Letter

Explosive seed dispersal

Introduction

Understanding the origin and evolution of complex traits is a major goal in biology. Here, we addressed how cellular novelties translate to trait innovation in the context of explosive seed dispersal. We followed a comparative approach by studying seed dispersal in the *Arabidopsis thaliana* relative, *Cardamine hirsuta*. This plant is a widespread, ruderal species that uses explosive seed dispersal to successfully colonize disturbed habitats. We recently presented a mathematical model that explains the mechanism of explosive dispersal in *C. hirsuta* (Hofhuis *et al.*, 2016). Here, we review the cellular innovations for the storage and rapid release of energy that underpin the evolution of this trait. The mechanism that we proposed for establishing pre-tension in the fruit depends on a particular cell shape in the exocarp layer. We analyze the phylogenetic distribution of exocarp cell shape in *Cardamine* species with explosive fruit, and other species in the Brassicaceae with nonexplosive fruit. We conclude that this was an enabling character for the origin of explosive seed dispersal, while a second character gain – asymmetric lignification of the endocarp *b* cell layer – was a driving character.

Dispersal of the next generation provides a valuable opportunity for plants to move. This evolutionary pressure has resulted in an enormous variety of seed dispersal mechanisms in flowering plants (van der Pijl, 1982). Explosive seed dispersal is an example of autochory, where seeds are dispersed by a plant's own mechanisms, and is found in various angiosperm lineages including the genus *Cardamine*. *Cardamine hirsuta* uses an explosive mechanism to launch its seeds at speeds in excess of $10 \, \mathrm{m \, s^{-1}}$ upon ballistic trajectories to land within a 2-m radius of the parent plant (Hofhuis *et al.*, 2016). During explosive pod shatter, the two valves of the *C. hirsuta* fruit pod coil rapidly, transferring kinetic energy to the seeds to fire them away. We showed that differential contraction of hydrated tissues in the fruit pod generates this coiling. Moreover, we demonstrated that this coiling becomes explosive through a rapid change in secondary cell wall geometry. To gain a quantitative and predictive understanding of this mechanism, we generated a multi-scale model that reproduces explosive pod shatter using interactions between cell and tissue-level processes (Hofhuis *et al.*, 2016). This innovation appears to have evolved only once in the Brassicaceae family, and here we explore the use of comparative studies to understand the origin of this trait.

Comparative logic for a mutant screen

Comparing a model species, such as *A. thaliana*, with a close relative, such as *C. hirsuta*, provides the experimental tools to identify and compare genetic mechanisms that underlie trait differences (Hay & Tsiantis, 2016). For example, the explosive fruit pods of *C. hirsuta* and the nonexplosive pods of *A. thaliana* are very similar in morphology but differ in the lignification of secondary cell walls (SCWs) in the valve (Fig. 1). We used this difference to design a genetic screen for *C. hirsuta* mutants with less lignified fruit valves that resemble *A. thaliana*. We reasoned that such mutants would tell us whether this character was required for explosive pod shatter and what genes controlled this character.

We identified *less lignin2* (*lig2*) as a recessive mutant with less lignified fruit valves, similar to *A. thaliana* (Fig. 1). We found that the lignified endocarp *b* cell layer was missing in *lig2* valves, causing the fruit pods to shatter non-explosively, like *A. thaliana* (Fig. 1). The mutated gene responsible for the *lig2* phenotype was the *C. hirsuta* ortholog of the DNA-binding protein BRASSINO-STEROID-INSENSITIVE4 (At5g24630; Breuer *et al.*, 2007; Kirik *et al.*, 2007; Hofhuis *et al.*, 2016). This result provides novel insight into the genetic regulation of endocarp *b* cell development and its importance for explosive pod shatter. Altogether, our findings showed that the geometry of the lignified secondary cell wall, deposited asymmetrically in endocarp *b* cells of the fruit valve, is required for explosive pod shatter (Fig. 1; Hofhuis *et al.*, 2016).

Phylogenetic comparisons

Comparing pod shatter between the explosive fruit of *C. hirsuta* and the nonexplosive fruit of *A. thaliana*, allowed us to uncover the
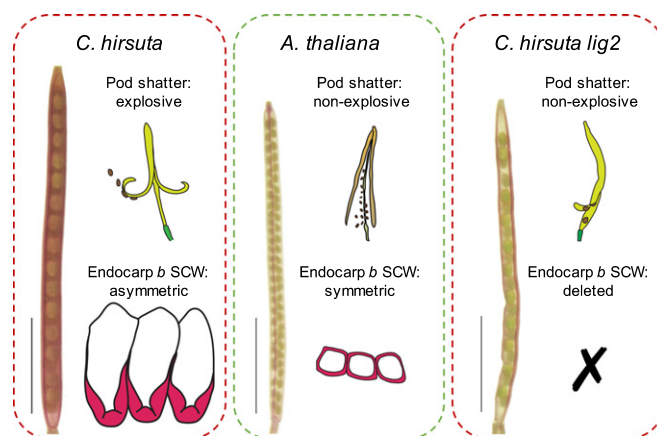

Fig. 1 Genetic screen for *Cardamine hirsuta* mutants with nonlignified fruit valves. Lignified secondary cell walls (SCWs) stain pink with phloroglucinol. In mature fruits, the valves stain pink in the explosive fruit of *C. hirsuta* but not in the nonexplosive fruit of *Arabidopsis thaliana*, while the valve margin and replum stain pink in both species. This staining method was used to screen for *C. hirsuta* mutants with nonlignified valves, similar to *A. thaliana*. The *less lignin2* (*lig2*) mutant was identified from this screen and has nonexplosive fruit valves. Lignified SCWs are asymmetrically localized in endocarp *b* cells of *C. hirsuta* but symmetric in *A. thaliana*. Valves of the *lig2* mutant lack lignin because the endocarp *b* cell layer is deleted. Bars, 5 mm.
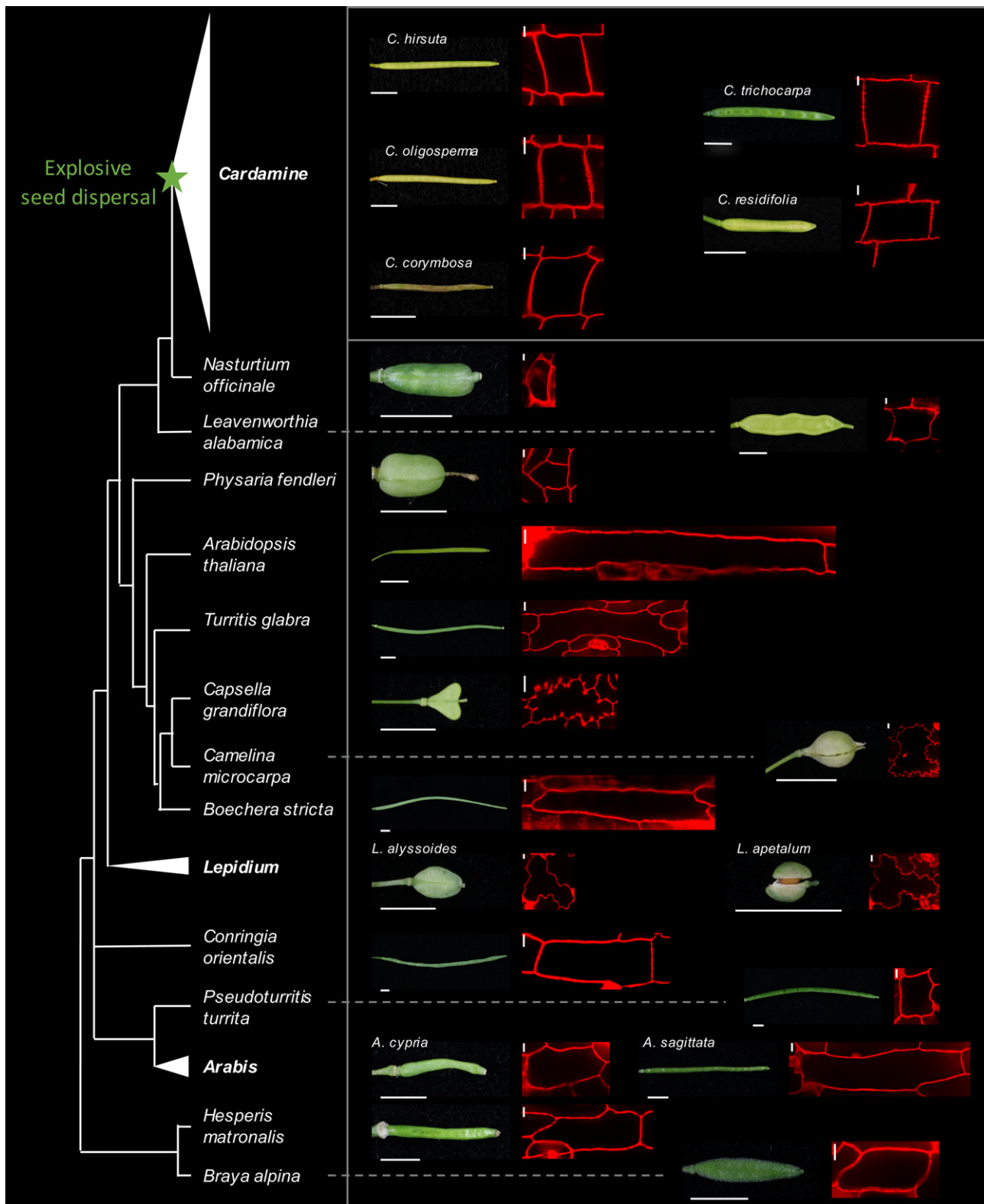
Fig. 2 Phylogenetic survey of fruit exocarp cell shape. Explosive seed dispersal evolved in *Cardamine* and all sampled species had a square cell shape. This character state was found in other species with nonexplosive seed dispersal sampled from the *Cardamineae* tribe (*Nasturtium officinale*, *Leavenworthia alabamica*). Exocarp cell shape varied among nonexplosive *Brassicaceae* species from elongated (e.g. *Arabidopsis thaliana*) to lobed (e.g. *Capsella grandiflora*) and at least two other instances of a square cell shape were found in *Physaria fendleri* and *Pseudoturritis turrita*. Cells were stained with propidium iodide and imaged by confocal laser scanning microscopy. All cells are aligned to the longitudinal axis of the accompanying fruit. Bars: 10 μm (cells), 2 mm (fruit).

developmental and genetic basis for this trait difference. However, to understand how explosive pod shatter evolved requires placing this character in a phylogenetic framework. We found that the geometry of endocarp *b* SCWs associated strictly with explosive vs nonexplosive seed dispersal across the Brassicaceae (Hofhuis *et al.*, 2016). Explosive seed dispersal evolved in *Cardamine* and all species sampled in this genus had the derived character state – asymmetric SCW geometry – similar to *C. hirsuta* (Fig. 1). Conversely, all sampled species with nonexplosive seed dispersal had the ancestral character state – symmetric SCW geometry – similar to *A. thaliana* (Fig. 1). Therefore, endocarp *b* SCW geometry appears to be a driving character for the trait innovation of explosive seed dispersal.

Another important component of explosive pod shatter is the differential contraction of fruit valves. Specifically, the exocarp tissue actively contracts in length while attached to the lignified endocarp *b* tissue, which is inextensible (Hofhuis *et al.*, 2016). This generates tension that is released by the valves coiling. We developed a mechanical model that recapitulated this active contraction of the exocarp, and found that the three-dimensional shape of these cells was critical for contraction (Hofhuis *et al.*, 2016). To understand how exocarp cell shape mapped on a phylogeny of the Brassicaceae, we imaged the top view of exocarp cells of mature fruit from 21 species (Fig. 2). We sampled five *Cardamine* species with explosive pod shatter and found a square cell shape in the exocarp of all species, similar to *C. hirsuta* (Fig. 2). However, we found that the exocarp of some other species with nonexplosive seed dispersal also had a square cell shape (Fig. 2). These included species sampled from the *Cardamineae* tribe, such as *Nasturtium officinale* and *Leavenworthia alabamica*, which indicates that this character evolved before explosive seed dispersal (Fig. 2). We also found square endocarp cells in the siliques of the more distantly related species *Physaria fendleri* and *Pseudoturritis turrita*, indicating that this character evolved independently outside of the *Cardamineae* tribe (Fig. 2). Moreover, we found that exocarp cell shape varied among nonexplosive Brassicaceae species. Elongated siliques usually had elongated cells, similar to *A. thaliana*, and short, broad silicles had lobed/jigsaw puzzle-shaped cells, such as in *Capsella grandiflora* (Fig. 2). Therefore, the square cell shape required for active tissue contraction is invariably present in *Cardamine* and likely enabled the innovation of explosive seed dispersal, but did not drive it (Fig. 3). By contrast, the gain of an asymmetric SCW geometry in endocarp *b* cells is likely to be the character that drove trait innovation (Fig. 3).

Conclusions

Comparisons made at different evolutionary scales are useful to understand how a trait arose and evolved. The experimental tractability of *A. thaliana* and *C. hirsuta*, including genetic tools and reference genome sequences (The Arabidopsis Genome Initiative, 2000; Hay *et al.*, 2014; Gan *et al.*, 2016), provides an excellent comparative system to investigate the cellular and genetic basis for trait differences (Hay & Tsiantis, 2006; Barkoulas *et al.*, 2008; Vlad *et al.*, 2014; Rast-Somssich *et al.*, 2015; Hofhuis *et al.*, 2016; Vuolo *et al.*, 2016). Placing such characters in a phylogenetic
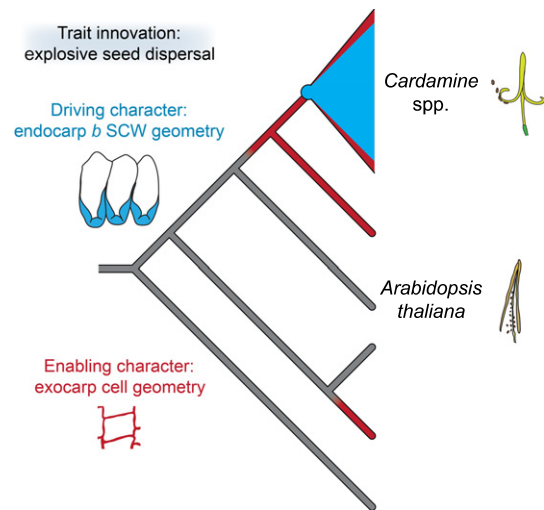

Fig. 3 Contribution of driving and enabling characters to trait innovation. Comparisons between *Cardamine hirsuta* and *Arabidopsis thaliana* identified two characters required for explosive seed dispersal that differed between these two species: endocarp *b* secondary cell wall (SCW) geometry and exocarp cell geometry. Comparing the distribution of these characters on a phylogeny showed that one character (endocarp *b* SCW geometry) gained a new state (hinged geometry, shown in blue) in association with the trait innovation of explosive seed dispersal in *Cardamine*. The other character (exocarp cell geometry) had multiple states, and the state found in *Cardamine* (square geometry, shown in red) occurred before the trait innovation of explosive seed dispersal, and occurred multiple times on the phylogeny. Therefore, endocarp *b* SCW geometry is a character driving trait innovation, while exocarp cell geometry is character enabling, rather than driving, this innovation.

context further aids our understanding of how trait differences evolved. Here, we found that two cellular innovations required for explosive seed dispersal associated differently with the appearance of this trait in *Cardamine*. Asymmetric SCW geometry in endocarp *b* cells associated strictly with the evolution of explosive seed dispersal (Fig. 3), suggesting that genes regulating SCW synthesis and patterning in this cell layer are likely targets of evolutionary modification. By contrast, species belonging to the *Cardamineae* tribe all had square rather than elongated cells in the fruit exocarp regardless of their mode of seed dispersal, suggesting that this character enabled, but did not drive, explosive seed dispersal (Fig. 3). The variation in exocarp cell shape in nonexplosive fruit pods (Fig. 2) indicates that this character alone is not sufficient to cause tissue contraction and consequent coiling of the pod. Based on our mechanical model, we predict that anisotropic cell wall properties in the exocarp are additionally required, together with three-dimensional cell shape, to produce tissue contraction (Hofhuis *et al.*, 2016). Future work will determine whether this combination of cellular innovations is sufficient to establish tension in the fruit pod. In summary, functional genetic studies, interpreted in an explicit phylogenetic framework, provide a useful path to study the evolution of morphological novelties.

Acknowledgements

Hugo Hofhuis and Angela Hay*

Max Planck Institute for Plant Breeding Research,
Carl-von-Linné-Weg 10, Köln 50829, Germany
(*Author for correspondence: tel +49 221 5062108;
email hay@mpipz.mpg.de)

References

Barkoulas M, Hay A, Kougioumoutzi E, Tsiantis M. 2008. A developmental framework for dissected leaf formation in the Arabidopsis relative *Cardamine hirsuta*. *Nature Genetics* 40: 1136–1141.

Breuer C, Stacey NJ, West CE, Zhao Y, Chory J, Tsukaya H, Azumi Y, Maxwell A, Roberts K, Sugimoto-Shirasu K. 2007. BIN4, a novel component of the plant DNA topoisomerase VI complex, is required for endoreduplication in Arabidopsis. *Plant Cell* 19: 3655–3668.

Gan X, Hay A, Kwantes M, Haberer G, Hallab A, Ioio RD, Hofhuis H, Pieper B, Cartolano M, Neumann U *et al.* 2016. The *Cardamine hirsuta* genome offers insight into the evolution of morphological diversity. *Nature Plants* 2: 16167.

Hay A, Pieper B, Cooke E, Mandakova T, Cartolano M, Tattersall AD, dello Ioio R, McGowan SJ, Barkoulas M, Galinha C *et al.* 2014. *Cardamine hirsuta*: a versatile genetic system for comparative studies. *Plant Journal* 78: 1–15.

Hay A, Tsiantis M. 2006. The genetic basis for differences in leaf form between *Arabidopsis thaliana* and its wild relative *Cardamine hirsuta*. *Nature Genetics* 38: 942–947.

Hay A, Tsiantis M. 2016. *Cardamine hirsuta*: a comparative view. *Current Opinion in Genetics & Development* 39: 1–7.

Hofhuis H, Moulton D, Lessinnes T, Routier-Kierzkowska AL, Bomphrey RJ, Mosca G, Reinhardt H, Sarchet P, Gan X, Tsiantis M *et al.* 2016. Morphomechanical innovation drives explosive seed dispersal. *Cell* 166: 222–233.

Kirik V, Schrader A, Uhrig JF, Hulskamp M. 2007. MIDGET unravels functions of the Arabidopsis topoisomerase VI complex in DNA endoreduplication, chromatin condensation, and transcriptional silencing. *Plant Cell* 19: 3100–3110.

van der Pijl L. 1982. *Principles of dispersal in higher plants*. Berlin, Germany: Springer-Verlag.

Rast-Somssich MI, Broholm S, Jenkins H, Canales C, Vlad D, Kwantes M, Bilsborough G, Dello Ioio R, Ewing RM, Laufs P *et al.* 2015. Alternate wiring of a *KNOXI* genetic network underlies differences in leaf development of *A. thaliana* and *C. hirsuta*. *Genes & Development* 29: 2391–2404.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796–815.

Vlad D, Kierzkowski D, Rast MI, Vuolo F, Dello Ioio R, Galinha C, Gan X, Hajheidari M, Hay A, Smith RS *et al.* 2014. Leaf shape evolution through duplication, regulatory diversification, and loss of a homeobox gene. *Science* 343: 780–783.

Vuolo F, Mentink RA, Hajheidari M, Bailey CD, Filatov DA, Tsiantis M. 2016. Coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. *Genes & Development* 30: 2370–2375.

About *New Phytologist*

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.

- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <28 days. There are **no page or colour charges** and a PDF version will be provided for each article.

- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.

- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)

- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**