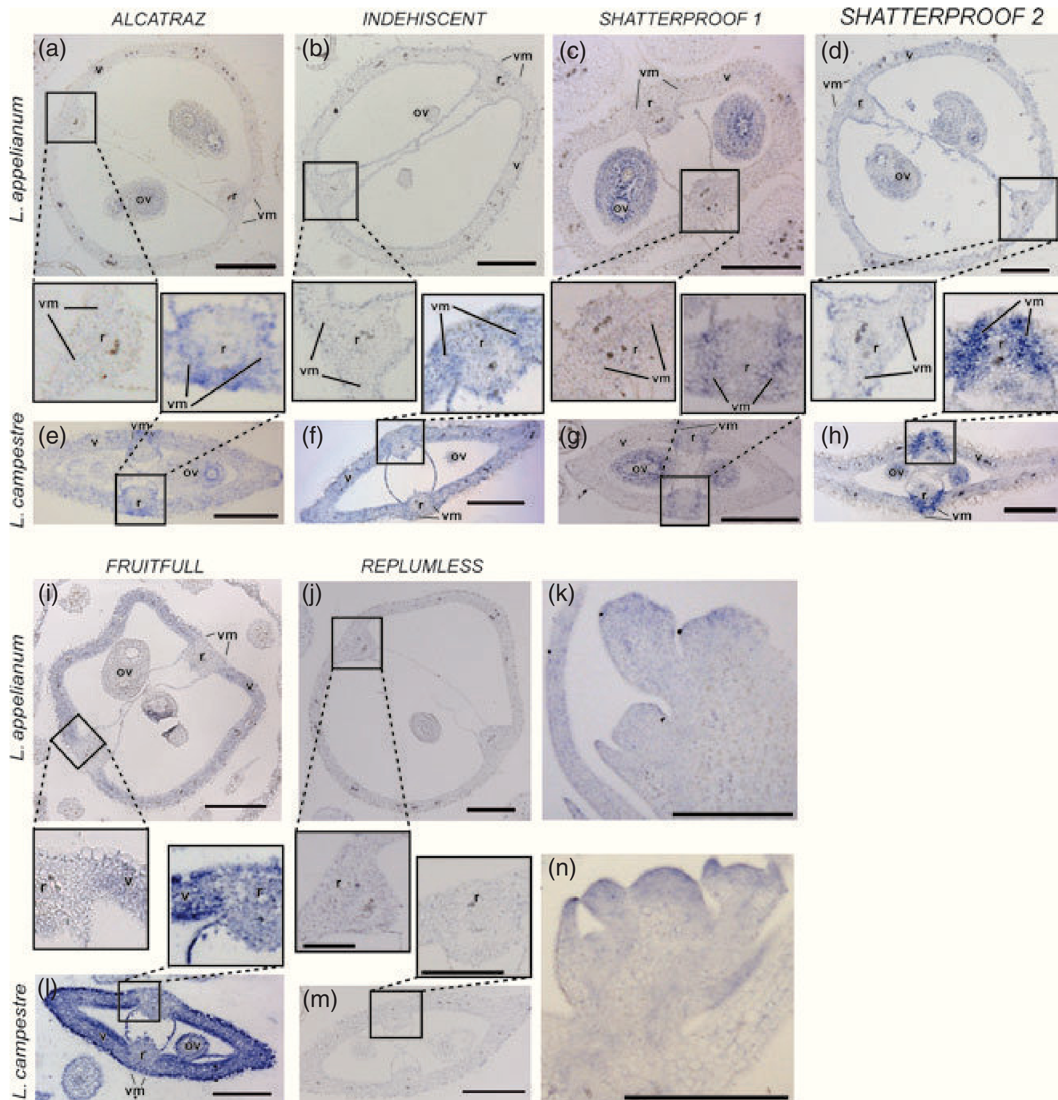


**Figure 4.** Gene expression analysis via *in situ* hybridization.
Comparison between valve margin identity genes *ALC* (a, e), *IND* (b, f), *SHP1* (c, g) and *SHP2* (d, h) and between upstream regulators *FUL* (i, l) and *RPL* (j, k, m, n) of *L. appelianum* (a–d, i–k) and *L. campestre* (e–h, l–n). Sections were prepared from flowers and fruits of stages 10-11 (a, c, e, g, i, l), 11–12 (b, j, m), 13–14 (f, h) or 15 (d), and from young inflorescences (k, n). Enlargements of valve/replum borders are shown in boxes. Expression of *ALC*, *IND*, *SHP1* and *SHP2* in the valve margins was exclusively found in sections of *L. campestre*, but expression patterns of *FUL* and *RPL* are not distinguishable between the two species. Scale bars = 200 μm. Abbreviations: ov, ovule; r, replum; v, valve; vm, valve margin.

previously reported for *AtRPL* over-expressing mutants (Cole *et al*., 2006), except that no early flowering was detected in our case. Ectopic expression of the *Lepidium FUL* orthologues frequently caused the formation of fruits that were more difficult or impossible to open manually (Figure 5e,f). When the half-life of dehiscence of such transformants was estimated using a random impact test, a significant increase was detected for both constructs expressing *FUL* orthologues compared to an empty vector control (Figure 5g). Therefore, over-expression of *FUL* cDNAs of both *Lepidium* species induces fruit indehiscence, as is also the case for *AtFUL* (Ferrandiz *et al*., 2000b).

## DISCUSSION

### Loss-of-function mutations in the coding region of classical fruit developmental genes are probably not responsible for indehiscence in *L. appelianum*

We isolated orthologues of six fruit developmental genes (*AtALC*, *AtFUL*, *AtIND*, *AtRPL*, *AtSHP1* and *AtSHP2*), which are known to be essential for pod shatter in *A. thaliana*, from *L. campestre* and *L. appelianum.* If mutations in these genes were involved in the evolution of indehiscence in *L. appelianum*, these may have led to changes in either the function of the encoded proteins or in gene expression patterns. However, the fact that, in addition to their roles in

© 2012 The Authors

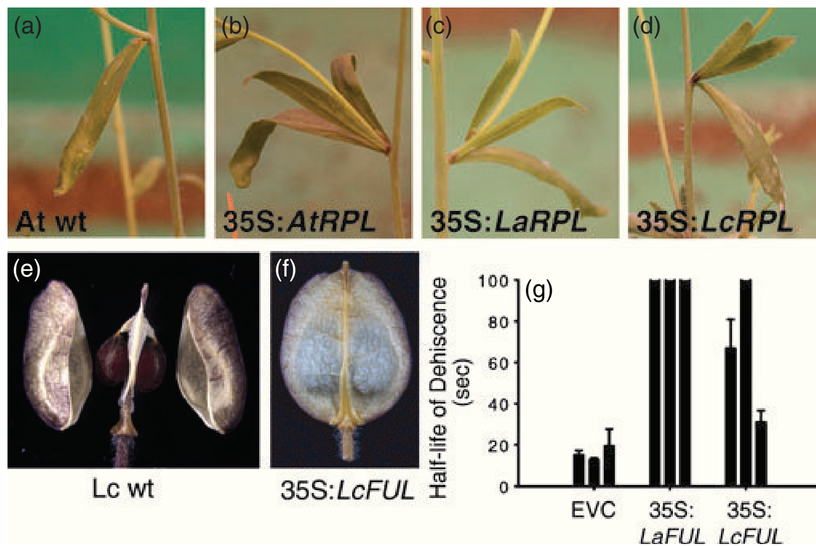


**Figure 5.** Over-expression phenotypes of *FUL* and *RPL* orthologues.
(a–d) Constitutive expression of *AtRPL* (b), *LaRPL* (c) or *LcRPL* (d) in *A. thaliana* results in formation of additional cauline leaves compared to the wild-type (a).
(e–g) Constitutive expression of *LcFUL* (f, g) or *LaFUL* (g) in *L. campestre* leads to formation of indehiscent fruits compared to dehiscent wild-type fruits (e). Three representative transformants per construct were analysed by the random impact test. Their half-life of fruit dehiscence was significantly higher than empty vector control plants (EVC). For (a), brightness and contrast were adjusted using PowerPoint 2007 (Microsoft Corporation, Redmond, WA, USA).

fruit dehiscence, all candidate proteins are also involved in other developmental functions in *A. thaliana* (Ferrandiz *et al.*, 2000a; Byrne *et al.*, 2003; Favaro *et al.*, 2003; Pagnussat *et al.*, 2005; Cai and Lashbrook, 2008) makes it unlikely, however, that dramatic changes in protein function (including total loss-of-function) contribute to evolution under conditions of natural selection due to pleiotropic effects. We found that amino acid sequences of all orthologous proteins are highly conserved between the *Lepidium* species and other members of the Brassicaceae (Figure S3). There is no evidence for dramatic changes in conceptual protein structure exclusively within *L. appelianum* that may explain the origin of fruit indehiscence. However, there are a number of small differences in amino acid sequences between analysed proteins of different Brassicaceae species, including a total of 20 amino acid changes that were exclusively detected in proteins of *L. appelianum* (Figure S3). We cannot completely rule out the possibility that any of these mutations is responsible for the switch from dehiscence to indehiscence in *Lepidium*, as it is well known that single amino acid substitutions may alter protein function dramatically. An example that is of interest in the broader context of our study is a single amino acid substitution in a predicted Myb3 transcription factor termed SH4 that caused reduced seed shattering during rice domestication (Li *et al.*, 2006). Nevertheless, a change in the expression pattern of a gene appears a more likely scenario. Due to the modularity of many promoter and enhancer regions, alterations in gene expression in one location without affecting the function in others are quite frequent processes during evolution, e.g. during sub-functionalization events (Force *et al.*, 1999).

### Absence of valve margin identity gene expression may cause indehiscence in *L. appelianum*

By analysing the expression patterns of the four valve margin identity gene orthologues from *L. appelianum* and *L. campestre*, we found that dehiscent fruits of *L. campestre* exhibit patterns very similar to those found in dehiscent *A. thaliana* fruits. In both fruit types, the valve margin identity genes are expressed within a thin stripe of cells at the valve/replum border, and, in addition, *ALC* and *SHP1/2* mRNA accumulation is found within ovules (Ma *et al.*, 1991; Savidge *et al.*, 1995; Flanagan *et al.*, 1996; Rajani and Sundaresan, 2001; Liljegren *et al.*, 2004). This conservation in spatial expression patterns suggests that gene functions may also be conserved between these two species. In *A. thaliana*, all four genes are important for establishing DZ tissue, which is an additional indication, together with the anatomical similarities, that fruit opening in *L. campestre* operates according to the same mechanisms as in *A. thaliana* (Liljegren *et al.*, 2000, 2004; Rajani and Sundaresan, 2001; Mummenhoff *et al.*, 2009). The function of *AtALC* in *A. thaliana* ovules is not yet known, but the *AtSHP* genes contribute redundantly to the floral organ identity 'D function' in *A. thaliana* together with *SEEDSTICK*, *AGAMOUS* and *BELL1* (Western and Haughn, 1999; Pinyopich *et al.*, 2003).

In indehiscent fruits of *L. appelianum*, *LaALC*, *LaIND*, *LaSHP1* and *LaSHP2* mRNAs were undetectable in the valve/replum border. It is already known that the lack of expression of either *AtALC* or *AtIND* or both *AtSHP1* and *AtSHP2* in *A. thaliana* knockout plants induces indehiscence (Liljegren *et al.*, 2000, 2004; Rajani and Sundaresan, 2001). These findings strongly suggest that the combined absence of the four valve margin identity genes is sufficient to cause the indehiscent fruit phenotype in *L. appelianum*. On the other hand, expression of *LaALC* and *LaSHP1/2* is retained in *L. appelianum* ovules, suggesting that it is not a complete gene knockout that causes the downregulation at the valve/replum border, but that more sophisticated genetic changes are involved. This ovule-specific expression also indicates that the function in ovule

© 2012 The Authors

development of these genes is not only conserved between *A. thaliana* and *L. campestre* but also in *L. appelianum*, although the significant increase in overall expression level for *LaALC* in *L. appelianum* fruits and for *LaSHP2* in *L. appelianum* flowers may indicate functional changes requiring an increased transcript abundance.

**Genetic cause of indehiscence in *L. appelianum*: evaluating various candidates**

After having established that combined absence of expression of four valve margin identity genes from the valve/replum border is probably involved in the development of indehiscence in *L. appelianum*, the question arises as to which genetic changes cause this simultaneous down-regulation. From studies of *A. thaliana* mutants, several mutations are known to induce formation of indehiscent fruits, but only a few of these fit the expression pattern found in *L. appelianum* fruits.

*Valve margin identity genes.* The first scenario involves mutations directly within the valve margin identity genes themselves. This requires at least two independent mutations within the loci of the *LaSHP* genes resulting in exclusion from the valve/replum border and subsequent non-activation of the downstream targets *LaIND* and *LaALC*, assuming that the regulatory network known from *A. thaliana* (Liljegren *et al*., 2004) is conserved in *Lepidium*. We consider this possibility unlikely because of the requirement for two independent mutations that do not involve simple gene knockouts but specific removal of expression from a certain region (the DZ) while remaining present at another region (the ovules). However, we cannot exclude at present that changes in the regulation of *SHP* gene expression may have occurred during evolution of *Lepidium*.

FUL *and* RPL. A scenario that appears more likely to us involves mutations in one of the upstream regulators *LaFUL* or *LaRPL*. These genes are known to restrict valve margin identity gene activity to a thin stripe at the valve/replum border (Ferrandiz *et al*., 2000b; Liljegren *et al*., 2000; Roeder *et al*., 2003; Dinneny and Yanofsky, 2005). Down-regulation of either of these genes induces indehiscent fruits in *A. thaliana* mutants, caused by expansion of valve margin identity gene expression into the valve or replum, respectively (Gu *et al*., 1998; Ferrandiz *et al*., 2000b; Roeder *et al*., 2003; Liljegren *et al*., 2004), thereby not matching the expression patterns found in *L. appelianum* fruits. On the other hand, ectopic expression of *FUL* throughout the whole fruit in 35S*::FUL* transgenic *A. thaliana* or *Brassica juncea* plants causes indehiscent fruit phenotypes by eliminating expression of valve margin identity genes from the valve/replum border while leaving *SHP1* and *SHP2* expression in ovules unaffected (Ferrandiz *et al*., 2000b; Ostergaard *et al*., 2006), a phenotype that is strikingly similar to that found in *L. appelianum*. Theoretically, for plants ectopically expressing *RPL*, a similar indehiscent fruit phenotype is expected, because as a down-regulator of valve margin identity genes ectopic expression should equally lead to elimination of DZ formation. However, transgenic *A. thaliana* plants in which *AtRPL* expression is controlled by a CaMV 35S promoter dd not show altered fruit development (Harley Smith, University of California, Riverside, CA, USA, personal communication). Additionally, overexpression of *BREVIPEDICELLUS*, an activator of *AtRPL*, leads to ectopic expression of *AtRPL* in valves and valve margins, resulting in plants with enlarged repla and a reduction in valve width but no changes in valve margin and lignification patterns (Alonso-Cantabrana *et al*., 2007). This may indicate that ectopic expression of *AtRPL* alone is not sufficient to eliminate the expression of valve margin identity genes from the valve/replum border, at least in *A. thaliana*.

In this study, the expression patterns of *FUL* and *RPL* were analysed in *L. appelianum* and *L. campestre* but did not provide a clear indication of their involvement in the evolution of fruit indehiscence. *FUL* expression was located in fruit valves in both *Lepidium* species and did not show any major expansion to other regions of the *L. appelianum* fruit, so a global up-regulation as in the 35S::*FUL A. thaliana* plants may be excluded. Nevertheless, expansion of the *LaFUL* expression domain by only a few cell layers towards the replum may already be sufficient to suppress valve margin identity gene expression and thereby to prevent DZ formation and induce indehiscence. Such a small expansion may not have been recognized in our data, because the fruit anatomy of the *Lepidium* species differs, especially at the valve/replum border, and therefore the *FUL* expression domains cannot be directly compared. One might even argue that such a subtle change in *FUL* expression is more likely to occur under natural evolutionary conditions compared to the global expansion seen in the 35S*::FUL* transgenic line, because it may avoid pleiotropic effects due to interference with other *FUL* functions. In addition to its function in fruit development, *AtFUL* is also known to act as a flowering time and meristem identity gene and to contribute to cauline leaf morphology (Gu *et al*., 1998; Ferrandiz *et al*., 2000a). 35S::*AtFUL A. thaliana* plants show a dramatically reduced time to flowering and form terminal flowers with increased seed weight in addition to their indehiscence phenotype (Ferrandiz *et al*., 2000a,b). For *RPL* expression, dramatic differences in transcript level may be excluded on the basis of our quantitative RT-PCR results, but spatial patterns could not be compared because *RPL* could not be detected via *in situ* hybridization. This is consistent with findings in *A. thaliana*, where *AtRPL* detection via *in situ* hybridization was also

© 2012 The Authors

very difficult in floral stages older than stage 8, and therefore GUS reporter systems had to be used to investigate *AtRPL* expression (Roeder *et al.*, 2003). Future experiments may include *RPL* expression studies in *Lepidium* with GUS reporter constructs as performed in *A. thaliana*, once an efficient transformation system for both species has been established.

Recently, the *RPL* gene has attracted quite some attention (Gasser and Simon, 2011; Wagner and Mitchell-Olds, 2011), because a nucleotide substitution at the same position within a conserved regulatory element (*Shattering element-like*, *Shl*) that causes loss of seed shattering in certain rice cultivars (Konishi *et al.*, 2006) was also found to have an effect on replum form in various Brassicaceae species (Arnaud *et al.*, 2011). Even though no evidence has been published so far showing that this substitution really affects fruit dehiscence in Brassicaceae (Arnaud *et al.*, 2011; Wagner and Mitchell-Olds, 2011), we investigated this regulatory element from *L. appelianum*. We found that the relevant nucleotide in *L. appelianum* is the same as in dehiscent *L. campestre* and *A. thaliana*, and thereby we can exclude its involvement in the evolution of indehiscence in our case (Figure S6).

During the analysis of conceptual amino acid sequences, one and seven amino acid changes were found exclusively in the *L. appelianum* LaFUL and LaRPL orthologues, respectively (Figure S3). As no alteration in expression pattern was detected for these genes, we analysed the possible effects of these amino acid changes on protein function by assessing their over-expression phenotypes. For both genes, these were found to be very similar for sequences of *L. campestre* and *L. appelianum*, and additionally correspond to over-expression phenotypes reported for the *A. thaliana* orthologues (Figure 5) (Ferrandiz *et al.*, 2000b; Cole *et al.*, 2006). Such similar phenotypic effects suggest a global conservation of protein function between both *Lepidium* species and *A. thaliana*, thereby indicating that a change in protein function is most probably not responsible for the evolution of indehiscence of *L. appelianum* fruits. Nevertheless, this possibility cannot be ruled out completely because (i) protein function was evaluated in a heterologous system and may differ in its natural environment, and (ii) *RPL* over-expression only causes changes in plant architecture but not in fruits. Therefore, any of the amino acid changes may compromise protein function exclusively in fruits.

AP2 *as a novel candidate gene.* During preparation of this paper, *AtAP2* was identified as an additional member of the fruit patterning pathway in *A. thaliana* (Ripoll *et al.*, 2011). As a negative regulator of *AtSHP1*, *AtSHP2* and *AtRPL*, it normally prevents over-expression of these genes in the replum and valve margin. Thus, if the regulatory network as proposed for *A. thaliana* by (Ripoll *et al.*, 2011) also holds in *Lepidium*, a simple increase in the *LaAP2* expression level may cause the observed elimination of expression of *L. appelianum* valve margin identity genes from the valve/replum border. We found a significant increase in *LaAP2* expression levels compared to *LcAP2* in all tissues except for roots (Figure 3g). Nevertheless, in *A. thaliana*, *AtAP2* is known to be regulated by miRNA172 at the translational level (Chen, 2004), implying that an increased mRNA level does not necessarily lead to an increased protein level. Due to the activity of miRNA172, 35S::*AtAP2* mutant plants have normal flowers or show only mild floral defects (Chen, 2004), but whether they exhibit changes in fruit dehiscence has, to the best of our knowledge, not been reported. Therefore, a thorough investigation of the effect of *AP2* over-expression on fruit opening in *A. thaliana* as well as in *Lepidium*, including a study of the *L. appelianum* miRNA172 pathway, is necessary in order to address whether the increased levels of *LaAP2* expression are responsible for the indehiscent fruit phenotype.

*Further candidate genes.* Other candidates that may cause the indehiscent fruit phenotype in *L. appelianum* (based on the fact that mutant alleles can induce indehiscent fruits in *A. thaliana*) are *FILAMENTOUS FLOWER* (*FIL*), *JAGGED* (*JAG*) and *YABBY3* (*YAB3*). These genes act redundantly in promoting valve and valve margin formation by activating *AtFUL* and *AtSHP1/2* (Sawa *et al.*, 1999; Siegfried *et al.*, 1999; Dinneny *et al.*, 2004, 2005; Ohno *et al.*, 2004). Because of their redundant function, each of the three *A. thaliana* single knockout mutants shows normal fruit opening, but *fil yab3* and *fil jag yab3* mutants show major defects in fruit dehiscence (Dinneny *et al.*, 2005). In these mutants, indehiscence is caused by a loss of *AtSHP* expression (and, as a consequence, probably also *ALC* and *IND* expression) in the valve margins, and an additional loss of *AtFUL* expression in valves (Dinneny *et al.*, 2005), which only partially resembles the situation found in *L. appelianum* fruits. Additionally, in *fil jag yab3* mutants, sepals, petals and stamens are replaced by filamentous structures with few floral characteristics (Dinneny *et al.*, 2005). Consequently, as described for *LaSHP1* and *LaSHP2*, complete loss-of-function mutations within these genes most likely do not cause indehiscence in *L. appelianum*, but slight shifts of expression domains may be involved. A retreat of *LaFIL* and *LaYAB3* expression from the valve margin may abolish *LaSHP* expression while leaving *LaFUL* expression unchanged.

**Outlook**

The absence of valve margin identity gene expression from the valve/replum border revealed by our data provides insights into the molecular cause of indehiscence in *L. appelianum*. However, it provides little direct indication

© 2012 The Authors

as to which genetic change led to the evolution of indehiscent fruits in this species. Several candidate genes were identified based on the fruit patterning pathway of *A. thaliana*, but the expression data do not favour one of these candidates in particular. Future studies should focus on isolating the genomic loci of all potential candidates and analysing their influence on fruit development by means of transformation into *A. thaliana* and *L. campestre.*

## EXPERIMENTAL PROCEDURES

### Plant material

Plants were grown in the greenhouse on loam/sand/pumice/compost (1:2:2:5) or seedling substrate (Kammlott, Kammlott GmbH, Erfurt, Germany)/sand/vermiculite (1–3 mm) (8:1:1), supplemented with 1 g $L^{-1}$ each of Osmocote mini (http://www.scotts.com,The Scotts Miracle-Gro Company, Marysville, OH, USA) and Triabon (http://www.compo-expert.com, COMPO Expert GmbH, Münster, Germany) under a photoperiod of 16 h at 20°C and 8 h without illumination at 15°C. Flowering was induced by at least 6 weeks (*L. campestre*) or 13 weeks (*L. appelianum*) at 4°C with illumination for 8 h $day^{-1}$. For morphological analysis, thin sections were prepared from paraplast-embedded fruits (http://www.leica-microsystems.com/, Leica Microsystems GmbH, Wetzlar, Germany) and stained with safranin-astra blue (http://www.sigmaaldrich.com, Sigma Aldrich Corporation, St. Louis, MO, USA).

### Cloning of candidate genes

RNA was isolated using RNAiso-G+ (segenetic, (www.segenetic.de, segenetic, Borken, Germany)). cDNA was prepared using RevertAid™ Premium reverse transcriptase (Fermentas, http://www.thermoscientificbio.com/fermentas/, ThermoFisher Scientific Corporation, Waltham, MA, USA). Primer details are given in Table S2. Isolation of candidate genes was performed by 3′ and 5′ RACE (Frohman *et al.*, 1988) or by specific PCR with primers binding to conserved regions of the *A. thaliana* sequences using Phusion™ high-fidelity DNA polymerase (Finnzymes, www.thermoscientificbio.com/finnzymes/, ThermoFisher Scientific Corporation, Waltham, MA, USA) and Taq DNA polymerase (Segenetic). For 5′ RACE, SP1 primers were used for gene-specific cDNA synthesis, followed by polyadenylation using terminal deoxynucleotidyl transferase (Fermentas) or terminal transferase (Roche, http://www.roche-applied-science.com Roche Applied Science, Penzberg, Germany). For nested PCR, oligo(dT) and SP2 primers (first step) and anchor primer with SP3 primer (second step) were used. Finally, full-length clones were PCR-amplified and ligated into pGEM-T vectors (Promega, www.promgega.com, Promega, Fitchburg, WI, USA).

### *In situ* hybridization

*In situ* hybridization was performed essentially as described by Zachgo (2002) using flower and fruit tissues at developmental stages 10–15 (according to Smyth *et al.*, 1990). Tissues were fixed in paraformaldehyde and embedded in paraplast. Probe templates were produced by linearizing the vector pGEM-T containing full-length clones of respective cDNAs in the correct orientation using *Xba*I. Antisense RNA probes were synthesized using T7 or SP6 RNA polymerases. Images were created using a stereomicroscope (Leica, DM5000B, http://www.leica-microsystems.com/, Leica Microsystems GmbH, Wetzlar, Germany) with an integrated digital camera (Leica, DCF 490); scale bars were generated using Leica Application Suite.

### QUANTITATIVE RT-PCR

Total RNA was extracted using Total RNA Isolation Reagent (Biomol, http://www.biomol.de/, Biomol GmbH, Hamburg, Germany), QIAzol lysis reagent (Qiagen, www.qiagen.com, Qiagen N.V., Hilden, Germany) or the RNeasy plant mini kit (Qiagen). RNA concentration and integrity were analysed before and after DNase I digestion using a NanoVue spectrophotometer (GE Healthcare, www.gelifesciences.com, GE Healthcare, Chalfont St Giles, UK) and by gel electrophoresis. Extracts containing up to 20 μg total RNA were digested using recombinant DNase I (Roche) and subsequently extracted with phenol/chloroform. Absence of genomic DNA was tested by PCR using primers (Table S3) designed to amplify the *SHP2* gene. cDNA synthesis was performed on 500 ng of DNase I-digested RNA using Transcriptor reverse transcriptase (Roche) and oligo$(dT)_{20}$ primers. Quantitative RT-PCR reactions were performed in triplicate in an Mx 3005P cycler (Stratagene, www.stratagene.com, Agilent Technologies, Santa Clara, CA, USA) using Maxima™ SYBR Green/Rox qPCR Master Mix (2×) (Fermentas) with 1 μl cDNA (1:5 diluted) as template and 0.3 μM of forward and reverse primers (Table S3). The following thermal profile was used: 95°C for 10 min, 40 cycles of 95°C for 15 sec, 62–64°C for 30 sec and 72°C for 30 sec. Raw data were analysed using LinRegPCR (Ramakers *et al.*, 2003; Ruijter *et al.*, 2009) to obtain sample $C_T$ values and PCR efficiencies ($E$) for each primer pair. $C_T$ values for triplicate reactions were averaged, and relative quantities of expression for each gene were calculated as $(1+E)^{(c_{TCal}-c_{TSOI})}$, where Cal is the sample with the lowest $C_T$ value, i.e. the highest expression level and SOI is the sample of interest [Correction added on 8 February 2013 after original online publication on 18 January 2013: (1+$E$)($C_{TCal}$ – $C_{TSOI}$) was changed to $(1+E)^{(c_{TCal}-c_{TSOI})}$]. For normalization, relative quantities of expression were divided by a normalization factor, the geometric mean of the relative quantities of expression of the normalization genes glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), ras-related gtp-binding nuclear protein 2 (*RAN2*), polyubiquitin 10 (*UBQ10*) and arabidopsis thaliana tip41-like protein (*TIP41*)-*like*. Inter-run calibration was performed by dividing normalized expression values by the geometric mean of the normalized expression values of three inter-run calibrator samples present on all plates to be compared.

### Ectopic expression of *FUL* and *RPL* orthologues

cDNA of *FUL* and *RPL* orthologues was PCR-amplified from plasmid clones using primers listed in Table S2, and placed under the control of the CaMV 35S promoter in pFGC5941 (www.chromdb.org) using restriction enzymes *Sma*I and *Nco*I. Plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101, and used for plant transformation via floral dip (Bartholmes *et al.*, 2008). *Agrobacteria* were resuspended in infiltration medium (5% sucrose; 0.02% Silwet L-77, http://www.arabidopsis.com/, LEHLE SEEDS, Round Rock, TX, USA) to an $OD_{600}$ of 2.0, and dipping was repeated three times at 1 week intervals. Ripe seeds were collected, and positively transformed $T_0$ offspring plants were selected by spraying seedlings with 0.1% (*A. thaliana*) or 0.01% (*L. campestre*) Basta solution (Aventis CropScience Deutschlang GmbH, Hattersheim, Germany). Ten surviving plants were phenotypically analysed per construct. In case of 35S:*FUL*, indehiscence was further quantified for three plants per construct applying a random impact test following the protocol described by Arnaud *et al.* (2010), with modifications. Shaking was performed in the grinding jar of an MM 400 mixer mill (Retsch, Retsch GmbH Germany, Haan, Germany) using six 5 mm steel balls (approximately 0.5 g each). Fruits were agitated at a frequency of 9 Hz for cumula-

© 2012 The Authors

tive times of 5, 10, 20, 40, 80 and 160 sec. After each interval, fruits for which at least one valve had completely detached were removed and counted as dehisced.

## ACKNOWLEDGEMENTS

We thank Ulrike Coja for technical assistance, the staff of the Botanical Garden Osnabrück for plant cultivation, and Lucille Schmieding for correcting style and grammar. We appreciated access to the *in situ* facilities of the Zachgo laboratory (Department of Biology/Chemistry, University of Osnabrück, Germany). Many thanks also to Pia Nutt and Dajana Lobbes for help with *in situ* hybridization analyses, to Mariana Mondragón-Palomino for introduction to quantitative RT-PCR, to Lydia Gramzow for support with bioinformatics questions, to Domenica Schnabelrauch (Departmant of Entomology, MPI for Chemical Ecology, Jena, Germany) for her sequencing efforts, and to Nicolas Arnaud and Robert Sablowski (Department of Cell & Developmental Biology, John Innes Centre, Norwich, UK) for valuable information. We are also very grateful to two anonymous reviewers for their helpful comments on a previous version of the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft to K.M. (MU 1137/8-1) and G.T. (TH 417/6-1), and by a grant from the Universitätsgesellschaft Osnabrück to K.M.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

**Figure S1.** Gene phylogenies.

**Figure S2.** Southern hybridization.

**Figure S3.** Amino acid alignments.

**Figure S4.** Northern hybridization.

**Figure S5.** 35S:*RPL* phenotypes.

**Figure S6.** *Shl* sequences.

**Table S1.** Accession numbers.

**Table S2.** Primer details.

**Table S3.** Quantitative RT–PCR primers.

## REFERENCES

**Alonso-Cantabrana, H., Ripoll, J.J., Ochando, I., Vera, A., Ferrandiz, C. and Martinez-Laborda, A.** (2007) Common regulatory networks in leaf and fruit patterning revealed by mutations in the *Arabidopsis ASYMMETRIC LEAVES1* gene. *Development*, **134**, 2663–2671.

**Al–Shehbaz, I.A. and Mummenhoff, K.** (2011) *Stubendorffia* and *Winklera* belong to the expanded *Lepidium* (Brassicaceae). *Edinb J. Bot.* **68**, 165–171.

**Al–Shehbaz, I.A., Mummenhoff, K. and Appel, O.** (2002) *Cardaria*, *Coronopus*, and *Stroganowia* are united with *Lepidium* (Brassicaceae). *Novon*, **12**, 5–11.

**Appel, O. and Al–Shehbaz, I.A.** (2003) Cruciferae. In *Families and Genera of Vascular Plants*, Vol. V (Kubitzki, K. and Bayer, C., eds). Berlin/Heidelberg: Springer-Verlag, pp. 75–174.

**Arnaud, N., Girin, T., Sorefan, K., Fuentes, S., Wood, T.A., Lawrenson, T., Sablowski, R. and Ostergaard, L.** (2010) Gibberellins control fruit patterning in *Arabidopsis thaliana*. *Genes Dev.* **24**, 2127–2132.

**Arnaud, N., Lawrenson, T., Ostergaard, L. and Sablowski, R.** (2011) The same regulatory point mutation changed seed-dispersal structures in evolution and domestication. *Curr. Biol.* **21**, 1–5.

**Bao, X.Z., Franks, R.G., Levin, J.Z. and Liu, Z.C.** (2004) Repression of *AGAMOUS* by *BELLRINGER* in floral and inflorescence meristems. *Plant Cell*, **16**, 1478–1489.

**Bartholmes, C., Nutt, P. and Theißen, G.** (2008) Germline transformation of Shepherd's purse (*Capsella bursa-pastoris*) by the 'floral dip' method as a tool for evolutionary and developmental biology. *Gene*, **409**, 11–19.

**Bhatt, A.A., Etchells, J.P., Canales, C., Lagodienko, A. and Dickinson, H.** (2004) *VAAMANA* – a BEL1-like homeodomain protein, interacts with KNOX proteins BP and STM and regulates inflorescence stem growth in *Arabidopsis*. *Gene*, **328**, 103–111.

**Bowman, J.L., Smyth, D.R. and Meyerowitz, E.M.** (1989) Genes directing flower development in *Arabidopsis*. *Plant Cell*, **1**, 37–52.

**Byrne, M.E., Groover, A.T., Fontana, J.R. and Martienssen, R.A.** (2003) Phyllotactic pattern and stem cell fate are determined by the *Arabidopsis* homeobox gene *BELLRINGER*. *Development*, **130**, 3941–3950.

**Cai, S.Q. and Lashbrook, C.C.** (2008) Stamen abscission zone transcriptome profiling reveals new candidates for abscission control: enhanced retention of floral organs in transgenic plants overexpressing *Arabidopsis ZINC FINGER PROTEIN2*. *Plant Physiol.* **146**, 1305–1321.

**Chen, X.M.** (2004) A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science*, **303**, 2022–2025.

**Cole, M., Nolte, C. and Werr, W.** (2006) Nuclear import of the transcription factor *SHOOT MERISTEMLESS* depends on heterodimerization with BLH proteins expressed in discrete sub-domains of the shoot apical meristem of *Arabidopsis thaliana*. *Nucleic Acids Res.* **34**, 1281–1292.

**Couvreur, T.L.P., Franzke, A., Al–Shehbaz, I.A., Bakker, F.T., Koch, M.A. and Mummenhoff, K.** (2010) Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). *Mol. Biol. Evol.* **27**, 55–71.

**Dinneny, J.R. and Yanofsky, M.F.** (2005) Drawing lines and borders: how the dehiscent fruit of Arabidopsis is patterned. *BioEssays*, **27**, 42–49.

**Dinneny, J.R., Yadegari, R., Fischer, R.L., Yanofsky, M.F. and Weigel, D.** (2004) The role of *JAGGED* in shaping lateral organs. *Development*, **131**, 1101–1110.

**Dinneny, J.R., Weigel, D. and Yanofsky, M.F.** (2005) A genetic framework for fruit patterning in *Arabidopsis thaliana*. *Development*, **132**, 4687–4696.

**Favaro, R., Pinyopich, A., Battaglia, R., Kooiker, M., Borghi, L., Ditta, G., Yanofsky, M.F., Kater, M.M. and Colombo, L.** (2003) MADS-box protein complexes control carpel and ovule development in *Arabidopsis*. *Plant Cell*, **15**, 2603–2611.

**Ferrandiz, C., Gu, Q., Martienssen, R. and Yanofsky, M.F.** (2000a) Redundant regulation of meristem identity and plant architecture by FRUITFULL, APETALA1 and CAULIFLOWER. *Development*, **127**, 725–734.

**Ferrandiz, C., Liljegren, S.J. and Yanofsky, M.F.** (2000b) Negative regulation of the SHATTERPROOF genes by FRUITFULL during Arabidopsis fruit development. *Science*, **289**, 436–438.

**Flanagan, C.A., Hu, Y. and Ma, H.** (1996) Specific expression of the AGL1 MADS-box gene suggests regulatory functions in Arabidopsis gynoecium and ovule development. *Plant J.* **10**, 343–353.

**Force, A., Lynch, M., Pickett, F.B., Amores, A., Yan, Y.L. and Postlethwait, J.** (1999) Preservation of duplicate genes by complementary, degenerative mutations. *Genetics*, **151**, 1531–1545.

**Franzke, A., Lysak, M.A., Al–Shehbaz, I.A., Koch, M.A. and Mummenhoff, K.** (2011) Cabbage family affairs: the evolutionary history of Brassicaceae. *Trends Plant Sci.* **16**, 108–116.

**Frohman, M.A., Dush, M.K. and Martin, G.R.** (1988) Rapid production of full-lenght cDNAs from rare transcripts – amplification using a single gene-specific oligonucleotide primer. *Proc. Natl Acad. Sci. USA*, **85**, 8998–9002.

**Gasser, C.S. and Simon, M.K.** (2011) Seed dispersal: same gene, different organs. *Curr. Biol.* **21**, R546–R548.

**Gu, Q., Ferrandiz, C., Yanofsky, M.F. and Martienssen, R.** (1998) The FRUITFULL MADS-box gene mediates cell differentiation during Arabidopsis fruit development. *Development*, **125**, 1509–1517.

**Hall, J.C., Sytsma, K.J. and Iltis, H.H.** (2002) Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *Am. J. Bot.* **89**, 1826–1842.

**Konishi, S., Izawa, T., Lin, S.Y., Ebana, K., Fukuta, Y., Sasaki, T. and Yano, M.** (2006) An SNP caused loss of seed shattering during rice domestication. *Science*, **312**, 1392–1396.

**Li, C.B., Zhou, A.L. and Sang, T.** (2006) Rice domestication by reducing shattering. *Science*, **311**, 1936–1939.

**Liljegren, S.J., Ditta, G.S., Eshed, H.Y., Savidge, B., Bowman, J.L. and Yanofsky, M.F.** (2000) SHATTERPROOF MADS-box genes control seed dispersal in Arabidopsis. *Nature*, **404**, 766–770.

**Liljegren, S.J., Roeder, A.H.K., Kempin, S.A., Gremski, K., Ostergaard, L., Guimil, S., Reyes, D.K. and Yanofsky, M.F.** (2004) Control of fruit patterning in Arabidopsis by INDEHISCENT. *Cell*, **116**, 843–853.

© 2012 The Authors

**Ma, H., Yanofsky, M.F. and Meyerowitz, E.M.** (1991) *AGL1–AGL6*, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes. *Genes Dev.* **5**, 484–495.

**Meakin, P.J. and Roberts, J.A.** (1990) Dehiscence of fruit in oilseed rape (*Brassica napus* L.). 2. The role of cell wall degrading enzymes and ethylene. *J. Exp. Bot.* **41**, 1003–1011.

**Meakin, P.J. and Roberts, J.A.** (1991) Anatomical and biochemical changes associated with the induction of oilseed rape (*Brassica napus*) pod dehiscence by *Dasineura brassicea* (Winn.). *Ann. Bot.* **67**, 193–197.

**Mummenhoff, K., Bruggemann, H. and Bowman, J.L.** (2001) Chloroplast DNA phylogeny and biogeography of Lepidium (Brassicaceae). *Am. J. Bot.* **88**, 2051–2063.

**Mummenhoff, K., Al–Shehbaz, I.A., Bakker, F.T., Linder, H.P. and Mühlhausen, A.** (2005) Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of southern Africa. *Ann. Mo. Bot. Gard.* **92**, 400–424.

**Mummenhoff, K., Polster, A., Muhlhausen, A. and Theissen, G.** (2009) Lepidium as a model system for studying the evolution of fruit development in Brassicaceae. *J. Exp. Bot.* **60**, 1503–1513.

**Ohno, C.K., Reddy, G.V., Heisler, M.G.B. and Meyerowitz, E.M.** (2004) The *Arabidopsis JAGGED* gene encodes a zinc finger protein that promotes leaf tissue development. *Development*, **131**, 1111–1122.

**Ostergaard, L., Kempin, S.A., Bies, D., Klee, H.J. and Yanofsky, M.F.** (2006) Pod shatter-resistant Brassica fruit produced by ectopic expression of the *FRUITFULL* gene. *Plant Biotechnol. J.* **4**, 45–51.

**Pagnussat, G.C., Yu, H.J., Ngo, Q.A., Rajani, S., Mayalagu, S., Johnson, C.S., Capron, A., Xie, L.F., Ye, D. and Sundaresan, V.** (2005) Genetic and molecular identification of genes required for female gametophyte development and function in Arabidopsis. *Development*, **132**, 603–614.

**Pinyopich, A., Ditta, G.S., Savidge, B., Liljegren, S.J., Baumann, E., Wisman, E. and Yanofsky, M.F.** (2003) Assessing the redundancy of MADS-box genes during carpel and ovule development. *Nature*, **424**, 85–88.

**Rajani, S. and Sundaresan, V.** (2001) The Arabidopsis myc/bHLH gene *ALCATRAZ* enables cell separation in fruit dehiscence. *Curr. Biol.* **11**, 1914–1922.

**Ramakers, C., Ruijter, J.M., Deprez, R.H.L. and Moorman, A.F.M.** (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci. Lett.* **339**, 62–66.

**Ripoll, J.J., Roeder, A.H.K., Ditta, G.S. and Yanofsky, M.F.** (2011) A novel role for the floral homeotic gene *APETALA2* during Arabidopsis fruit development. *Development*, **138**, 5167–5176.

**Roeder, A.H.K., Ferrandiz, C. and Yanofsky, M.F.** (2003) The role of the REPLUMLESS homeodomain protein in patterning the Arabidopsis fruit. *Curr. Biol.* **13**, 1630–1635.

**Ruijter, J.M., Ramakers, C., Hoogaars, W.M.H., Karlen, Y., Bakker, O., van den Hoff, M.J.B. and Moorman, A.F.M.** (2009) Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res.* **37**, e45.

**Savidge, B., Rounsley, S.D. and Yanofsky, M.F.** (1995) Temporal relationships between the transcription of two *Arabidopsis* MADS box genes and the floral organ identity genes. *Plant Cell*, **7**, 721–733.

**Sawa, S., Watanabe, K., Goto, K., Kanaya, E., Morita, E.H. and Okada, K.** (1999) *FILAMENTOUS FLOWER*, a meristem and organ identity gene of *Arabidopsis*, encodes a protein with a zinc finger and HMG-related domains. *Genes Dev.* **13**, 1079–1088.

**Siegfried, K.R., Eshed, Y., Baum, S.F., Otsuga, D., Drews, G.N. and Bowman, J.L.** (1999) Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis*. *Development*, **126**, 4117–4128.

**Smith, H.M.S. and Hake, S.** (2003) The interaction of two homeobox genes, *BREVIPEDICELLUS* and *PENNYWISE*, regulates internode patterning in the *Arabidopsis* inflorescence. *Plant Cell*, **15**, 1717–1727.

**Smyth, D.R., Bowman, J.L. and Meyerowitz, E.M.** (1990) Early flower development in Arabidopsis. *Plant Cell*, **2**, 755–767.

**Sorefan, K., Girin, T., Liljegren, S.J., Ljung, K., Robles, P., Galvan-Ampudia, C.S., Offringa, R., Friml, J., Yanofsky, M.F. and Ostergaard, L.** (2009) A regulated auxin minimum is required for seed dispersal in Arabidopsis. *Nature*, **459**, 583–586.

**Spence, J., Vercher, Y., Gates, P. and Harris, N.** (1996) 'Pod shatter' in *Arabidopsis thaliana*, *Brassica napus* and *B. juncea*. *J. Microsc.* **181**, 195–203.

**Wagner, M. and Mitchell-Olds, T.** (2011) Repeated phenotypic changes highlight molecular targets of convergent evolution. *Genome Biol.* **12**, 124.

**Warwick, S.I., Mummenhoff, K., Sauder, C.A., Koch, M.A. and Al–Shehbaz, I.A.** (2010) Closing the gaps: phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS region. *Plant Syst. Evol.* **285**, 209–232.

**Western, T.L. and Haughn, G.W.** (1999) *BELL1* and *AGAMOUS* genes promote ovule identity in *Arabidopsis thaliana*. *Plant J.* **18**, 329–336.

**Wu, H., Mori, A., Jiang, X.S., Wang, Y.X. and Yang, M.** (2006) The INDEHISCENT protein regulates unequal cell divisions in Arabidopsis fruit. *Planta*, **224**, 971–979.

**Zachgo, S.** (2002) *In situ* hybridization. In *Molecular Plant Biology: A Practical Approach*, Vol. 2 (Gillmartin, P. and Bowler, C., eds). Oxford, UK: IRL Press, pp. 41–63.

© 2012 The Authors

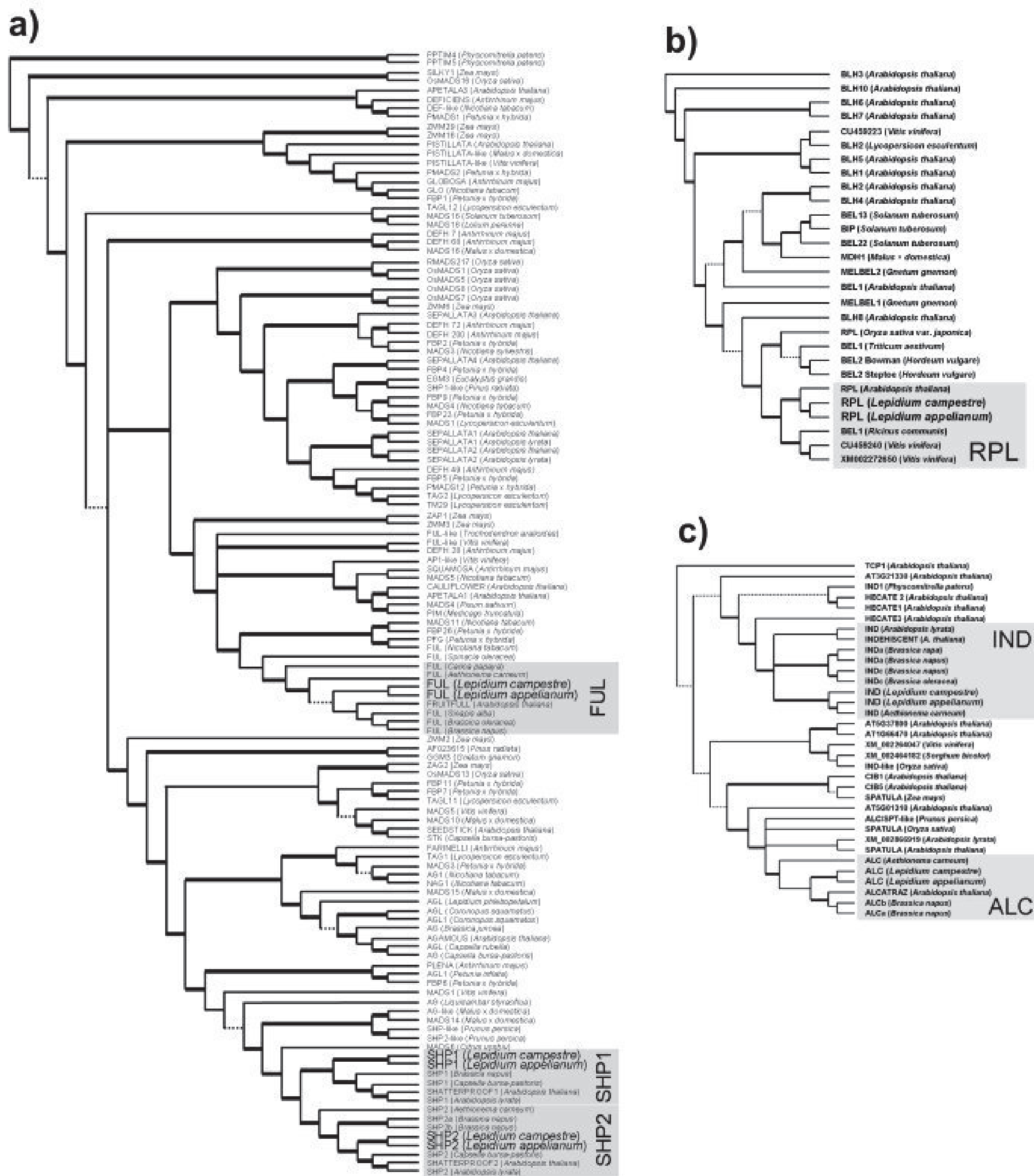


**Figure S1 - Phylogenetic relationships of *Lepidium* and *Arabidopsis* fruit developmental genes based on Bayesian analysis:** *Lepidium* genes (highlighted in grey) are sister to those of other Brassicaceae and thus seem to represent orthologs of *Arabidopsis* genes. (a) MADS-box genes including *SHATTERPROOF1,2* and *FRUITFULL*, (b) BELL homeobox genes including *REPLUMLESS*, and (c) bHLH genes including *INDEHISCENT* and *ALCATRAZ*. Thick branches indicate posterior probability (PP) values > 0.95, PP values < 0.80 are indicated by dashed lines.

Nucleotide sequences were conceptionally translated into amino acids sequences, aligned using the ClustalW software (Larkin *et al.,* 2007) and edited manually. Subsequently, the alignment was translated back to nucleotide sequences. Nucleotide sequence similarities (*p*-distances) and amino acid similarities and identities were calculated in PAUP* 4.0 b 10 (Swofford 2003) and MatGAT 2.01 (Campanella *et al.,* 2003), respectively. Best substitution models were established using Mrmodeltest2 (Nyylander 2004). Datasets were implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Bayesian analyses were run for 1.000.000 generations at a sample frequency of 100. The 50% majority rule consensus trees were established after a burn in of 250.000 generations. Ratios of non-synonymous to synonymous substitution rates ($K_a/K_{s,}$ ω) were determined using the KaKs-Calculator (Zhang *et al.,* 2006).

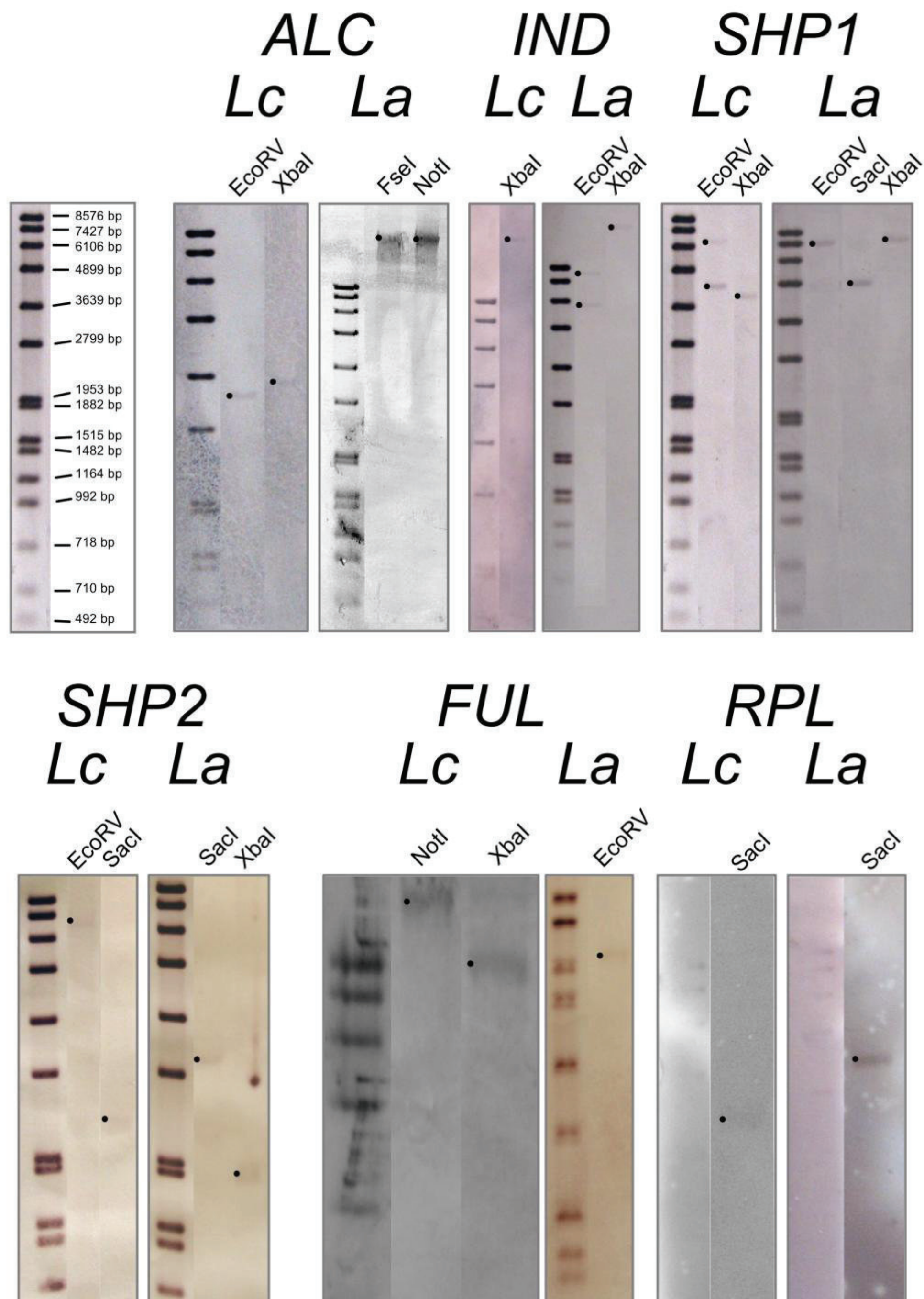


**Figure S2 - Southern blot hybridisation to analyse gene copy number of *Lepidium* fruit developmental gene orthologs:** DNA gel blot analysis was generally conducted as described by Southern (1975) but employing the non-radioactive DIG-labelling system and using Biodyne B membranes (Pall, USA). A few analyses were performed using $^{32}$P labelled probes and Zeta Probe nylon membranes (BioRad, Germany). Probes were labelled using the PCR-DIG-labelling mix (Roche, Germany) and the NEBlot-Kit (NEB, England) using Klenow fragments for radiolabelling of probes. Probe templates were produced using primers given in the supplementary data (Table S2). Hybridisation signals of gene-specific probes on digested genomic DNA of *L. appelianum* and *L. campestre* are indicated by black dots. Fragment sizes of the molecular ladder (DNA Molecular Weight Marker VII, Roche) are indicated. Abbreviations: La, *Lepidium appelianum*; Lc, *Lepidium campestre*. All genes seem to be single copy genes.

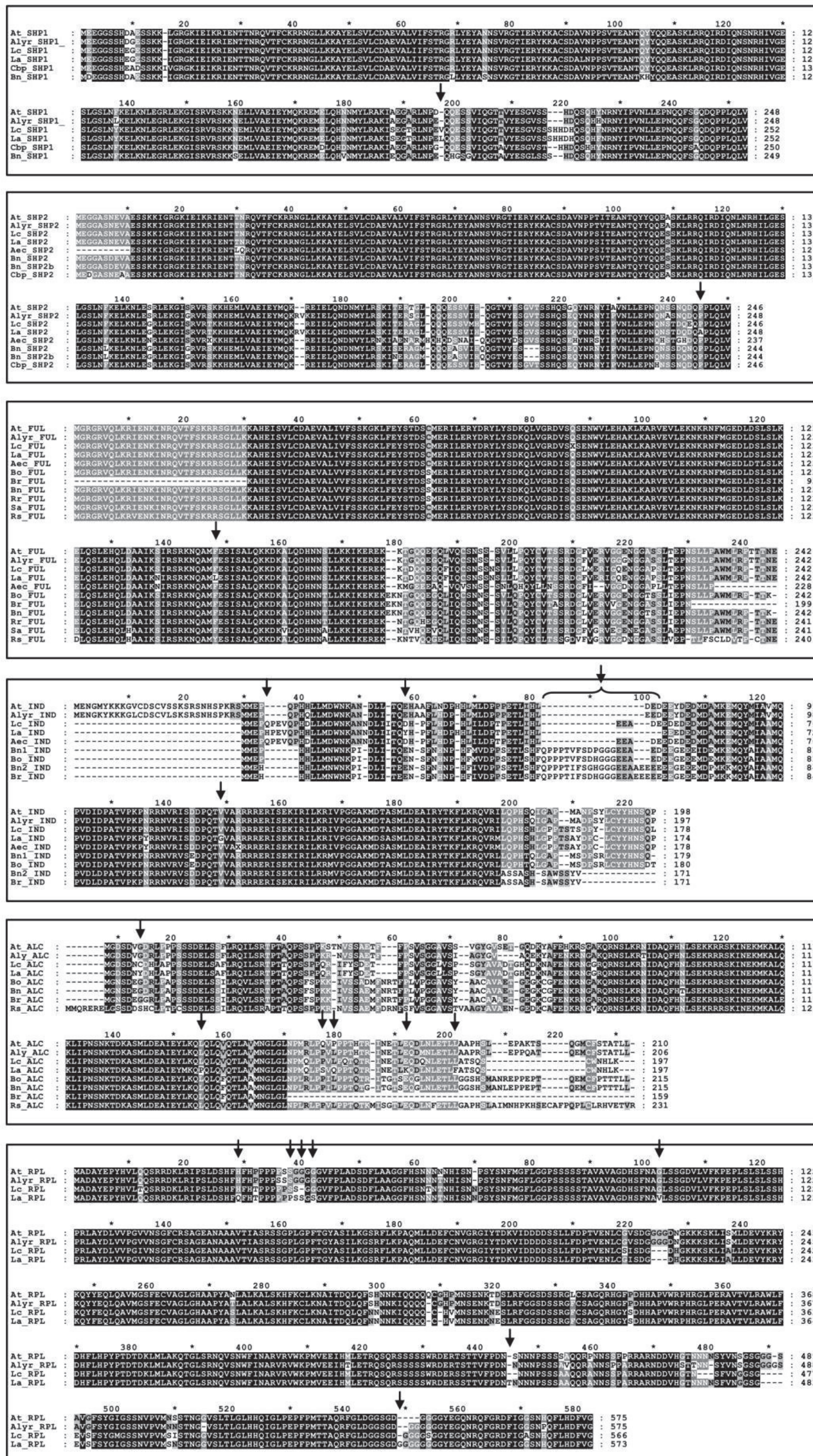

**Figure S3 - Amino acid alignments of genes under study including sequences of further Brassicaceae species with dehiscent fruits:** In total, 20 amino acids changes were detected solely in genes of indehiscent *L. appelianum* and they are marked by arrows.

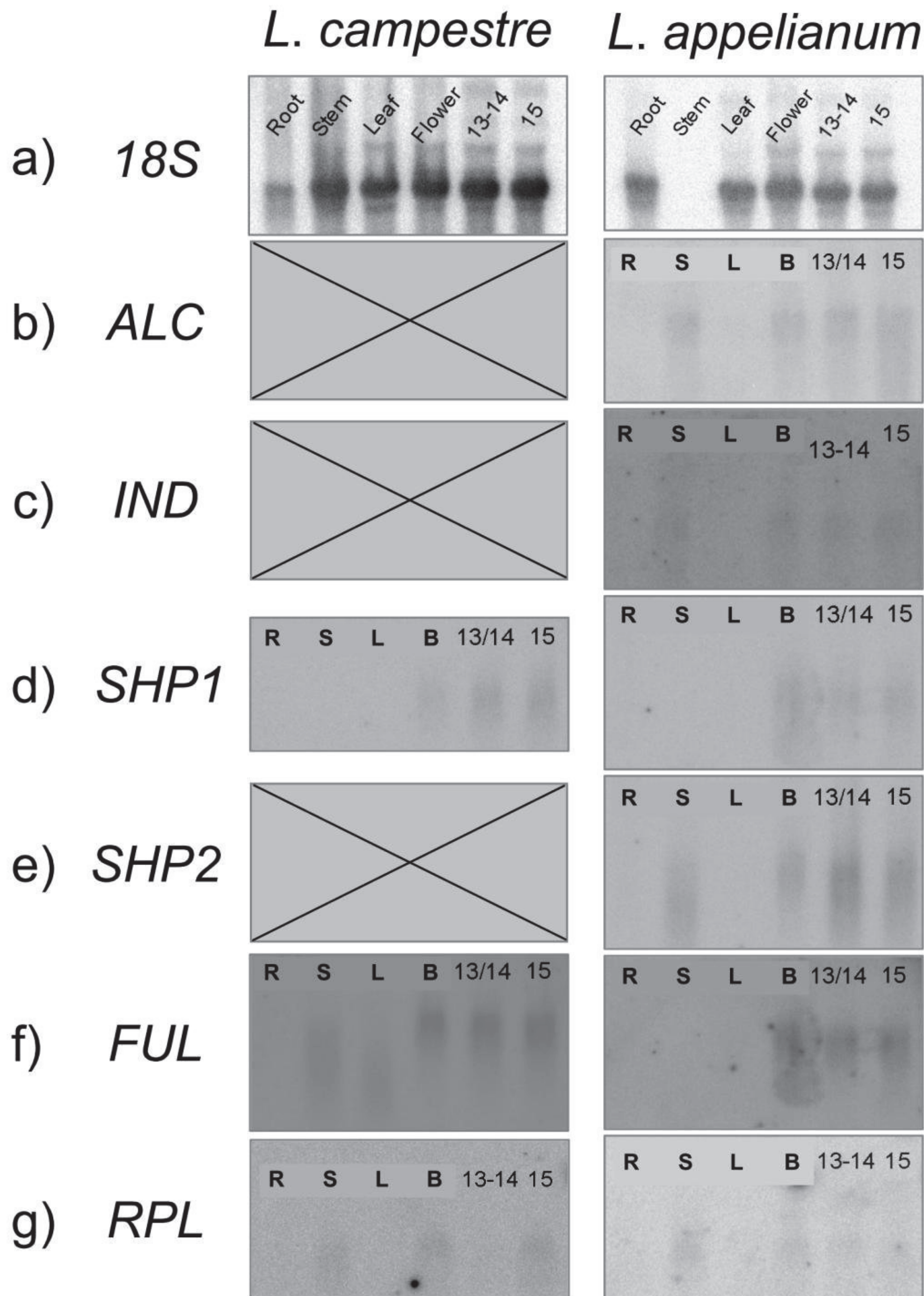


**Figure S4 - Gene expression analysis of *Lepidium* fruit developmental genes in different tissues via Northern blot hybridisation:** RNA was isolated using RNAiso-G+ (Segenetic, Germany). 15 µg of total RNA were separated on a 0.8% (w/v) agarose gel and transferred under denaturing conditions to Zeta Probe nylon membranes (BioRad, Germany) via a vacuum blotter. Probes were radiolabelled with a NEBlot-Kit (NEB, England) using Klenow fragments. Primer information is given in the supplementary data (Table S2). Hybridisation was performed following the manufacturer's instruction manual of the Zeta Probe nylon membrane (BioRad, Germany). An 18S rRNA specific probe has been used as a positive control. Northern hybridisation on distinct plant tissues of *L. campestre* and *L. appelianum* using radiolabelled ($\alpha^{32}$P-(d)CTP) DNA-probes. (a) positive control using an 18S gene specific probe. (b-g) gene expression of putative fruit developmental genes in total RNA of distinct tissues. No hybridisation results could be obtained for *LcALC*, *LcIND*, and *LcSHP2*. Abbreviations: R, Root; S, Stem; L, Leaf; B, buds; 13-14, Flower stage 13-14; 15, Flower stage 15.

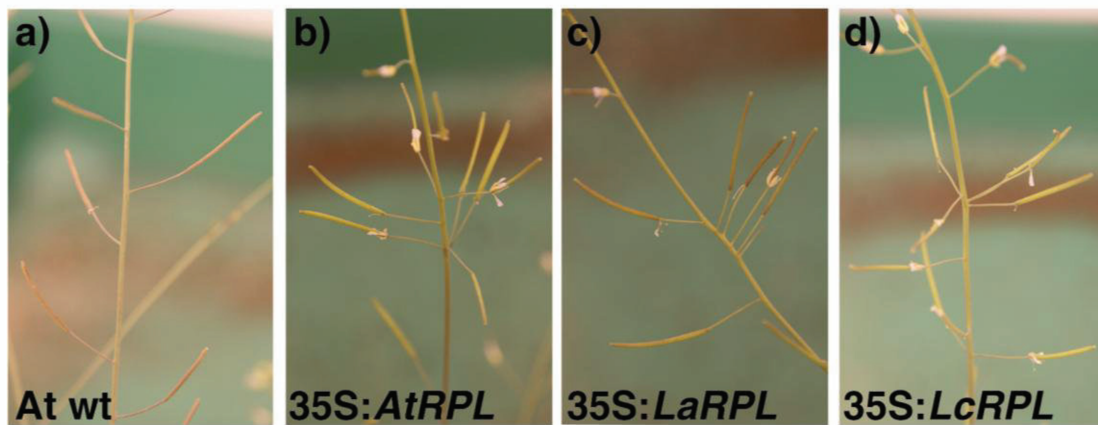


**Figure S5 - Overexpression phenotypes of *RPL* orthologs.** Constitutive expression of *AtRPL* (b), *LaRPL* (c), or *LcRPL* (d) in *A. thaliana* results in alterations in phyllotaxy and irregular elongation of internodes in some transformants compared to the wild-type (a).

| | | | |
|---|---|---|---|
| *Arabidopsis thaliana* | TTACCCGTGATG | C | AATGTAAAA |
| *Arabidopsis lyrata* | TTACCCGTGATG | C | AATGTTAAA |
| *Capsella rubella* | TTACCCGTGATG | C | AATGTAAAA |
| *Lepidium campestre* | TTACCCGTGATG | C | AATGTAAAA |
| *Lepidium appelianum* | TTACCCGTGATG | C | AATGTAAAA |
| | AAATCCGTGATG | T | AATGCGAGA |
| *Brassica rapa* | ATGTCCGTGATG | T | AATGTATAA |
| | AAATCCGTGATG | T | AATGTAAAA |
| *Brassica nigra* | AAATCCGTGATG | T | AATGTAATA |
| *Sinapis alba* | AAACCCGTGATG | T | AATGTGATG |

**Figure S6 - *Shl* sequences of dehiscent Brassicaceae species (green) and indehiscent *Lepidium appelianum* (blue):** The boxed nucleotide, responsible for replum differentiation in *Brassica* and *Sinapis*, seems not to have any effect on the evolution of indehiscence in *Lepidium appelianum*. All sequences except that of *L. appelianum* are from (Arnaud *et al.,* 2011).

Amplification of the *Shl* region was performed using Phusion™ DNA polymerase. Specific primers with an optimised annealing temperature of 52°C were designed basing on sequence information from *Arabidopsis lyrata, A. thaliana* and *Capsella rubella* (accessions given in Table S1). PCR products of about 600 bp were gel eluted using NucleoSpin® Gel and PCR Clean-up (Macherey & Nagel) and directly sequenced.

**Table S1** GenBank accession numbers of all genes used for this manuscript.

| Species | Gene name | GenBank number |
|---|---|---|
| *Aethionema carneum* B.Fedtsch. | *ALC* [1] | FR727242 |
| | *FRUITFULL* [1] | FR727244 |
| | *IND* [1] | FR727243 |
| | *SHATTERPROOF2* [1] | FR727241 |
| *Antirrhinum majus* L. | *DEFICIENS* [2] | X62810 |
| | *DEHF7* [2] | AJ311828 |
| | *DEFH28* [2] | AY040247 |
| | *DEFH49* [2] | AJ311828 |
| | *DEFH68* [2] | AJ311827 |
| | *DEFH72* [2] | X95468 |
| | *DEFH200* [2] | X95469 |
| | *FARINELLI* [2] | AJ239057 |
| | *GLOBOSA* [2] | X68831 |
| | *PLENA* [2] | S53900 |
| | *SQUAMOSA* [2] | X63701 |
| *Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz | *IND* [2] | XM_002872923 |
| | *SEPALLATA1* [2] | AY727598 |
| | *SEPALLATA2* [2] | AY727621 |
| | *SHATTERPROOF1* [2] | AY727645 |
| | *SHATTERPROOF2* [2] | AY727670 |
| | *Shl* [2] | ti\|1543093320 |
| | [2] | XM_002866919 |
| *Arabidopsis thaliana* (L.) Heynh. | *AGAMOUS* [2] | AT4G18960 |
| | *ALCATRAZ* [2] | AT5G67110 |
| | *APETALA1* [2] | AT1G69120 |
| | *APETALA3* [2] | AT3G54340 |
| | *BEL1* [2] | AT5G41410 |
| | *BLH1* [2] | AT2G35940 |
| | *BLH2* [2] | AT4G36870 |
| | *BLH3* [2] | AT1G75410 |
| | *BLH4* [2] | AT2G23760 |
| | *BLH5* [2] | AT2G27220 |
| | *BLH6* [2] | AT4G34610 |
| | *BLH7* [2] | AT2G16400 |
| | *BLH8* [2] | AT2G27990 |
| | *BLH10* [2] | AT1G19700 |
| | *CAULIFLOWER* [2] | AT1G26310 |
| | *CIB5* [2] | AT1G26260 |
| | *FRUITFUL* [2] | AT5G60910 |
| | *HECATE1* [2] | AT5G67060 |
| | *HECATE2* [2] | AT3G50330 |
| | *HECATE3* [2] | AT5G09750 |
| | *INDEHISCENT* [2] | AT4G00120 |
| | *PISTILLATA* [2] | AT5G20240 |
| | *REPLUMLESS* [2] | AT5G02030 |
| | *SEEDSTICK* [2] | AT4G09960 |
| | *SEPALLATA1* [2] | AT5G15800 |
| | *SEPALLATA2* [2] | AT3G02310 |
| | *SEPALLATA3* [2] | AT1G24260 |
| | *SEPALLATA4* [2] | AT2G03710 |
| | *SHATTERPROOF1* [2] | AT3G58780 |
| | *SHATTERPROOF2* [2] | AT2G42830 |
| | *Shl* [2] | AL162508 |
| | *SPATULA* [2] | AT4G36930 |
| | *TCP1* [2] | AT1G67260 |
| | [2] | AT5G01310 |
| | [2] | AT3G21330 |
| | [2] | AT1G66470 |
| | [2] | AT5G37800 |
| | [2] | AT4G34530 |
| *Brassica juncea* (L.) Czern. | *AGAMOUS* [2] | DQ060334 |
| *Brassica napus L.* | *ALC_A* [2] | Hua *et al*. 2009 |
| | *ALC_C* [2] | Hua *et al*. 2009 |
| | *FRUITFUL* [2] | DQ414534 |
| | *IND_A* [2] | HB416515 |
| | *IND_C* [2] | HB416517 |
| | *SHATTERPROOF1* [2] | AF226865 |
| | *SHATTERPROOF2_A* [2] | EU424342 |
| | *SHATTERPROOF2_B* [2] | EU424343 |
| *Brassica oleracea L.* | *FRUITFUL* [2] | AJ505844 |
| | *IND* [2] | GU386356 |
| *Brassica rapa* ssp. *pekinensis* | *IND* [2] | GU386357 |
| *Capsella bursa-pastoris* (L.) Medik. | *AGAMOUS*-like_A [2] | EU551772 |
| | *SEEDSTICK*_A [2] | EU662264 |
| | *SHATTERPROOF1*_A [2] | EU662256 |
| | *SHATTERPROOF2*_A [2] | EU662260 |
| *Capsella rubella* Reut. | *AGAMOUS*-like [2] | AY277679 |
| | *Shl* [2] | ti\|2141861974 |
| | *Shl* [2] | ti\|2141999946 |
| *Carica papaya* L. | *FRUITFUL* [2] | EU141967 |
| *Citrus unshiu* (Swingle) Marcow. | *CitMADS6* [2] | AB218613 |
| *Coronopus squamatus* (Forssk.) Asch. | *AGAMOUS*-like [2] | AY277684 |
| | *AGAMOUS*-like [2] | AY277685 |
| *Eucalyptus grandis* W. Hill | *EGM3* [2] | AF029977 |
| *Gnetum gnemon* L. | *GGM3* [2] | AJ132209 |
| | *MELBEL1* [2] | AJ318871 |
| | *MELBEL2* [2] | AJ318872 |
| *Hordeum vulgare* L. | *BEL2* Bow [2] | GQ853057 |
| | *BEL2* Step [2] | GQ853058 |
| *Lepidium campestre* (L.) R. Br. | *18S* [1] | FR728673 |
| | *ALC* [1] | FR727240 |
| | *APETALA2* [1] | JX674032 |
| | *FRUITFUL* [1] | FR727237 |
| | *GAPDH* [1] | FR728677 |
| | *IND* [1] | FR727239 |
| | *RAN2* [1] | FR728678 |
| | *REPLUMLESS* [1] | FR727238 |
| | *SHATTERPROOF1* [1] | FR727235 |
| | *SHATTERPROOF2* [1] | FR727236 |
| | *Tip41 like* [1] | FR728679 |
| | *UBQ10* [1] | FR733641 |
| *Lepidium appelianum* Al-Shehbaz | *18S* [1] | FR728672 |
| | *ALC* [1] | FR727234 |
| | *APETALA2* [1] | JX674031 |
| | *FRUITFUL* [1] | FR727231 |
| | *GAPDH* [1] | FR728674 |
| | *IND* [1] | FR727233 |
| | *RAN2* [1] | FR728675 |
| | *REPLUMLESS* [1] | FR727232 |
| | *SHATTERPROOF1* [1] | FR727229 |
| | *SHATTERPROOF2* [1] | FR727230 |
| | *TIPlike41* [1] | FR728676 |
| | *UBQ10* [1] | FR733640 |
| *Lepidium phlebopetalum* F.Muell. | *AGAMOUS*-like [2] | AY277686 |
| *Liquidambar styraciflua* L. | *AGAMOUS* [2] | AF103903 |
| *Lolium perenne* L. | *MADS16* [2] | DQ110011 |
| *Lycopersicon esculentum* Mill. | *BL2* [2] | AF375966 |
| | *MADS1* [2] | AY294329 |
| | *TAG1* [2] | L26295 |
| | *TAG2* [2] | AY098738 |
| | *TAGL11* [2] | AY098736 |
| | *TAGL12* [2] | AY09873 |
| | *TM29* [2] | AJ302015 |
| *Malus x domestica* Baumg. | *AG*-like [2] | AF401637 |
| | *MADS10* [2] | AJ000762 |
| | *MADS14* [2] | AJ251117 |
| | *MADS15* [2] | AJ251118 |
| | *MADS16* [2] | AB370212 |
| | *MDH1* [2] | AF053769 |
| | *PI*-like [2] | AJ291490 |
| *Medicago truncatula* Gaertn. | *PIM* [2] | DQ139345 |
| *Nicotiana tabacum* L. | *AG1* [2] | L23925 |
| | *DEF* [2] | X96428 |
| | *FUL* [2] | DQ534202 |
| | *GLO* [2] | X67959 |
| | *MADS4* [2] | AF068723 |
| | *MADS5* [2] | AF068724 |
| | *MADS11* [2] | AF385746 |
| | *NAG1* [2] | L23925 |
| *Nicotiana sylvestris* Speg. | *MADS3* [2] | AF068722 |
| *Oryza sativa* L. | *IND* [2] | NM_001057202 |
| | *MADS16* [2] | AF077760 |
| | *OsMADS1* [2] | AF204063 |
| *Oryza sativa* L. | *OsMADS5* [2] | U78890 |
| | *OsMADS7* [2] | U78891 |
| | *OsMADS8* [2] | U78892 |
| | *OsMADS13* [2] | AF151693 |
| | *RMADS217* [2] | AY551922 |
| | *RPL* [2] | Os01g0848400 |
| | *SPATULA* [2] | AC069158 |
| *Petunia inflata* R.E.Fr. | *AGL1* [2] | L33973 |
| *Petunia* x *hybrida* E. Vilm. | *FBP1* [2] | M91190 |
| | *FBP2* [2] | M91666 |
| | *FBP4* [2] | AF335234 |
| | *FBP5* [2] | AF335235 |
| | *FBP6* [2] | X68675 |
| | *FBP7* [2] | X81651 |
| | *FBP9* [2] | AF335236 |
| | *FBP11* [2] | X81852 |
| | *FBP23* [2] | AF335241 |
| | *FBP26* [2] | AF176783 |
| | *MADS3* [2] | X72912 |
| | *PFG* [2] | AF176782 |
| | *PMADS1* [2] | DQ539416 |
| | *PMADS2* [2] | X69947 |
| | *PMADS12* [2] | AY370527 |
| *Physcomitrella patens* (Hedw.) Bruch & Schimp. | *IND1* [2] | EF156400 |
| | *PPTIM4* [2] | XM_001775895 |
| | *PPTIM5* [2] | XM_001775855 |
| *Pinus radiata* D. Don. | *MADS* [2] | AF023615 |
| | *SHP1*-like [2] | U42399 |
| *Pisum sativum* L. | *MADS4* [2] | AF461740 |
| *Prunus persica* (L.) Batsch. | *SHP*-like [2] | DQ777635 |
| | *ALC/SPT*-like [2] | ADG56590 |
| | *SHP2* [2] | EU072119 |
| *Ricinus communis* L. | *BEL1* [2] | XM_002529809 |
| *Solanum tuberosum* L. | *BEL13* [2] | AF406699 |
| | *BEL22* [2] | AF406701 |
| | *BIP* [2] | EU352654 |
| | *MADS16* [2] | AY643733 |
| *Sorghum bicolor* (L.) Moench | [2] | XM_002464182 |
| *Spinacia oleracea* L. | *FUL* [2] | EU726486 |
| *Triticunm aestivum* L. | *BEL1* [2] | AB546646 |
| *Trochodendron aralioides* P.F.Siebold & Zucc. | *FUL*-like [2] | EF436258 |
| *Vitis vinifera* L. | *AP1*-like [2] | AY538746 |
| | *FUL*-like [2] | AY538747 |
| | *MADS1* [2] | AF265562 |
| | *MADS5* [2] | AF373604 |
| | *PI*-like [2] | DQ059750 |
| | [2] | CU459223 |
| | [2] | CU459240 |
| | [2] | XM_002272650 |
| | [2] | XM_002264047 |
| *Zea mays* L. | *SILKY1* [2] | AF181479 |
| | *ZAG2* [2] | NM_001111908 |
| | *ZAP1* [2] | NM_001111863 |
| | *ZMM2* [2] | NM_001111456 |
| | *ZMM3* [2] | AF112150 |
| | *ZMM6* [2] | NM_001111683 |
| | *ZMM16* [2] | NM_001111666 |
| | *ZMM29* [2] | NM_001111667 |
| | *SPATULA* [2] | NM_001156937 |

[1] Genes isolated for this study
[2] Sequences taken from GeneBank

**Table S2** Details of primers used for cloning and preparation of probes for Northern and Southern blot hybridisation.

| *Gene name*[a] | *Accession no.* | *Primer sequence* |
|---|---|---|
| *La18S* | FR728672 | 5'-AGGAATTGACGGAAGGGCAC-3'[6] |
| *Lc18S* | FR728673 | 5'-GGACATCTAAGGGCATCACA-3'[6] |
| *LaGAPDH* | FR728674 | 5'-GAAGGACTGGAGAGGTGG-3' |
| *LcGAPDH* | FR728677 | 5'-GCTYGACCTGTTGCACC-3' |
| *LaRAN2* | FR728675 | 5'-GTCATCGTTGGTGATGGAGG-3' |
| *LcRAN2* | FR728678 | 5'-CTCCAGCCAGTTTCCTAGC-3' |
| *LaUBQ10* | FR733640 | 5'-GTGGAGAGTTCTGACACCATTGATAACGTG-3'[1] |
| | | 5'-GCGTGGAGGTATGCAGATCTTCG-3'[7] |
| | | 5'-AAAGAGATAACAGGAACGGAAACATAGT-3'[7] |
| *LcUBQ10* | FR733641 | 5'-GTGGAGAGTTCTGACACCATTGATAACGTG-3'[1] |
| | | 5'-GCGTGGAGGTATGCAGATCTTCG-3'[7] |
| | | 5'-AAAGAGATAACAGGAACGGAAACATAGT-3'[7] |
| *LaTIP41-like* | FR728676 | 5'-GCAMAATGGAAATTCAGRAGCAARCC-3'[7] |
| | | 5'-GGAAGCCTCTGRCTGATGRWGC-3'[7] |
| *LcTIP41-like* | FR728679 | 5'-GCAMAATGGAAATTCAGRAGCAARCC-3'[7] |
| | | 5'-GGAAGCCTCTGRCTGATGRWGC-3'[7] |
| *LaSHP1* | FR727229 | 5'-CGACGCAATGGTCTTCTCAAG-3'[1] |
| | | 5'-GCTTCAGTGACAGAAGGAGGATTG-3'[2] |
| | | 5'-CTCTCACACTGTTGTTGGC-3'[2] |
| | | 5'-CGAGTGGAGAAGATAATAAGAGC-3'[2] |
| | | 5'-GAGGTAAATAATAATAAAATAGC-3'[4] |
| | | 5'-GAATATAATAAGTCGAATTACAG-3'[4] |
| | | 5'-GGTACGAGATTGAATCCAG-3'[5] |
| | | 5'-GTTGAGGGTAGAAACAAG-3'[5] |
| | | 5'-GTCTTGTGTGATGCTGAAG-3'[6] |
| | | 5'-CTCTCAATTGTTGAGGGTAG-3'[6] |
| *LcSHP1* | FR727235 | 5'-CGACGCAATGGTCTTCTCAAG-3'[1] |
| | | 5'-GCTTCAGTGACAGAAGGAGGATTG-3'[2] |
| | | 5'-CTCTCACACTGTTGTTGGC-3'[2] |
| | | 5'-CGAGTGGAGAAGATAATAAGAGC-3'[2] |
| | | 5'- GCTATAGCAGTATCTCTTCAAC-3'[4] |
| | | 5'-GATTATTATAAAAAGCAAAACC-3'[4] |
| | | 5'-GGTACGAGATTGAATCCAG-3'[5] |
| | | 5'-GTTGAGGGTAGAAACAAG-3'[5] |
| | | 5'-GTCTTGTGTGATGCTGAAG-3'[6] |
| | | 5'-CTCTCAATTGTTGAGGGTAG-3'[6] |
| *LaSHP2* | FR727230 | 5'-GAGAGCAGCAAGAAGATAGGG-3'[1] |
| | | 5'-CTCTAGTCTACCTTCGAGGTTC-3'[2] |
| | | 5'-GTGATGGAAGGAGGATTAACG-3'[2] |
| | | 5'-GTGGAGAAGATGACAAGAGC-3'[2] |
| | | 5'-GCAGCAGTTAAGTGGTGCTGAG-3'[4] |
| | | 5'-CTTATATTATTAATCTTTTAGCC-3'[4] |
| | | 5'-CTTCAAGGAACTCAAGAAC-3'[5] |
| | | 5'-GAAGACACACCCGACTCG-3'[5] |
| | | 5'-CCAAGATTACTGAAAGAGC-3'[6] |
| | | 5'-GCCTTGAATAGCTAGAAGG-3'[6] |
| *LcSHP2* | FR727236 | 5'-GAGAGCAGCAAGAAGATAGGG-3'[1] |
| | | 5'-CTCTAGTCTACCTTCGAGGTTC-3'[2] |
| | | 5'-GTGATGGAAGGAGGATTAACG-3'[2] |
| | | 5'-GTGGAGAAGATGACAAGAGC-3'[2] |
| | | 5'-GTAAGAGCAGCAGTTAAGTG-3'[4] |
| | | 5'-GCTATGCATCCAACGCGTTG-3'[4] |
| | | 5'-CTTCAAGGAACTCAAGAAC-3'[5] |
| | | 5'-GAAGACACACCCGACTCG-3'[5] |
| | | 5'-CCAAGATTACTGAAAGAGC-3'[6] |
| | | 5'-CTATGCATCCAACGCGTTG-3'[6] |
| *LaALC* | FR727234 | 5'-CCACCGGTTCTACCACAAACACAG-3'[1] |
| | | 5'-CTGAGGTGGTGATGAAGGTTGAGTTG-3'[2] |
| | | 5'-CTGCCGGAGAAAGGCCGAG-3'[2] |
| | | 5'-CGTCGGAAGAAGATGGAGG-3'[2] |
| | | 5'-GATTAGCCACAGTGTAGAGAGAGTG-3'[4] |
| | | 5'-GTAATAATCATCTCATGTAAATAAATTATT-3'[4] |
| | | 5'-CGTCGGTGATCATCTTGC-3'[5] |
| | | 5'-GTGGAATTGTGCATCAATG-3'[5] |
| | | 5'-YWMATAYAATAGCGAAG-3'[6] |
| | | 5'-CAGACAAGGATCAATGAG-3'[6] |
| | | 5'-CCTCCATCTTCTTCCGACG-3'[7] |
| | | 5'-TGCTCTAAGGTCTCATTGATCCT-3'[7] |
| *LcALC* | FR727240 | 5'-CCACCGGTTCTACCACAAACACAG-3'[1] |
| | | 5'-CTGAGGTGGTGATGAAGGTTGAGTTG-3'[2] |
| | | 5'-CTGCCGGAGAAAGGCCGAG-3'[2] |
| | | 5'-CGTCGGAAGAAGATGGAGG-3'[2] |
| | | 5'-GATTAGCCACAGAGTAGAGAGAG-3'[4] |
| | | 5'-AACTAAATTATTCTTTTATATTCATATAATAGCG-3'[4] |
| | | 5'-CGTCGGTGATCATCTTGC-3'[5] |
| | | 5'-GTGGAATTGTGCATCAATG-3'[5] |
| | | 5'-YWMATAYAATAGCGAAG-3'[6] |
| | | 5'-CAGACAAGGATCAATGAG-3'[6] |
| | | 5'-ATGGGTGATTCTGACGTCGGTGATC-3'[7] |
| | | 5'-TCAAAGCAGAGTGGCTGTGGAAAAGC-3'[7] |
| *LaFUL* | FR727231 | 5'-GCTCAAGGCAAGAGTTGAGG-3'[1] |
| | | 5'-CTTCTGAAGGGCAGATATGGATTC-3'[2] |
| | | 5'-GATAGCAGCATCAAGCTG-3'[2] |
| | | 5'-GATCTTCCCCCATAAAATTCC-3'[2] |
| | | 5'-CATAATACTTTGGTCATTTCAGG-3'[4] |
| | | 5'-GAATCTAATGATAACTTAAGTAC-3'[4] |
| | | 5'-GGGGGAAGATCTTGATTC-3'[5] |
| | | 5'-GAAGCGAATTATTGTGATCC-3'[5] |
| | | 5'-GGAATTTTATGGGGGAAGATC-3'[6] |
| | | 5'-CTACTCATTGGTGGTCGGAC-3'[6] |
| | | 5'- AACCATGGCAGGGTTGTCGTTTCTCTC -3'[10] |
| | | 5'- AACCCGGGAGTGAATCTAATGATAACTTAAGTACG -3'[10] |
| *LcFUL* | FR727237 | 5'-GCTCAAGGCAAGAGTTGAGG-3'[1] |
| | | 5'-CTTCTGAAGGGCAGATATGGATTC-3'[2] |
| | | 5'-GATAGCAGCATCAAGCTG-3'[2] |
| | | 5'-GATCTTCCCCCATAAAATTCC-3'[2] |
| | | 5'-CAGGGTTGTCGTTTCTCTCTC-3'[4] |
| | | 5'-CAATGATGAGTACAAGCCACC-3'[4] |
| | | 5'-GGGGGAAGATCTTGATTC-3'[5] |
| | | 5'-GAAGCGAATTATTGTGATCC-3'[5] |
| | | 5'-GGAATTTTATGGGGGAAGATC-3'[6] |
| | | 5'-CTACTCATTGGTGGTCGGAC-3'[6] |
| | | 5'- AACCATGGCAGGGTTGTCGTTTCTCTC -3'[10] |
| | | 5'- AACCCGGGATGAACTAAACATATATATAAGTTCAATGATGAG -3'[10] |
| *LcIND* | FR727239 | 5'-GGCGTAACGTAAGAATAAGCGACG-3'[1] |
| | | 5'-CCGTCTGAGGATCGTCGCTTATTC-3'[2] |
| | | 5'-CTTAGGGACAGTGGCTGGATCG-3'[2] |
| | | 5'-CAGGCTGCATGGCGGCAATC-3'[2] |
| | | 5'-GGTATGTATAAAAAGAAAGGTGTGTGCGAC-3'[4] |
| | | 5'-CAAATGATGTCGAGCGAGCAAGC-3'[4] |
| | | 5'-GATGCAGTACATGATTGCC-3'[5] |
| | | 5'-CAGGGTTGGGAGTTGTGG-3'[5] |
| | | 5'-CTCCTCATGGATTGGAAC-3'[6] |
| | | 5'-GCTTATTCTTACGTTACGC-3'[6] |
| | | 5'-GTGTGCGACTCTTGTGTCTCGTCC-3'[7] |
| | | 5'-TCAGGGTTGGGAGTTGTGGTAA-3'[7] |
| | | 5'-TCCACCTCCCGAAACCCTAATTC-3'[7] |
| *LaIND* | FR727233 | 5'-CCGTCTGAGGATCGTCGCTTATTC-3'[2] |
| | | 5'-CTTAGGGACAGTGGCTGGATCG-3'[2] |
| | | 5'-CAGGCTGCATGGCGGCAATC-3'[2] |
| | | 5'-GATGCAGTACATGATTGCC-3'[5] |
| | | 5'-CAGGGTTGGGAGTTGTGG-3'[5] |
| | | 5'-CTCCTCATGGATTGGAAC-3'[6] |
| | | 5'-GCTTATTCTTACGTTACGC-3'[6] |
| | | 5'-TCCACCTCCCGAAACCCTAATTC-3'[7] |
| | | 5'-TCAGGGTTGGGAGTTGTGGTAA-3'[7] |
| *LcRPL* | FR727238 | 5'-CAGGTCTCTCCAGAAATCAGG-3'[1] |
| | | 5'-CTGCTACGTTCGTCTCTCCAAGAGG-3'[2] |
| | | 5'-CCAACATATGAATCTCTTCC-3'[2] |
| | | 5'-CTCGTGCGTTTATGAACC-3'[2] |
| | | 5'-CCACCGAGAAATCCCATG-3'[3] |
| | | 5'-GGAGTGGAAGCCACCTGC-3'[3] |
| | | 5'-GAGAGAGGGAATTCGAAG-3'[3] |
| | | 5'-ATGGCTGATGCATACGAGCC-3'[4] |
| | | 5'-GTTTAAAAGTTTCTAAATATACAC-3'[4] |
| | | 5'-GGTTCATAAACGCACGAG-3'[5] |
| | | 5'-CTGACGATTTTGCCCTTCG-3'[5] |
| | | 5'-CGCACGAGTTAGGGTTTG-3'[6] |
| | | 5'-CCTCTGAGCAGTTGTCATTG-3'[6] |
| | | 5'-AACCATGGATGGCTGATGCATACGAGCC-3'[10] |
| | | 5'-AACCCGGGTCATCCTACAAAATCATGTAGAAACTG -3'[10] |
| *LaRPL* | FR727232 | 5'-CAGGTCTCTCCAGAAATCAGG-3'[1] |
| | | 5'-CTGCTACGTTCGTCTCTCCAAGAGG-3'[2] |
| | | 5'-CCAACATATGAATCTCTTCC-3'[2] |
| | | 5'-CTCGTGCGTTTATGAACC-3'[2] |
| | | 5'-CCACCGAGAAATCCCATG-3'[3] |
| | | 5'-GGAGTGGAAGCCACCTGC-3'[3] |
| | | 5'-GAGAGAGGGAATTCGAAG-3'[3] |
| | | 5'-ATGGCTGATGCATACGAGCC-3'[4] |
| | | 5'-AAATTTTGTAGTTTAAAAG-3'[4] |
| | | 5'-GGTTCATAAACGCACGAG-3'[5] |
| | | 5'-CTGACGATTTTGCCCTTCG-3'[5] |
| | | 5'-CGCACGAGTTAGGGTTTG-3'[6] |
| | | 5'-CCTCTGAGCAGTTGTCATTG-3'[6] |
| | | 5'-AACCATGGATGGCTGATGCATACGAGCC-3'[10] |
| | | 5'-AACCCGGGTCATCCTACAAAATCATGTAGAAACTG -3'[10] |
| *LcAP2* | JX674032 | 5'-GTAAACGGGTCGGATCTTTCTC-3'[7] |
| | | 5'- GAAGCCATTKGTCTGCAGCC-3'[7] |
| *LaAP2* | JX674031 | 5'-ATGTGGGATCTAAACGACGCACC-3'[7] |
| | | 5'-GAAGCCATTKGTCTGCAGCC-3'[7] |
| *LepidiumShl* | | 5'-CACYGCAARATCYYGATC-3'[8] |
| | | 5'-CGACTGTACTGTAATAAT CG-3'[8] |
| oligo d(T) primer | | 5'-GACTCGAGTCGACATCTG(T)$_{14}$-3'[9] |
| anchor primer | | 5'- GACTCGAGTCGACATCTG -3'[9] |

a: Based on homology to *Arabidopsis* genes

[1] Primers for 3´RACE
[2] Primers for 5´RACE; 1st: SP1, 2nd: SP2, 3rd: SP3
[3] Second step primers for *RPL* gene
[4] Primers for full length clones; 1st: fwd, 2nd: rev
[5] Primers for Southern probes; 1st: fwd, 2nd: rev
[6] Primers for Northern probes; 1st: fwd, 2nd: rev
[7] *Arabidopsis* gene specific primers; 1st: fwd, 2nd: rev
[8] Primers for amplification of *Lepidium appelianum Shl* sequence; 1st: fwd, 2nd: rev
[9] Primers for reverse transcription and 3´- and 5´RACE analyses
[10] Primers for cloning into pFGC5941

**Table S3** Details of qRT-PCR primers and amplicons for each of the 20 analyzed genes

| *Gene name*[a] | *Accession no.* | *Primer sequence*[b] | *Amplicon length (bp)* | *Mean PCR efficiency*[c] |
|---|---|---|---|---|
| *LaGAPDH* | FR728674 | 5'-GCTATCAAGAAGGAATCTGAAGGCAAAC-3'<br>5'-ACGAAGTCAGTTGAGACAACATCATC-3' | 79 | 0.931 |
| *LcGAPDH* | FR728677 | 5'-CTATCAAGGAGGAATCTGAAGGCAAAC-3'<br>5'-ACGAAGTCAGTTGAGACGACATCATC-3' | 78 | 0.939 |
| *LaRAN2*<br>*LcRAN2* | FR728675<br>FR728678 | 5'-CTGCTGGGATACTGCTGGAC-3'<br>5'-GTAACTTGCTTTGCCTTCACTTGC-3' | 224 | 0.936 |
| *LaUbq10*<br>*LcUbq10* | FR733640<br>FR733641 | 5'-ACCAGCAGCGTCTCATCTTC-3'<br>5'-TTCTGAATGTTGTAGTCGGCTAAGG-3' | 72 | 0.926 |
| *LaTip41 like*<br>*LcTip41 like* | FR728676<br>FR728679 | 5'-GCTTATGAGATTGAGAGAGACGAGAA-3'<br>5'-GGATACCCTTTCGCAGATAGAGAC-3' | 122 | 0.922 |
| *LaALC*<br>*LcALC* | FR727234<br>FR727240 | 5'-GAACCACCTCAAGTAAGTCAAG-3'<br>5'-ACGCTGGCTAATCAATGTC-3' | 76 | 0.916 |
| *LaAP2*<br>*LcAP2* | JX674031<br>JX674032 | 5'- CACTTATTACCCATCAGTTTTTCCCTG-3'<br>5'- CAAGACCACCACCATCTCCTC-3' | 69 | 0.931 |
| *LaFUL* | FR727231 | 5'-AATACTGCGTTACCTCCTCCAGAG-3'<br>5'-AACATCCAAGCCGGAAGAAGAGAG-3' | 108 | 0.942 |
| *LcFUL* | FR727237 | 5'-GATGGTTTGATGGAGAGAATCGGAC-3'<br>5'-CTACTCATTGGTGGTCGGACG-3' | 110 | 0.946 |
| *LaIND*<br>*LcIND* | FR727233<br>FR727239 | 5'-CTCATCTTAGATCCAACTCCTGAAACC-3'<br>5'-GCTTAGGGACAGTGGCTGG-3' | 150 | 0.933 |
| *LaRPL*<br>*LcRPL* | FR727232<br>FR727238 | 5'-TGGACTTGACGGTGGTAGTG-3'<br>5'-CCAATAAAATCTCTCCCAAACTGACG-3' | 89 | 0.917 |
| *LaSHP1* | FR727229 | 5'-AGAAGAGAGAAATGGAGTTGCAGC-3'<br>5'-ACACTTGATTCCTGTTGTAGTTCTGG-3' | 99 | 0.934 |
| *LcSHP1* | FR727235 | 5'-AGAAGAGAGAAATGGAGTTGCAGC-3'<br>5'-ACACTTGATTCCTGTTGTACTTCTGG-3' | 99 | 0.933 |
| *LaSHP2* | FR727230 | 5'-GGTGTGTCTTCTTCTCATCAATCGG-3'<br>5'-GAGGTGCTTGGTCTTGGTCG-3' | 102 | 0.941 |
| *LcSHP2* | FR727236 | 5'-GTGTGTCTTCTTCTCATCAGTCGG-3'<br>5'-GAGGTGGTTGCTCTTGGTCG-3' | 101 | 0.935 |

a: Based on homology to Arabidopsis genes
b: Forward (upper line) and reverse (lower line) primer sequences
c: Average PCR efficiency for a certain amplicon group as calculated by LinRegPCR (11.0)

## 2.4 Manuscript III

**Teresa Lenser and Günter Theißen** (2013) Molecular mechanisms involved in convergent crop domestication. *Trends in Plant Science*, DOI: 10.1016/j.tplants.2013.08.007, *in press*

Review

Cell PRESS

# Molecular mechanisms involved in convergent crop domestication

**Teresa Lenser and Günter Theißen**

Department of Genetics, Friedrich Schiller University Jena, Philosophenweg 12, D-07743 Jena, Germany

**Domestication has helped to understand evolution. We argue that, vice versa, novel insights into evolutionary principles could provide deeper insights into domestication. Molecular analyses have demonstrated that convergent phenotypic evolution is often based on molecular changes in orthologous genes or pathways. Recent studies have revealed that during plant domestication the causal mutations for convergent changes in key traits are likely to be located in particular genes. These insights may contribute to defining candidate genes for genetic improvement during the domestication of new plant species. Such efforts may help to increase the range of arable crops available, thus increasing crop biodiversity and food security to help meet the predicted demands of the continually growing global population under rapidly changing environmental conditions.**

## Crop domestication – a genetic perspective

During the process of crop domestication (see Glossary) and improvement, many morphological and physiological traits underwent dramatic modifications to meet the fastidious needs of humans (for recent reviews, see [1–5]). Some of these modifications, such as an increase in fruit number and size or a reduction of fruit abscission, were necessary to enable efficient cultivation by humans, whereas other changes were brought about merely to satisfy culinary or esthetic preferences, for example, alterations in fruit color, taste, or texture. Similar human demands led to similar adaptations of many domestication traits over a wide range of plant species (Figure 1), thereby providing numerous examples of convergent phenotypic evolution {for simplicity we use the term 'convergence' so as to include 'parallelism' following [6], even though the issue of how to define and determine convergent and parallel evolution is still actively discussed in the literature (see, e.g., [7])}. In this context, a key question is which genetic changes underlie this phenotypic convergence and, more precisely, whether it is mainly mutations at orthologous or distinct genomic loci that are involved. The term 'molecular convergence' is often used in this context if changes at orthologous loci underlie phenotypic convergence and we use it accordingly; however, care must be taken because in other studies the same term sometimes happens to be restricted to identical mutations within the same locus or expanded to include mutations within the same regulatory pathway [8,9]. For natural adaptation in animal systems it is already accepted that convergent phenotypic changes rely strikingly often on mutations at orthologous loci [6,9–11], and recently an elaborate meta-analysis gathering genetic loci of repeated evolution also found this to be the case in plant systems [12]. There has been some lively discussion regarding which factors favor the emergence of these so-called genetic hotspots [13–17]. By contrast, in crop domestication research, the importance of molecular convergence is still a controversial topic [18]. Although an often cited early study attached great importance to the role of molecular convergence [19], subsequent analyses have often dismissed or diminished its relevance because they have revealed some examples of non-orthologous loci at the basis of convergent phenotypic changes [4,20–22].

## Glossary

**Abscission**: controlled shedding of plant organs (e.g., leaves, flowers, fruits) by means of cell–cell separation processes in specialized tissue layers (abscission zones).
***Cis*-regulatory mutation**: mutation within a non-protein coding part of a gene influencing its level of expression.
**Convergence**: independent emergence of the same phenotypic trait in distinct lineages.
**Determinate growth**: naturally self-limited growth, for example, by the formation of a terminal flower at a predetermined developmental stage.
**Domestication**: process of genetic adaptation of wild species to human needs, typically including changes in appearance and lifestyle.
**Fruit dehiscence**: concerted process of fruit opening by cell–cell separation to enable seed dispersal.
**Homology**: similarity owing to common ancestry.
**Indeterminate growth**: plant growth continues indefinitely.
**Molecular convergence**: phenotypic convergence caused by changes at orthologous genetic loci.
**Orthologs**: genes that arose from a common ancestor sequence via speciation.
**Parallelism**: independent emergence of the same phenotypic trait from the same original phenotypic state in distinct lineages.
**Paralogs**: genes that arose from a common ancestor sequence via duplication.
**Pleiotropic effects**: phenomenon in which a single gene influences more than one phenotypic trait.
**Polygenic**: a phenotypic trait is controlled by more than one gene.
**Quantitative trait locus (QTL)**: genomic region containing one or more genes contributing to phenotypic variation of a quantitative trait.
**Standing genetic variation**: a genomic locus has more than one allelic version within a population.
**Stochastic process**: non-deterministic, chance-dependent process whereby progression can only be predicted in terms of probability.
**Vernalization**: induction of flowering through exposure to low temperatures for a certain period of time.

*Corresponding author:* Theißen, G. (guenter.theissen@uni-jena.de).

*Keywords:* crop improvement; genetic hotspots; molecular convergence; molecular evolution; plant domestication.

1360-1385/$ – see front matter

© 2013 Elsevier Ltd. All rights reserved. http://dx.doi.org/10.1016/j.tplants.2013.08.007

Review
Trends in Plant Science xxx xxxx, Vol. xxx, No. x

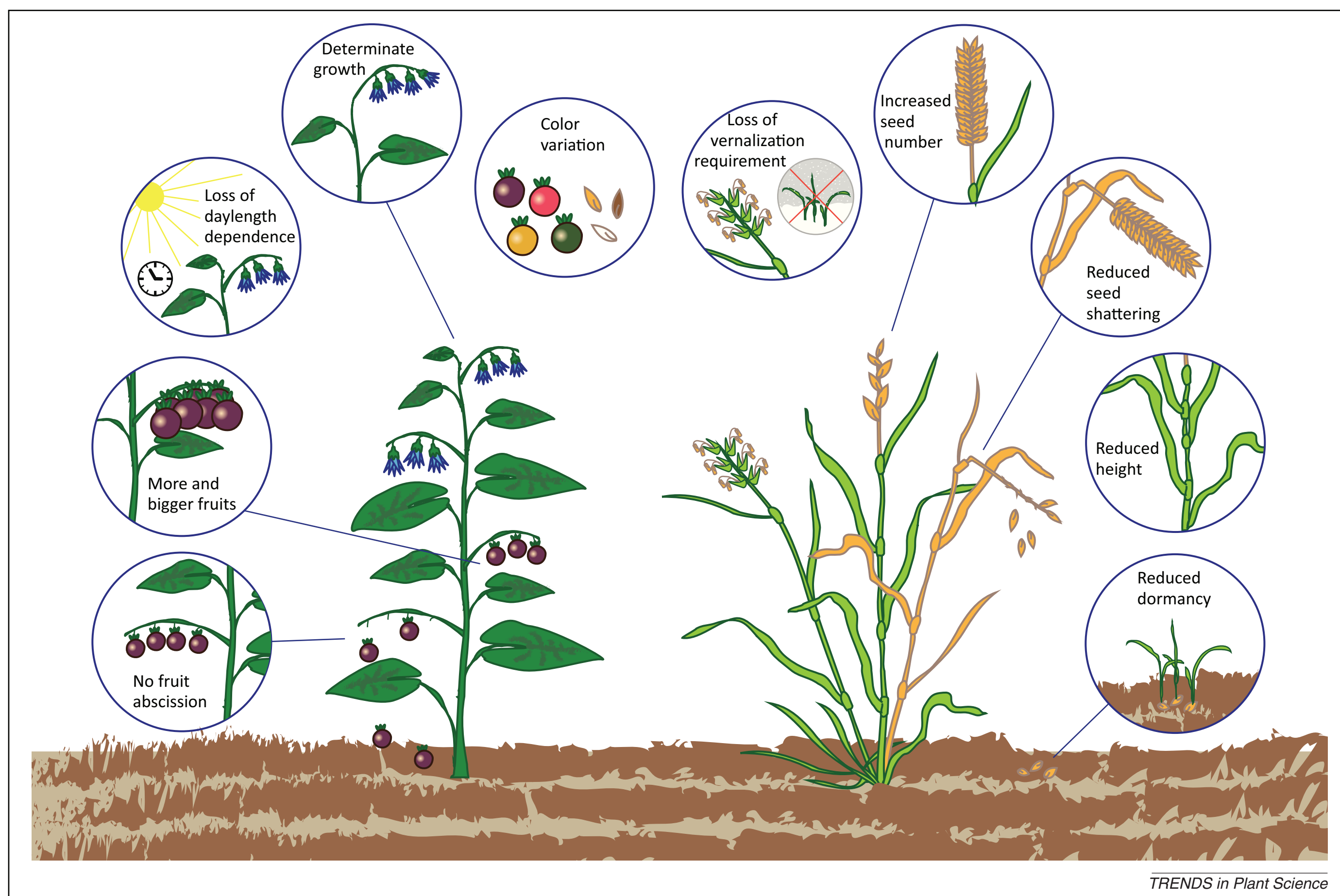


**Figure 1**. Convergent domestication. Convergent phenotypic changes are frequently observed in many different crops because systematic human cultivation often brings about similar demands. Attempts to maximize yield cause selective pressure for an increase in size and number of edible plant parts on the one hand and for a decrease in natural seed and fruit dispersal mechanisms to reduce yield loss on the other hand. Shifts in cultivation area often require changes in day length dependence or in the vernalization requirement and a reduction in seed dormancy is needed for synchronous germination. Small plants with a determinate growth habit are often selected because they are more robust, have a better yield to overall biomass ratio, and are better suited to mechanical harvesting methods. Finally, satisfying esthetic preferences often drives convergent adaptations, a prominent example being changes in color. Stylized examples of the major angiosperm plant lineages from which current crops originated are shown (eudicot, left; monocot, right) featuring traits of typical wild species. Characters that convergently evolved in various domesticated crops are depicted in circles.

In recent years, our knowledge about the genes involved in crop domestication has increased dramatically, enabling more in-depth questions to be asked regarding the molecular basis of domestication in a wide variety of species. In this review, we try to incorporate such recent molecular insights into the framework of genes that are already known to control domestication traits in plants. Unlike previous review articles [1,4,5], we do not distinguish between domestication and improvement genes because classification can be ambiguous. Instead, we equally consider all loci that have been artificially selected to discriminate crops from their wild ancestors as determining factors of domestication. We discuss recent findings that suggest that convergent molecular evolution played an important role in plant domestication and the suggestion that, as postulated for adaptive evolution, certain genes are particularly likely to become the target of domestication-relevant mutations. An understanding of the factors influencing this susceptibility in evolutionary biology might enable the likely course of molecular domestication to be predicted and, thus, might have great potential in the facilitation of future crop domestication and breeding procedures.

## How molecular convergence contributed to crop domestication

Our knowledge of the genetic loci controlling diverse domestication phenotypes in crops is increasing. However, the picture is still far from complete, and the possibility of bias owing to the preferential investigation of candidate genes has to be taken into account when trying to evaluate the importance of molecular convergence in this context [13]. In this section, we concentrate on examining a few selected traits that have been characterized at the molecular level particularly well in several species in order to present a picture of the extent to which molecular convergence might contribute to shaping crop plants.

### *Plant growth*

Controlling plant growth is an important aspect of domestication. Under the influence of systematic nitrogen fertilization, most wild species would grow excessively tall, making them more prone to damage by wind and rain. Moreover, the development of mechanized harvesting methods required the cultivation of plants of defined height and stature. Thus, many crop species were convergently selected for a determinate and ‘dwarfing’ growth

habit that increases yield at the expense of overall biomass. Although the number of effector loci as detected by quantitative trait locus (QTL) studies might differ greatly between species, plant height is a polygenic trait [23]. Some genes, such as *Ghd7* in rice (*Oryza sativa*) or *Q* in wheat (*Triticum aestivum*), have been reported to affect plant height in a certain species but to date have not been reported to influence growth habit in any other crop [4,24]. Despite this seeming plurality in domestication targets, two strategies have been repeatedly applied to cause dwarfing habit in different species: interference with hormone metabolism or signaling [25] and alteration of meristem identity causing determinate growth [26].

Loss-of-function mutations in two distinct genes of the gibberellic acid (GA) metabolic pathway cause dwarfism in rice. A change within the *ent*-kaurene oxidase gene *OsKO2* has been exclusively detected in a single Japanese cultivar [27], whereas at least nine distinct alleles of the *OsGA20 oxidase-2* (*OsGA20ox-2*) gene of varying severity were used for breeding semi-dwarfing varieties during both early domestication and the ‘green revolution’ in the 1960s [28,29]. The orthologous *HvGA20ox-2* gene from barley (*Hordeum vulgare*) has also been identified as an important semi-dwarfing gene in many barley varieties [30]. Contrasting this repeated occurrence of loss-of-function mutations disrupting GA biosynthesis in rice and barley, dwarfism in wheat is caused by independent gain-of-function mutations at two paralogous *Reduced height-B1*/*D1* loci coding GA-regulated growth repressors [1]. The mutant loci both produce proteins that lack an important GA signaling domain that normally directs GA-dependent degradation, thus becoming constitutively active growth repressors [25]. In addition to modulation of GA signaling, interference with brassinosteroid signal transduction and with polar auxin transport was shown to cause semi-dwarfing growth habit in agronomically important varieties of barley or sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*), respectively [31–33].

To date, all the alleles that have been associated with the domestication-related switch from indeterminate to determinate growth habit are characterized by loss-of-function mutations in genes orthologous to the *Arabidopsis* (*Arabidopsis thaliana*) meristem identity gene *TERMINAL FLOWER 1* (*TFL1*) [34–36]. Furthermore, in soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*), four and eight recessive alleles, respectively, were apparently selected independently [37,38], presenting an intriguing example of how orthologous genes can be repeatedly involved in convergent domestication of a given trait.

### *Flowering time*

*TFL1* orthologs are not only involved in defining the growth habit of a plant but have also been reported to induce early flowering in species as varied as barley, pea (*Pisum sativum*), and strawberry (*Fragaria vesca*) [39–41]. In the case of *TFL1*, this simultaneous influence on flowering time and plant height is probably due to its dual function in controlling the length of both the vegetative and floral phase [40]. Flowering time and plant height are also not independent of each other *per se*, because early flowering automatically reduces the time for vegetative growth, thus leading to smaller plants, whereas late flowering increases it resulting in bigger plants. This is reflected by several domestication loci that likewise influence both traits, such as *Q* (wheat), *Ghd7* (rice), and possibly *Vgt1* in maize (*Zea mays*) [4,24,42,43]. Nevertheless, the size reduction resulting from early flowering clearly has to be distinguished from that of plants with dwarfing or determinate growth habit because it does not increase overall yield and has thus to be considered a mere side effect of the adaptation of flowering time.

Adaptation of flowering time is especially important for the global success of a particular crop species because expansion of the geographic range often also means a shift in climatic conditions and in the length of the photoperiod. A common effect observed in domesticated plants is that the onset of flowering becomes less dependent on environmental stimuli such as day length or vernalization, resulting in shorter growth cycles [39,44–46].

The molecular network controlling floral induction is rather complex and the comparative studies performed to date suggest that an ancient core pathway is conserved between distantly related plant lineages but has been modified by the recruitment of family-specific genes or pathways (see [47–49] and references therein). As a consequence of this limited conservation it has been noted that during adaptation of flowering time, molecular convergence occurs more often between closely related species (Figure 2) [49]. In the monocot lineage, for example, the *AP1*-like transcription factor (TF) and vernalization regulator gene *VRN1* was repeatedly found to induce a spring growth habit owing to dominant mutations disrupting potential repressor binding sites [49–51]. A possible candidate for the respective repressor is the zinc finger–CCT domain TF coding gene *VRN2*; loss-of-function mutations within this locus also cause a spring growth habit exclusively in monocots [49]. A member of the pseudo-response regulator (PRR) gene family in *Arabidopsis*, *PRR7*, is known to function in the circadian clock pathway [52]. Nevertheless, domestication-relevant alleles in closely related paralogs have only been reported in crops which are distantly related to *Arabidopsis*; in the closely related cereals rice [53], barley [54,55], wheat [56–58], and sorghum [45], mutations in *PRR37* orthologs influence flowering time by altering the photoperiod response, whereas in sugar beet (*Beta vulgaris* ssp. *vulgaris*) a paralogous *PRR* gene has been involved in the switch from annual to biennial growth habit [59]. By contrast, domestication-related flowering time variation by means of mutations in orthologs of *FLOWERING LOCUS C* (*FLC*) or its upstream regulator *FRIGIDA* (*FRI*) is typical for members of the Brassicaceae lineage [44,60–62].

Adaptation of flowering time in distantly related species has been reported to rely on mutations in genes that are non-homologous but that occupy analogous network positions within the floral induction pathway: for example, *FLC* and *VRN2*, which are both repressors of flowering and become downregulated by vernalization [49]. A similar non-homologous pair is *Ghd7* (rice) and *E1* (soybean), which are both photoperiod-dependent repressors of *FLOWERING LOCUS T* (*FT*) orthologs whose loss-of-function alleles induce early flowering [24,63]. However, some key genes also exist that convergently alter flowering time

Review
Trends in Plant Science xxx xxxx, Vol. xxx, No. x

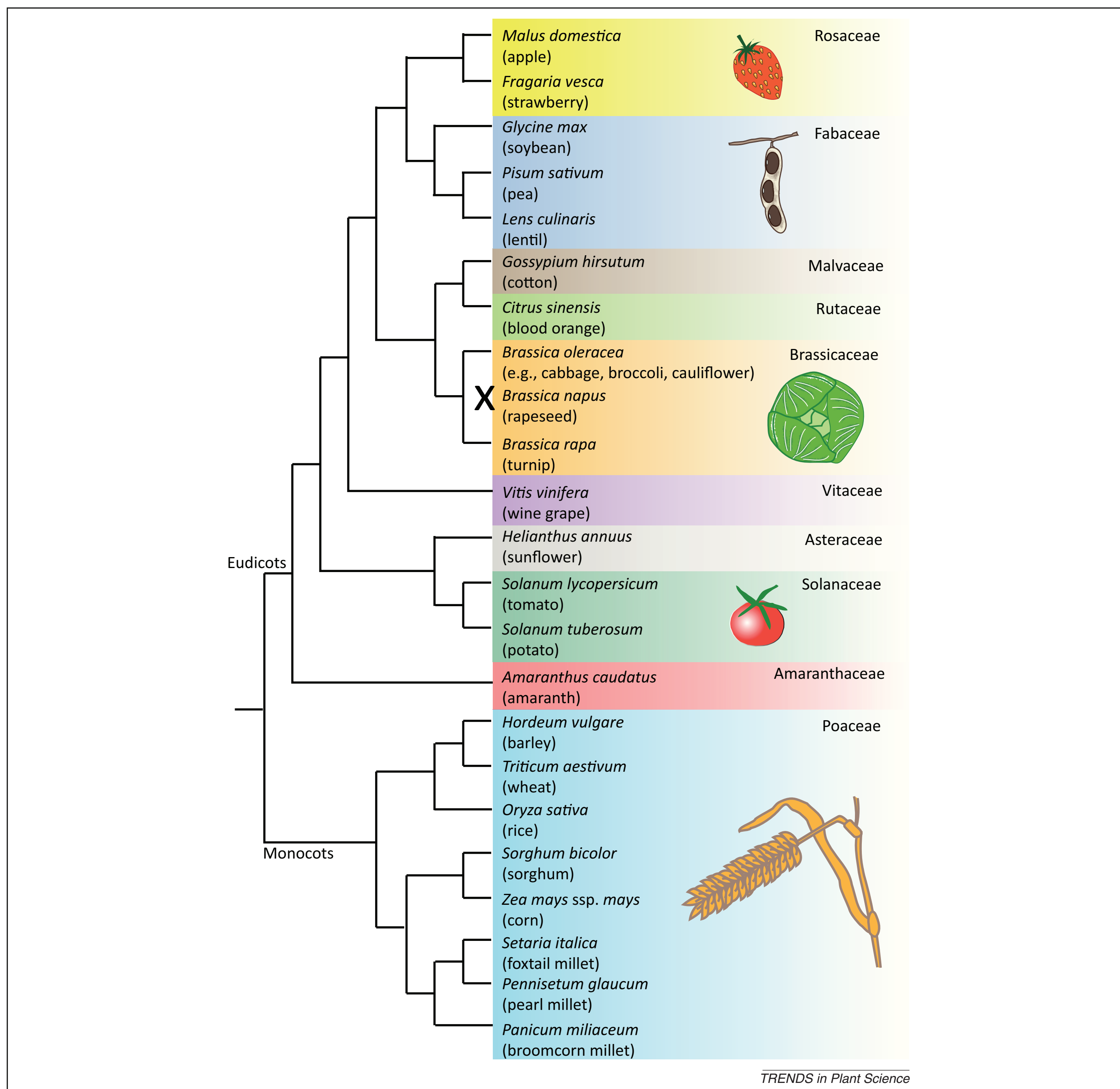


**Figure 2.** Phylogenetic relationship of major crop plants. The topology of the tree is mainly based on data from [110] supplemented with the results of some additional recent studies. Family affiliation is indicated by colors. Branch lengths are not drawn to scale. The '×' represents a hybridization event that happened between *Brassica oleracea* and turnip (*Brassica rapa*) giving rise to rapeseed (*Brassica napus*).

in a more distantly related set of species. Besides the above-mentioned *TFL1* orthologs, loss-of-function mutations in orthologs of the *Arabidopsis* circadian clock regulator gene *EARLY FLOWERING 3* (*ELF3*) have been repeatedly involved in generating photoperiod-insensitive cultivars in grasses and legumes [46,64–66], and orthologs of the highly conserved floral inducer gene *FT* exhibit allelic variants that change flowering time across great phylogenetic distances [67–69].

### *Fruit and seed dispersal*

Given that the evolutionary success of wild plant species relies heavily on their ability to spread their offspring, most of them possess elaborate mechanisms to separate from their fruits and seeds. From an agronomic perspective, this is a highly undesirable trait that hampers harvest and causes considerable yield loss. As a result, there has been a convergent emergence of crop species with reduced seed dispersal capability. Fruit abscission or dehiscence is implemented by cell–cell separation at different anatomical structures, including the base of the spikelet in wheat and barley, the juncture between lemma and pedicel in rice, and the pod valve margins in soybean and *Brassica* species [22,70–72]. Thus, separation happens mostly at non-homologous structures, which appears to be associated with a high level of molecular variability.

Two tightly linked loci (*Btr1* and *Btr2*) controlling the brittle rachis phenotype have been identified in cultivated barley to date, and recessive mutations in either of these loci represent the independent emergence of shatter-resistant barley on two occasions [22,73]. In domesticated wheat species, three loci (*Br1–3*) bear recessive mutations conferring a loss of spikelet disarticulation [21,22]. None of these genes has been molecularly cloned yet and whether wheat and barley loci carry homologous genes is the subject of debate [21,22]. However, some data suggest that they are not orthologous to shattering loci identified in other grasses [22]. Equally unrelated to each other are the genes that suppress pedicel abscission in tomato (*Solanum lycopersicum*) (*jointless* [74]), pod dehiscence in soybean (*qPDH1* [70]), and the major non-shattering locus *sh4*, which is fixed in all rice cultivars [72,75]. Recently, a new shattering resistance locus was identified upstream of the rice gene *LIGULELESS* mediating the formation of closed panicles [76].

To date, only two instances of molecular convergence possibly underlying the domestication of shattering behavior have been reported. Three independent loss-of-function mutations within the YABBY-like TF gene *Sh1* were found to be responsible for a disruption in abscission layer formation and loss of seed shattering in domesticated sorghum; orthologous genes in rice and maize carry mutations that are similarly associated with shattering reduction [77]. Remarkably, single nucleotide substitutions at the same position in an upstream regulatory sequence of genes orthologous to the *Arabidopsis* gene *REPLUMLESS*, which are known to disrupt abscission layer formation in *japonica* rice were also found to alter fruit morphology in dehiscence-relevant tissues in different Brassicaceae species [78,79].

## Why molecular domestication repeats itself

The question of whether or not crop plants develop similar phenotypic traits via changes at orthologous loci has been the subject of debate since investigators started to identify the genetic loci of domestication [4,18–22]. Research over the past few years has added a wealth of new genes to our list, thereby providing a solid base for further evaluation of this question. Surveying these data indicates that there is no clear-cut answer because convergent domestication at all phylogenetic levels might occur via mutations at orthologous or non-orthologous loci (Table 1). There are many examples that show that molecular convergence plays an important role during domestication (Table 1) [12]; however, why are certain genes more likely to become involved in domestication than others? To define factors that promote convergent molecular crop domestication (Figure 3), it is worth having a more in-depth look at convergent evolution because this topic has already been examined to some extent in the context of natural adaptation in animal systems [10,12,14–16].

### *Nodal positioning*

Nodal positioning of a TF protein within a certain regulatory network has been reported to increase the probability of it becoming involved in natural adaptation [13–15]. Such evolutionary hotspot genes typically collect regulatory input from several upstream regulators and control a whole set of target genes necessary to guide the formation of an entire developmental module, such as a floral organ. Mutations in upstream regulators or downstream targets often alter only specific aspects within the developmental module, thus making the nodal gene particularly predestined for changes concerning the entire module. In addition, changes in upstream regulators might result in pleiotropic effects that might be deleterious for the organism (see section on 'minimal pleiotropic effects' below). Orthologs of the floral homeotic C-class gene *AGAMOUS* (*AG*) occupy such a nodal position in terms of controlling the formation of stamens, carpels, and determinate flowers across all angiosperms [80] (Figure 3). Changes in *AG* expression are involved in all cases of domesticated plants with double flowers that have been molecularly characterized to date, although the causative mutations have only been identified in two of these studies as loss-of-function mutations within the *AG* locus: in the ornamental 'Double White' cultivar of Rue anemone (*Thalictrum thalictroides*) and the Japanese cherry (*Prunus lannesiana*) variant 'Albo rosea' [80–83]. Double flower phenotypes have been convergently selected in various ornamental plants because the numerous extra petals increase their attractiveness for humans. As a link between upstream sensory modules and floral induction, *FT* orthologs likewise take up a nodal position in the gene regulatory network leading to flowering, which might explain their repeated involvement in flowering time variation [67–69,84]. However, given that *FT* genes are not the only mutational hotspots for flowering time manipulation, other factors have to be considered when evaluating predisposition for convergent molecular domestication, such as pathway size and complexity.

### *Simple pathways*

Simple pathways involving only a few gene products offer limited targets for domestication-relevant mutations unlike complex pathways with a multitude of players, such as the flowering time pathway. This is likely to reflect back on the probability of orthologous genes becoming involved in the repeated occurrence of a certain phenotype [16]. Glutinous cereal varieties whose seeds develop a sticky texture when cooked were domesticated repeatedly in certain areas because of cultural culinary preferences. The glutinous character derives from seed endosperms with a reduced content of amylose, a starch molecule that is produced from a glucose precursor by a single catalytic process mediated by a granule-bound starch synthase encoded by the *Waxy* locus (Figure 3) [85]. In all studies reported to date, the glutinous character of cereal varieties has been brought about by mutations in *Waxy* orthologs [85–90]. Likewise, the loss of betaine aldehyde dehydrogenase (BADH2) enzyme activity in a metabolic pathway leading to the aroma compound 2-acetyl-1-pyrroline has repeatedly caused the occurrence of fragrant varieties in rice and soybean [91,92].

### *Minimal pleiotropic effects*

Minimal pleiotropic effects of adaptive mutations have been reported to raise their chances of becoming evolutionarily fixed [10,12–15]. Not only might this requirement be

Review
*Trends in Plant Science* xxx xxxx, Vol. xxx, No. x

**Table 1. Examples of molecular convergence underlying domestication-related phenotypic changes[a]**

| Crop species | Phylogenetic distribution[c] | Orthologous gene(s) | Class of gene product | Phenotypic effect | Causative changes[d] | Refs |
|---|---|---|---|---|---|---|
| Rice[b], barley | Species/family | *OsGA20ox-2* (*GA20 oxidase-2*), *HvGA20ox-2* | Metabolic enzyme | Dwarfism | Coding | [28–30] |
| Wheat[b] | Species | *Rht-1* (*reduced height-1*) | SH2-TF | Dwarfism | Coding | [1][e] |
| Sorghum, pearl millet | Family | *dw3* (*dwarfing3*), *d2* | Transporter protein | Dwarfism | Coding | [32,33] |
| Tomato, soybean[b], common bean[b] | Family/above family | *SP* (*SELF-PRUNING*), *Dt1* (determinate stem locus 1), *PvTFL1y* (*TERMINAL FLOWER 1*) | Signaling protein | Determinate growth | Coding | [1][e], [35–38] |
| Barley, pea, strawberry | Above family | *HvCEN* (*CENTRORADIALIS*), *PsTFL1c*, *FvTFL1* | Signaling protein | Variation in flowering time | Mixed | [39–41] |
| Barley, wheat[b], ryegrass (*Lolium perenne*) | Species/family | *VRN1* (*BM5*, *TmAP1*, *WAP1*, *LpVRN1*) | MADS domain TF | Variation in flowering time | Non-coding | [51][e] |
| Barley, wheat[b] | Species/family | *VRN2* (*ZCCT1*) | Zinc finger–CCT domain TF | Variation in flowering time | Mixed | [49][e] |
| Rice, barley, wheat, sorghum[b], sugar beet | Species/family/above family | *OsPRR37* (*pseudoresponse regulator protein 37*), *Ppd-H1*, *Ppd1*, *SbPRR37*, *BvBTC1* | Regulator of the circadian clock pathway | Variation in flowering time | Mixed | [53–58] |
| Turnip, *Brassica oleracea* | Family | *BrFLC2* (*FLOWERING LOCUS C*), *BoFLC2* | MADS domain TF | Variation in flowering time | Mixed | [44,60,62] |
| Rice, barley, pea, lentil | Family/above family | *Hd17* (*Heading date 17*), *EAM8* (*EARLY MATURITY 8*)/*Mat-a* (*Praematurum-a*), *HR* (*HIGH RESPONSE TO PHOTOPERIOD*), *LcELF3* (*EARLY FLOWERING 3*) | Regulator of the circadian clock pathway | Variation in flowering time | Coding | [46,64–66] |
| Rice, wheat, sunflower, barley | Family/above family | *Hd3a* (*Heading date 3a*), *VRN3*/*TaFT* (*FLOWERING LOCUS T*), *HaFT1*, *HvFT* | Signaling protein | Variation in flowering time | Mixed | [67–69] |
| Rice[b] | Species | *Hd1* (*Heading date 1*) | Zinc finger TF | Variation in flowering time | Coding | [12][e] |
| Sorghum, rice, corn | Family | *Sh1* (*Shattering 1*), *OsSh1*, *ZmSh1* | YABBY-like TF | Shatter resistance | Mixed | [77] |
| Rice, wheat[b], corn[b], foxtail millet[b], barley[b], amaranth, sorghum[b], broomcorn millet | Species/family/above family | *GBSSI* (*granule-bound starch synthase I*)/*Waxy* | Metabolic enzyme | Glutinous seeds | Mixed | [85][e], [86–90] |
| Rice[b], soybean | Species/family | *BADH2* (*betaine aldehyde dehydrogenase gene 2*), *GmBADH2* | Metabolic enzyme | Fragrance | Coding | [91,92] |
| Rice[b], potato | Species/above family | *Rd*/*DFR* (*dihydroflavonol-4-reductase*), *DFR* | Metabolic enzyme | Coloration | Coding | [111,112] |
| Blood orange[b] | Species | *Ruby* | MYB-TF | Coloration | Non-coding | [97] |
| Rice[b] | Species | *Bh4* (*Black hull4*) | Transporter protein | Coloration | Coding | [113] |
| Soybean[b] | Species | *R* | MYB-TF | Coloration | Coding | [96] |
| Pea[b], potato | Above family | *F3′5′H* (*flavonoid 3′,5′-hydroxylase*) | Metabolic enzyme | Coloration | Mixed | [12][e] |
| Rice[b] | Species | *Rc* | bHLH-TF | Coloration | Coding | [12][e] |
| Grapevine[b] | Species | *VvMYBA1-3* | MYB-TF | Coloration | Mixed | [12][e] |
| Corn, pearl millet, barley | Family | *tb1* (*teosinte branched 1*), *Pgtb1*, *INT-C* (*INTERMEDIUM-C*) | TCP-TF | Plant architecture | Mixed | [101,114,115] |
| Barley[b] | Species | *VRS1* (*six-rowed spike 1*) | Homeodomain-TF | Plant architecture | Coding | [12][e] |
| Rice, corn | Family | *GS3* (*QTL for grain size and length on chromosome 3*), *ZmGS3* | Putative transmembrane protein | Grain size | Mixed | [4][e], [116] |
| Rice[b] | Species | *GS5* (*QTL for grain size and length on chromosome 5*) | Metabolic enzyme | Grain size | Non-coding | [117] |
| Rice, corn, wheat | Family | *GW2* (*QTL for grain weight on chromosome 2*), *ZmGW2-CHR4*/*5*, *TaGW2* | Metabolic enzyme | Grain size | Mixed | [4][e], [118,119] |
| Rice[b], wheat | Species/family | *Gn1a* (*QTL for grain number on chromosome 1, a*)/*OsCKX2* (*cytokinin oxidase*/*dehydrogenase*), *TaCKX6-D1* | Metabolic enzyme | Grain number | Mixed | [4][e], [120] |
| Corn[b] | Species | *Opaque2* | bZIP-TF | Grain quality | Mixed | [12][e] |

Review
Trends in Plant Science xxx xxxx, Vol. xxx, No. x

**Table 1 (*Continued*)**

| Crop species | Phylogenetic distribution[c] | Orthologous gene(s) | Class of gene product | Phenotypic effect | Causative changes[d] | Refs |
|---|---|---|---|---|---|---|
| Rice[b] | Species | *GW8* (*QTL for grain weight on chromosome 8*)/*OsSPL16* (*squamosa promoter-binding protein-like 16*) | SBP-TF | Grain size and shape | Non-coding | [12][e] |
| Wheat, rye (*Secale cereale*) | Family | *TaALMT1* (*Al-activated malate transporter 1*), *ScALMT1* | Transporter protein | Metal tolerance | Mixed | [12][e] |
| Sorghum, corn | Family | *SbMATE1* (*multidrug and toxic compound extrusion 1*), *ZmMATE1* | Transporter protein | Metal tolerance | Mixed | [12][e], [121] |

[a]This list is not intended to be exhaustive.

[b]Multiple independent alleles present in this species.

[c]Species: molecular convergence within the same species, Family: molecular convergence between plants of one plant family, Above family: molecular convergence between species of different plant families.

[d]Discrimination between mutations detected exclusively in coding regions (coding), non-coding regions (non-coding) or both (mixed).

[e]Review containing original references.

fulfilled by the preferential occurrence of *cis*-regulatory mutations [13,14,93] but also by the preferential usage of mutational target genes of limited functionality [94]. The flavonoid biosynthetic pathway produces anthocyanins, thereby giving rise to blue, purple, and red coloration of plant organs among angiosperms [94,95]. Three types of TFs coordinate the activity of this pathway and it has been argued that owing to their high copy number and often tissue-specific function, evolutionary changes in members of the R2R3-MYB (MYB) TF family are likely to have fewer pleiotropic effects compared with basic helix–loop–helix (bHLH) or WD40 repeat family members, which function more broadly (Figure 3) [94,95]. Indeed, a preference for mutations in MYB-TF genes has been observed in studies dealing with the natural adaptation of floral pigment intensity [94]. During plant domestication, changes in

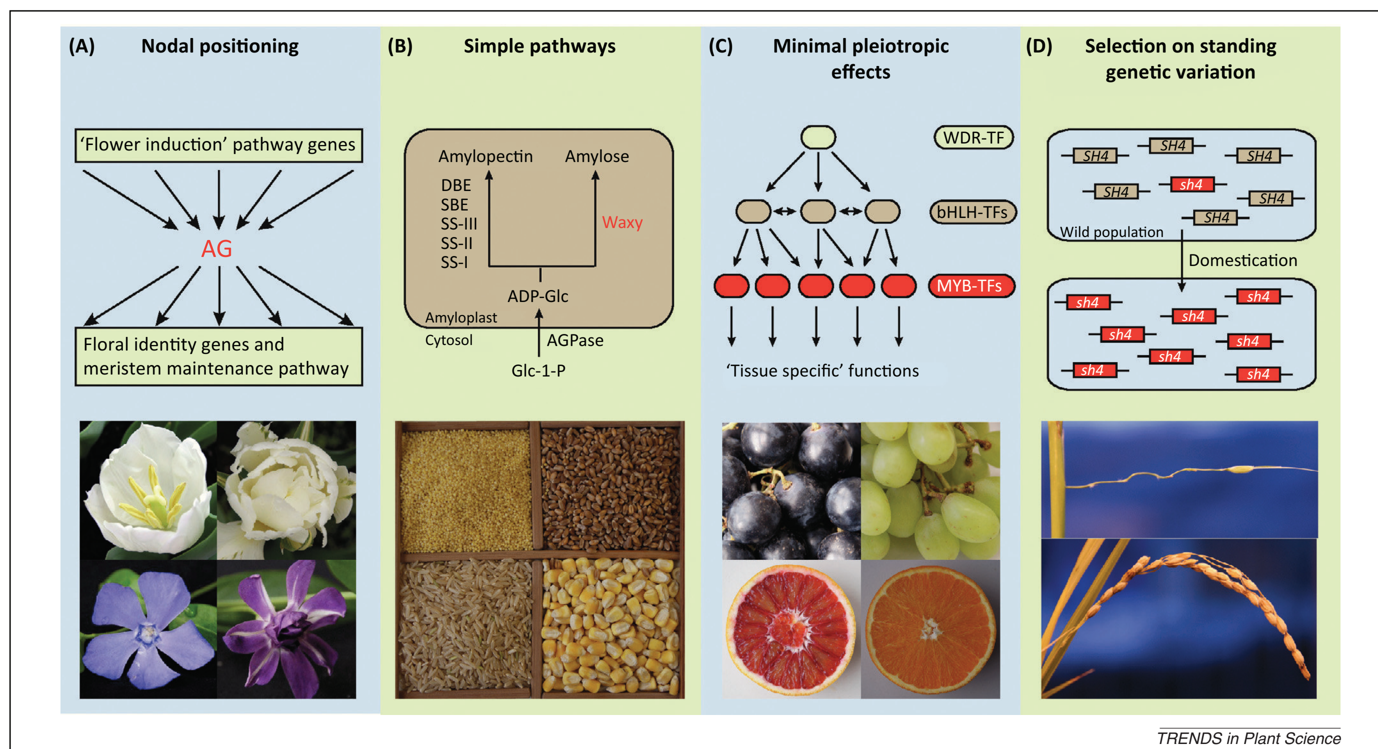


**Figure 3**. Factors promoting convergent molecular domestication. Convergent phenotypic changes in the course of plant domestication are often caused by mutations within orthologous genes. There are several factors explaining why mutations in such hotspot genes are more likely to become fixed in a population than mutations in other genes. **(A)** Genes occupying nodal positions within a given regulatory pathway incorporate inputs of several upstream regulators and in turn regulate several downstream genes, thereby often controlling self-contained developmental units. Mutations within such an input–output gene might alter a parameter value in a way that could otherwise only be achieved by concerted mutations within several upstream or downstream genes simultaneously. Mutations involving the nodal gene *AGAMOUS* (*AG*) were found at the basis of all molecularly characterized cases of domesticated plants with double flowers. **(B)** Simple metabolic pathways might also favor convergent molecular changes because only a minimal set of genes serves as a potential mutational target to change a given trait. Low amylose content because of mutations within *Waxy* gene orthologs is responsible for the domestication of glutinous seeds in many cereal variants. **(C)** Changes in fruit or seed color are often caused by mutations within MYB transcription factors because within the anthocyanin pathway they mostly have tissue-specific functions, thereby minimizing pleiotropic effects. **(D)** Finally, if domestication-related alleles are already present at low frequency within a wild population, as is the case for the non-shattering allele *sh4* in rice, independent selection on this standing genetic variation is likely to drive the same allele towards fixation repeatedly. Abbreviations: bHLH, basic helix–loop–helix; DBE, starch debranching enzyme; Glc, glucose; SBE, starch branching enzyme; SS, starch synthase; TF, transcription factor; WDR, WD40 repeat.

plant coloration, particularly of edible parts, also play an important role and molecular data likewise point towards mutations in MYB-TF genes as the overrepresented cause, probably to avoid unwanted pleiotropic effects [95–98]. In the same vein, the superior qualities and domestication-related success of *OsGA20ox-2* compared with *OsKO2* mutations to induce a semi-dwarfing growth habit in rice has been attributed to a difference between the pleiotropic effects of both genes [27,28]. Consequences of a defective *OsGA20ox-2* gene are restricted to stem tissue because in all other plant parts redundancy with other genes compensates for the impaired gene function, whereas in the case of *OsKO2* expression of the redundant *OsKO1* is restricted to flower organs, thus resulting in pleiotropic effects in the rest of the plant [27]. Furthermore, difficulties in breeding rape (*Brassica napus*) varieties that were resistant to pod shatter have been hypothesized to derive from fruit developmental genes acting pleiotropically in anther development [99].

### *Selection on standing genetic variation*

Selection on the standing genetic variation might cause the repeated involvement of the same locus in independent domestication events because the selection of favorable alleles that are already present in a wild population usually proceeds faster than new mutations can arise (Figure 3) [9–12,15,100]. Naturally, this only applies in some cases because domestication-related alleles might have strong deleterious effects on wild plants; however, various domestication-related alleles with a moderate negative effect on plant fitness are known to be present in wild populations at low frequency [39,55,72,101]. Additionally, introgression of wild alleles of adaptive value into already established crop species was recently discussed as another mechanism for the evolution of adaptive changes during domestication [102]. Thus, the potential to form viable alleles with altered functionality in a wild plant population might be viewed as an additional factor designating certain genes as likely domestication targets.

Having shed light on some factors that promote molecular convergence, another important variable acting in addition with these factors seems to be phylogenetic distance between species. It was already noted elsewhere that mutations at orthologous loci cause convergent evolution more frequently within the same or between closely related species compared with distantly related ones [6,11,16,49]. Concentrating on data about plant domestication (dataset described in [12] and Table 1 from this study), we observe the same trend counting less than ten cases of molecular convergence involving species from different plant families, whereas more than 50 cases were reported for species belonging to the same family. This higher frequency of molecular convergence is probably because of higher levels of similarity on average between close relatives, both at a molecular and morphological level, thus providing more possible targets for convergent mutations affecting domestication traits. With regard to morphology, non-homologous structures at the base of a domestication trait (as for seed dispersal in soybean and rice [70,72]) should diminish (although not remove) the probability of orthologous genes controlling this trait. However, the more evolutionary distance there is separating the respective species the more likely it is that even homologous traits are governed by a divergent set of genes (as seen for control of flowering time) [11,47–49,103]. In extreme cases, orthologs of a domestication-related gene in one species may even be absent in other species, making molecular convergence impossible. However, in most cases that we have compiled (Table 1) where different genes underlie the same domestication trait in distinct plants orthologous genes are nevertheless present in all species considered, suggesting that absence of orthologs is usually not the reason for non-convergent molecular evolution. In any case, the phenomenon of molecular convergence decreases with increasing phylogenetic distance, although this can only be considered as a probability statement given that domestication, like evolution, proceeds largely as a stochastic process.

## From evolution back to domestication – on the origin of new crop species

Domestication has inspired evolutionary biologists: famous examples of domestication are outlined in Charles Darwin's seminal book *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* [104]. One of the most intriguing phenomena studied by evolutionary biology is convergent evolution, which is often based on mutations in closely related genes. In this review, we have compiled recent evidence showing that convergent evolution also applies to plant domestication and is governed by similar factors. Thus, knowledge about evolution has led to a better understanding of domestication.

However, not only can our improved understanding of domestication at the molecular level be used to improve existing crops but it can also be used to facilitate the domestication of new crops. Although several thousand plant species have been cultivated for consumption during the course of human history, at present 95% of human food energy is derived from only approximately 30 crop species (http://www.fao.org/biodiversity/components/plants/en/). Relying on this small basis of crop biodiversity seems risky and we may need more or better adapted crops to meet the challenges posed by plant pests, global warming, and the growing human population. Classical domestication and breeding is a slow process and may be too slow to meet the predicted demands of the global population. Therefore, it is good news that domestication is based on genetic hotspots and, thus, is predictable to a certain degree. Rather than starting domestication of wild plant species in a naïve way from scratch, promising hotspot genes (e.g., *TFL1* orthologs for determinate growth, *ELF3* orthologs for early flowering) might be targeted via marker-assisted breeding or transgenic technology to generate desired phenotypic changes. In principle, this rationale has already been used for crop improvement in some straightforward cases. For example, downregulating the expression of *Waxy* orthologs has been used to artificially generate potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), and cassava (*Manihot esculenta*) lines containing optimized starches for culinary and industrial applications [105–107]. Other desirable traits appear to be more complex or labile. For example, although salt tolerance has evolved more than

70 times in a wide range of grass species, few commercially viable salt tolerant crops have been released [108]. However, there is evidence that genes encoding membrane transporters of the HKT family (transporting sodium and potassium) can be used to generate salt tolerant cereal grasses (reviewed in [109]). Knowing why certain genes are repeatedly successfully involved in domestication might help to predict the most promising mutational targets from such newly discovered genes or pathways.

In conclusion, domestication has been a tremendous help in understanding evolution. However, now the time is ripe to use the knowledge gleaned from evolutionary biology to adapt domesticated plants to our changing environment.

### Acknowledgments
We apologize to colleagues whose work was not included owing to space constraints. We thank the University of Jena for general support. Support by a grant from the Deutsche Forschungsgemeinschaft (DFG) to G.T. (TH 417/6-1) is acknowledged.

### References
1 Doebley, J.F. *et al.* (2006) The molecular genetics of crop domestication. *Cell* 127, 1309–1321
2 Miller, A.J. (2007) Crop plants: evolution. In *eLS* (Hetherington, A.M., ed.), pp. 1–7, John Wiley & Sons
3 Purugganan, M.D. and Fuller, D.Q. (2009) The nature of selection during plant domestication. *Nature* 457, 843–848
4 Gross, B.L. and Olsen, K.M. (2010) Genetic perspectives on crop domestication. *Trends Plant Sci.* 15, 529–537
5 Olsen, K.M. and Wendel, J.F. (2013) A bountiful harvest: genomic insights into crop domestication phenotypes. *Annu. Rev. Plant Biol.* 64, 47–70
6 Arendt, J. and Reznick, D. (2008) Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? *Trends Ecol. Evol.* 23, 26–32
7 Scotland, R.W. (2011) What is parallelism? *Evol. Dev.* 13, 214–227
8 Yoon, H.S. and Baum, D.A. (2004) Transgenic study of parallelism in plant morphological evolution. *Proc. Natl. Acad. Sci. U.S.A.* 101, 6524–6529
9 Elmer, K.R. and Meyer, A. (2011) Adaptation in the age of ecological genomics: insights from parallelism and convergence. *Trends Ecol. Evol.* 26, 298–306
10 Nadeau, N.J. and Jiggins, C.D. (2010) A golden age for evolutionary genetics? Genomic studies of adaptation in natural populations. *Trends Genet.* 26, 484–492
11 Conte, G.L. *et al.* (2012) The probability of genetic parallelism and convergence in natural populations. *Proc. R. Soc. B: Biol. Sci.* 279, 5039–5047
12 Martin, A. and Orgogozo, V. (2013) The loci of repeated evolution: a catalog of genetic hotspots of phenotypic variation. *Evolution* 67, 1235–1250
13 Stern, D.L. and Orgogozo, V. (2008) The loci of evolution: How predictable is genetic evolution? *Evolution* 62, 2155–2177
14 Stern, D.L. and Orgogozo, V. (2009) Is genetic evolution predictable? *Science* 323, 746–751
15 Gompel, N. and Prud'homme, B. (2009) The causes of repeated genetic evolution. *Dev. Biol.* 332, 36–47
16 Christin, P.A. *et al.* (2010) Causes and evolutionary significance of genetic convergence. *Trends Genet.* 26, 400–405
17 Lobkovsky, A.E. and Koonin, E.V. (2012) Replaying the tape of life: quantification of the predictability of evolution. *Front. Genet.* 3, 246
18 Tang, H.B. *et al.* (2010) Domestication and plant genomes. *Curr. Opin. Plant Biol.* 13, 160–166
19 Paterson, A.H. *et al.* (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic-loci. *Science* 269, 1714–1718
20 Sood, S. *et al.* (2009) The major threshability genes soft glume (*sog*) and tenacious glume (*Tg*), of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. *Theor. Appl. Genet.* 119, 341–351
21 Li, W. and Gill, B. (2006) Multiple genetic pathways for seed shattering in the grasses. *Funct. Integr. Genomics* 6, 300–309
22 Sang, T. (2009) Genes and mutations underlying domestication transitions in grasses. *Plant Physiol.* 149, 63–70
23 Salas Fernandez, M.G. *et al.* (2009) From dwarves to giants? Plant height manipulation for biomass yield. *Trends Plant Sci.* 14, 454–461
24 Xue, W.Y. *et al.* (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nat. Genet.* 40, 761–767
25 Salamini, F. (2003) Hormones and the green revolution. *Science* 302, 71–72
26 McGarry, R.C. and Ayre, B.G. (2012) Manipulating plant architecture with members of the CETS gene family. *Plant Sci.* 188, 71–81
27 Itoh, H. *et al.* (2004) A rice semi-dwarf gene, *Tan-Ginbozu* (D35), encodes the gibberellin biosynthesis enzyme, *ent*-kaurene oxidase. *Plant Mol. Biol.* 54, 533–547
28 Asano, K. *et al.* (2007) Genetic and molecular analysis of utility of *sd1* alleles in rice breeding. *Breed. Sci.* 57, 53–58
29 Asano, K. *et al.* (2011) Artificial selection for a green revolution gene during *japonica* rice domestication. *Proc. Natl. Acad. Sci. U.S.A.* 108, 11034–11039
30 Jia, Q.J. *et al.* (2009) GA-20 oxidase as a candidate for the semidwarf gene *sdw1/denso* in barley. *Funct. Integr. Genomics* 9, 255–262
31 Chono, M. *et al.* (2003) A semidwarf phenotype of barley uzu results from a nucleotide substitution in the gene encoding a putative brassinosteroid receptor. *Plant Physiol.* 133, 1209–1219
32 Multani, D.S. *et al.* (2003) Loss of an MDR transporter in compact stalks of maize *br2* and sorghum *dw3* mutants. *Science* 302, 81–84
33 Parvathaneni, R.K. *et al.* (2013) Fine-mapping and identification of a candidate gene underlying the *d2* dwarfing phenotype in pearl millet, *Cenchrus americanus* (L.) Morrone. *G3 (Bethesda)* 3, 563–572
34 Pnueli, L. *et al.* (1998) The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. *Development* 125, 1979–1989
35 Repinski, S.L. *et al.* (2012) The common bean growth habit gene *PvTFL1y* is a functional homolog of *Arabidopsis TFL1*. *Theor. Appl. Genet.* 124, 1539–1547
36 Liu, B.H. *et al.* (2010) The soybean stem growth habit gene *Dt1* Is an ortholog of *Arabidopsis TERMINAL FLOWER1*. *Plant Physiol.* 153, 198–210
37 Kwak, M. *et al.* (2012) Multiple origins of the determinate growth habit in domesticated common bean (*Phaseolus vulgaris*). *Ann. Bot.* 110, 1573–1580
38 Tian, Z.X. *et al.* (2010) Artificial selection for determinate growth habit in soybean. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8563–8568
39 Comadran, J. *et al.* (2012) Natural variation in a homolog of *Antirrhinum CENTRORADIALIS* contributed to spring growth habit and environmental adaptation in cultivated barley. *Nat. Genet.* 44, 1388–1392
40 Foucher, F. *et al.* (2003) *DETERMINATE* and *LATE FLOWERING* are two *TERMINAL FLOWER1/CENTRORADIALIS* homologs that control two distinct phases of flowering initiation and development in pea. *Plant Cell* 15, 2742–2754
41 Koskela, E.A. *et al.* (2012) Mutation in *TERMINAL FLOWER1* reverses the photoperiodic requirement for flowering in the wild strawberry *Fragaria vesca*. *Plant Physiol.* 159, 1043–1054
42 Salvi, S. *et al.* (2011) Genetic dissection of maize phenology using an intraspecific introgression library. *BMC Plant Biol.* 11, 4
43 Salvi, S. *et al.* (2007) Conserved noncoding genomic sequences associated with a flowering-time quantitative trait locus in maize. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11376–11381
44 Wu, J. *et al.* (2012) A naturally occurring InDel variation in *BraA.FLC.b* (*BrFLC2*) associated with flowering time variation in *Brassica rapa*. *BMC Plant Biol.* 12, 151
45 Murphy, R.L. *et al.* (2011) Coincident light and clock regulation of *pseudoresponse regulator protein 37* (*PRR37*) controls photoperiodic flowering in sorghum. *Proc. Natl. Acad. Sci. U.S.A.* 108, 16469–16474
46 Weller, J.L. *et al.* (2012) A conserved molecular basis for photoperiod adaptation in two temperate legumes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 21158–21163

47 Higgins, J.A. *et al.* (2010) Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. *PLoS ONE* 5, e10065
48 Jung, C. and Muller, A.E. (2009) Flowering time control and applications in plant breeding. *Trends Plant Sci.* 14, 563–573
49 Alonso-Blanco, C. *et al.* (2009) What has natural variation taught us about plant development, physiology, and adaptation? *Plant Cell* 21, 1877–1896
50 Trevaskis, B. *et al.* (2003) MADS box genes control vernalization-induced flowering in cereals. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13099–13104
51 Asp, T. *et al.* (2011) Comparative sequence analysis of *VRN1* alleles of *Lolium perenne* with the co-linear regions in barley, wheat, and rice. *Mol. Genet. Genomics* 286, 433–447
52 Salome, P.A. and McClung, C.R. (2005) *PSEUDO-RESPONSE REGULATOR 7* and *9* are partially redundant genes essential for the temperature responsiveness of the *Arabidopsis* circadian clock. *Plant Cell* 17, 791–803
53 Murakami, M. *et al.* (2005) Circadian-associated rice pseudo response regulators (*Os*PRRs): insight into the control of flowering time. *Biosci. Biotechnol. Biochem.* 69, 410–414
54 Turner, A. *et al.* (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. *Science* 310, 1031–1034
55 Jones, H. *et al.* (2008) Population-based resequencing reveals that the flowering time adaptation of cultivated barley originated east of the fertile crescent. *Mol. Biol. Evol.* 25, 2211–2219
56 Beales, J. *et al.* (2007) A *Pseudo-Response Regulator* is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 115, 721–733
57 Wilhelm, E.P. *et al.* (2009) Photoperiod insensitive *Ppd-A1a* mutations in tetraploid wheat (*Triticum durum* Desf.). *Theor. Appl. Genet.* 118, 285–294
58 Diaz, A. *et al.* (2012) Copy number variation affecting the *Photoperiod-B1* and *Vernalization-A1* genes is associated with altered flowering time in wheat (*Triticum aestivum*). *PLoS ONE* 7, e33234
59 Pin, P.A. *et al.* (2012) The role of a pseudo-response regulator gene in life cycle adaptation and domestication of beet. *Curr. Biol.* 22, 1095–1101
60 Yuan, Y.X. *et al.* (2009) A naturally occurring splicing site mutation in the *Brassica rapa FLC1* gene is associated with variation in flowering time. *J. Exp. Bot.* 60, 1299–1308
61 Wang, N.A. *et al.* (2011) Flowering time variation in oilseed rape (*Brassica napus* L.) is associated with allelic variation in the *FRIGIDA* homologue *BnaA.FRI.a*. *J. Exp. Bot.* 62, 5641–5658
62 Okazaki, K. *et al.* (2007) Mapping and characterization of *FLC* homologs and QTL analysis of flowering time in *Brassica oleracea*. *Theor. Appl. Genet.* 114, 595–608
63 Xia, Z.J. *et al.* (2012) Positional cloning and characterization reveal the molecular basis for soybean maturity locus *E1* that regulates photoperiodic flowering. *Proc. Natl. Acad. Sci. U.S.A.* 109, E2155–E2164
64 Matsubara, K. *et al.* (2012) Natural variation in *Hd17*, a homolog of *Arabidopsis ELF3* that is involved in rice photoperiodic flowering. *Plant Cell Physiol.* 53, 709–716
65 Zakhrabekova, S. *et al.* (2012) Induced mutations in circadian clock regulator *Mat-a* facilitated short-season adaptation and range extension in cultivated barley. *Proc. Natl. Acad. Sci. U.S.A.* 109, 4326–4331
66 Faure, S. *et al.* (2012) Mutation at the circadian clock gene *EARLY MATURITY 8* adapts domesticated barley (*Hordeum vulgare*) to short growing seasons. *Proc. Natl. Acad. Sci. U.S.A.* 109, 8328–8333
67 Yan, L. *et al.* (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19581–19586
68 Takahashi, Y. *et al.* (2009) Variations in Hd1 proteins, *Hd3a* promoters, and *Ehd1* expression levels contribute to diversity of flowering time in cultivated rice. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4555–4560
69 Blackman, B.K. *et al.* (2010) The role of recently derived *FT* paralogs in sunflower domestication. *Curr. Biol.* 20, 629–635
70 Suzuki, M. *et al.* (2010) Fine mapping and development of DNA markers for the *qPDH1* locus associated with pod dehiscence in soybean. *Mol. Breed.* 25, 407–418
71 Spence, J. *et al.* (1996) 'Pod shatter' in *Arabidopsis thaliana*, *Brassica napus* and *B. juncea*. *J. Microsc.* 181, 195–203
72 Lin, Z.W. *et al.* (2007) Origin of seed shattering in rice (*Oryza sativa* L.). *Planta* 226, 11–20
73 Azhaguvel, P. and Komatsuda, T. (2007) A phylogenetic analysis based on nucleotide sequence of a marker linked to the brittle rachis locus indicates a diphyletic origin of barley. *Ann. Bot.* 100, 1009–1015
74 Mao, L. *et al.* (2000) *JOINTLESS* is a MADS-box gene controlling tomato flower abscission zone development. *Nature* 406, 910–913
75 Li, C.B. *et al.* (2006) Rice domestication by reducing shattering. *Science* 311, 1936–1939
76 Ishii, T. *et al.* (2013) *OsLG1* regulates a closed panicle trait in domesticated rice. *Nat. Genet.* 45, 462–465
77 Lin, Z.W. *et al.* (2012) Parallel domestication of the *Shattering1* genes in cereals. *Nat. Genet.* 44, 720–724
78 Konishi, S. *et al.* (2006) A SNP caused the loss of seed shattering during rice domestication. *Plant Cell Physiol.* 47, S14
79 Arnaud, N. *et al.* (2011) The same regulatory point mutation changed seed-dispersal structures in evolution and domestication. *Curr. Biol.* 21, 1215–1219
80 Galimba, K.D. *et al.* (2012) Loss of deeply conserved C-class floral homeotic gene function and C- and E-class protein interaction in a double-flowered ranunculid mutant. *Proc. Natl. Acad. Sci. U.S.A.* 109, E2267–E2275
81 Dubois, A. *et al.* (2010) Tinkering with the C-function: a molecular frame for the selection of double flowers in cultivated roses. *PLoS ONE* 5, e9288
82 Akita, Y. *et al.* (2011) Effect of the expression level of an *AGAMOUS*-like gene on the petaloidy of stamens in the double-flowered lily, 'Elodie'. *Sci. Hortic. (Amst.)* 128, 48–53
83 Liu, Z. *et al.* (2013) Exon skipping of *AGAMOUS* homolog *PrseAG* in developing double flowers of *Prunus lannesiana* (Rosaceae). *Plant Cell Rep.* 32, 227–237
84 Pin, P.A. and Nilsson, O. (2012) The multifaceted roles of FLOWERING LOCUS T in plant development. *Plant Cell Environ.* 35, 1742–1755
85 Jeon, J.S. *et al.* (2010) Starch biosynthesis in cereal endosperm. *Plant Physiol. Biochem.* 48, 383–392
86 Fan, L.J. *et al.* (2008) Molecular evidence for post-domestication selection in the *Waxy* gene of Chinese waxy maize. *Mol. Breed.* 22, 329–338
87 Kawahigashi, H. *et al.* (2013) A novel *waxy* allele in sorghum landraces in East Asia. *Plant Breed.* 132, 305–310
88 Kawase, M. *et al.* (2005) Diverse origins of waxy foxtail millet crops in East and Southeast Asia mediated by multiple transposable element insertions. *Mol. Genet. Genomics* 274, 131–140
89 Hunt, H.V. *et al.* (2013) Waxy phenotype evolution in the allotetraploid cereal broomcorn millet: mutations at the *GBSSI* locus in their functional and phylogenetic context. *Mol. Biol. Evol.* 30, 109–122
90 Park, Y.J. *et al.* (2012) The molecular basis of mutations at the *Waxy* locus from *Amaranthus caudatus* L.: evolution of the waxy phenotype in three species of grain amaranth. *Mol. Breed.* 30, 511–520
91 Kovach, M.J. *et al.* (2009) The origin and evolution of fragrance in rice (*Oryza sativa* L.). *Proc. Natl. Acad. Sci. U.S.A.* 106, 14444–14449
92 Juwattanasomran, R. *et al.* (2011) A SNP in *GmBADH2* gene associates with fragrance in vegetable soybean variety "Kaori" and SNAP marker development for the fragrance. *Theor. Appl. Genet.* 122, 533–541
93 Carroll, S.B. (2008) Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* 134, 25–36
94 Streisfeld, M.A. and Rausher, M.D. (2011) Population genetics, pleiotropy, and the preferential fixation of mutations during adaptive evolution. *Evolution* 65, 629–642
95 Petroni, K. and Tonelli, C. (2011) Recent advances on the regulation of anthocyanin synthesis in reproductive organs. *Plant Sci.* 181, 219–229
96 Gillman, J.D. *et al.* (2011) Loss-of-function mutations affecting a specific *Glycine max* R2R3 MYB transcription factor result in brown hilum and brown seed coats. *BMC Plant Biol.* 11, 155

97 Butelli, E. *et al.* (2012) Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. *Plant Cell* 24, 1242–1255
98 Cockram, J. *et al.* (2010) Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21611–21616
99 Jarvis, M.C. *et al.* (2003) Intercellular adhesion and cell separation in plants. *Plant Cell Environ.* 26, 977–989
100 Tsiantis, M. (2011) A transposon in *tb1* drove maize domestication. *Nat. Genet.* 43, 1048–1050
101 Studer, A. *et al.* (2011) Identification of a functional transposon insertion in the maize domestication gene *tb1*. *Nat. Genet.* 43, 1160–1163
102 Hufford, M.B. *et al.* (2013) The genomic signature of crop-wild introgression in maize. *PLoS Genet.* 9, e1003477
103 Wagner, M.R. and Mitchell-Olds, T. (2011) Repeated phenotypic changes highlight molecular targets of convergent evolution. *Genome Biol.* 12, 124
104 Darwin, C. (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. J. Murray
105 Noda, T. *et al.* (2002) Physicochemical properties of amylose-free starch from transgenic sweet potato. *Carbohydr. Polym.* 49, 253–260
106 Muth, J. *et al.* (2008) Precision breeding for novel starch variants in potato. *Plant Biotechnol. J.* 6, 576–584
107 Zhao, S.S. *et al.* (2011) Development of waxy cassava with different biological and physico-chemical characteristics of starches for industrial applications. *Biotechnol. Bioeng.* 108, 1925–1935
108 Bennett, T.H. *et al.* (2013) Repeated evolution of salt-tolerance in grasses. *Biol. Lett.* 9, 20130029
109 Schroeder, J.I. *et al.* (2013) Using membrane transporters to improve crops for sustainable food production. *Nature* 497, 60–66
110 Hedges, S.B. (2002) The origin and evolution of model organisms. *Nat. Rev. Genet.* 3, 838–849
111 Furukawa, T. *et al.* (2007) The *Rc* and *Rd* genes are involved in proanthocyanidin synthesis in rice pericarp. *Plant J.* 49, 91–102
112 Zhang, Y.F. *et al.* (2009) The potato *R* locus codes for dihydroflavonol 4-reductase. *Theor. Appl. Genet.* 119, 931–937
113 Zhu, B.F. *et al.* (2011) Genetic control of a transition from black to straw-white seed hull in rice domestication. *Plant Physiol.* 155, 1301–1311
114 Remigereau, M.S. *et al.* (2011) Cereal domestication and evolution of branching: evidence for soft selection in the *Tb1* orthologue of pearl millet (*Pennisetum glaucum* [L.] R. Br.). *PLoS ONE* 6, e22404
115 Ramsay, L. *et al.* (2011) *INTERMEDIUM-C*, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene *TEOSINTE BRANCHED 1*. *Nat. Genet.* 43, 169–172
116 Li, Q. *et al.* (2010) Cloning and characterization of a putative *GS3* ortholog involved in maize kernel development. *Theor. Appl. Genet.* 120, 753–763
117 Li, Y.B. *et al.* (2011) Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. *Nat. Genet.* 43, 1266–1269
118 Yang, Z.B. *et al.* (2012) SNP identification and allelic-specific PCR markers development for *TaGW2*, a gene linked to wheat kernel weight. *Theor. Appl. Genet.* 125, 1057–1068
119 Li, Q. *et al.* (2010) Relationship, evolutionary fate and function of two maize co-orthologs of rice *GW2* associated with kernel size and weight. *BMC Plant Biol.* 10, 143
120 Zhang, L. *et al.* (2012) *TaCKX6-D1*, the ortholog of rice *OsCKX2*, is associated with grain weight in hexaploid wheat. *New Phytol.* 195, 574–584
121 Maron, L.G. *et al.* (2013) Aluminum tolerance in maize is associated with higher MATE1 gene copy number. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5241–5246

# 3. Discussion

Up to now, the results of my thesis have been presented and discussed in three self-contained manuscripts. In the following discussion, I will illuminate several superordinate aspects arising from the combined consideration of all my data. I start with an evaluation to what extent the two Lepidium species studied in this thesis are suitable as scientific model organisms. The high conservation of the gene regulatory network guiding fruit development in the Brassicaceae is a major finding of this thesis, and I proceed by discussing whether this conservation may also apply to plants of more distant phylogenetic levels. Another major topic is the relative role of molecular convergence during convergent evolution, and I evaluate how the individual manuscripts contribute new insights to this topic. Finally, I point out the agronomic value of my data and conclude by presenting possible future issues, including the ecological significance of indehiscent fruits in natural environments and the search for alternative approaches to study the genetic background of fruit evolution in the Brassicaceae.

## 3.1 Lepidium species as models for fruit evolution – the ups and downs

The importance of new model species for biological research has been emphasized before (Mandoli and Olmstead, 2000; Milinkovitch and Tzika, 2007; Abzhanov *et al.*, 2008). One argument for the use of alternative models is that specific traits may not be accessible in the preexisting models. For example, studying photoperiodic responses in metabolism and behavior will not reflect genuine patterns if it is performed in the laboratory mouse (*Mus musculus*), a classical animal model which is, due to human selection, by now well adapted to year-round breeding under laboratory conditions (Rissman, 2004). Likewise, the classical plant model *A. thaliana* is not well suited to study traits such as perennial life cycle or self-incompatibility (Clauss and Koch, 2006). Another argument for more model species is specific to evo-devo research, where a clear picture of evolution will only emerge as more taxa are studied in comparison (Abzhanov *et al.*, 2008). Species which are placed at key branches of the tree of life have to be studied in order to give representative information about the millions of species on earth, and suitable models have to be selected individually to study the evolutionary transition of specific characters (Abzhanov *et al.*, 2008).

In this thesis, *L. campestre* and *L. appelianum* have been chosen as model systems to study fruit evolution with special focus on changes in the molecular pathway leading to fruit dehiscence (manuscript I, II). They have been selected because of a recent switch from dehiscent to indehiscent fruits in the lineage leading to *L. appelianum* and because their diploid genomes and close relatedness with *A. thaliana* have been promising aspects concerning the establishment of molecular techniques (Mummenhoff *et al.*, 2009). Efforts to make these species accessible to this purpose included the determination of suitable controlled growth conditions, the cloning of putative fruit developmental genes, the establishment of an effective protocol for *in situ* hybridization and the determination of adequate genes for normalization during qRT-PCR analyses. Additionally, for *L. campestre* a floral dip based transformation system has been established as a reverse genetic tool, making this plant species accessible for functional analyses (manuscript I).

Compared to common protocols for floral-dip transformation in *A. thaliana*, the generation of transgenic *L. campestre* plants as described in manuscript I takes longer because the life cycle of this typically biennial species includes a long vernalization period. But flowering time and vernalization requirement are known to vary in natural populations of many plant species including other members of the Brassicaceae family (Michaels and Amasino, 2000; Okazaki *et al.*, 2007; Slotte *et al.*, 2007; Kuittinen *et al.*, 2008; Wang *et al.*, 2011; Coustham *et al.*, 2012). Since *L. campestre* is distributed over a wide range of different habitats, it might be possible to isolate accessions from temperate or subtropical regions with reduced flowering time or vernalization requirement, thereby significantly shortening the process of transformation. Two additional factors that might be investigated in order to further improve the transformation protocol of *L. campestre* are the actual requirement of repeated dipping and the use of vacuum to increase transformation efficiency. In case of repeated dipping it was only assumed but not systematically tested that it might increase transformation efficiency as it does in *A. thaliana* (Clough and Bent, 1998). However, considerable effort could be spared if fewer repeats were comparably effective. On the other hand vacuum is often used in order to enhance the infectivity of *Agrobacteria* (see de Oliveira *et al.*, 2009 and references therein) and was shown to improve efficiency during floral-dip transformation of *A. lasiocarpa* (Tague, 2001).

In summary of my experience with both Lepidium species and as a result of this work, *L. campestre* can now be used as a plant model organism which is easy to cultivate, transformable and accessible by various molecular techniques. For *L. appelianum*, the same molecular techniques can be applied, and its value as a research model becomes obvious because the molecular background of indehiscent fruits has been successfully studied in this species (manuscript II). Nevertheless, I would also like to point out some pitfalls of working with *L. appelianum*, because it was explicitly criticized that the practice of not reporting negative results may slow down the emergence of new model systems (Mandoli and Olmstead, 2000). Despite of extensive trials, induction of flowering in *L. appelianum* under laboratory conditions proved to be very unreliable and asynchronous in my hands. On top of that, the amount of emerging flowers was very low compared to *L. campestre* or *A. thaliana* and the amount of viable seeds was negligible (probably due to self-incompatibility). Thus, *L. appelianum* is not susceptible to floral-dip transformation, this lack of a reverse genetic tool being a major disadvantage when studying gene functions. Transformation via tissue culture based methods, as also applied for other Brassicaceae species including *L. campestre* (Barfield and Pua, 1991; Li *et al.*, 2010c; Chhikara *et al.*, 2012; Ivarson *et al.*, 2013), might be a promising way to solve this problem but was not further explored in the frame of this thesis. Switching to one of the other two species with indehiscent fruits that are phylogenetically close to *L. campestre* (Figure 3) does not seem promising, because these species are also reported to be self-incompatible and, additionally, harbor polyploid genomes (Gaskin *et al.*, 2005).

## 3.2 Conservation of fruit development – Brassicaceae and beyond

In this thesis, I present evidence from functional and gene expression analyses of fruit developmental genes in *L. campestre* that, upon comparison with data from *A. thaliana*, point towards a high conservation of gene functions and pathway connectivity during Brassicaceae fruit development, *per se* (manuscript I, II). This statement is also supported by some sporadic insights into the molecular basis of fruit development in other Brassicaceae species. In *Brassica juncea*, *BjSHATTERPROOF1* (*BjSHP1*), which is normally expressed at the valve/replum border in a similar pattern as its *A. thaliana* ortholog, was shown to be downregulated by heterologous over-expression of *FRUITFULL* (*FUL*) (Ostergaard *et al.*, 2006). The *INDEHISCENT* (*IND*) orthologs of *Brassica rapa* and *Brassica oleracea* are responsible for dehiscence zone formation since downregulation leads to loss of both

separation layer and lignified layer (Girin *et al.*, 2010). It is further known that orthologous enzymes are involved in cell separation in the separation layer of *A. thaliana* and *Brassica napus* (Petersen *et al.*, 1996; Ogawa *et al.*, 2009) and that in both plants dehiscence goes along with a decrease in auxin level (Chauvaux *et al.*, 1997; Sorefan *et al.*, 2009). In addition, gene expression studies reveal conserved expression patterns of different dehiscence zone identity gene and *FUL* orthologs in dehiscent fruits of *Erucaria erucarioides* as compared to *A. thaliana* (Avino *et al.*, 2012).

Although high conservation of developmental pathways between closely related species might seem natural, it can by far not be taken for granted. Functional studies in the Ranunculales have revealed that while two paralogous *FUL*-like genes play key roles in fruit development of *Papaver somniferum* and *Eschscholzia californica*, the respective orthologous pair is not involved in fruit development of *Aquilegia coerulea* (Pabon-Mora *et al.*, 2012; Pabon-Mora *et al.*, 2013). These studies not only highlight that gene functions and corresponding developmental pathways might change dramatically within one phylogenetic order but also point out a strikingly conserved role of *FUL*-like genes in fruit development of Brassicaceae and Papaveraceae, an early-diverging family of basal eudicots separated from the core eudicots by approximately 125 million years of evolution (Fawcett *et al.*, 2009). Could this conserved role of *FUL*-like genes be part of an ancestral fruit developmental pathway that might predate the split between core eudicots and basal eudicots or even trace back to the evolution of fruits as a novel character at the base of the angiosperm lineage?

Some genes that guide Brassicaceae fruit development cannot be part of such a hypothetical ancient pathway because they are Brassicaceae specific. Recent duplication events at the base of the Brassicaceae lineage gave rise to the paralogous gene pairs *SHP1* and *SHP2*, *ALCATRAZ* (*ALC*) and *SPATULA* (*SPT*), and *IND* and *HECATE3*, respectively (Kramer *et al.*, 2004; Groszmann *et al.*, 2008; Kay *et al.*, 2013). However, co-orthologs of *SHP1/2* and *ALC/SPT* have been implicated in fruit development in a variety of species (Figure 4) (Tani *et al.*, 2007; Vrebalov *et al.*, 2009; Tisza *et al.*, 2010; Tani *et al.*, 2011; Fourquin and Ferrandiz, 2012; Araujo *et al.*, 2013; Daminato *et al.*, 2013). Also orthologs (or co-orthologs) of *FUL* and *APETALA2* (*AP2*) have been found to participate in fruit development in various eudicot species (Müller *et al.*, 2001; Smykal *et al.*, 2007; Tani *et al.*, 2007; Xu *et al.*, 2008; Cevik *et al.*, 2010; Chung *et al.*, 2010; Jaakola *et al.*, 2010; Karlova *et al.*, 2011; Bemer *et al.*, 2012; Pabon-Mora *et al.*, 2012; Araujo *et al.*, 2013; Pabon-Mora *et al.*, 2013; Shima *et al.*, 2013) and there are even two reported parallels between fruit developmental genes of *Arabidopsis*

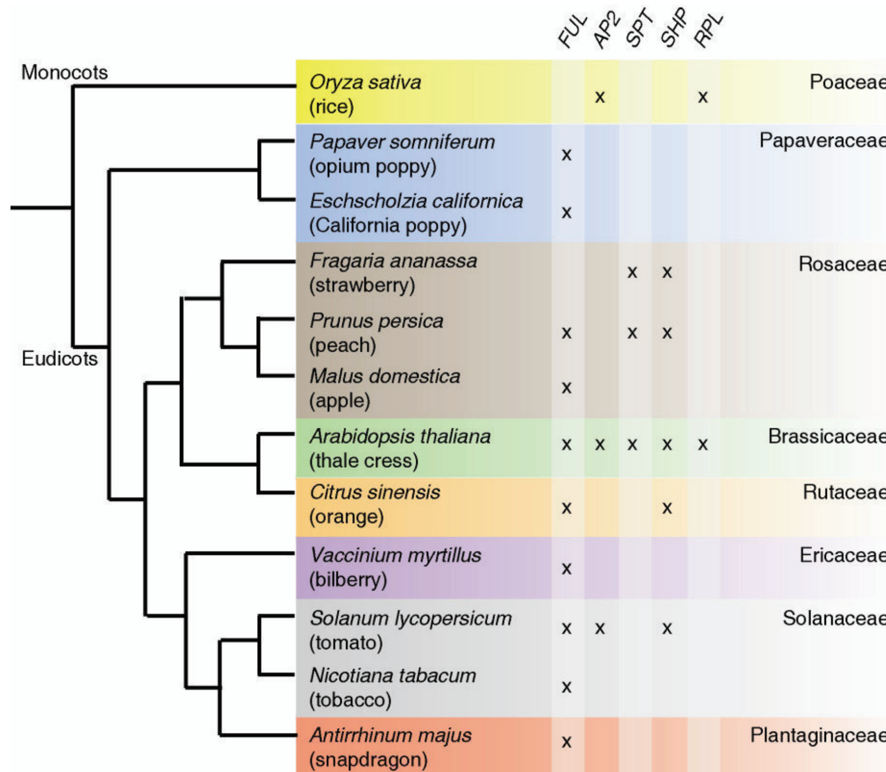


**Figure 4: Orthologs of *A. thaliana* fruit developmental genes function in fruit development over large phylogenetic distances.** A species phylogeny is shown including angiosperm species where orthologs of *FRUITFULL* (*FUL*), *APETALA2* (*AP2*), *SPATULA* (*SPT*), *SHATTERPROOF* (*SHP*), or *REPLUMLESS* (*RPL*) have been reported to play a role in fruit development (indicated by an "x"). They all control fruit development in quite distantly related species, thus indicating that ancestral versions of these genes may have been part of a basal gene regulatory network driving fruit development early during angiosperm evolution.

and rice, a monocotyledonous species (Figure 4) (Konishi *et al.*, 2006; Arnaud *et al.*, 2011; Zhou *et al.*, 2012). In some of these cases, the genes participate in the formation of fruit tissues that are necessary for dehiscence-like processes similar as in *Arabidopsis* (Müller *et al.*, 2001; Konishi *et al.*, 2006; Smykal *et al.*, 2007; Pabon-Mora *et al.*, 2012; Zhou *et al.*, 2012). However, they are also associated with developmental processes of fleshy fruits, especially with fruit ripening (Tani *et al.*, 2007; Vrebalov *et al.*, 2009; Cevik *et al.*, 2010; Chung *et al.*, 2010; Jaakola *et al.*, 2010; Tisza *et al.*, 2010; Karlova *et al.*, 2011; Bemer *et al.*, 2012; Daminato *et al.*, 2013; Shima *et al.*, 2013). Notably, orthologous genes are even reported to guide similar developmental processes in anatomically non-homologous structures like ripening in fleshy fruits of tomato and in false fruits of strawberry (Vrebalov *et al.*, 2009; Daminato *et al.*, 2013) or fruit dehiscence in *Arabidopsis* and seed shattering in rice (Roeder *et al.*, 2003; Konishi *et al.*, 2006; Ripoll *et al.*, 2011; Zhou *et al.*, 2012).

All these data support the idea that a basal gene regulatory network involving ancestral versions of *FUL*, *SHP*, *SPT*, and *AP2* was already present very early during angiosperm evolution and forms the basis of fruit development in the immense diversity of fruit forms we observe today. It even seems to underlie very distinct developmental programs, like those involved in fruit dehiscence of dry or ripening of fleshy fruits (Fourquin and Ferrandiz, 2012). Nevertheless, it remains largely unknown how exactly this basal network diverged or got recruited to different fruit structures during evolution. By analyzing and comparing the fruit developmental pathways of Brassicaceae species with fruits of different shape (manuscript I) and of different dehiscence behavior (manuscript II), this thesis contributes towards the general goal of understanding molecular fruit evolution at a shallow phylogenetic level. However, for a deeper understanding more studies like this are needed ideally covering various phylogenetic levels and changes in diverse fruit characters.

## 3.3 The role of molecular convergence during plant evolution

Assessing the role of molecular convergence during plant evolution from different angles was a central goal of this thesis. On the background of convergent evolution of indehiscence in the Brassicaceae family, comparative gene expression analyses between dehiscent fruits of *L. campestre* and indehiscent fruits of *L. appelianum* revealed that the loss of expression of *SHP1/2*, *ALC*, and *IND* orthologs at the valve-replum border of *L. appelianum* fruits is the likely cause underlying this particular case of character evolution (manuscript II). A similar case study analyzing the evolutionary origin of indehiscent fruits in *Cakile lanceolata*, another member of the Brassicaceae family, reports similar changes in expression patterns of orthologs of the same fruit developmental genes (Avino *et al.*, 2012). Thus, the emergence of indehiscent fruits is molecularly caused by a loss of dehiscence zone identity gene expression at the valve-replum border in both indehiscent Brassicaceae species that have been analyzed to date. These data give a striking example of how the same molecular changes may underlie convergent character evolution and provide a basis to speculate that this particular change in gene expression pattern may represent a common mechanism for the evolution of indehiscent fruits in the Brassicaceae, in general. However, based on the strict definition provided in the introductory part of this thesis, evolution of indehiscence within the Brassicaceae can to date not be considered as an example of molecular convergence, because the genetic changes leading to the observed alterations in gene expression have not been elucidated in any of the

two cases. This lack of genetic evidence also prevents these studies from contributing to the debate on other important questions in evolutionary biology, like the relative importance of cis-regulatory versus protein-coding mutations or the relative contribution of mutations in few genes with large phenotypic effect versus mutations in many genes of small phenotypic effect. Thus, future efforts should concentrate on identifying the genetic changes that cause *L. appelianum* and *C. lanceolata* to produce indehiscent fruits. Additionally, also more Brassicaceae species with indehiscent fruits representing independent adaptive events have to be studied in order to make a strong statement about the relative contribution of molecular convergence to this particular example of convergent character evolution.

Even though determining the exact role of molecular convergence during the convergent evolution of indehiscence in the Brassicaceae family remains a future challenge, reviewing data on genes involved in convergent crop domestication clearly demonstrates that this phenomenon represents an important principle in the evolution of plants under artificial selection (manuscript III). Besides its general importance during crop domestication, I also point out that molecular convergence often involves genes and their respective protein products fulfilling certain criteria with regard to their genetic background or position within a molecular pathway, similar to what has been reported for natural adaptation in animal systems. Thus the genetic changes underlying crop domestication seem to be somewhat predictable, at least in terms of an estimation of probabilities. However, cases of molecular convergence in plant systems are by far not restricted to domestic backgrounds and in future studies, more effort should be put into combining genetic data on artificial and natural adaptation of plants. Some genes are implicated in convergent changes in both, crop and wild species, as for example reported for the variation in flowering time caused by mutations in *FLC* (turnip, *B. oleracea*, *A. thaliana*, *Capsella rubella*) (Gazzani *et al.*, 2003; Michaels *et al.*, 2003; Okazaki *et al.*, 2007; Yuan *et al.*, 2009; Guo *et al.*, 2012; Wu *et al.*, 2012), or *FT* (rice, wheat, barley, sunflower, *A. thaliana*, *Lolium perenne*, *Boechera stricta*) (Yan *et al.*, 2006; Schwartz *et al.*, 2009; Takahashi *et al.*, 2009; Blackman *et al.*, 2010; Anderson *et al.*, 2011; Skot *et al.*, 2011). Mutations in other genes are repeatedly involved in convergent changes occurring specifically in wild plant species, as for *LEAFY* orthologs in the convergent evolution of rosette flowering (Yoon and Baum, 2004), for *GLABROUS1* orthologs in the convergent evolution of reduced resistance to insect herbivores (Kivimäki *et al.*, 2007), for *RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1* in the convergent loss of resistance to bacterial infections (Rose *et al.*, 2012), or for *ANTHOCYANIN2* in convergent

shifts in flower color and associated pollinator attraction (Hoballah *et al.*, 2007). In some spectacular cases, the convergent evolution of new traits was even shown to depend on convergent recruitment of genes or whole pathways into a new molecular context. The $C_4$ photosynthetic pathway evolved independently more than 45 times in various angiosperm families (Sage, 2004) and key enzymes of this pathway have been shown to have originated convergently several times from the same progenitor genes (Christin *et al.*, 2007; Christin *et al.*, 2008; Besnard *et al.*, 2009). During the independent evolution of leaves, apparently the same developmental pathway involving *KNOX* (*knotted1*-like homeobox) and *ARP* genes (MYB orthologs of *ASYMMETRIC LEAVES*, *ROUGH SHEATH2*, and *PHANTASTICA*) has been repeatedly recruited in different plant lineages (Harrison *et al.*, 2005), and convergent recruitment of *CYCLOIDEA* homologs has been implicated in independent evolutionary transitions to zygomorphy in distantly related core eudicot lineages (Preston and Hileman, 2009). These examples only represent the tip of an iceberg of excellent studies on the molecular background of convergent plant adaptation in natural environments. Summarizing these multifaceted data with the objective of comparing them with molecular data on crop domestication and animal evolution promises to make a valuable contribution towards an overall understanding of molecular evolutionary principles.

## 3.4 Beyond the ivory tower – practical contributions of this thesis

The results presented in this thesis are not only of relevance from an evolutionary point of view but may also be of considerable agronomic importance. *L. campestre* is currently developed into an oilseed crop for industrial applications (Eriksson, 2009). It has useful seed oil qualities and excellent winter hardiness which may allow an expansion of planting regions of oil crops to more northern latitudes (Andersson *et al.*, 1999; Ivarson *et al.*, 2013). In addition, it has the potential to serve as a biennial catch crop to reduce leaching of nutrients into ground and surface water during autumn and winter periods (Eriksson, 2009). Towards the adaption of *L. campestre* as a highly productive crop, the efficient and reliable transformation system described in this thesis (manuscript I) will be of great benefit in order to improve certain agronomic traits as for example seed oil content and composition. Reduction of seed shattering in order to reduce yield loss is another challenge of the domestication process that will profit from knowledge gained in this thesis. Insights into the *L. campestre* molecular pathway leading to fruit dehiscence will facilitate choosing targets for

genetic improvement of this particular trait and the correlation detected between gene expression level of *LcALC* and *LcIND* and the level of fruit dehiscence may further allow fine-tuning dehiscence capability (manuscript I+II).

The high conservation of the fruit developmental pathway that is demonstrated within the Brassicaceae family may also allow the transfer of knowledge from model species like *A. thaliana* and *L. campestre*, that are well studied in terms of fruit development, to other species of this family. Reducing pod dehiscence is an important issue for all those Brassicaceae that are (or may in the future be) grown for their seeds. This topic is most prominently discussed for oil-seed rape (*B. napus*), where under unfavorable climatic conditions up to 50% of seeds are lost due to premature pod shattering (MacLeod, 1981; Price *et al.*, 1996). Another problem is the growth of volunteer plants that derive from the seeds of shattered pods and contaminate future crops (Spence *et al.*, 1996; Ostergaard *et al.*, 2006). Unlike many other domesticated plant species that have been successfully selected for reduced seed dispersal (e.g. rice, wheat, sorghum, pea, or soybean) no shatter resistant rape varieties could be established so far, probably due to pleiotropic effects of certain genes in cell separation of both, fruit and anther dehiscence (Jarvis *et al.*, 2003). Thus, deeper insights into the developmental processes leading to fruit dehiscence in Brassicaceae are essential for developing improved strategies to overcome such problems using genetic technology (Liljegren *et al.*, 2004; Ostergaard *et al.*, 2006; Girin *et al.*, 2010). Other established Brassicaceae crops could benefit in their productivity from a reduction of seed dispersal. Indian mustard (*B. juncea*) is the oilseed crop of choice in India and Australia due to its high heat and drought tolerance (Ostergaard *et al.*, 2006). Like *B. napus*, cultivation of this species also suffers from high yield loss due to premature pod shattering. Further examples of Brassicaceae species producing seeds of agronomic importance include white mustard (*Sinapis alba*) , black mustard (*Brassica nigra*), different subspecies of *B. rapa*, Ethiopian mustard (*Brassica carinata*), gold-of-pleasure (*Camelina sativa*), *Crambe abyssinica*, and arugula (*Eruca vesicaria*) (Warwick *et al.*, 2006; Warwick, 2011). Besides, many family members are, like *L. campestre*, currently examined regarding their potential as new oilseed crops for various applications, including pennycress (*Thlaspi arvense*), honesty (*Lunaria annua*), hoary stock (*Matthiola incana*), Fendler's bladderpod (*Lesquerella fendleri*), or garden cress (*Lepidium sativum*) (Mathews *et al.*, 1993; Yaniv *et al.*, 1997; Marvin *et al.*, 2000; Mastebroek and Marvin, 2000; Dierig *et al.*, 2004; Gokavi *et al.*, 2004; Salywon *et al.*, 2005; Warwick, 2011; Dorn *et al.*, 2013). This orientation towards the development of

alternative crops is very commendable because it eases human dependency on only a few agriculturally highly used species, a situation that may become problematic in light of newly emerging plant pests, the continuous growth of the world's population, and changing climatic conditions (Conway and Toenniessen, 1999; Gressel, 2008; de Ribou *et al.*, 2013). Knowledge that speeds up the domestication of new plant species, for example by reducing fruit dehiscence, may considerably contribute to shaping a modern diversity-oriented farming system.

However, knowledge about the molecular background of a given trait is only one aspect when it comes to changing its parameter value in a concerted way. Other aspects are (i) the technical capability to screen for or introduce desired genetic alterations and (ii) a general understanding of the molecular principles that may designate certain genes as more promising targets for the introduction of domestication relevant mutations than others. The former aspect has to be approached in a species-specific manner, as for example by the development of a transformation system for *L. campestre* like is has been presented in this thesis (manuscript I). Concerning the latter aspect, it is proposed in manuscript III that genetically studying natural adaptation and previous domestication events may identify molecular characteristics of established (and thus successful) target genes of adaptive evolution. Such characteristics may guide the choice of target genes for the deliberate domestication of new species, resulting for example in the preferred employment of genes which occupy nodal positions within their molecular pathways or which exhibit little pleiotropic effects.

To sum up, the results presented in this thesis may be of agronomic importance on several levels: they may facilitate the domestication process of the new oilseed crop *L. campestre* by providing an easy and reliable transformation protocol; they may contribute to developing shatter-resistant Brassicaceae crops through various insights into the molecular background and conservation of fruit development in this family; and they may facilitate the genetic improvement of new and preexisting crop plants in general by identifying preferable characteristics of domestication target genes.

## 3.5 A fruity taste of things to come

In the frame of this thesis, the evolution of indehiscent fruits has been discussed with respect to its molecular and genetic background and its relevance for crop domestication. However,

the ecological significance of fruit indehiscence in contrast to dehiscence in natural habitats remains elusive. From research on heterocarpic species that form both, dehiscent and indehiscent fruits, within the same inflorescence, it has been shown that the seeds of these two fruit types may differ in ecologically important traits like seed size, dispersal capability, dormancy, or viability (Takeno and Yamaguchi, 1991; Venable *et al.*, 1995; Mandak and Pysek, 2001; Lu *et al.*, 2010). Because the phenomenon of heterocarpy has mainly been found in annual species adapted to unpredictable environments such as frequently disturbed habitats and arid and semi-arid regions, it is considered as a strategy to reduce the risk of extinction by escaping from unfavorable conditions either in space (differential seed dispersal) or in time (fractional germination) (Venable and Levin, 1985; Venable *et al.*, 1995; Imbert, 2002; Lu *et al.*, 2010). However, possible advantages of the exclusive production of indehiscent fruits have not been investigated to date, although the repeated emergence of this character in the Brassicaceae suggests it to provide a selective advantage under certain environmental conditions. Comparative evolutionary studies (Harvey and Pagel, 1991; Martins, 2000) may be one way to approach this question, either by searching for correlated changes between fruit dehiscence capability and other phenotypic or environmental traits in an unbiased way or by directly testing certain hypotheses, as for example a correlation between indehiscence and arid environments as it was proposed previously (Mühlhausen *et al.*, 2008). Other studies employing this kind of strategy for example found a correlation between cordate leaf shape and a climbing growth habit (Goodwillie *et al.*, 2004), evaluated the correlation of morphological evolution with habitat shifts in the Amblystegiaceae (Vanderpoorten *et al.*, 2002), or analyzed factors that may determine the length of non-coding organelle DNA spacers in plants (Duminil *et al.*, 2008). Because comparative studies rely strongly on the inclusion of reliable phylogenetic information (Felsenstein, 1985; Huelsenbeck *et al.*, 2000), existing Brassicaceae-phylogenies that do not include many indehiscent members (Beilstein *et al.*, 2010; Couvreur *et al.*, 2010; Warwick *et al.*, 2010) should be expanded or combined in order to comprise as many indehiscent species as possible.

Although the work presented in this thesis elucidates the loss of dehiscence zone identity gene expression at the valve-replum border of *L. appelianum* as the molecular mechanism that determines the development of indehiscent fruits in this plant, a future challenge will be to identify the exact genetic mutations underlying this adaptive change. So far, only candidate loci, which have been predicted from the fruit developmental pathway of *A. thaliana,* have been included in the search for such causative mutations. The upstream regulators of the

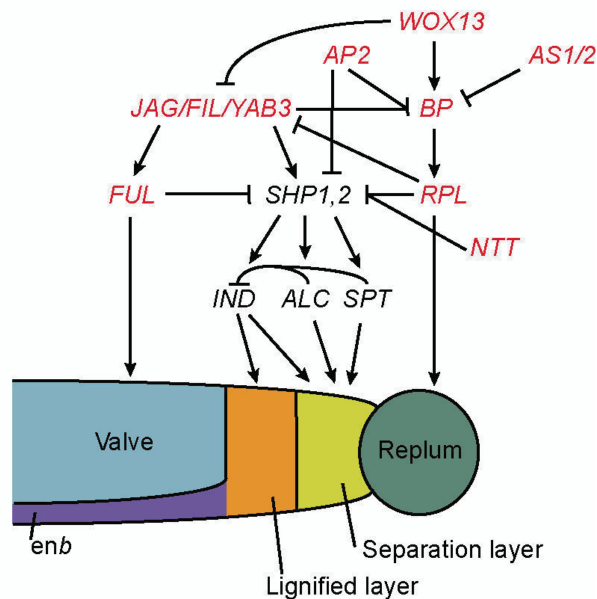


**Figure 5: Regulatory pathway controlling fruit dehiscence in *A. thaliana*.** The regulatory connections have been compiled based on several studies (Dinneny *et al.*, 2005; Dinneny and Yanofsky, 2005; Alonso-Cantabrana *et al.*, 2007; Girin *et al.*, 2011; Groszmann *et al.*, 2011; Ripoll *et al.*, 2011; Gonzalez-Reig *et al.*, 2012; Chung *et al.*, 2013; Romera-Branchat *et al.*, 2013). Candidate genes, whose orthologs may harbor mutations that lead to the elimination of *LaSHP* expression at the valve-replum border of *L. appelianum* fruits, are highlighted in red.

*LaSHP* genes, *LaFUL*, *LaREPLUMLESS* (*LaRPL*), and *LaAP2*, have been experimentally analyzed concerning their sequence conservation, gene expression patterns and protein function and a possible involvement of the *L. appelianum* orthologs of *JAGGED* (*JAG*), *YABBY3* (*YAB3*), and *FILAMENTOUS FLOWER* (*FIL*) in the development of the indehiscence phenotype has been discussed (manuscript II). However, new candidate genes are continuously discovered (Figure 5). The class I *KNOX* gene *BREVIPEDICELLUS* (*BP*) as well as the MYB transcription factor-encoding gene *ASYMMETRIC LEAVES1* (*AS1*) have previously been shown to participate in determining strength and position of the valve margin related *SHP* expression domain in fruits of *A. thaliana* (Gonzalez-Reig *et al.*, 2012). Besides, *WUSCHEL-LIKE HOMEOBOX13* (*WOX13*), a member of the plant-specific WOX family of transcription factor coding genes, has been identified as a negative regulator of valve margin identity genes with the ability to increase dehiscence capability (*wox13* mutants) or to cause indehiscence (35S::*WOX13* plants) (Romera-Branchat *et al.*, 2013). Similarly, *NO TRANSMITTING TRACT* (*NTT*) has been identified as yet another replum factor suppressing the expression of valve margin identity genes and inducing the formation of indehiscent fruits when ectopically expressed in 35S::*NTT* transgenic plants (Chung *et al.*, 2013). This increased number of candidate genes significantly reduces the chance of success of this approach and complementary or alternative strategies should be taken into account.

One major obstacle is the lack of sequence information from the two Lepidium species under study. In the course of this work, all genomic regions that were to be compared between *L. campestre* and *L. appelianum* had to be cloned and sequenced from scratch, a constraint that

has restricted the search for genetic differences to a very limited number of candidate sites. New sequencing technologies may provide new possibilities for this project (Schuster, 2008). A transcriptome analysis of *L. campestre* is currently under way (http://www.mistra.org/download/18.3ca3f3b513cd5907aea793/1378682217696/MistraBiotech_AR2012_Webb.pdf) and doing the same for *L. appelianum* may be a future option gaining lots of useful sequence information with relatively low effort. With rapidly decreasing costs for sequencing services, even gaining whole genome information for both species may become possible in the near future.

Hybridizing *L. campestre* and *L. appelianum* could form the basis for alternative approaches which are independent of previously identified candidate genes. Analyzing the patterns of inheritance of the indehiscent fruit phenotype in respective hybrid plants may be used to identify the number of loci that are involved in this phenotypic difference between both species. Furthermore, hybrid offspring populations may be created in order to fine-map the genomic location of causative mutations, resulting in the identification of candidate genes via conserved synteny with other Brassicaceae or of candidate sequence polymorphisms via positional cloning. Hybridization, both naturally occurring and artificially induced, is very common within the Brassicaceae, having resulted for example in the emergence of *B. napus* as an interspecific hybrid between *B. rapa* and *B. oleracea* (U, 1935; FitzJohn *et al.*, 2007; Allender and King, 2010). Preliminary attempts to hybridize *L. campestre* and *L. appelianum* have been unsuccessful (Andreas Mühlhausen, personal communication). However, embryo rescue (Sharma *et al.*, 1996; Reed, 2004; Cisneros and Tel-Zur, 2010) or protoplast fusion (Navrátilová, 2004) are techniques that have been repeatedly used to facilitate crosses between Brassicaceae species that do not readily hybridize (see for example Toriyama *et al.*, 1987; Hansen and Earle, 1995; Brown *et al.*, 1997; Bang *et al.*, 2003; Bang *et al.*, 2007).

As discussed above, one future desire may be to expand studying the genetic origin of indehiscent fruits from *L. appelianum* to additional non-shattering species in order to address more far-reaching questions concerning, for example, the relative contribution of molecular convergence to this adaptive event. Especially in this case, a classical candidate gene approach may prove too labor-intensive in light of the rising number of possible candidates and exploiting alternative strategies should be considered.

# 4. Summary

Identifying and understanding the molecular changes that accompany phenotypic adaptation are central goals in evolutionary biology. This thesis contributes to these goals by presenting two detailed case studies comparing the molecular network leading to fruit dehiscence in three Brassicaceae species that differ in prominent fruit traits and by featuring a higher level approach summarizing data on the molecular background of convergent crop domestication.

Fruits of *Lepidium campestre* and *Arabidopsis thaliana* share a common mechanism of dehiscence but differ dramatically in overall morphology. Data presented in this thesis highlight that despite this morphological variability the molecular pathway leading to fruit dehiscence is highly conserved between both species. My results further contribute to a better understanding of Brassicaceae fruit development in general by identifying the transcription factors ALCATRAZ and SPATULA to be repressors of *INDEHISCENT* gene expression.

*Lepidium appelianum* is a close relative of *L. campestre* and, in this thesis, serves as a representative of the many species within the Brassicaceae that have evolved indehiscent fruits. The molecular change causing this phenotypic adaptation is identified to be a loss of expression of dehiscence zone identity genes at the valve-replum border of *L. appelianum* fruits.

Plant domestication is often accompanied by dramatic phenotypic changes, presenting an excellent model to study molecular principles of adaptation. A survey of genes known to carry causative mutations responsible for domestication-related changes reveals that convergent plant domestication is often based on mutations at orthologous loci. Furthermore, factors are identified that influence the probability of a certain gene to serve as a target for mutations driving phenotypic adaptation.

Taken together, this thesis combines studies targeting the molecular background of phenotypic conservation as well as phenotypic change and searches for general patterns that may allow explaining or even predicting the likely course of molecular evolution. The results presented are of relevance for basic evolutionary biology and future agronomic applications, alike.

# Zusammenfassung

Zusammenhänge zwischen molekularen Veränderungen und phänotypischer Adaption zu erkennen und zu verstehen ist ein zentrales Ziel der Evolutionsbiologie. Die vorliegende Arbeit trägt zum Erreichen dieses Ziels bei, indem sie in zwei detaillierten Fallstudien die für die Fruchtöffnung wichtigen molekularen Netzwerke drei verschiedener Arten der Gattung Brassicaceae miteinander vergleicht und außerdem Ergebnisse über den molekularen Hintergrund konvergenter Domestikation von Nutzpflanzen überblickshaft darstellt.

Früchte der Arten *Lepidium campestre* und *Arabidopsis thaliana* haben zwar den gleichen Öffnungsmechanismus, unterscheiden sich aber stark in ihrem äußeren Erscheinungsbild. Die hier präsentierten Daten zeigen, dass die molekulare Regulation des Fruchtöffnungsprozesses trotz dieser morphologischen Variabilität zwischen beiden Spezies stark konserviert ist. Darüber hinaus tragen die Ergebnisse zu einem besseren allgemeinen Verständnis der Fruchtentwicklung in Brassicaceen bei, indem sie die Transkriptionsfaktoren ALCATRAZ und SPATULA als Repressoren des *INDEHISCENT* Gens identifizieren.

*Lepidium appelianum* ist nahe verwandt mit *L. campestre* und steht in dieser Arbeit stellvertretend für all die Brassicaceen, die im Laufe ihrer Evolution aus Öffnungsfrüchten Schließfrüchte entwickelt haben. Es wird gezeigt, dass in *L. appelianum* die molekulare Ursache dieser Merkmalsänderung ein Expressionsverlust von Genen ist, deren Orthologe in Arten mit Öffnungsfrüchten die Entwicklung der Dehiszenzzone steuern.

Domestikation von Pflanzen geht oft mit dramatischen phänotypischen Änderungen einher und ist daher ein hervorragendes Modell, um molekulare Grundsätze von Adaption zu erforschen. Die Analyse von Genen, die bekanntermaßen Mutationen als Ursache domestikationsbedingter Merkmalsänderungen tragen, zeigt, dass Konvergenz während pflanzlicher Domestikation oft durch Mutationen in orthologen Loci verursacht wird. Weiterhin werden Faktoren identifiziert, die die Wahrscheinlichkeit beeinflussen, mit der Änderungen in einem bestimmten Gen phänotypischer Adaption zugrunde liegen.

Das verbindende Element dieser Arbeit ist die Suche nach den molekularen Hintergründen phänotypischer Ausprägung sowie nach möglichen Gesetzmäßigkeiten, anhand derer der Verlauf molekularer Evolution erklärt oder sogar vorhergesagt werden könnte. Die Ergebnisse sind sowohl aus evolutionsbiologischer als auch aus agronomischer Sicht relevant.

# 5. Bibliography


**Abzhanov, A., Extavour, C.G., Groover, A., Hodges, S.A., Hoekstra, H.E., Kramer, E.M. and Monteiro, A.** (2008) Are we there yet? Tracking the development of new model systems. *Trends Genet.*, **24**, 353-360.

**Akita, Y., Nakada, M. and Kanno, A.** (2011) Effect of the expression level of an *AGAMOUS*-like gene on the petaloidy of stamens in the double-flowered lily, 'Elodie'. *Sci. Hortic. (Amst.)*, **128**, 48-53.

**Al-Shehbaz, I.A.** (2001) Brassicaceae (mustard family). In *eLS*. Chichester, UK: John Wiley & Sons, Ltd. DOI: 10.1038/npg.els.0003690.

**Al-Shehbaz, I.A. and Mummenhoff, K.** (2011) *Stubendorffia* and *Winklera* belong to the expanded *Lepidium* (Brassicaceae). *Edinb. J. Bot.*, **68**, 165-171.

**Al-Shehbaz, I.A., Mummenhoff, K. and Appel, O.** (2002) Cardaria, Coronopus, and Stroganowia are united with Lepidium (Brassicaceae). *Novon*, **12**, 5-11.

**Allender, C.J. and King, G.J.** (2010) Origins of the amphiploid species *Brassica napus* L. investigated by chloroplast and nuclear molecular markers. *BMC Plant Biol.*, **10**, 54.

**Alonso-Blanco, C., Aarts, M.G.M., Bentsink, L., Keurentjes, J.J.B., Reymond, M., Vreugdenhil, D. and Koornneef, M.** (2009) What has natural variation taught us about plant development, physiology, and adaptation? *Plant Cell*, **21**, 1877-1896.

**Alonso-Cantabrana, H., Ripoll, J.J., Ochando, I., Vera, A., Ferrandiz, C. and Martinez-Laborda, A.** (2007) Common regulatory networks in leaf and fruit patterning revealed by mutations in the *Arabidopsis ASYMMETRIC LEAVES1* gene. *Development*, **134**, 2663-2671.

**Alvarez, J. and Smyth, D.R.** (1999) *CRABS CLAW* and *SPATULA*, two *Arabidopsis* genes that control carpel development in parallel with *AGAMOUS*. *Development*, **126**, 2377-2386.

**Anderson, J.T., Lee, C.R. and Mitchell-Olds, T.** (2011) Life-history QTLs and natural selection on flowering time in *Boechera stricta*, a perennial relative of *Arabidopsis*. *Evolution*, **65**, 771-787.

**Andersson, A.A.M., Merker, A., Nilsson, P., Sorensen, H. and Aman, P.** (1999) Chemical composition of the potential new oilseed crops *Barbarea vulgaris*, *Barbarea verna* and *Lepidium campestre*. *J. Sci. Food Agric.*, **79**, 179-186.

**Appel, O. and Al-Shehbaz, I.A.** (2003) Cruciferae. In *Flowering Plants · Dicotyledons*, Kubitzki, K. and Bayer, C. eds: Springer Berlin Heidelberg, pp. 75-174. DOI: 10.1007/978-3-662-07255-4_17.

**Araujo, P., Cesarino, I., Carmello-Guerreiro, S.M. and Dornelas, M.C.** (2013) *Citrus sinensis* L. Osbeck orthologs of *FRUITFULL* and *SHATTERPROOF* are differentially expressed during fruit development. *Plant Growth Regul.*, **70**, 1-13.

**Arendt, J. and Reznick, D.** (2008) Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? *Trends Ecol. Evol.*, **23**, 26-32.

**Arnaud, N., Girin, T., Sorefan, K., Fuentes, S., Wood, T.A., Lawrenson, T., Sablowski, R. and Ostergaard, L.** (2010) Gibberellins control fruit patterning in *Arabidopsis thaliana*. *Genes Dev.*, **24**, 2127-2132.

**Arnaud, N., Lawrenson, T., Ostergaard, L. and Sablowski, R.** (2011) The same regulatory point mutation changed seed-dispersal structures in evolution and domestication. *Curr. Biol.*, **21**, 1215-1219.

**Asano, K., Takashi, T., Miura, K., Qian, Q., Kitano, H., Matsuoka, M. and Ashikari, M.** (2007) Genetic and molecular analysis of utility of *sd1* alleles in rice breeding. *Breed. Sci.*, **57**, 53-58.

**Asano, K., Yamasaki, M., Takuno, S., Miura, K., Katagiri, S., Ito, T., Doi, K., Wu, J.Z., Ebana, K., Matsumoto, T., Innan, H., Kitano, H., Ashikari, M. and Matsuoka, M.** (2011) Artificial selection for a green revolution gene during *japonica* rice domestication. *Proc. Natl. Acad. Sci. U. S. A.*, **108**, 11034-11039.

**Asp, T., Byrne, S., Gundlach, H., Bruggmann, R., Mayer, K.F.X., Andersen, J.R., Xu, M.L., Greve, M., Lenk, I. and Lubberstedt, T.** (2011) Comparative sequence analysis of *VRN1* alleles of *Lolium perenne* with the co-linear regions in barley, wheat, and rice. *Mol. Genet. Genomics*, **286**, 433-447.

**Avino, M., Kramer, E., Donohue, K., Hammel, A. and Hall, J.** (2012) Understanding the basis of a novel fruit type in Brassicaceae: conservation and deviation in expression patterns of six genes. *EvoDevo*, **3**, 20.

**Azhaguvel, P. and Komatsuda, T.** (2007) A phylogenetic analysis based on nucleotide sequence of a marker linked to the brittle rachis locus indicates a diphyletic origin of barley. *Ann. Bot.*, **100**, 1009-1015.

**Bailey, C.D., Koch, M.A., Mayer, M., Mummenhoff, K., O'Kane, S.L., Warwick, S.I., Windham, M.D. and Al-Shehbaz, I.A.** (2006) Toward a global phylogeny of the Brassicaceae. *Mol. Biol. Evol.*, **23**, 2142-2160.

**Bang, S.W., Mizuno, Y., Kaneko, Y., Matsuzawa, Y. and Bang, K.S.** (2003) Production of intergeneric hybrids between the C3-C4 intermediate species *Diplotaxis tenuifolia* (L.) DC. and *Raphanus sativus* L. *Breed. Sci.*, **53**, 231-236.

**Bang, S.W., Sugihara, K., Jeung, B.H., Kaneko, R., Satake, E., Kaneko, Y. and Matsuzawa, Y.** (2007) Production and characterization of intergeneric hybrids between *Brassica oleracea* and a wild relative *Moricandia arvensis*. *Plant Breed.*, **126**, 101-103.

**Bao, X.Z., Franks, R.G., Levin, J.Z. and Liu, Z.C.** (2004) Repression of *AGAMOUS* by *BELLRINGER* in floral and inflorescence meristems. *Plant Cell*, **16**, 1478-1489.

**Barfield, D.G. and Pua, E.C.** (1991) Gene transfer in plants of *Brassica juncea* using *Agrobacterium tumefaciens*-mediated transformation. *Plant Cell Rep.*, **10**, 308-314.

**Bartholmes, C., Nutt, P. and Theissen, G.** (2008) Germline transformation of Shepherd's purse (*Capsella bursa-pastoris*) by the 'floral dip' method as a tool for evolutionary and developmental biology. *Gene*, **409**, 11-19.

**Beales, J., Turner, A., GriYths, S., Snape, J.W. and Laurie, D.A.** (2007) A *Pseudo-Response Regulator* is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.*, **115**, 721-733.

**Bechtold, N., Jaudeau, B., Jolivet, S., Maba, B., Vezon, D., Voisin, R. and Pelletier, G.** (2000) The maternal chromosome set is the target of the T-DNA in the *in planta* transformation of *Arabidopsis thaliana*. *Genetics*, **155**, 1875-1887.

**Beilstein, M.A., Al-Shehbaz, I.A. and Kellogg, E.A.** (2006) Brassicaceae phylogeny and trichome evolution. *Am. J. Bot.*, **93**, 607-619.

**Beilstein, M.A., Nagalingum, N.S., Clements, M.D., Manchester, S.R. and Mathews, S.** (2010) Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.*, **107**, 18724-18728.

**Bemer, M., Karlova, R., Ballester, A.R., Tikunov, Y.M., Bovy, A.G., Wolters-Arts, M., Rossetto Pde, B., Angenent, G.C. and de Maagd, R.A.** (2012) The tomato FRUITFULL homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-independent aspects of fruit ripening. *Plant Cell*, **24**, 4437-4451.

**Bennett, T.H., Flowers, T.J. and Bromham, L.** (2013) Repeated evolution of salt-tolerance in grasses. *Biol. Lett.*, **9**, 20130029.

**Besnard, G., Muasya, A.M., Russier, F., Roalson, E.H., Salamin, N. and Christin, P.A.** (2009) Phylogenomics of C4 photosynthesis in sedges (Cyperaceae): multiple appearances and genetic convergence. *Mol. Biol. Evol.*, **26**, 1909-1919.

**Bharathan, G., Goliber, T.E., Moore, C., Kessler, S., Pham, T. and Sinha, N.R.** (2002) Homologies in leaf form inferred from KNOXI gene expression during development. *Science*, **296**, 1858-1860.

**Bhatt, A.M., Etchells, J.P., Canales, C., Lagodienko, A. and Dickinson, H.** (2004) *VAAMANA* - a BEL1-like homeodomain protein, interacts with KNOX proteins BP and STM and regulates inflorescence stem growth in *Arabidopsis*. *Gene*, **328**, 103-111.

**Blackman, B.K., Strasburg, J.L., Raduski, A.R., Michaels, S.D. and Rieseberg, L.H.** (2010) The role of recently derived *FT* paralogs in sunflower domestication. *Curr. Biol.*, **20**, 629-635.

**Bolmgren, K. and Eriksson, O.** (2005) Fleshy fruits - origins, niche shifts, and diversification. *Oikos*, **109**, 255-272.

**Bowman, J.L.** (2006) Molecules and morphology: comparative developmental genetics of the Brassicaceae. *Plant Syst. Evol.*, **259**, 199-215.

**Bowman, J.L., Smyth, D.R. and Meyerowitz, E.M.** (1989) Genes directing flower development in *Arabidopsis*. *Plant Cell*, **1**, 37-52.

**Bradford, J.C. and Barnes, R.W.** (2001) Phylogenetics and classification of Cunoniaceae (Oxalidales) using chloroplast DNA sequences and morphology. *Syst. Bot.*, **26**, 354-385.

**Bremer, B. and Eriksson, O.** (1992) Evolution of fruit characteristics and dispersal modes in the tropical family Rubiaceae. *Biol. J. Linn. Soc.*, **47**, 79-95.

**Brown, J., Brown, A.P., Davis, J.B. and Erickson, D.** (1997) Intergeneric hybridization between *Sinapis alba* and *Brassica napus*. *Euphytica*, **93**, 163-168.

**Bruce, D.M., Farrent, J.W., Morgan, C.L. and Child, R.D.** (2002) Determining the oilseed rape pod strength needed to reduce seed loss due to pod shatter. *Biosyst. Eng.*, **81**, 179-184.

**Butelli, E., Licciardello, C., Zhang, Y., Liu, J.J., Mackay, S., Bailey, P., Reforgiato-Recupero, G. and Martin, C.** (2012) Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. *Plant Cell*, **24**, 1242-1255.

**Byrne, M.E., Groover, A.T., Fontana, J.R. and Martienssen, R.A.** (2003) Phyllotactic pattern and stem cell fate are determined by the *Arabidopsis* homeobox gene *BELLRINGER*. *Development*, **130**, 3941-3950.

**Cai, S. and Lashbrook, C.C.** (2008) Stamen abscission zone transcriptome profiling reveals new candidates for abscission control: enhanced retention of floral organs in transgenic plants overexpressing Arabidopsis *ZINC FINGER PROTEIN2*. *Plant Physiol.*, **146**, 1305-1321.

**Campanella, J.J., Bitincka, L. and Smalley, J.** (2003) MatGAT: An application that generates similarity/identity matrices using protein or DNA sequences. *BMC Bioinformatics*, **4**, DOI: 10.1186/1471-2105-1184-1129.

**Carroll, S.B.** (2008) Evo-devo and an expanding evolutionary synthesis: A genetic theory of morphological evolution. *Cell*, **134**, 25-36.

**Cevik, V., Ryder, C.D., Popovich, A., Manning, K., King, G.J. and Seymour, G.B.** (2010) A *FRUITFULL-like* gene is associated with genetic variation for fruit flesh firmness in apple (*Malus domestica* Borkh.). *Tree Genet. Genomes*, **6**, 271-279.

**Chauvaux, N., Child, R., John, K., Ulvskov, P., Borkhardt, B., Prinsen, E. and VanOnckelen, H.A.** (1997) The role of auxin in cell separation in the dehiscence zone of oilseed rape pods. *J. Exp. Bot.*, **48**, 1423-1429.

**Chen, X.M.** (2004) A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science*, **303**, 2022-2025.

**Chhikara, S., Dutta, I., Paulose, B., Jaiwal, P.K. and Dhankher, O.P.** (2012) Development of an *Agrobacterium*-mediated stable transformation method for industrial oilseed crop *Crambe abyssinica* 'BelAnn'. *Ind. Crop. Prod.*, **37**, 457-465.

**Chono, M., Honda, I., Zeniya, H., Yoneyama, K., Saisho, D., Takeda, K., Takatsuto, S., Hoshino, T. and Watanabe, Y.** (2003) A semidwarf phenotype of barley uzu results from a nucleotide substitution in the gene encoding a putative brassinosteroid receptor. *Plant Physiol.*, **133**, 1209-1219.

**Christin, P.A., Salamin, N., Muasya, A.M., Roalson, E.H., Russier, F. and Besnard, G.** (2008) Evolutionary switch and genetic convergence on *rbcL* following the evolution of C4 photosynthesis. *Mol. Biol. Evol.*, **25**, 2361-2368.

**Christin, P.A., Salamin, N., Savolainen, V., Duvall, M.R. and Besnard, G.** (2007) C4 photosynthesis evolved in grasses via parallel adaptive genetic changes. *Curr. Biol.*, **17**, 1241-1247.

**Christin, P.A., Weinreich, D.M. and Besnard, G.** (2010) Causes and evolutionary significance of genetic convergence. *Trends Genet.*, **26**, 400-405.

**Chung, K.S., Lee, J.H., Lee, J.S. and Ahn, J.H.** (2013) Fruit indehiscence caused by enhanced expression of *NO TRANSMITTING TRACT* in *Arabidopsis thaliana*. *Mol. Cells*, **35**, 519-525.

**Chung, M.Y., Vrebalov, J., Alba, R., Lee, J., McQuinn, R., Chung, J.D., Klein, P. and Giovannoni, J.** (2010) A tomato (*Solanum lycopersicum*) *APETALA2/ERF* gene, *SlAP2a*, is a negative regulator of fruit ripening. *Plant J.*, **64**, 936-947.

**Cisneros, A. and Tel-Zur, N.** (2010) Embryo rescue and plant regeneration following interspecific crosses in the genus *Hylocereus* (Cactaceae). *Euphytica*, **174**, 73-82.

**Clausing, G., Meyer, K. and Renner, S.S.** (2000) Correlations among fruit traits and evolution of different fruits within Melastomataceae. *Bot. J. Linn. Soc.*, **133**, 303-326.

**Clauss, M.J. and Koch, M.A.** (2006) Poorly known relatives of *Arabidopsis thaliana*. *Trends Plant Sci.*, **11**, 449-459.

**Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibe, B., Bouix, J., Caiment, F., Elsen, J.M., Eychenne, F., Larzul, C., Laville, E., Meish, F., Milenkovic, D., Tobin, J., Charlier, C. and Georges, M.** (2006) A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nat. Genet.*, **38**, 813-818.

**Clough, S.J. and Bent, A.F.** (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.*, **16**, 735-743.

**Cockram, J., White, J., Zuluaga, D.L., Smith, D., Comadran, J., Macaulay, M., Luo, Z.W., Kearsey, M.J., Werner, P., Harrap, D., Tapsell, C., Liu, H., Hedley, P.E., Stein, N., Schulte, D., Steuernagel, B., Marshall, D.F., Thomas, W.T.B., Ramsay, L., Mackay, I., Balding, D.J., Waugh, R., O'Sullivan, D.M. and The AGOUEB Consortium.** (2010) Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. *Proc. Natl. Acad. Sci. U. S. A.*, **107**, 21611-21616.

**Cole, M., Nolte, C. and Werr, W.** (2006) Nuclear import of the transcription factor *SHOOT MERISTEMLESS* depends on heterodimerization with BLH proteins expressed in discrete sub-domains of the shoot apical meristem of *Arabidopsis thaliana*. *Nucleic Acids Res.*, **34**, 1281-1292.

**Colosimo, P.F., Hosemann, K.E., Balabhadra, S., Villarreal, G., Dickson, M., Grimwood, J., Schmutz, J., Myers, R.M., Schluter, D. and Kingsley, D.M.** (2005) Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*, **307**, 1928-1933.

**Comadran, J., Kilian, B., Russell, J., Ramsay, L., Stein, N., Ganal, M., Shaw, P., Bayer, M., Thomas, W., Marshall, D., Hedley, P., Tondelli, A., Pecchioni, N., Francia, E., Korzun, V., Walther, A. and Waugh, R.** (2012) Natural variation in a homolog of *Antirrhinum CENTRORADIALIS* contributed to spring growth habit and environmental adaptation in cultivated barley. *Nat. Genet.*, **44**, 1388-1392.

**Conte, G.L., Arnegard, M.E., Peichel, C.L. and Schluter, D.** (2012) The probability of genetic parallelism and convergence in natural populations. *P. Roy. Soc. B-Biol. Sci.*, **279**, 5039-5047.

**Conway, G. and Toenniessen, G.** (1999) Feeding the world in the twenty-first century. *Nature*, **402**, C55-C58.

**Coustham, V., Li, P.J., Strange, A., Lister, C., Song, J. and Dean, C.** (2012) Quantitative modulation of polycomb silencing underlies natural variation in vernalization. *Science*, **337**, 584-587.

**Couvreur, T.L.P., Franzke, A., Al-Shehbaz, I.A., Bakker, F.T., Koch, M.A. and Mummenhoff, K.** (2010) Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). *Mol. Biol. Evol.*, **27**, 55-71.

**Curtis, I.S. and Nam, H.G.** (2001) Transgenic radish (*Raphanus sativus* L. *longipinnatus* Bailey) by floral-dip method - plant development and surfactant are important in optimizing transformation efficiency. *Transgenic Res.*, **10**, 363-371.

**Daminato, M., Guzzo, F. and Casadoro, G.** (2013) A *SHATTERPROOF*-like gene controls ripening in non-climacteric strawberries, and auxin and abscisic acid antagonistically affect its expression. *J. Exp. Bot.*, **64**, 3775-3786.

**Darwin, C.** (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* 1st edn. London,: J. Murray.

**Davis, C.C., Anderson, W.R. and Donoghue, M.J.** (2001) Phylogeny of Malpighiaceae: Evidence from chloroplast *ndhF* and *trnL-F* nucleotide sequences. *Am. J. Bot.*, **88**, 1830-1846.

**De Block, M., Debrouwer, D. and Moens, T.** (1997) The development of a nuclear male sterility system in wheat. Expression of the *barnase* gene under the control of tapetum specific promoters. *Theor. Appl. Genet.*, **95**, 125-131.

**De Buck, S., Van Montagu, M. and Depicker, A.** (2001) Transgene silencing of invertedly repeated transgenes is released upon deletion of one of the transgenes involved. *Plant Mol. Biol.*, **46**, 433-445.

**De Buck, S., Windels, P., De Loose, M. and Depicker, A.** (2004) Single-copy T-DNAs integrated at different positions in the *Arabidopsis* genome display uniform and comparable *ß*-glucuronidase accumulation levels. *Cell. Mol. Life Sci.*, **61**, 2632-2645.

**de Oliveira, M.L.P., Febres, V.J., Costa, M.G.C., Moore, G.A. and Otoni, W.C.** (2009) High-efficiency *Agrobacterium*-mediated transformation of citrus via sonication and vacuum infiltration. *Plant Cell Rep.*, **28**, 387-395.

**De Paepe, A., De Buck, S., Hoorelbeke, K., Nolf, J., Peck, I. and Depicker, A.** (2009) High frequency of single-copy T-DNA transformants produced by floral dip in *CRE*-expressing Arabidopsis plants. *Plant J.*, **59**, 517-527.

**de Ribou, S.D., Douam, F., Hamant, O., Frohlich, M.W. and Negrutiu, J.** (2013) Plant science and agricultural productivity: Why are we hitting the yield ceiling? *Plant Sci.*, **210**, 159-176.

**Desfeux, C., Clough, S.J. and Bent, A.F.** (2000) Female reproductive tissues are the primary target of *Agrobacterium*-mediated transformation by the Arabidopsis floral-dip method. *Plant Physiol.*, **123**, 895-904.

**Diaz, A., Zikhali, M., Turner, A.S., Isaac, P. and Laurie, D.A.** (2012) Copy number variation affecting the *Photoperiod-B1* and *Vernalization-A1* genes is associated with altered flowering time in wheat (*Triticum aestivum*). *Plos One*, **7**, e33234.

**Dierig, D.A., Tomasi, P.M., Salywon, A.M. and Ray, D.T.** (2004) Improvement in hydroxy fatty acid seed oil content and other traits from interspecific hybrids of three *Lesquerella* species: *Lesquerella fendleri*, *L. pallida*, and *L. lindheimeri*. *Euphytica*, **139**, 199-206.

**Dinneny, J.R., Weigel, D. and Yanofsky, M.F.** (2005) A genetic framework for fruit patterning in *Arabidopsis thaliana*. *Development*, **132**, 4687-4696.

**Dinneny, J.R., Yadegari, R., Fischer, R.L., Yanofsky, M.F. and Weigel, D.** (2004) The role of *JAGGED* in shaping lateral organs. *Development*, **131**, 1101-1110.

**Dinneny, J.R. and Yanofsky, M.F.** (2005) Drawing lines and borders: how the dehiscent fruit of *Arabidopsis* is patterned. *BioEssays*, **27**, 42-49.

**Doebley, J.F., Gaut, B.S. and Smith, B.D.** (2006) The molecular genetics of crop domestication. *Cell*, **127**, 1309-1321.

**Dorn, K.M., Fankhauser, J.D., Wyse, D.L. and Marks, M.D.** (2013) De novo assembly of the pennycress (*Thlaspi arvense*) transcriptome provides tools for the development of a winter cover crop and biodiesel feedstock. *Plant J.*, **75**, 1028-1038.

**Dubois, A., Raymond, O., Maene, M., Baudino, S., Langlade, N.B., Boltz, V., Vergne, P. and Bendahmane, M.** (2010) Tinkering with the C-function: A molecular frame for the selection of double flowers in cultivated roses. *Plos One*, **5**, e9288.

**Duminil, J., Grivet, D., Ollier, S., Jeandroz, S. and Petit, R.J.** (2008) Multilevel control of organelle DNA sequence length in plants. *J. Mol. Evol.*, **66**, 405-415.

**Elmer, K.R. and Meyer, A.** (2011) Adaptation in the age of ecological genomics: insights from parallelism and convergence. *Trends Ecol. Evol.*, **26**, 298-306.

**Eriksson, D.** (2009) Towards the domestication of *Lepidium campestre* as an undersown oilseed crop. In *Department of Plant Breeding and Biotechnology*. Alnarp: Swedish University of Agricultural Sciences, pp. 42.

**Fan, L.J., Quan, L.Y., Leng, X.D., Guo, X.Y., Hu, W.M., Ruan, S.L., Ma, H.S. and Zeng, M.Q.** (2008) Molecular evidence for post-domestication selection in the *Waxy* gene of Chinese waxy maize. *Mol. Breed.*, **22**, 329-338.

**Faure, S., Turner, A.S., Gruszka, D., Christodoulou, V., Davis, S.J., von Korff, M. and Laurie, D.A.** (2012) Mutation at the circadian clock gene *EARLY MATURITY 8* adapts domesticated barley (*Hordeum vulgare*) to short growing seasons. *Proc. Natl. Acad. Sci. U. S. A.*, **109**, 8328-8333.

**Favaro, R., Pinyopich, A., Battaglia, R., Kooiker, M., Borghi, L., Ditta, G., Yanofsky, M.F., Kater, M.M. and Colombo, L.** (2003) MADS-box protein complexes control carpel and ovule development in *Arabidopsis*. *Plant Cell*, **15**, 2603-2611.

**Fawcett, J.A., Maere, S. and Van de Peer, Y.** (2009) Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event. *Proc. Natl. Acad. Sci. U. S. A.*, **106**, 5737-5742.

**Feldman, C.R., Brodie, E.D., Brodie, E.D. and Pfrender, M.E.** (2012) Constraint shapes convergence in tetrodotoxin-resistant sodium channels of snakes. *Proc. Natl. Acad. Sci. U. S. A.*, **109**, 4556-4561.

**Felsenstein, J.** (1985) Phylogenies and the comparative method. *Am. Nat.*, **125**, 1-15.

**Ferrandiz, C.** (2011) Fruit Structure and Diversity. In *eLS*. Chichester, UK: John Wiley & Sons, Ltd. DOI: 10.1002/9780470015902.a0002044.pub2.

**Ferrandiz, C., Gu, Q., Martienssen, R. and Yanofsky, M.F.** (2000a) Redundant regulation of meristem identity and plant architecture by FRUITFULL, APETALA1 and CAULIFLOWER. *Development*, **127**, 725-734.

**Ferrandiz, C., Liljegren, S.J. and Yanofsky, M.F.** (2000b) Negative regulation of the *SHATTERPROOF* genes by FRUITFULL during *Arabidopsis* fruit development. *Science*, **289**, 436-438.

**FitzJohn, R.G., Armstrong, T.T., Newstrom-Lloyd, L.E., Wilton, A.D. and Cochrane, M.** (2007) Hybridisation within *Brassica* and allied genera: evaluation of potential for transgene escape. *Euphytica*, **158**, 209-230.

**Flagel, L.E. and Wendel, J.F.** (2009) Gene duplication and evolutionary novelty in plants. *New Phytol.*, **183**, 557-564.

**Flanagan, C.A., Hu, Y. and Ma, H.** (1996) Specific expression of the *AGL1* MADS-box gene suggests regulatory functions in *Arabidopsis* gynoecium and ovule development. *Plant J.*, **10**, 343-353.

**Force, A., Lynch, M., Pickett, F.B., Amores, A., Yan, Y.L. and Postlethwait, J.** (1999) Preservation of duplicate genes by complementary, degenerative mutations. *Genetics*, **151**, 1531-1545.

**Foucher, F., Morin, J., Courtiade, J., Cadioux, S., Ellis, N., Banfield, M.J. and Rameau, C.** (2003) *DETERMINATE* and *LATE FLOWERING* are two *TERMINAL FLOWER1/CENTRORADIALIS* homologs that control two distinct phases of flowering initiation and development in pea. *Plant Cell*, **15**, 2742-2754.

**Fourquin, C., Del Cerro, C., Victoria, F.C., Vialette-Guiraud, A., de Oliveira, A.C. and Ferrandiz, C.** (2013) A change in SHATTERPROOF protein lies at the origin of a fruit morphological novelty and a new strategy for seed dispersal in Medicago genus. *Plant Physiol.*, **162**, 907-917.

**Fourquin, C. and Ferrandiz, C.** (2012) Functional analyses of AGAMOUS family members in *Nicotiana benthamiana* clarify the evolution of early and late roles of C-function genes in eudicots. *Plant J.*, **71**, 990-1001.

**Franzke, A., Lysak, M.A., Al-Shehbaz, I.A., Koch, M.A. and Mummenhoff, K.** (2011) Cabbage family affairs: the evolutionary history of Brassicaceae. *Trends Plant Sci.*, **16**, 108-116.

**Frohman, M.A., Dush, M.K. and Martin, G.R.** (1988) Rapid production of full-length cDNAs from rare transcripts - amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. U. S. A.*, **85**, 8998-9002.

**Furukawa, T., Maekawa, M., Oki, T., Suda, I., Iida, S., Shimada, H., Takamure, I. and Kadowaki, K.I.** (2007) The *Rc* and *Rd* genes are involved in proanthocyanidin synthesis in rice pericarp. *Plant J.*, **49**, 91-102.

**Galimba, K.D., Tolkin, T.R., Sullivan, A.M., Melzer, R., Theissen, G. and Di Stilio, V.S.** (2012) Loss of deeply conserved C-class floral homeotic gene function and C- and E-class protein interaction in a double-flowered ranunculid mutant. *Proc. Natl. Acad. Sci. U. S. A.*, **109**, E2267-E2275.

**Gaskin, J.F., Zhang, D.Y. and Bon, M.C.** (2005) Invasion of *Lepidium draba* (Brassicaceae) in the western United States: distributions and origins of chloroplast DNA haplotypes. *Mol. Ecol.*, **14**, 2331-2341.

**Gasser, C.S. and Simon, M.K.** (2011) Seed dispersal: same gene, different organs. *Curr. Biol.*, **21**, R546-R548.

**Gazzani, S., Gendall, A.R., Lister, C. and Dean, C.** (2003) Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. *Plant Physiol.*, **132**, 1107-1114.

**Gerlach, D.** (1984) *Botanische Mikrotechnik, eine Einführung, 2. Aufl.* Stuttgart: Georg Thieme Verlag.

**Gillman, J.D., Tetlow, A., Lee, J.D., Shannon, J.G. and Bilyeu, K.** (2011) Loss-of-function mutations affecting a specific *Glycine max* R2R3 MYB transcription factor result in brown hilum and brown seed coats. *BMC Plant Biol.*, **11**.

**Girin, T., Paicu, T., Stephenson, P., Fuentes, S., Korner, E., O'Brien, M., Sorefan, K., Wood, T.A., Balanza, V., Ferrandiz, C., Smyth, D.R. and Ostergaard, L.** (2011) INDEHISCENT and SPATULA interact to specify carpel and valve margin tissue and thus promote seed dispersal in *Arabidopsis*. *Plant Cell*, **23**, 3641-3653.

**Girin, T., Sorefan, K. and Ostergaard, L.** (2009) Meristematic sculpting in fruit development. *J. Exp. Bot.*, **60**, 1493-1502.

**Girin, T., Stephenson, P., Goldsack, C.M.P., Kempin, S.A., Perez, A., Pires, N., Sparrow, P.A., Wood, T.A., Yanofsky, M.F. and Ostergaard, L.** (2010) Brassicaceae *INDEHISCENT* genes specify valve margin cell fate and repress replum formation. *Plant J.*, **63**, 329-338.

**Gokavi, S.S., Malleshi, N.G. and Guo, M.R.** (2004) Chemical composition of garden cress (*Lepidium sativum*) seeds and its fractions and use of bran as a functional ingredient. *Plant Food Hum. Nutr.*, **59**, 105-111.

**Gompel, N. and Prud'homme, B.** (2009) The causes of repeated genetic evolution. *Dev. Biol.*, **332**, 36-47.

**Gompel, N., Prud'homme, B., Wittkopp, P.J., Kassner, V.A. and Carroll, S.B.** (2005) Chance caught on the wing: cis-regulatory evolution and the origin of pigment patterns in Drosophila. *Nature*, **433**, 481-487.

**Gonzalez-Reig, S., Ripoll, J.J., Vera, A., Yanofsky, M.F. and Martinez-Laborda, A.** (2012) Antagonistic gene activities determine the formation of pattern elements along the mediolateral axis of the *Arabidopsis* fruit. *Plos Genet*, **8**, e1003020.

**Goodwillie, C., May, M.K., West, J.W. and McKeon, C.S.** (2004) Convergence in the leaf shape of vines: A test of the Carolina flora using phylogenetic comparative methods. *Southeast. Nat.*, **3**, 277-288.

**Gouy, M., Guindon, S. and Gascuel, O.** (2010) SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.*, **27**, 221-224.

**Gressel, J.** (2008) *Genetic glass ceilings: transgenics for crop biodiversity* Baltimore, Maryland: Johns Hopkins University Press.

**Grieneisen, V.A., Maree, A.F. and Ostergaard, L.** (2013) Juicy stories on female reproductive tissue development: coordinating the hormone flows. *J. Integr. Plant Biol.*, **55**, 847-863.

**Grobet, L., Martin, L.J.R., Poncelet, D., Pirottin, D., Brouwers, B., Riquet, J., Schoeberlein, A., Dunner, S., Menissier, F., Massabanda, J., Fries, R., Hanset, R. and Georges, M.** (1997) A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. *Nat. Genet.*, **17**, 71-74.

**Gross, B.L. and Olsen, K.M.** (2010) Genetic perspectives on crop domestication. *Trends Plant Sci.*, **15**, 529-537.

**Gross, J.B., Borowsky, R. and Tabin, C.J.** (2009) A novel role for *Mc1r* in the parallel evolution of depigmentation in independent populations of the cavefish *Astyanax mexicanus*. *Plos Genet*, **5**, e1000326.

**Groszmann, M., Paicu, T., Alvarez, J.P., Swain, S.M. and Smyth, D.R.** (2011) *SPATULA* and *ALCATRAZ*, are partially redundant, functionally diverging bHLH genes required for Arabidopsis gynoecium and fruit development. *Plant J.*, **68**, 816-829.

**Groszmann, M., Paicu, T. and Smyth, D.R.** (2008) Functional domains of SPATULA, a bHLH transcription factor involved in carpel and fruit development in Arabidopsis. *Plant J.*, **55**, 40-52.

**Gu, Q., Ferrandiz, C., Yanofsky, M.F. and Martienssen, R.** (1998) The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. *Development*, **125**, 1509-1517.

**Guo, Y.-L., Todesco, M., Hagmann, J., Das, S. and Weigel, D.** (2012) Independent *FLC* mutations as causes of flowering-time variation in *Arabidopsis thaliana* and *Capsella rubella*. *Genetics*, **192**, 729-739.

**Hall, J.C., Sytsma, K.J. and Iltis, H.H.** (2002) Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *Am. J. Bot.*, **89**, 1826-1842.

**Hall, J.C., Tisdale, T.E., Donohue, K. and Kramer, E.M.** (2006) Developmental basis of an anatomical novelty: Heteroarthrocarpy in *Cakile lanceolata* and *Erucaria erucarioides* (Brassicaceae). *Int. J. Plant Sci.*, **167**, 771-789.

**Hansen, L.N. and Earle, E.D.** (1995) Transfer of resistance to *Xanthomonas campestris* pv *campestris* into *Brassica oleracea* L by protoplast fusion. *Theor. Appl. Genet.*, **91**, 1293-1300.

**Harrison, C.J., Corley, S.B., Moylan, E.C., Alexander, D.L., Scotland, R.W. and Langdale, J.A.** (2005) Independent recruitment of a conserved developmental mechanism during leaf evolution. *Nature*, **434**, 509-514.

**Harvey, P.H. and Pagel, M.D.** (1991) *Comparative method in evolutionary biology* Oxford: Oxford University Press.

**Hedges, S.B.** (2002) The origin and evolution of model organisms. *Nat. Rev. Genet.*, **3**, 838-849.

**Heim, M.A., Jakoby, M., Werber, M., Martin, C., Weisshaar, B. and Bailey, P.C.** (2003) The basic helix-loop-helix transcription factor family in plants: A genome-wide study of protein structure and functional diversity. *Mol. Biol. Evol.*, **20**, 735-747.

**Higgins, J.A., Bailey, P.C. and Laurie, D.A.** (2010) Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. *Plos One*, **5**, e10065.

**Hoballah, M.E., Gübitz, T., Stuurman, J., Broger, L., Barone, M., Mandel, T., Dell'Olivo, A., Arnold, M. and Kuhlemeier, C.** (2007) Single gene–mediated shift in pollinator attraction in *Petunia*. *The Plant Cell Online*, **19**, 779-790.

**Hoekema, A., Hirsch, P.R., Hooykaas, P.J.J. and Schilperoort, R.A.** (1983) A binary plant vector strategy based on separation of *vir*- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. *Nature*, **303**, 179-180.

**Hoekstra, H.E. and Coyne, J.A.** (2007) The locus of evolution: Evo devo and the genetics of adaptation. *Evolution*, **61**, 995-1016.

**Hoekstra, H.E., Hirschmann, R.J., Bundey, R.A., Insel, P.A. and Crossland, J.P.** (2006) A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science*, **313**, 101-104.

**Hua, S.J., Shamsi, I.H., Guo, Y., Pak, H., Chen, M.X., Shi, C.G., Meng, H.B. and Jiang, L.X.** (2009) Sequence, expression divergence, and complementation of homologous *ALCATRAZ* loci in *Brassica napus*. *Planta*, **230**, 493-503.

**Huelsenbeck, J.P., Rannala, B. and Masly, J.P.** (2000) Accommodating phylogenetic uncertainty in evolutionary studies. *Science*, **288**, 2349-2350.

**Huelsenbeck, J.P. and Ronquist, F.** (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754-755.

**Hufford, M.B., Lubinksy, P., Pyhajarvi, T., Devengenzo, M.T., Ellstrand, N.C. and Ross-Ibarra, J.** (2013) The genomic signature of crop-wild introgression in maize. *Plos Genet*, **9**, e1003477.

**Hunt, H.V., Moots, H.M., Graybosch, R.A., Jones, H., Parker, M., Romanova, O., Jones, M.K., Howe, C.J. and Trafford, K.** (2013) Waxy phenotype evolution in the allotetraploid cereal broomcorn millet: Mutations at the *GBSSI* locus in their functional and phylogenetic context. *Mol. Biol. Evol.*, **30**, 109-122.

**Imbert, E.** (2002) Ecological consequences and ontogeny of seed heteromorphism. *Perspect. Plant Ecol. Evol. Syst.*, **5**, 13-36.

**Innan, H. and Kondrashov, F.** (2010) The evolution of gene duplications: classifying and distinguishing between models. *Nat. Rev. Genet.*, **11**, 97-108.

**Ishii, T., Numaguchi, K., Miura, K., Yoshida, K., Thanh, P.T., Htun, T.M., Yamasaki, M., Komeda, N., Matsumoto, T., Terauchi, R., Ishikawa, R. and Ashikari, M.** (2013) *OsLG1* regulates a closed panicle trait in domesticated rice. *Nat. Genet.*, **45**, 462-465.

**Ito, T. and Meyerowitz, E.M.** (2000) Overexpression of a gene encoding a cytochrome p450, *CYP78A9*, induces large and seedless fruit in Arabidopsis. *Plant Cell*, **12**, 1541-1550.

**Itoh, H., Tatsumi, T., Sakamoto, T., Otomo, K., Toyomasu, T., Kitano, H., Ashikari, M., Ichihara, S. and Matsuoka, M.** (2004) A rice semi-dwarf gene, *Tan-Ginbozu* (*D35*), encodes the gibberellin biosynthesis enzyme, *ent*-kaurene oxidase. *Plant Mol. Biol.*, **54**, 533-547.

**Ivarson, E., Ahlman, A., Li, X.Y. and Zhu, L.H.** (2013) Development of an efficient regeneration and transformation method for the new potential oilseed crop *Lepidium campestre*. *BMC Plant Biol.*, **13**.

**Jaakola, L., Poole, M., Jones, M.O., Kamarainen-Karppinen, T., Koskimaki, J.J., Hohtola, A., Haggman, H., Fraser, P.D., Manning, K., King, G.J., Thomson, H. and Seymour, G.B.** (2010) A SQUAMOSA MADS box gene involved in the regulation of anthocyanin accumulation in bilberry fruits. *Plant Physiol.*, **153**, 1619-1629.

**Jacob, F.** (1977) Evolution and tinkering. *Science*, **196**, 1161-1166.

**Jarvis, M.C., Briggs, S.P.H. and Knox, J.P.** (2003) Intercellular adhesion and cell separation in plants. *Plant Cell Environ.*, **26**, 977-989.

**Jeon, J.S., Ryoo, N., Hahn, T.R., Walia, H. and Nakamura, Y.** (2010) Starch biosynthesis in cereal endosperm. *Plant Physiol. Biochem.*, **48**, 383-392.

**Jia, Q.J., Zhang, J.J., Westcott, S., Zhang, X.Q., Bellgard, M., Lance, R. and Li, C.D.** (2009) GA-20 oxidase as a candidate for the semidwarf gene *sdw1/denso* in barley. *Funct. Integr. Genomics*, **9**, 255-262.

**Jones, H., Leigh, F.J., Mackay, I., Bower, M.A., Smith, L.M.J., Charles, M.P., Jones, G., Jones, M.K., Brown, T.A. and Powell, W.** (2008) Population-based resequencing reveals that the flowering time adaptation of cultivated barley originated east of the fertile crescent. *Mol. Biol. Evol.*, **25**, 2211-2219.

**Jung, C. and Muller, A.E.** (2009) Flowering time control and applications in plant breeding. *Trends Plant Sci.*, **14**, 563-573.

**Juwattanasomran, R., Somta, P., Chankaew, S., Shimizu, T., Wongpornchai, S., Kaga, A. and Srinives, P.** (2011) A SNP in *GmBADH2* gene associates with fragrance in vegetable soybean variety "Kaori" and SNAP marker development for the fragrance. *Theor. Appl. Genet.*, **122**, 533-541.

**Kaessmann, H.** (2010) Origins, evolution, and phenotypic impact of new genes. *Genome Res.*, **20**, 1313-1326.

**Karlova, R., Rosin, F.M., Busscher-Lange, J., Parapunova, V., Do, P.T., Fernie, A.R., Fraser, P.D., Baxter, C., Angenent, G.C. and de Maagd, R.A.** (2011) Transcriptome and metabolite profiling show that APETALA2a is a major regulator of tomato fruit ripening. *Plant Cell*, **23**, 923-941.

**Kawahigashi, H., Oshima, M., Nishikawa, T., Okuizumi, H., Kasuga, S. and Yonemaru, J.** (2013) A novel *waxy* allele in sorghum landraces in East Asia. *Plant Breed.*, **132**, 305-310.

**Kawase, M., Fukunaga, K. and Kato, K.** (2005) Diverse origins of waxy foxtail millet crops in East and Southeast Asia mediated by multiple transposable element insertions. *Mol. Genet. Genomics*, **274**, 131-140.

**Kay, P., Groszmann, M., Ross, J.J., Parish, R.W. and Swain, S.M.** (2013) Modifications of a conserved regulatory network involving INDEHISCENT controls multiple aspects of reproductive tissue development in Arabidopsis. *New Phytol.*, **197**, 73-87.

**King, M.C. and Wilson, A.C.** (1975) Evolution at two levels in humans and chimpanzees. *Science*, **188**, 107-116.

**Kivimäki, M., Kärkkäinen, K., Gaudeul, M., Loe, G. and Agren, J.** (2007) Gene, phenotype and function: *GLABROUS1* and resistance to herbivory in natural populations of *Arabidopsis lyrata*. *Mol. Ecol.*, **16**, 453-462.

**Knapp, S.** (2002) Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. *J. Exp. Bot.*, **53**, 2001-2022.

**Koncz, C. and Schell, J.** (1986) The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of *Agrobacterium* binary vector. *Mol. Gen. Genet.*, **204**, 383-396.

**Konishi, S., Lin, S.Y., Ebana, K., Fukuta, Y., Izawa, T., Sasaki, T. and Yano, M.** (2006) A SNP caused the loss of seed shattering during rice domestication. *Plant Cell Physiol.*, **47**, S14-S14.

**Kopp, A.** (2009) Metamodels and phylogenetic replication: a systematic approach to the evolution of developmental pathways. *Evolution*, **63**, 2771-2789.

**Koskela, E.A., Mouhu, K., Albani, M.C., Kurokura, T., Rantanen, M., Sargent, D.J., Battey, N.H., Coupland, G., Elomaa, P. and Hytonen, T.** (2012) Mutation in *TERMINAL FLOWER1* reverses the photoperiodic requirement for flowering in the wild strawberry *Fragaria vesca*. *Plant Physiol.*, **159**, 1043-1054.

**Kovach, M.J., Calingacion, M.N., Fitzgerald, M.A. and McCouch, S.R.** (2009) The origin and evolution of fragrance in rice (*Oryza sativa* L.). *Proc. Natl. Acad. Sci. U. S. A.*, **106**, 14444-14449.

**Kramer, E.M., Jaramillo, M.A. and Di Stilio, V.S.** (2004) Patterns of gene duplication and functional evolution during the diversification of the *AGAMOUS* subfamily of MADS box genes in angiosperms. *Genetics*, **166**, 1011-1023.

**Kuittinen, H., Niittyvuopio, A., Rinne, P. and Savolainen, O.** (2008) Natural variation in *Arabidopsis lyrata* vernalization requirement conferred by a *FRIGIDA* indel polymorphism. *Mol. Biol. Evol.*, **25**, 319-329.

**Kwak, M., Toro, O., Debouck, D.G. and Gepts, P.** (2012) Multiple origins of the determinate growth habit in domesticated common bean (*Phaseolus vulgaris*). *Ann. Bot.*, **110**, 1573-1580.

**Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G.** (2007) Clustal W and clustal X version 2.0. *Bioinformatics*, **23**, 2947-2948.

**Lazo, G.R., Stein, P.A. and Ludwig, R.A.** (1991) A DNA transformation-competent *Arabidopsis* genomic library in *Agrobacterium*. *Nat. Biotechnol.*, **9**, 963-967.

**Leander, B.S.** (2008) Different modes of convergent evolution reflect phylogenetic distances: a reply to Arendt and Reznick. *Trends Ecol. Evol.*, **23**, 481-482.

**Lease, K.A., Wen, J.Q., Li, J., Doke, J.T., Liscum, E. and Walker, J.C.** (2001) A mutant Arabidopsis heterotrimeric G-protein ß subunit affects leaf, flower, and fruit development. *Plant Cell*, **13**, 2631-2641.

**Li, C.B., Zhou, A.L. and Sang, T.** (2006) Rice domestication by reducing shattering. *Science*, **311**, 1936-1939.

**Li, Q., Li, L., Yang, X.H., Warburton, M.L., Bai, G.H., Dai, J.R., Li, J.S. and Yan, J.B.** (2010a) Relationship, evolutionary fate and function of two maize co-orthologs of rice *GW2* associated with kernel size and weight. *BMC Plant Biol.*, **10**.

**Li, Q., Yang, X.H., Bai, G.H., Warburton, M.L., Mahuku, G., Gore, M., Dai, J.R., Li, J.S. and Yan, J.B.** (2010b) Cloning and characterization of a putative *GS3* ortholog involved in maize kernel development. *Theor. Appl. Genet.*, **120**, 753-763.

**Li, S.J., Liu, Y.J., Zheng, L.Y., Chen, L.L., Li, N., Corke, F., Lu, Y.R., Fu, X.D., Zhu, Z.G., Bevan, M.W. and Li, Y.H.** (2012) The plant-specific G protein γ subunit AGG3 influences organ size and shape in *Arabidopsis thaliana*. *New Phytol.*, **194**, 690-703.

**Li, W. and Gill, B.** (2006) Multiple genetic pathways for seed shattering in the grasses. *Funct. Integr. Genomics*, **6**, 300-309.

**Li, X.Y., Ahlman, A., Yan, X.F., Lindgren, H. and Zhu, L.H.** (2010c) Genetic transformation of the oilseed crop *Crambe abyssinica*. *Plant Cell Tissue Organ Cult.*, **100**, 149-156.

**Li, Y.B., Fan, C.C., Xing, Y.Z., Jiang, Y.H., Luo, L.J., Sun, L., Shao, D., Xu, C.J., Li, X.H., Xiao, J.H., He, Y.Q. and Zhang, Q.F.** (2011) Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. *Nat. Genet.*, **43**, 1266-1269.

**Liljegren, S.J., Ditta, G.S., Eshed, H.Y., Savidge, B., Bowman, J.L. and Yanofsky, M.F.** (2000) *SHATTERPROOF* MADS-box genes control seed dispersal in *Arabidopsis*. *Nature*, **404**, 766-770.

**Liljegren, S.J., Roeder, A.H.K., Kempin, S.A., Gremski, K., Ostergaard, L., Guimil, S., Reyes, D.K. and Yanofsky, M.F.** (2004) Control of fruit patterning in *Arabidopsis* by INDEHISCENT. *Cell*, **116**, 843-853.

**Lin, Z.W., Griffith, M.E., Li, X.R., Zhu, Z.F., Tan, L.B., Fu, Y.C., Zhang, W.X., Wang, X.K., Xie, D.X. and Sun, C.Q.** (2007) Origin of seed shattering in rice (*Oryza sativa* L.). *Planta*, **226**, 11-20.

**Lin, Z.W., Li, X.R., Shannon, L.M., Yeh, C.T., Wang, M.L., Bai, G.H., Peng, Z., Li, J.R., Trick, H.N., Clemente, T.E., Doebley, J., Schnable, P.S., Tuinstra, M.R., Tesso, T.T., White, F. and Yu, J.M.** (2012) Parallel domestication of the *Shattering1* genes in cereals. *Nat. Genet.*, **44**, 720-724.

**Liu, B.H., Watanabe, S., Uchiyama, T., Kong, F.J., Kanazawa, A., Xia, Z.J., Nagamatsu, A., Arai, M., Yamada, T., Kitamura, K., Masuta, C., Harada, K. and Abe, J.** (2010a) The soybean stem growth habit gene *Dt1* Is an ortholog of Arabidopsis *TERMINAL FLOWER1*. *Plant Physiol.*, **153**, 198-210.

**Liu, Y., Cotton, J.A., Shen, B., Han, X.Q., Rossiter, S.J. and Zhang, S.Y.** (2010b) Convergent sequence evolution between echolocating bats and dolphins. *Curr. Biol.*, **20**, R53-R54.

**Liu, Z., Zhang, D., Liu, D., Li, F. and Lu, H.** (2013) Exon skipping of *AGAMOUS* homolog *PrseAG* in developing double flowers of *Prunus lannesiana* (Rosaceae). *Plant Cell Rep.*, **32**, 227-237.

**Lobkovsky, A.E. and Koonin, E.V.** (2012) Replaying the tape of life: quantification of the predictability of evolution. *Front. Genet.*, **3**, 246.

**Lorts, C.M., Briggeman, T. and Sang, T.** (2008) Evolution of fruit types and seed dispersal: A phylogenetic and ecological snapshot. *J. Syst. Evol.*, **46**, 396-404.

**Lu, C.F. and Kang, J.L.** (2008) Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by *Agrobacterium*-mediated transformation. *Plant Cell Rep.*, **27**, 273-278.

**Lu, J.J., Tan, D.Y., Baskin, J.M. and Baskin, C.C.** (2010) Fruit and seed heteromorphism in the cold desert annual ephemeral *Diptychocarpus strictus* (Brassicaceae) and possible adaptive significance. *Ann. Bot.*, **105**, 999-1014.

**Ma, H., Yanofsky, M.F. and Meyerowitz, E.M.** (1991) *AGL1-AGL6*, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes. *Genes Dev.*, **5**, 484-495.

**MacLeod, J.** (1981) Harvesting in oilseed rape. In *Oilseed rape book. A manual for growers, farmers and advisors*, Green, C. ed. Cambridge, UK: Cambridge Agricultural Publishing, pp. 107-120.

**Majerus, M.E.N. and Mundy, N.I.** (2003) Mammalian melanism: natural selection in black and white. *Trends Genet.*, **19**, 585-588.

**Manceau, M., Domingues, V.S., Linnen, C.R., Rosenblum, E.B. and Hoekstra, H.E.** (2010) Convergence in pigmentation at multiple levels: mutations, genes and function. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **365**, 2439-2450.

**Mandak, B. and Pysek, P.** (2001) Fruit dispersal and seed banks in *Atriplex sagittata*: the role of heterocarpy. *J. Ecol.*, **89**, 159-165.

**Mandoli, D.F. and Olmstead, R.** (2000) The importance of emerging model systems in plant biology. *J. Plant Growth Regul.*, **19**, 249-252.

**Mao, L., Begum, D., Chuang, H.W., Budiman, M.A., Szymkowiak, E.J., Irish, E.E. and Wing, R.A.** (2000) *JOINTLESS* is a MADS-box gene controlling tomato flower abscission zone development. *Nature*, **406**, 910-913.

**Maron, L.G., Guimaraes, C.T., Kirst, M., Albert, P.S., Birchler, J.A., Bradbury, P.J., Buckler, E.S., Coluccio, A.E., Danilova, T.V., Kudrna, D., Magalhaes, J.V., Pineros, M.A., Schatz, M.C., Wing, R.A. and Kochian, L.V.** (2013) Aluminum tolerance in maize is associated with higher *MATE1* gene copy number. *Proc. Natl. Acad. Sci. U. S. A.*, **110**, 5241-5246.

**Marsch-Martinez, N., Ramos-Cruz, D., Irepan Reyes-Olalde, J., Lozano-Sotomayor, P., Zuniga-Mayo, V.M. and de Folter, S.** (2012) The role of cytokinin during *Arabidopsis* gynoecia and fruit morphogenesis and patterning. *Plant J.*, **72**, 222-234.

**Martin, A. and Orgogozo, V.** (2013) The loci of repeated evolution: a catalog of genetic hotspots of phenotypic variation. *Evolution*, **67**, 1235-1250.

**Martinez-Trujillo, M., Limones-Briones, V., Cabrera-Ponce, J.L. and Herrera-Estrella, L.** (2004) Improving transformation efficiency of *Arabidopsis thaliana* by modifying the floral dip method. *Plant Mol. Biol. Report.*, **22**, 63-70.

**Martins, E.P.** (2000) Adaptation and the comparative method. *Trends Ecol. Evol.*, **15**, 296-299.

**Marvin, H.J.P., Mastebroek, H.D., Becu, D.M.S. and Janssens, R.J.J.** (2000) Investigation into the prospects of five novel oilseed crops within Europe. *Outlook Agric.*, **29**, 47-53.

**Mastebroek, H.D. and Marvin, H.J.P.** (2000) Breeding prospects of *Lunaria annua* L. *Ind. Crop. Prod.*, **11**, 139-143.

**Mathews, S., Singhal, R. and Kulkarni, P.** (1993) Some physicochemical characteristics of *Lepidium sativum* (haliv) seeds. *Food/Nahrung*, **37**, 69-71.

**Matsubara, K., Ogiso-Tanaka, E., Hori, K., Ebana, K., Ando, T. and Yano, M.** (2012) Natural variation in *Hd17*, a homolog of *Arabidopsis ELF3* that is involved in rice photoperiodic flowering. *Plant Cell Physiol.*, **53**, 709-716.

**McGarry, R.C. and Ayre, B.G.** (2012) Manipulating plant architecture with members of the CETS gene family. *Plant Sci.*, **188**, 71-81.

**Meakin, P.J. and Roberts, J.A.** (1990) Dehiscence of fruit in oilseed rape (*Brassica napus* L.) 2. The role of cell-wall degrading enzymes and ethylene. *J. Exp. Bot.*, **41**, 1003-1011.

**Meakin, P.J. and Roberts, J.A.** (1991) Anatomical and biochemical changes associated with the induction of oilseed rape (*Brassica napus*) pod dehiscence by *Dasineura brassicae* (Winn.). *Ann. Bot.*, **67**, 193-197.

**Michaels, S.D. and Amasino, R.M.** (2000) Memories of winter: vernalization and the competence to flower. *Plant Cell Environ.*, **23**, 1145-1153.

**Michaels, S.D., He, Y., Scortecci, K.C. and Amasino, R.M.** (2003) Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of summer-annual flowering behavior in *Arabidopsis*. *P. Natl. Acad. Sci. USA*, **100**, 10102-10107.

**Milinkovitch, M.C. and Tzika, A.** (2007) Escaping the mouse trap: The selection of new evo-devo model species. *J. Exp. Zool. Part B*, **308B**, 337-346.

**Miller, A.J.** (2007) *Crop plants: evolution* Chichester, UK: John Wiley & Sons. DOI: 10.1002/9780470015902.a0003360.

**Morgan, C.L., Bruce, D.M., Child, R., Ladbrooke, Z.L. and Arthur, A.E.** (1998) Genetic variation for pod shatter resistance among lines of oilseed rape developed from synthetic *B. napus*. *Field Crops Res.*, **58**, 153-165.

**Morgan, D.R., Soltis, D.E. and Robertson, K.R.** (1994) Systematic and evolutionary implications of rbcL sequence variation in Rosaceae. *Am. J. Bot.*, **81**, 890-903.

**Mosher, D.S., Quignon, P., Bustamante, C.D., Sutter, N.B., Mellersh, C.S., Parker, H.G. and Ostrander, E.A.** (2007) A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *Plos Genet*, **3**, 779-786.

**Mühlhausen, A., Polster, A., Theissen, G. and Mummenhoff, K.** (2008) Evolution of fruit dehiscence in *Brassicaceae* - examples from *Aethionema* and *Lepidium*. In *V International Symposium on Brassicas and XVI International Crucifer Genetics Workshop, Brassica 2008 867*, pp. 207-220.

**Müller, B.M., Saedler, H. and Zachgo, S.** (2001) The MADS-box gene *DEFH28* from *Antirrhinum* is involved in the regulation of floral meristem identity and fruit development. *Plant J.*, **28**, 169-179.

**Multani, D.S., Briggs, S.P., Chamberlin, M.A., Blakeslee, J.J., Murphy, A.S. and Johal, G.S.** (2003) Loss of an MDR transporter in compact stalks of maize *br2* and sorghum *dw3* mutants. *Science*, **302**, 81-84.

**Mummenhoff, K., Al-Shehbaz, I.A., Bakker, F.T., Linder, H.P. and Muhlhausen, A.** (2005) Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape Flora of southern Africa. *Ann. Mo. Bot. Gard.*, **92**, 400-424.

**Mummenhoff, K., Bruggemann, H. and Bowman, J.L.** (2001) Chloroplast DNA phylogeny and biogeography of Lepidium (Brassicaceae). *Am. J. Bot.*, **88**, 2051-2063.

**Mummenhoff, K., Polster, A., Muhlhausen, A. and Theissen, G.** (2009) *Lepidium* as a model system for studying the evolution of fruit development in Brassicaceae. *J. Exp. Bot.*, **60**, 1503-1513.

**Mundy, N.I.** (2005) A window on the genetics of evolution: *MC1R* and plumage colouration in birds. *P. Roy. Soc. B-Biol. Sci.*, **272**, 1633-1640.

**Murakami, M., Matsushika, A., Ashikari, M., Yamashino, T. and Mizuno, T.** (2005) Circadian-associated rice pseudo response regulators (*Os*PRRs): Insight into the control of flowering time. *Biosci. Biotechnol. Biochem.*, **69**, 410-414.

**Murphy, R.L., Klein, R.R., Morishige, D.T., Brady, J.A., Rooney, W.L., Miller, F.R., Dugas, D.V., Klein, P.E. and Mullet, J.E.** (2011) Coincident light and clock regulation of *pseudoresponse regulator protein 37* (*PRR37*) controls photoperiodic flowering in sorghum. *Proc. Natl. Acad. Sci. U. S. A.*, **108**, 16469-16474.

**Muskens, M.W.M., Vissers, A.P.A., Mol, J.N.M. and Kooter, J.M.** (2000) Role of inverted DNA repeats in transcriptional and post-transcriptional gene silencing. *Plant Mol. Biol.*, **43**, 243-260.

**Muth, J., Hartje, S., Twyman, R.M., Hofferbert, H.R., Tacke, E. and Prufer, D.** (2008) Precision breeding for novel starch variants in potato. *Plant Biotechnol. J.*, **6**, 576-584.

**Nadeau, N.J. and Jiggins, C.D.** (2010) A golden age for evolutionary genetics? Genomic studies of adaptation in natural populations. *Trends Genet.*, **26**, 484-492.

**Narita, N.N., Moore, S., Horiguchi, G., Kubo, M., Demura, T., Fukuda, H., Goodrich, J. and Tsukaya, H.** (2004) Overexpression of a novel small peptide ROTUNDIFOLIA4 decreases cell proliferation and alters leaf shape in *Arabidopsis thaliana*. *Plant J.*, **38**, 699-713.

**Navrátilová, B.** (2004) Protoplast cultures and protoplast fusion focused on Brassicaceae—a review. *Hort. Sci.*, **31**, 140-157.

**Noda, T., Kimura, T., Otani, M., Ideta, O., Shimada, T., Saito, A. and Suda, I.** (2002) Physicochemical properties of amylose-free starch from transgenic sweet potato. *Carbohydr. Polym.*, **49**, 253-260.

**Nyylander, J.A.A.** (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

**Ogawa, M., Kay, P., Wilson, S. and Swain, S.M.** (2009) ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1 (ADPG1), ADPG2, and QUARTET2 are polygalacturonases required for cell separation during reproductive development in *Arabidopsis*. *Plant Cell*, **21**, 216-233.

**Ohno, C.K., Reddy, G.V., Heisler, M.G.B. and Meyerowitz, E.M.** (2004) The *Arabidopsis JAGGED* gene encodes a zinc finger protein that promotes leaf tissue development. *Development*, **131**, 1111-1122.

**Okazaki, K., Sakamoto, K., Kikuchi, R., Saito, A., Togashi, E., Kuginuki, Y., Matsumoto, S. and Hirai, M.** (2007) Mapping and characterization of *FLC* homologs and QTL analysis of flowering time in *Brassica oleracea*. *Theor. Appl. Genet.*, **114**, 595-608.

**Olsen, K.M. and Wendel, J.F.** (2013) A bountiful harvest: Genomic insights into crop domestication phenotypes. *Annu. Rev. Plant Biol.*, **64**, 47-70.

**Onate-Sanchez, L. and Vicente-Carbajosa, J.** (2008) DNA-free RNA isolation protocols for *Arabidopsis thaliana*, including seeds and siliques. *BMC Res. Notes*, **1**, 93.

**Orr, H.A.** (2005) The genetic theory of adaptation: A brief history. *Nat. Rev. Genet.*, **6**, 119-127.

**Ostergaard, L.** (2009) Don't 'leaf' now. The making of a fruit. *Curr. Opin. Plant Biol.*, **12**, 36-41.

**Ostergaard, L., Kempin, S.A., Bies, D., Klee, H.J. and Yanofsky, M.F.** (2006) Pod shatter-resistant *Brassica* fruit produced by ectopic expression of the *FRUITFULL* gene. *Plant Biotechnol. J.*, **4**, 45-51.

**Pabon-Mora, N., Ambrose, B.A. and Litt, A.** (2012) Poppy *APETALA1/FRUITFULL* orthologs control flowering time, branching, perianth identity, and fruit development. *Plant Physiol.*, **158**, 1685-1704.

**Pabon-Mora, N., Sharma, B., Holappa, L.D., Kramer, E.M. and Litt, A.** (2013) The *Aquilegia FRUITFULL*-like genes play key roles in leaf morphogenesis and inflorescence development. *Plant J.*, **74**, 197-212.

**Pagnussat, G.C., Yu, H.J., Ngo, Q.A., Rajani, S., Mayalagu, S., Johnson, C.S., Capron, A., Xie, L.F., Ye, D. and Sundaresan, V.** (2005) Genetic and molecular identification of genes required for female gametophyte development and function in Arabidopsis. *Development*, **132**, 603-614.

**Papp, B., Notebaart, R.A. and Pal, C.** (2011) Systems-biology approaches for predicting genomic evolution. *Nat. Rev. Genet.*, **12**, 591-602.

**Park, Y.J., Nishikawa, T., Tomooka, N. and Nemoto, K.** (2012) The molecular basis of mutations at the *Waxy* locus from *Amaranthus caudatus* L.: evolution of the waxy phenotype in three species of grain amaranth. *Mol. Breed.*, **30**, 511-520.

**Parvathaneni, R.K., Jakkula, V., Padi, F.K., Faure, S., Nagarajappa, N., Pontaroli, A.C., Wu, X.M., Bennetzen, J.L. and Devos, K.M.** (2013) Fine-mapping and identification of a candidate gene underlying the *d2* dwarfing phenotype in pearl millet, *Cenchrus americanus* (L.) Morrone. *G3 (Bethesda)*, **3**, 563-572.

**Paterson, A.H., Lin, Y.R., Li, Z.K., Schertz, K.F., Doebley, J.F., Pinson, S.R.M., Liu, S.C., Stansel, J.W. and Irvine, J.E.** (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science*, **269**, 1714-1718.

**Patterson, T.B. and Givnish, T.J.** (2002) Phylogeny, concerted convergence, and phylogenetic niche conservatism in the core Liliales: Insights from rbcL and ndhF sequence data. *Evolution*, **56**, 233-252.

**Payne, T., Johnson, S.D. and Koltunow, A.M.** (2004) *KNUCKLES* (*KNU*) encodes a C2H2 zinc-finger protein that regulates development of basal pattern elements of the *Arabidopsis* gynoecium. *Development*, **131**, 3737-3749.

**Petersen, M., Sander, L., Child, R., vanOnckelen, H., Ulvskov, P. and Borkhardt, B.** (1996) Isolation and characterisation of a pod dehiscence zone-specific polygalacturonase from *Brassica napus*. *Plant Mol. Biol.*, **31**, 517-527.

**Petroni, K. and Tonelli, C.** (2011) Recent advances on the regulation of anthocyanin synthesis in reproductive organs. *Plant Sci.*, **181**, 219-229.

**Pin, P.A. and Nilsson, O.** (2012) The multifaceted roles of FLOWERING LOCUS T in plant development. *Plant Cell Environ.*, **35**, 1742-1755.

**Pin, P.A., Zhang, W.Y., Vogt, S.H., Dally, N., Buttner, B., Schulze-Buxloh, G., Jelly, N.S., Chia, T.Y.P., Mutasa-Gottgens, E.S., Dohm, J.C., Himmelbauer, H., Weisshaar, B., Kraus, J., Gielen, J.J.L., Lommel, M., Weyens, G., Wahl, B., Schechert, A., Nilsson, O., Jung, C., Kraft, T. and Muller, A.E.** (2012) The role of a pseudo-response regulator gene in life cycle adaptation and domestication of beet. *Curr. Biol.*, **22**, 1095-1101.

**Pinyopich, A., Ditta, G.S., Savidge, B., Liljegren, S.J., Baumann, E., Wisman, E. and Yanofsky, M.F.** (2003) Assessing the redundancy of MADS-box genes during carpel and ovule development. *Nature*, **424**, 85-88.

**Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D. and Lifschitz, E.** (1998) The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. *Development*, **125**, 1979-1989.

**Preston, J.C. and Hileman, L.C.** (2009) Developmental genetics of floral symmetry evolution. *Trends Plant Sci.*, **14**, 147-154.

**Price, J.S., Hobson, R.N., Neale, M.A. and Bruce, D.M.** (1996) Seed losses in commercial harvesting of oilseed rape. *J. Agr. Eng. Res.*, **65**, 183-191.

**Pritchard, J.K. and Di Rienzo, A.** (2010) Adaptation – not by sweeps alone. *Nat. Rev. Genet.*, **11**, 665-667.

**Protas, M.E., Hersey, C., Kochanek, D., Zhou, Y., Wilkens, H., Jeffery, W.R., Zon, L.I., Borowsky, R. and Tabin, C.J.** (2006) Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. *Nat. Genet.*, **38**, 107-111.

**Purugganan, M.D. and Fuller, D.Q.** (2009) The nature of selection during plant domestication. *Nature*, **457**, 843-848.

**Qing, C.M., Fan, L., Lei, Y., Bouchez, D., Tourneur, C., Yan, L. and Robaglia, C.** (2000) Transformation of Pakchoi (*Brassica rapa* L. ssp *chinensis*) by *Agrobacterium* infiltration. *Mol. Breed.*, **6**, 67-72.

**Rajani, S. and Sundaresan, V.** (2001) The *Arabidopsis* myc/bHLH gene *ALCATRAZ* enables cell separation in fruit dehiscence. *Curr. Biol.*, **11**, 1914-1922.

**Ramakers, C., Ruijter, J.M., Deprez, R.H.L. and Moorman, A.F.M.** (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci. Lett.*, **339**, 62-66.

**Ramsay, L., Comadran, J., Druka, A., Marshall, D.F., Thomas, W.T.B., Macaulay, M., MacKenzie, K., Simpson, C., Fuller, J., Bonar, N., Hayes, P.M., Lundqvist, U., Franckowiak, J.D., Close, T.J., Muehlbauer, G.J. and Waugh, R.** (2011) *INTERMEDIUM-C*, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene *TEOSINTE BRANCHED 1*. *Nat. Genet.*, **43**, 169-172.

**Reed, S.M.** (2004) Embryo rescue. In *Plant development and biotechnology*, Trigiano, R.N. and Gray, D.J. eds: CRC Press, pp. 235-239.

**Remigereau, M.S., Lakis, G., Rekima, S., Leveugle, M., Fontaine, M.C., Langin, T., Sarr, A. and Robert, T.** (2011) Cereal domestication and evolution of branching: Evidence for soft selection in the *Tb1* orthologue of pearl millet (*Pennisetum glaucum* [L.] R. Br.). *Plos One*, **6**, e22404.

**Repinski, S.L., Kwak, M. and Gepts, P.** (2012) The common bean growth habit gene *PvTFL1y* is a functional homolog of *Arabidopsis TFL1*. *Theor. Appl. Genet.*, **124**, 1539-1547.

**Ripoll, J.J., Roeder, A.H., Ditta, G.S. and Yanofsky, M.F.** (2011) A novel role for the floral homeotic gene *APETALA2* during *Arabidopsis* fruit development. *Development*, **138**, 5167-5176.

**Rissman, E.F.** (2004) Thinking outside the mouse box: The importance of comparative laboratory animal models in research. *ILAR J.*, **45**, 1-3.

**Rockman, M.V.** (2012) The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution*, **66**, 1-17.

**Roeder, A.H.K., Ferrandiz, C. and Yanofsky, M.F.** (2003) The role of the REPLUMLESS homeodomain protein in patterning the *Arabidopsis* fruit. *Curr. Biol.*, **13**, 1630-1635.

**Romera-Branchat, M., Ripoll, J.J., Yanofsky, M.F. and Pelaz, S.** (2013) The *WOX13* homeobox gene promotes replum formation in the *Arabidopsis thaliana* fruit. *Plant J.*, **73**, 37-49.

**Römpler, H., Rohland, N., Lalueza-Fox, C., Willerslev, E., Kuznetsova, T., Rabeder, G., Bertranpetit, J., Schöneberg, T. and Hofreiter, M.** (2006) Nuclear gene indicates coat-color polymorphism in mammoths. *Science*, **313**, 62-62.

**Ronquist, F. and Huelsenbeck, J.P.** (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572-1574.

**Rose, L.E., Atwell, S., Grant, M. and Holub, E.B.** (2012) Parallel loss-of-function at the *RPM1* bacterial resistance locus in *Arabidopsis thaliana*. *Front. Plant Sci.*, **3**.

**Rosenblum, E.B., Rompler, H., Schoneberg, T. and Hoekstra, H.E.** (2010) Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proc. Natl. Acad. Sci. U. S. A.*, **107**, 2113-2117.

**Ruijter, J.M., Ramakers, C., Hoogaars, W.M.H., Karlen, Y., Bakker, O., van den Hoff, M.J.B. and Moorman, A.F.M.** (2009) Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res.*, **37**.

**Sage, R.F.** (2004) The evolution of C4 photosynthesis. *New Phytol.*, **161**, 341-370.

**Salamini, F.** (2003) Hormones and the green revolution. *Science*, **302**, 71-72.

**Salas Fernandez, M.G., Becraft, P.W., Yin, Y. and Lubberstedt, T.** (2009) From dwarves to giants? Plant height manipulation for biomass yield. *Trends Plant Sci.*, **14**, 454-461.

**Salome, P.A. and McClung, C.R.** (2005) *PSEUDO-RESPONSE REGULATOR 7* and *9* are partially redundant genes essential for the temperature responsiveness of the Arabidopsis circadian clock. *Plant Cell*, **17**, 791-803.

**Salvi, S., Corneti, S., Bellotti, M., Carraro, N., Sanguineti, M.C., Castelletti, S. and Tuberosa, R.** (2011) Genetic dissection of maize phenology using an intraspecific introgression library. *BMC Plant Biol.*, **11**.

**Salvi, S., Sponza, G., Morgante, M., Tomes, D., Niu, X., Fengler, K.A., Meeley, R., Ananiev, E.V., Svitashev, S., Bruggemann, E., Li, B., Hainey, C.F., Radovic, S., Zaina, G., Rafalski, J.A., Tingey, S.V., Miao, G.H., Phillips, R.L. and Tuberosa, R.** (2007) Conserved noncoding genomic sequences associated with a flowering-time quantitative trait locus in maize. *Proc. Natl. Acad. Sci. U. S. A.*, **104**, 11376-11381.

**Salywon, A.M., Dierig, D.A., Rebman, J.P. and De Rodriguez, D.J.** (2005) Evaluation of new *Lesquerella* and *Physaria* (Brassicaceae) oilseed germplasm. *Am. J. Bot.*, **92**, 53-62.

**Sang, T.** (2009) Genes and mutations underlying domestication transitions in grasses. *Plant Physiol.*, **149**, 63-70.

**Savidge, B., Rounsley, S.D. and Yanofsky, M.F.** (1995) Temporal relationships between the transcription of two *Arabidopsis* MADS box genes and the floral organ identity genes. *Plant Cell*, **7**, 721-733.

**Sawa, S., Watanabe, K., Goto, K., Kanaya, E., Morita, E.H. and Okada, K.** (1999) *FILAMENTOUS FLOWER*, a meristem and organ identity gene of *Arabidopsis*, encodes a protein with a zinc finger and HMG-related domains *Genes Dev.*, **13**, 2337-2337.

**Schroeder, J.I., Delhaize, E., Frommer, W.B., Guerinot, M.L., Harrison, M.J., Herrera-Estrella, L., Horie, T., Kochian, L.V., Munns, R., Nishizawa, N.K., Tsay, Y.F. and Sanders, D.** (2013) Using membrane transporters to improve crops for sustainable food production. *Nature*, **497**, 60-66.

**Schubert, D., Lechtenberg, B., Forsbach, A., Gils, M., Bahadur, S. and Schmidt, R.** (2004) Silencing in *Arabidopsis* T-DNA transformants: The predominant role of a gene-specific RNA sensing mechanism versus position effects. *Plant Cell*, **16**, 2561-2572.

**Schuster, S.C.** (2008) Next-generation sequencing transforms today's biology. *Nat. Meth.*, **5**, 16-18.

**Schwartz, C., Balasubramanian, S., Warthmann, N., Michael, T.P., Lempe, J., Sureshkumar, S., Kobayashi, Y., Maloof, J.N., Borevitz, J.O., Chory, J. and Weigel, D.** (2009) Cis-regulatory changes at *FLOWERING LOCUS T* mediate natural variation in flowering responses of *Arabidopsis thaliana*. *Genetics*, **183**, 723-732.

**Scotland, R.W.** (2011) What is parallelism? *Evol Dev*, **13**, 214-227.

**Scutt, C.P., Vinauger-Douard, M., Fourquin, C., Finet, C. and Dumas, C.** (2006) An evolutionary perspective on the regulation of carpel development. *J. Exp. Bot.*, **57**, 2143-2152.

**Seymour, G., Poole, M., Manning, K. and King, G.J.** (2008) Genetics and epigenetics of fruit development and ripening. *Curr. Opin. Plant Biol.*, **11**, 58-63.

**Seymour, G.B., Ostergaard, L., Chapman, N.H., Knapp, S. and Martin, C.** (2013) Fruit development and ripening. *Annu. Rev. Plant Biol.*, **64**, 219-241.

**Sharma, D.R., Kaur, R. and Kumar, K.** (1996) Embryo rescue in plants - a review. *Euphytica*, **89**, 325-337.

**Shima, Y., Kitagawa, M., Fujisawa, M., Nakano, T., Kato, H., Kimbara, J., Kasumi, T. and Ito, Y.** (2013) Tomato FRUITFULL homologues act in fruit ripening via forming MADS-box transcription factor complexes with RIN. *Plant Mol. Biol.*, **82**, 427-438.

**Siegfried, K.R., Eshed, Y., Baum, S.F., Otsuga, D., Drews, G.N. and Bowman, J.L.** (1999) Members of the YABBY gene family specify abaxial cell fate in *Arabidopsis*. *Development*, **126**, 4117-4128.

**Skot, L., Sanderson, R., Thomas, A., Skot, K., Thorogood, D., Latypova, G., Asp, T. and Armstead, I.** (2011) Allelic variation in the perennial ryegrass *FLOWERING LOCUS T* gene is associated with changes in flowering time across a range of populations. *Plant Physiol.*, **155**, 1013-1022.

**Slotte, T., Holm, K., McIntyre, L.M., Lagercrantz, U. and Lascoux, M.** (2007) Differential expression of genes important for adaptation in *Capsella bursa-pastoris* (Brassicaceae). *Plant Physiol.*, **145**, 160-173.

**Smith, H.M.S. and Hake, S.** (2003) The interaction of two homeobox genes, *BREVIPEDICELLUS* and *PENNYWISE*, regulates internode patterning in the *Arabidopsis* inflorescence. *Plant Cell*, **15**, 1717-1727.

**Smykal, P., Gennen, J., De Bodt, S., Ranganath, V. and Melzer, S.** (2007) Flowering of strict photoperiodic *Nicotiana* varieties in non-inductive conditions by transgenic approaches. *Plant Mol. Biol.*, **65**, 233-242.

**Smyth, D.R., Bowman, J.L. and Meyerowitz, E.M.** (1990) Early flower development in *Arabidopsis*. *Plant Cell*, **2**, 755-767.

**Sood, S., Kuraparthy, V., Bai, G.H. and Gill, B.S.** (2009) The major threshability genes soft glume (*sog*) and tenacious glume (*Tg*), of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. *Theor. Appl. Genet.*, **119**, 341-351.

**Sorefan, K., Girin, T., Liljegren, S.J., Ljung, K., Robles, P., Galvan-Ampudia, C.S., Offringa, R., Friml, J., Yanofsky, M.F. and Ostergaard, L.** (2009) A regulated auxin minimum is required for seed dispersal in *Arabidopsis*. *Nature*, **459**, 583-U114.

**Southern, E.M.** (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, **98**, 503-&.

**Spence, J., Vercher, Y., Gates, P. and Harris, N.** (1996) 'Pod shatter' in *Arabidopsis thaliana*, *Brassica napus* and *B. juncea*. *J. Microsc.-Oxf.*, **181**, 195-203.

**Srivastava, V., Anderson, O.D. and Ow, D.W.** (1999) Single-copy transgenic wheat generated through the resolution of complex integration patterns. *P. Natl. Acad. Sci. USA*, **96**, 11117-11121.

**Stern, D.L. and Orgogozo, V.** (2008) The loci of evolution: How predictable is genetic evolution ? *Evolution*, **62**, 2155-2177.

**Stern, D.L. and Orgogozo, V.** (2009) Is genetic evolution predictable? *Science*, **323**, 746-751.

**Streisfeld, M.A. and Rausher, M.D.** (2011) Population genetics, pleiotropy, and the preferential fixation of mutations during adaptive evolution. *Evolution*, **65**, 629-642.

**Studer, A., Zhao, Q., Ross-Ibarra, J. and Doebley, J.** (2011) Identification of a functional transposon insertion in the maize domestication gene *tb1*. *Nat. Genet.*, **43**, 1160-1163.

**Suzuki, M., Fujino, K., Nakamoto, Y., Ishimoto, M. and Funatsuki, H.** (2010) Fine mapping and development of DNA markers for the *qPDH1* locus associated with pod dehiscence in soybean. *Mol. Breed.*, **25**, 407-418.

**Swofford, D.L.** (2003) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. *Sinauer Associates, Sunderland, Massachusetts*.

**Tague, B.W.** (2001) Germ-line transformation of *Arabidopsis lasiocarpa*. *Transgenic Res.*, **10**, 259-267.

**Takahashi, Y., Teshima, K.M., Yokoi, S., Innan, H. and Shimamoto, K.** (2009) Variations in Hd1 proteins, *Hd3a* promoters, and *Ehd1* expression levels contribute to diversity of flowering time in cultivated rice. *Proc. Natl. Acad. Sci. U. S. A.*, **106**, 4555-4560.

**Takeno, K. and Yamaguchi, H.** (1991) Diversity in seed germination behavior in relation to heterocarpy in *Salsola komarovii* Iljin. *Bot Mag Tokyo*, **104**, 207-215.

**Tang, H.B., Sezen, U. and Paterson, A.H.** (2010) Domestication and plant genomes. *Curr. Opin. Plant Biol.*, **13**, 160-166.

**Tani, E., Polidoros, A.N. and Tsaftaris, A.S.** (2007) Characterization and expression analysis of *FRUITFULL*- and *SHATTERPROOF*-like genes from peach (*Prunus persica*) and their role in split-pit formation. *Tree Physiol.*, **27**, 649-659.

**Tani, E., Tsaballa, A., Stedel, C., Kalloniati, C., Papaefthimiou, D., Polidoros, A., Darzentas, N., Ganopoulos, I., Flemetakis, E., Katinakis, P. and Tsaftaris, A.** (2011) The study of a *SPATULA*-like bHLH transcription factor expressed during peach (*Prunus persica*) fruit development. *Plant Physiol. Biochem.*, **49**, 654-663.

**Theißen, G.** (2009) Saltational evolution: hopeful monsters are here to stay. *Theory Biosci.*, **128**, 43-51.

**Tian, Z.X., Wang, X.B., Lee, R., Li, Y.H., Specht, J.E., Nelson, R.L., McClean, P.E., Qiu, L.J. and Ma, J.X.** (2010) Artificial selection for determinate growth habit in soybean. *Proc. Natl. Acad. Sci. U. S. A.*, **107**, 8563-8568.

**Tisza, V., Kovacs, L., Balogh, A., Heszky, L. and Kiss, E.** (2010) Characterization of *FaSPT*, a *SPATULA* gene encoding a bHLH transcriptional factor from the non-climacteric strawberry fruit. *Plant Physiol. Biochem.*, **48**, 822-826.

**Toledo-Ortiz, G., Huq, E. and Quail, P.H.** (2003) The *Arabidopsis* basic/helix-loop-helix transcription factor family. *Plant Cell*, **15**, 1749-1770.

**Toriyama, K., Hinata, K. and Kameya, T.** (1987) Production of somatic hybrid plants, Brassicomoricandia, through protoplast fusion between *Moricandia arvensis* and *Brassica oleracea*. *Plant Sci.*, **48**, 123-128.

**Trevaskis, B., Bagnall, D.J., Ellis, M.H., Peacock, W.J. and Dennis, E.S.** (2003) MADS box genes control vernalization-induced flowering in cereals. *Proc. Natl. Acad. Sci. U. S. A.*, **100**, 13099-13104.

**Trieu, A.T., Burleigh, S.H., Kardailsky, I.V., Maldonado-Mendoza, I.E., Versaw, W.K., Blaylock, L.A., Shin, H.S., Chiou, T.J., Katagi, H., Dewbre, G.R., Weigel, D. and Harrison, M.J.** (2000) Transformation of *Medicago truncatula* via infiltration of seedlings or flowering plants with *Agrobacterium*. *Plant J.*, **22**, 531-541.

**Tsiantis, M.** (2011) A transposon in *tb1* drove maize domestication. *Nat. Genet.*, **43**, 1048-1050.

**Turner, A., Beales, J., Faure, S., Dunford, R.P. and Laurie, D.A.** (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. *Science*, **310**, 1031-1034.

**U, N.** (1935) Genome analysis in Brassica with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jap. J. Bot.*, **7**, 389-452.

**Van de Peer, Y., Maere, S. and Meyer, A.** (2009) The evolutionary significance of ancient genome duplications. *Nat. Rev. Genet.*, **10**, 725-732.

**Van Larebeke.N, Engler, G., Holsters, M., Vandenel.S, Zaenen, I., Schilper.Ra and Schell, J.** (1974) Large plasmid in *Agrobacterium tumefaciens* essential for crown gall-inducing ability. *Nature*, **252**, 169-170.

**Vanderpoorten, A., Hedenas, L., Cox, C.J. and Shaw, A.J.** (2002) Phylogeny and morphological evolution of the Amblystegiaceae (Bryopsida). *Mol. Phylogenet. Evol.*, **23**, 1-21.

**Venable, D.L., Dyreson, E. and Morlaes, E.** (1995) Population dynamic consequences and evolution of seed traits of *Heterosperma pinnatum* (Asteraceae). *Am. J. Bot.*, **82**, 410-420.

**Venable, D.L. and Levin, D.A.** (1985) Ecology of achene dimorphism in *Heterotheca latifolia*. 1. Achene structure, germination and dispersal. *J. Ecol.*, **73**, 133-145.

**Vrebalov, J., Pan, I.L., Arroyo, A.J.M., McQuinn, R., Chung, M., Poole, M., Rose, J.K.C., Seymour, G., Grandillo, S., Giovannoni, J. and Irish, V.F.** (2009) Fleshy fruit expansion and ripening are regulated by the tomato *SHATTERPROOF* gene *TAGL1*. *Plant Cell*, **21**, 3041-3062.

**Wagner, M.R. and Mitchell-Olds, T.** (2011) Repeated phenotypic changes highlight molecular targets of convergent evolution. *Genome Biol.*, **12**.

**Wagstaff, S.J. and Olmstead, R.G.** (1997) Phylogeny of Labiatae and Verbenaceae inferred from rbcL sequences. *Syst. Bot.*, **22**, 165-179.

**Wang, M.B. and Waterhouse, P.M.** (2000) High-efficiency silencing of a *ß*-glucuronidase gene in rice is correlated with repetitive transgene structure but is independent of DNA methylation. *Plant Mol. Biol.*, **43**, 67-82.

**Wang, N.A., Qian, W., Suppanz, I., Wei, L.J., Mao, B.Z., Long, Y., Meng, J.L., Muller, A.E. and Jung, C.** (2011) Flowering time variation in oilseed rape (*Brassica napus* L.) is associated with allelic variation in the *FRIGIDA* homologue *BnaA.FRI.a*. *J. Exp. Bot.*, **62**, 5641-5658.

**Wang, W.C., Menon, G. and Hansen, G.** (2003) Development of a novel *Agrobacterium*-mediated transformation method to recover transgenic *Brassica napus* plants. *Plant Cell Rep.*, **22**, 274-281.

**Warwick, S., Mummenhoff, K., Sauder, C., Koch, M. and Al-Shehbaz, I.** (2010) Closing the gaps: phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS region. *Plant Syst. Evol.*, **285**, 209-232.

**Warwick, S.I.** (2011) Brassicaceae in agriculture. In *Genetics and Genomics of the Brassicaceae*, Schmidt, R. and Bancroft, I. eds: Springer New York, pp. 33-65. DOI: 10.1007/978-1-4419-7118-0_2.

**Warwick, S.I., Gugel, R.K., McDonald, T. and Falk, K.C.** (2006) Genetic variation of Ethiopian mustard (*Brassica carinata* A. Braun) germplasm in western Canada. *Genet. Resour. Crop Evol.*, **53**, 297-312.

**Weller, J.L., Liew, L.C., Hecht, V.F.G., Rajandran, V., Laurie, R.E., Ridge, S., Wenden, B., Vander Schoor, J.K., Jaminon, O., Blassiau, C., Dalmais, M., Rameau, C., Bendahmane, A., Macknight, R.C. and Lejeune-Henaut, I.** (2012) A conserved molecular basis for photoperiod adaptation in two temperate legumes. *Proc. Natl. Acad. Sci. U. S. A.*, **109**, 21158-21163.

**Wen, J.Q., Lease, K.A. and Walker, J.C.** (2004) DVL, a novel class of small polypeptides: overexpression alters *Arabidopsis* development. *Plant J.*, **37**, 668-677.

**Western, T.L. and Haughn, G.W.** (1999) *BELL1* and *AGAMOUS* genes promote ovule identity in *Arabidopsis thaliana*. *Plant J.*, **18**, 329-336.

**Wilhelm, E.P., Turner, A.S. and Laurie, D.A.** (2009) Photoperiod insensitive *Ppd-A1a* mutations in tetraploid wheat (*Triticum durum* Desf.). *Theor. Appl. Genet.*, **118**, 285-294.

**Wood, T.E., Burke, J.M. and Rieseberg, L.H.** (2005) Parallel genotypic adaptation: when evolution repeats itself. *Genetica*, **123**, 157-170.

**Wray, G.A.** (2007) The evolutionary significance of *cis*-regulatory mutations. *Nat. Rev. Genet.*, **8**, 206-216.

**Wu, H., Mori, A., Jiang, X.S., Wang, Y.X. and Yang, M.** (2006) The INDEHISCENT protein regulates unequal cell divisions in Arabidopsis fruit. *Planta*, **224**, 971-979.

**Wu, J., Wei, K.Y., Cheng, F., Li, S.K., Wang, Q., Zhao, J.J., Bonnema, G. and Wang, X.W.** (2012) A naturally occurring InDel variation in *BraA.FLC.b* (*BrFLC2*) associated with flowering time variation in *Brassica rapa*. *BMC Plant Biol.*, **12**.

**Xia, Z.J., Watanabe, S., Yamada, T., Tsubokura, Y., Nakashima, H., Zhai, H., Anai, T., Sato, S., Yamazaki, T., Lu, S.X., Wu, H.Y., Tabata, S. and Harada, K.** (2012) Positional cloning and characterization reveal the molecular basis for soybean maturity locus *E1* that regulates photoperiodic flowering. *Proc. Natl. Acad. Sci. U. S. A.*, **109**, E2155-E2164.

**Xu, Y., Zhang, L. and Ma, R.C.** (2008) Functional characterization and mapping of two MADS box genes from peach (*Prunus persica*). *Chin. Sci. Bull.*, **53**, 853-859.

**Xue, W.Y., Xing, Y.Z., Weng, X.Y., Zhao, Y., Tang, W.J., Wang, L., Zhou, H.J., Yu, S.B., Xu, C.G., Li, X.H. and Zhang, Q.F.** (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nat. Genet.*, **40**, 761-767.

**Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S. and Dubcovsky, J.** (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proc. Natl. Acad. Sci. U. S. A.*, **103**, 19581-19586.

**Yang, Z.B., Bai, Z.Y., Li, X.L., Wang, P., Wu, Q.X., Yang, L., Li, L.Q. and Li, X.J.** (2012) SNP identification and allelic-specific PCR markers development for *TaGW2*, a gene linked to wheat kernel weight. *Theor. Appl. Genet.*, **125**, 1057-1068.

**Yaniv, Z., Schafferman, D., Zur, M. and Shamir, I.** (1997) Evaluation of *Matthiola incana* as a source of omega-3-linolenic acid. *Ind. Crop. Prod.*, **6**, 285-289.

**Yoon, H.S. and Baum, D.A.** (2004) Transgenic study of parallelism in plant morphological evolution. *Proc. Natl. Acad. Sci. U. S. A.*, **101**, 6524-6529.

**Yuan, Y.X., Wu, J., Sun, R.F., Zhang, X.W., Xu, D.H., Bonnema, G. and Wang, X.W.** (2009) A naturally occurring splicing site mutation in the *Brassica rapa FLC1* gene is associated with variation in flowering time. *J. Exp. Bot.*, **60**, 1299-1308.

**Zachgo, S.** (2002) *In situ* hybridization. In *Molecular plant biology: A Practival Approach*, Gillmartin, P., Bolwer, C. ed. Oxford, UK: Oxford University Press, pp. 41-63.

**Zakhrabekova, S., Gough, S.P., Braumann, I., Muller, A.H., Lundqvist, J., Ahmann, K., Dockter, C., Matyszczak, I., Kurowska, M., Druka, A., Waugh, R., Graner, A., Stein, N., Steuernagel, B., Lundqvist, U. and Hansson, M.** (2012) Induced mutations in circadian clock regulator *Mat-a* facilitated short-season adaptation and range extension in cultivated barley. *Proc. Natl. Acad. Sci. U. S. A.*, **109**, 4326-4331.

**Zhang, L., Zhao, Y.L., Gao, L.F., Zhao, G.Y., Zhou, R.H., Zhang, B.S. and Jia, J.Z.** (2012) *TaCKX6-D1*, the ortholog of rice *OsCKX2*, is associated with grain weight in hexaploid wheat. *New Phytol.*, **195**, 574-584.

**Zhang, R., Guo, C.C., Zhang, W.G., Wang, P.P., Li, L., Duan, X.S., Du, Q.G., Zhao, L., Shan, H.Y., Hodges, S.A., Kramer, E.M., Ren, Y. and Kong, H.Z.** (2013) Disruption of the petal identity gene *APETALA3-3* is highly correlated with loss of petals within the buttercup family (Ranunculaceae). *Proc. Natl. Acad. Sci. U. S. A.*, **110**, 5074-5079.

**Zhang, X.R., Henriques, R., Lin, S.S., Niu, Q.W. and Chua, N.H.** (2006a) *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nature Protocols*, **1**, 641-646.

**Zhang, Y.F., Cheng, S.P., De Jong, D., Griffiths, H., Halitschke, R. and De Jong, W.** (2009) The potato *R* locus codes for dihydroflavonol 4-reductase. *Theor. Appl. Genet.*, **119**, 931-937.

**Zhang, Z., Li, J., Zhao, X.-Q., Wang, J., Wong, G.K.-S. and Yu, J.** (2006b) KaKs_calculator: calculating Ka and Ks through model selection and model averaging. *Genomics, Proteomics & Bioinformatics*, **4**, 259-263.

**Zhao, S.S., Dufour, D., Sanchez, T., Ceballos, H. and Zhang, P.** (2011) Development of waxy cassava with different biological and physico-chemical characteristics of starches for industrial applications. *Biotechnol. Bioeng.*, **108**, 1925-1935.

**Zhou, Y., Lu, D.F., Li, C.Y., Luo, J.H., Zhu, B.F., Zhu, J.J., Shangguan, Y.Y., Wang, Z.X., Sang, T., Zhou, B. and Han, B.** (2012) Genetic control of seed shattering in rice by the APETALA2 transcription factor *SHATTERING ABORTION1*. *Plant Cell*, **24**, 1034-1048.

**Zhu, B.F., Si, L.Z., Wang, Z.X., Zhou, Y., Zhu, J.J., Shangguan, Y.Y., Lu, D.F., Fan, D.L., Li, C.Y., Lin, H.X., Qian, Q.A., Sang, T., Zhou, B., Minobe, Y.Z. and Han, B.** (2011) Genetic control of a transition from black to straw-white seed hull in rice domestication. *Plant Physiol.*, **155**, 1301-1311.

**Zjhra, M.L., Sytsma, K.J. and Olmstead, R.G.** (2004) Delimitation of Malagasy tribe Coleeae and implications for fruit evolution in Bignoniaceae inferred from a chloroplast DNA phylogeny. *Plant Syst. Evol.*, **245**, 55-67.

# 6. Danksagung

An der Entstehung dieser Doktorarbeit waren sehr viele Menschen beteiligt. Sie haben mir unzählige Dinge beigebracht, mir neue Blickwinkel und Denkansätze geboten, mich vor Herausforderungen gestellt, mit mir diskutiert, meine Fragen beantwortet, mich technisch und organisatorisch unterstützt, mich vor Fehlern bewahrt, mir Material zur Verfügung gestellt, meine Texte gelesen und verbessert, sind für mich eingesprungen, haben mit mir gejubelt und gelitten, für vieles Verständnis gezeigt, mich motiviert oder einfach nur an mich geglaubt. Ohne diese Unterstützung wäre diese Arbeit nicht möglich gewesen und ich bedanke mich für all die Hilfsbereitschaft von ganzem Herzen.

Einige Personen möchte ich besonders erwähnen:

Günter Theißen danke ich für die großen Freiheiten bei der inhaltlichen und zeitlichen Gestaltung dieser Arbeit, für konstruktive Diskussionen und hilfreiche Lösungsansätze bei allen auftretenden Problemen, für die Entschachtelung meiner Schachtelsätze und für die humorvolle Herangehensweise an… eigentlich alles.

Alle aktiven und ehemaligen Mitarbeiter des Lehrstuhls für Genetik: Andrea Härter, Andrea Hoffmeier, Anja Werther, Anna Beyerlein, Anna Brinckmann, Carolin Kloß, Christian Gafert, Christiane Ritz, Christoph Thieme, Cornelius Eibner, Dajana Lobbes, Dörte Kasten, Florian Rümpler, Fred Ferber, Hannelore Simon, Heidi Kressler, Janine Ziermann, Kerstin Pohl, Khushboo Jetha, Lisa Haslauer, Lisa Weilandt, Lydia Gramzow, Maren Fräger, Maria Unger, Mariana Mondragon-Palomino, Markus Ritz, Nina Kottenhagen, Pia Nutt, Rainer Melzer, Sabine Schein, Sandra Gusewski, Sophia Walter, Susanne Nolden, Susanne Schilling, Ulrike Wrazidlo, Wim Damen, Yong-Qiang Wang. Danke für die tolle Arbeitsatmosphäre und die vielen allzeit offenen Ohren. Es war echt super mit Euch und für Euch Waffeln zu backen war mir immer eine besondere Freude!

Andreas Mühlhausen und Klaus Mummenhoff. Die Zusammenarbeit mit Euch kann man nur als äußerst FRUITFULL bezeichnen! Vielen Dank dafür!

Meine Eltern. Danke für die lebenslange und rückhaltlose Unterstützung.

Meine erweiterte Familie: Anke, Dirk, Martina, Mathias, Mathilde, Niklas, Wiebke. Ihr seid legen…wait for it…!!!

Meine Spitzenmädels Malin, Frieda und Ronja. Niemand hat mich so aktiv vom Arbeiten abgehalten und mich trotzdem so viel weitergebracht.

Thorsten. Für einfach alles!

# 7. Ehrenwörtliche Erklärung

Hiermit erkläre ich ehrenwörtlich, dass mir die geltende Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena bekannt ist und ich die vorliegende Arbeit selbst angefertigt habe. Alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen habe ich in meiner Arbeit angegeben. Bei der Auswertung des Materials sowie beim Verfassen des Manuskriptes haben mich die in der Danksagung dieser Arbeit genannten Personen unterstützt.

Ferner erkläre ich ehrenwörtlich, für die Anfertigung der Arbeit keinen Promotionsberater in Anspruch genommen zu haben und dass Dritte weder mittelbar noch unmittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen. Ich habe die vorliegende Dissertation bisher nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung vorgelegt. Auch habe ich weder diese Dissertation noch eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung bei einer anderen Hochschule als Dissertation eingereicht.

Teresa Lenser

Jena, 12. November 2013

# 8. *Curriculum vitae*

***Persönliche Daten***

| | |
|---|---|
| **Name:** | Teresa Lenser, geb. Keining |
| **Geburt:** | 06.07.1981 in Bottrop / NRW |
| **Familienstand:** | verheiratet, drei Kinder |

***Schulbildung***

| | |
|---|---|
| **1988-1992** | Droste-Hülshoff-Schule in Bottrop |
| **1992-2001** | Josef-Albers-Gymnasium in Bottrop<br>*Abschluss mit dem Abitur* |

***Studium***

| | |
|---|---|
| **2001-2007** | Biologie-Studium an der Friedrich-Schiller-Universität in Jena<br>*Abschluss als Diplom-Biologin (Abschlussnote 1,0)* |
| **2003** | Abschluss des Vordiploms (Note 1,2) |
| **2004-2005** | Auslandsstudium an der University of York, UK<br>Forschungspraktikum im Labor von Dr. Louise Jones<br>*Thema: Study of argonaute proteins that are involved in the process of RNA silencing* |
| **2006-2007** | Diplomarbeit am Leibniz Institut für Altersforschung in Jena in der Gruppe Einzelzell- und Einzelmolekültechniken<br>*Thema: Early birds in DNA-repair: Aspects of real-time accumulation kinetics of Ku80 and XRCC4* |
| **2008-heute** | Promotionsstudium am Lehrstuhl für Genetik an der FSU Jena<br>*Thema: Tracking the molecular background of phenotypic change in fruit evolution and plant domestication* |

# 9. Konferenzbeiträge und Publikationen

***Publikationen***

**Jones, L., Keining, T., Eamens, A., and Vaistij, F.E.** (2006) Virus-induced gene silencing of *Argonaute* genes in *Nicotiana benthamiana* demonstrates that extensive systemic silencing requires *Argonaute1-like* and *Argonaute4-like* genes. *Plant Physiol*., **141**, 598-606

**Mühlhausen, A., Lenser, T., Mummenhoff, K., and Theißen, G.** (2013) Evidence that an evolutionary transition from dehiscent to indehiscent fruits in *Lepidium* (Brassicaceae) was caused by a change in the control of valve margin identity genes. *Plant J*., **73**, 824-835

**Lenser, T. and Theißen, G.** (2013) Conservation of fruit dehiscence pathways between *Lepidium campestre* and *Arabidopsis thaliana* sheds light on the regulation of *INDEHISCENT*. *Plant J.,* **76**, 545-556

**Lenser, T. and Theißen, G.** (2013) Molecular mechanisms involved in convergent crop domestication. *Trends Plant Sci.,* DOI: 10.1016/j.tplants.2013.08.007, *in press*

***Poster***

**Lenser, T., Mühlhausen, A., Mummenhoff, K., and Theißen, G.**: Unequal sisters: Evolution of fruit dehiscence in *Lepidium* (Brassicaceae). 2nd International PhD Conference on Plant Development, Retzbach, Deutschland (2009)

**Lenser, T., Mühlhausen, A., Mummenhoff, K., and Theißen, G.**: Evolutionary studies of fruit development in the genus Lepidium (Brassicaceae). Workshop on molecular mechanisms controlling flower development, Presqu’ile de Giens, Frankreich (2013)