Developmental Control and Plasticity of Fruit and Seed Dimorphism in *Aethionema arabicum*[1][CC-BY]

Teresa Lenser[2], Kai Graeber[2], Özge Selin Cevik, Nezaket Adigüzel, Ali A. Dönmez, Christopher Grosche, Marcel Kettermann, Sara Mayland-Quellhorst, Zsuzsanna Mérai, Setareh Mohammadin, Thu-Phuong Nguyen, Florian Rümpler, Christina Schulze, Katja Sperber, Tina Steinbrecher, Nils Wiegand, Miroslav Strnad, Ortrun Mittelsten Scheid, Stefan A. Rensing, Michael Eric Schranz, Günter Theißen[3]*, Klaus Mummenhoff[3]*, and Gerhard Leubner-Metzger[3]*

Department of Genetics, Friedrich Schiller University, 07743 Jena, Germany (T.L., F.R., G.T.); School of Biological Sciences, Plant Molecular Science and Centre for Systems and Synthetic Biology, Royal Holloway University of London, Egham, Surrey TW20 0EX, United Kingdom (K.G., C.S., T.S., G.L.-M.); Department of Physiology, Faculty of Medicine, Mersin University, 33343 Mersin, Turkey (Ö.S.C.); Department of Biology, Science Faculty, Gazi University, 06500 Teknikokullar, Ankara, Turkey (N.A.); Department of Botany, Faculty of Science, Hacettepe University, 06800 Beytepe, Ankara, Turkey (A.A.D.); Plant Cell Biology, Faculty of Biology, University of Marburg, 35043 Marburg, Germany (C.G., S.A.R.); Department of Biology, Botany, University of Osnabrück, 49076 Osnabrueck, Germany (M.K., S.M.-Q., K.S., N.W., K.M.); Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna Biocenter, 1030 Vienna, Austria (Z.M., O.M.S.); Biosystematics Group, Wageningen University, 6708 PB Wageningen, The Netherlands (S.M., T.-P.N., M.E.S.); and Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University and Institute of Experimental Botany, Academy of Sciences of the Czech Republic, 78371 Olomouc, Czech Republic (M.S., G.L.-M.)

ORCID IDs: 0000-0003-2948-0856 (K.G.); 0000-0002-2211-7600 (Ö.S.C.); 0000-0002-2048-1628 (Z.M.); 0000-0003-3492-1062 (T.-P.N.); 0000-0003-2103-8879 (C.S.); 0000-0003-3282-6029 (T.S.); 0000-0002-2806-794X (M.S.); 0000-0002-7757-4809 (O.M.S.); 0000-0002-0225-873X (S.A.R.); 0000-0001-6777-6565 (M.E.S.); 0000-0003-4854-8692 (G.T.); 0000-0002-8449-1593 (K.M.); 0000-0002-6045-8713 (G.L.-M.).

Understanding how plants cope with changing habitats is a timely and important topic in plant research. Phenotypic plasticity describes the capability of a genotype to produce different phenotypes when exposed to different environmental conditions. In contrast, the constant production of a set of distinct phenotypes by one genotype mediates bet hedging, a strategy that reduces the temporal variance in fitness at the expense of a lowered arithmetic mean fitness. Both phenomena are thought to represent important adaptation strategies to unstable environments. However, little is known about the underlying mechanisms of these phenomena, partly due to the lack of suitable model systems. We used phylogenetic and comparative analyses of fruit and seed anatomy, biomechanics, physiology, and environmental responses to study fruit and seed heteromorphism, a typical morphological basis of a bet-hedging strategy of plants, in the annual Brassicaceae species *Aethionema arabicum*. Our results indicate that heteromorphism evolved twice within the Aethionemeae, including once for the monophyletic annual *Aethionema* clade. The dimorphism of *Ae. arabicum* is associated with several anatomic, biomechanical, gene expression, and physiological differences between the fruit and seed morphs. However, fruit ratios and numbers change in response to different environmental conditions. Therefore, the life-history strategy of *Ae. arabicum* appears to be a blend of bet hedging and plasticity. Together with the available genomic resources, our results pave the way to use this species in future studies intended to unravel the molecular control of heteromorphism and plasticity.

Fruits and seeds with very specific properties evolved as typical propagation and dispersal units to support the angiosperm life cycle in adaptation to the prevailing environment (Donohue et al., 2010; Linkies et al., 2010; Ferrandiz, 2011). Seeds provide the receptacle for the embryo and nutrients to aid germination and early seedling establishment. Innate morphological and physiological seed properties define the environmental conditions suitable for germination timing through various dormancy mechanisms (Finch-Savage and Leubner-Metzger, 2006) and thereby greatly influence seedling survival and plant fitness. Fruits are unique structures that enclose and protect angiosperm seeds (Scutt et al., 2006; Seymour et al., 2008). Like seeds, they have a large impact on the fate of a plant's offspring by providing various mechanisms for seed dispersal (Ferrandiz, 2011). Most plant species commit themselves to the propagation strategy of homomorphism, producing seeds and fruits of a single type that is optimally adapted to the respective habitat.

However, several angiosperm families independently evolved heteromorphism, characterized by the production of two or more distinct fruit and seed morphs on individual plants (Imbert, 2002). These differ in various

properties, including fruit and seed size, shape, color, mechanisms of dispersal, dormancy, germination, and mucilage production upon imbibition (Takeno and Yamaguchi, 1991; Mandak and Pysek, 2001; Lu et al., 2010; Dubois and Cheptou, 2012; Baskin et al., 2014; Yang et al., 2015, and refs. therein). Heteromorphic plants thus can produce offspring with different fates, determined by the distinct properties of their fruits and seeds. Consequently, heteromorphism has been interpreted as a bet-hedging strategy in adaptation to unpredictable environments, where flexibility in terms of propagation is an important fitness advantage (Venable and Lawlor, 1980; Philippi and Seger, 1989; Evans and Dennehy, 2005; Abley et al., 2016). The facts that heteromorphic species occur primarily in stressful and frequently disturbed habitats (such as arid and semiarid environments) and that they mostly consist of annual species further support this conclusion (Venable et al., 1995; Imbert, 2002).

Heteromorphism represents a classical tradeoff. It may increase long-term reproductive success by reducing the risk of extinction, but it comes at the cost of decreasing the immediate fitness because only a fraction of propagules are optimally adapted to any given environment (Venable, 2007). Several studies suggest that at least some heteromorphic species diminish this problem by means of phenotypic plasticity, defined as the ability of a genotype to produce different phenotypes when exposed to different environmental conditions (Via et al., 1995; Sultan, 2000; Pigliucci et al., 2006; Abley et al., 2016). The fruit-morph ratio of heteromorphic species may vary in response to herbivory (Imbert and Ronce, 2001), nutrient availability and plant density (Mandák and Pyšek, 1999; Sadeh et al., 2009; Lu et al., 2013a), germination time (Yang et al., 2015), and soil moisture (Lu et al., 2013a), indicating that these plants can adjust their fruit development in response to certain environmental parameters. However, so far, very little is known about the molecular determinants of heteromorphism in general and the plastic developmental modulation of this phenotype in particular.

The monogeneric tribe Aethionemeae (genus *Aethionema*), the sister group to the rest of the Brassicaceae (core Brassicaceae), comprises 57 species with a distributional hotspot in the Middle East and Eastern Europe (Al-Shehbaz et al., 2006; Beilstein et al., 2010; Franzke et al., 2011). Heteromorphism in this lineage has been reported for six species (Solms-Laubach, 1901; Hedge, 1965), including *Aethionema arabicum*, a small diploid, annual, herbaceous plant whose genome sequence was published recently (Haudry et al., 2013). Analysis of the genome has shown that the genus *Aethionema* shares the ancient whole-genome duplication "At-alpha" with the crown-group Brassicaceae (Schranz et al., 2012); thus, it has been used for comparative molecular evolutionary analyses for several gene families (Hofberger et al., 2015; Mohammadin et al., 2015; Simon et al., 2015). The species has been described as forming two distinct fruit and seed morphs that may influence their propagation strategy (Solms-Laubach, 1901). We have now systematically analyzed the dimorphic phenotype of *Ae. arabicum* throughout the plant's life cycle. Our data characterize morphological and physiological features of the two distinct fruit and seed morphs and provide evidence for phenotypic plasticity. These findings, together with its phylogenetic position, available genome sequence, and life-history traits, make *Ae. arabicum* attractive for future research on both the proximate causes (molecular mechanisms) and the ultimate causes (selection regimes or genetic drift) of heteromorphism.

RESULTS

Heteromorphism Evolved at Least Twice within the Aethionemeae

Phylogenetic analysis (Fig. 1) of the genus *Aethionema* gives a tree topology with a well-supported backbone splitting in three clades: A, B, and C. Both Bayesian (Supplemental Fig. S1) and RaxML (Supplemental Fig. S2) phylogenetic analyses with the chloroplast *rbcL-a* gene and the *trnL-F* intergenic spacer support this topology. Our results also confirmed that the four *Noccaea* spp. are not part of a monophyletic *Aethionema* (Khosravi et al., 2009; Al-Shehbaz, 2012). Five annual species form a monophyletic group within clade A, suggesting a single origin of the annual life history. The five annual species, as well as the perennial *Aethionema saxatile*, are heteromorphic. However, *Ae. saxatile* is not monophyletic with the annual species, suggesting an independent origin of its heteromorphism.

[1] This work is part of the ERA-CAPS SeedAdapt consortium project (www.seedadapt.eu) and was supported by the Deutsche Forschungsgemeinschaft (grant no. TH 417/10–1 to G.T., grant no. MU 1137/12–1 to K.M., and grant no. RE 1697/8–1 to S.A.R.); by the Austrian Science Fund (grant no. FWF I1477 to O.M.S.); by the Netherlands Organization for Scientific Research (grant no. 849. 13.004 to M.E.S.); by the National Program of Sustainability of the Czech Republic (grant no. LO1204 to M.S.); by the Biotechnology and Biological Sciences Research Council (grant nos. BB/M00192X/1 and BB/M000583/1 to G.L.-M.); and by the Scientific and Technological Research Council of Turkey (grant no. TUBITAK–TBAG 1958–100 T 125 to N.A. and A.A.D.).

[2] These authors contributed equally to the article.

[3] These authors contributed equally to the article.

* Address correspondence to gerhard.leubner@rhul.ac.uk.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Gerhard Leubner-Metzger (gerhard.leubner@rhul.ac.uk). 'The Seed Biology Place' - www.seedbiology.eu

G.L.-M., G.T., K.G., K.M., K.S., M.E.S., M.K., M.S., N.W., O.M.S., S.M.Q., S.A.R., T.L., T.-P.N., T.S., and Z.M. planned and designed the research; C.S., K.G., K.S., M.K., N.W., S.M., S.M.Q., T.L., and Z.M. performed experiments; A.A.D., C.G., N.A., Ö.S.C., and T.S. provided material or analytical tools; F.R., G.L.M., G.T., K.G., K.M., K.S., M.E.S., M.K., N.W., O.M.S., S.M., S.M.Q., T.L., T.S., and Z.M. analyzed and interpreted the data; G.L.-M., G.T., K.G., K.M., O.M.S., S.M., and T.L. drafted the article; all authors revised and approved the final article.

www.plantphysiol.org/cgi/doi/10.1104/pp.16.00838

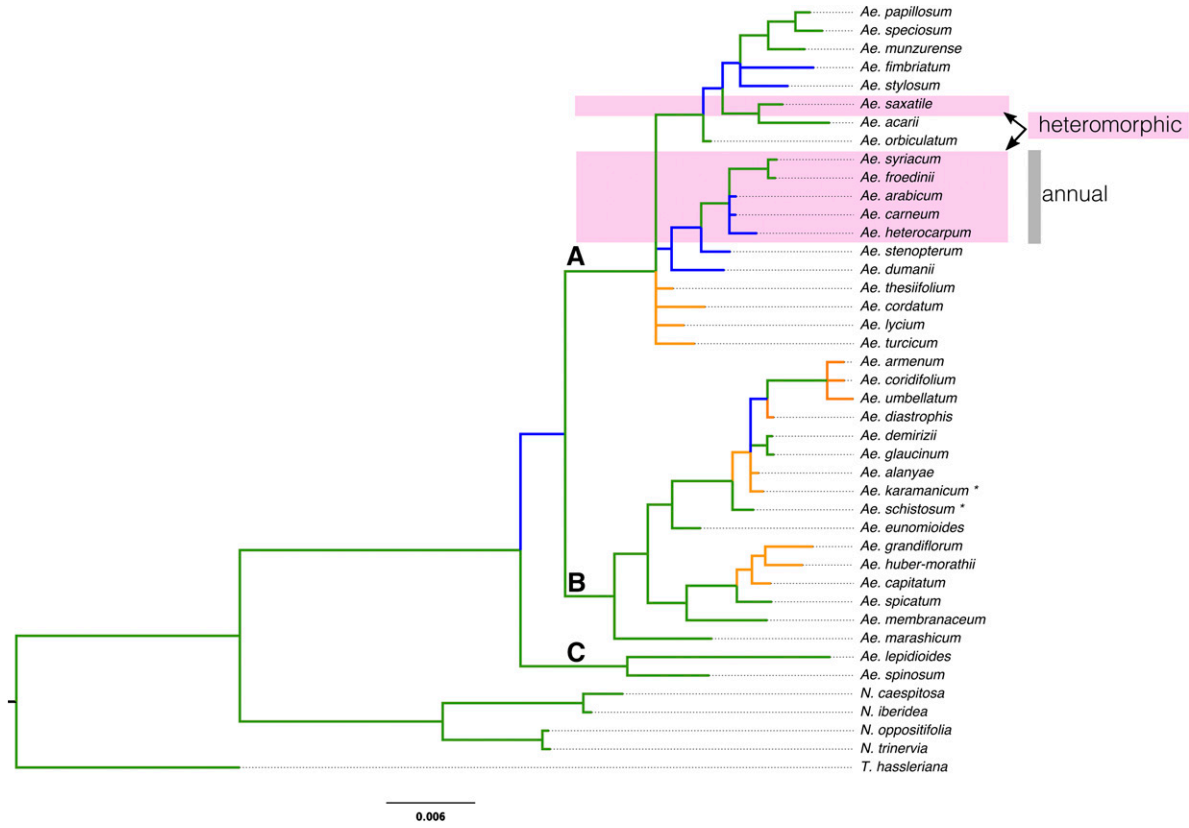

Figure 1. Phylogenetic tree for the tribe Aethionemeae. Phylogenetic analysis of 38 *Aethionema* species identifies three well-supported clades (A, B, and C). The corresponding RaxML and MrBayes analyses had the same topology except for the species marked with stars (for exact differences, see Supplemental Figs. S1 and S2). The topology suggests a single origin of the annual life-history strategy (gray bar) but indicates two independent origins of heteromorphism (highlighted in purple). Green brackets, posterior $P > 0.8$ and bootstrap percentage greater than 80; orange brackets, posterior $P < 0.5$ and bootstrap percentage less than 80; blue brackets, posterior $P > 0.8$ and bootstrap percentage less than 80.

Ae. arabicum Produces Two Clearly Distinct Fruit Morphs

To test whether fruits of *Ae. arabicum* cluster into two distinct fruit morphs (Solms-Laubach, 1901; Hedge, 1965), we performed a morphometric analysis that incorporated data for fruit width and fruit length, the presence or absence of a septum, the number of seeds per fruit, and the production of seed mucilage. Plotting the width and length of ripe fruits against each other confirmed two clusters corresponding to the distinct fruit types (Fig. 2). This is further supported by hierarchical and two-step cluster analyses based on fruit width, fruit length, and the number of seeds per fruit (Supplemental Fig. S3). The clustering correlates to 100% with the presence of a septum and the production of seed mucilage in the large, two- to six-seed-containing fruit morph, as opposed to the absence of a septum and nonmucilaginous seeds in the small, single-seeded fruit morph. The two clearly distinct fruit morphs characterize *Ae. arabicum* as a dimorphic species.

Dehiscence, Abscission, and Movement of Dimorphic Fruits

Fruits of heteromorphic plant species have often been reported to differ in their dispersal mechanisms (Sorensen, 1986; Mandak and Pysek, 2001; Lu et al., 2010, 2013; Dubois and Cheptou, 2012). To investigate whether this also holds true for the two *Ae. arabicum* fruit morphs, we studied their dehiscence and detachment behavior (Fig. 3). A random-impact test performed on ripe fruits revealed significant differences in dehiscence half-life between the two morphs (Fig. 3A). The many-seeded larger morph dehisced readily upon mechanical stimulation (mean half-life of 15 s) and is henceforth called the dehiscent fruit morph. The single-seeded smaller morph took approximately 10 times longer to open (mean half-life of 158 s) and is henceforth called the indehiscent fruit morph. In contrast, a fruit detachment force test (Fig. 3B) revealed that a slight touch (140 mN on average) was already sufficient for the abscission of ripe indehiscent fruits from the mother plant, while a significantly higher force (885 mN on average) was required to detach ripe dehiscent fruits. The moisture-induced movement of fruits or fruit parts often helps desert plant species to relate seed dispersal to rain events, so as to ensure optimal germination conditions (Gutterman, 1993). Therefore, we exposed dry *Ae. arabicum* infructescences to different degrees of humidity and measured changes in the angle between the pedicel and infructescence axis (Fig. 3C). The exposure

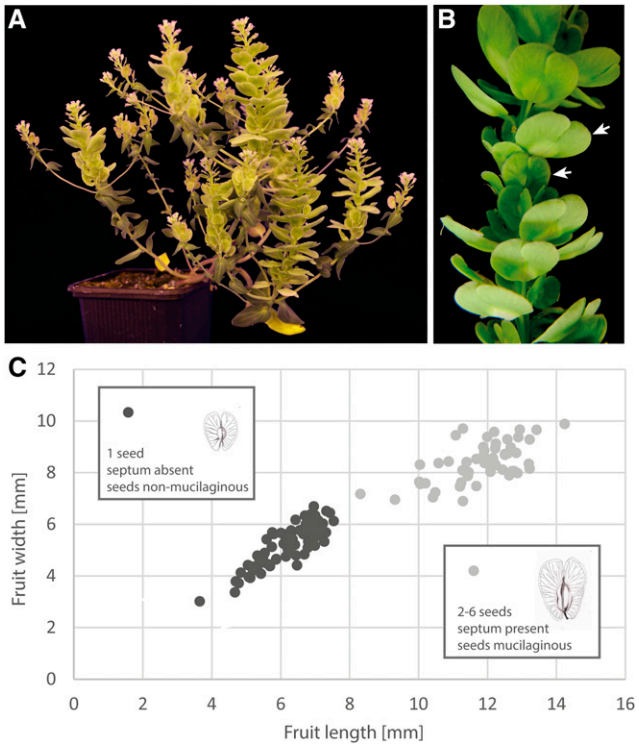

Figure 2. *Ae. arabicum* shows fruit dimorphism. Two distinct fruit morphs of different size are produced by *Ae. arabicum*. A, Entire plant. B, Closeup of a single infructescence with one representative of each morph marked by white arrows. C, Plot of length and width of 140 ripe *Ae. arabicum* fruits. Small fruits contain a single nonmucilaginous seed and no septum (dark gray circles), while large fruits contain two to six mucilaginous seeds and a septum (light gray circles).

to moisture resulted in a noticeable outward bending of pedicels, starting from an angle of approximately 30° in dry conditions (Fig. 3D) up to 90° after 30 min of exposure to water spraying (Fig. 3E; Supplemental Video S1). This spatiotemporal movement pattern was similar for both fruit morphs (Fig. 3C), suggesting that rain may aid the dispersal of both morphs. Taken together, this shows that *Ae. arabicum* applies two alternative strategies, fruit dehiscence versus abscission, to disperse its seeds.

Comparative Morphology of the Dimorphic Fruits

In our search for anatomical features underlying the observed mechanical differences between the two fruit morphs, we compared their dehiscence and abscission zones (Fig. 4). Dehiscent fruits contain two to six seeds and a septum dividing their inside into two locules (Fig. 4, A and B), whereas indehiscent fruits contain a single seed tightly enclosed by the unilocular fruit without a septum (Fig. 4, A and C). Scanning electron microscopy (SEM) images of ripe fruits revealed the beginning of tissue separation, indicative for the presence of a dehiscence zone at the valve-replum transition in the dehiscent morph (Fig. 4, D, E, and G), while valve and replum remain tightly connected in the indehiscent morph (Fig. 4, F, H, and I). Cross sections of the valve-replum border of green silicles just prior to the onset of ripening-induced yellowing revealed the presence of two stripes of nonlignified cells separating the lignified cells of the replum and endocarp layer *b* (en*b*) in the dehiscent morph (Fig. 4J), thus resembling the dehiscence zones of other Brassicaceae species with dehiscent fruits, including Arabidopsis (*Arabidopsis thaliana*; Ferrándiz et al., 2000; Østergaard et al., 2006; Arnaud et al., 2011; Mühlhausen et al., 2013). In contrast, in the indehiscent morph, the lignified cells of the en*b* and the replum are not separated, thus forming a continuous lignified band at the inside of the fruit valve completely enclosing the seed (Fig. 4K). Therefore, a dehiscence zone (dz and white arrow in Fig. 4, G and J) is present only in the dehiscent morph.

Programmed organ abscission often is mediated by the formation of an abscission zone, characterized by several layers of small, densely cytoplasmic cells at the base of the respective organ (Li et al., 2006; Estornell et al., 2013). An abscission zone is evident at the fruit-pedicel junction of the indehiscent fruit morph, separating the lignified cells at the fruit base from those of the pedicel (az in Fig. 4M). In contrast, the dehiscent fruit morph is tightly connected with the pedicel through a continuous bridge of lignified cells (Fig. 4L).

The *Ae. arabicum INDEHISCENT* Ortholog Is Down-Regulated in the Indehiscent Fruit Morph

In order to characterize the molecular pathway underlying fruit morph development in *Ae. arabicum*, we performed homology searches to identify *Ae. arabicum* orthologs of the best characterized fruit regulatory genes in Arabidopsis, namely *ALCATRAZ* (*ALC*), *APETALA2* (*AP2*), *FILAMENTOUS FLOWER* (*FIL*), *FRUITFULL* (*FUL*), *INDEHISCENT* (*IND*), *REPLUMLESS* (*RPL*), *SHATTERPROOF1* (*SHP1*), and *SHP2* (Dinneny et al., 2005; Ripoll et al., 2011). These also include genes that are known to show changes in expression pattern in other indehiscent Brassicaceae fruits (Avino et al., 2012; Mühlhausen et al., 2013). Phylogenetic analyses showed that, for each of these Arabidopsis fruit regulators, a single ortholog is present in the *Ae. arabicum* genome, henceforth called *AearALC*, *AearAP2*, *AearFIL*, *AearFUL*, *AearIND*, *AearRPL*, *AearSHP1*, and *AearSHP2*, respectively (Table I; Supplemental Fig. S4). Gene expression analyses via quantitative reverse transcription-PCR on outgrown, green fruits revealed that all genes were expressed substantially in the dehiscent morph. Comparing expression levels between the two fruit morphs showed that, of the investigated genes, only *AearIND* expression was significantly different and approximately 7-fold lower in indehiscent compared with dehiscent fruits (Fig. 5). This finding indicates that differential regulation of *AearIND* might be one of the key molecular mechanisms for the establishment of morph-specific differences during *Ae. arabicum* fruit development.

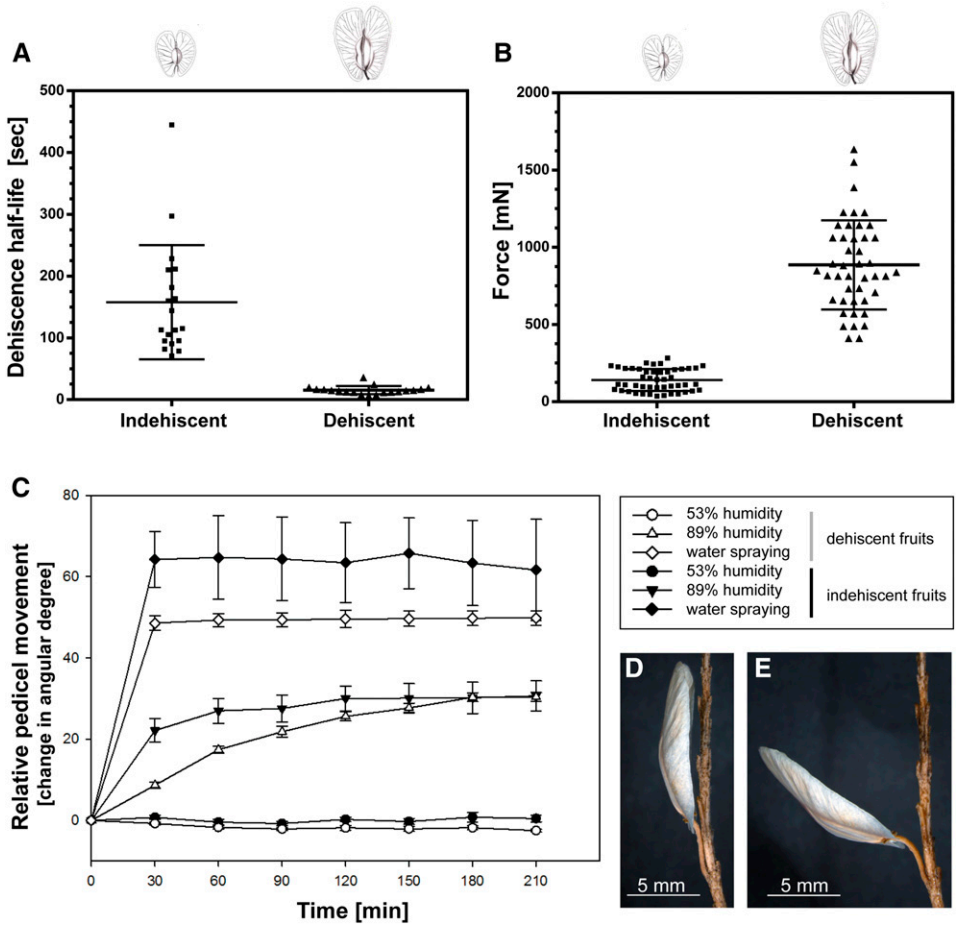

Figure 3. Comparison of fruit dehiscence, abscission, and movement between the two fruit morphs. A to C, Small and large fruit morphs of *Ae. arabicum* differ in dehiscence half-life (A) and detachment force (B) but have similar movement patterns in response to humidity (C). Values shown are means ± SD for $n = 19$ (A), $n = 47$ (B; indehiscent), $n = 44$ (B; dehiscent), and $n = 8$ (C) replicate measurements. D and E, Fruit movement is further illustrated by exemplary images of a ripe dehiscent fruit under dry conditions (D; relative humidity less than 50%) and humid conditions (E; water spraying).

Comparative Morphology of the Dimorphic Seeds

In addition to the strong dimorphism of *Ae. arabicum* fruits, the seeds developing within indehiscent or dehiscent fruits also exhibit remarkable morphophysiological differences (Fig. 6). The most obvious difference is the production of mucilage upon imbibition in mature seeds from dehiscent fruits (Fig. 6, C–H), henceforth referred to as the mucilaginous seed morph (M^+). This mucilage is mostly lacking in imbibed mature seeds from indehiscent fruits (Fig. 6, A and B), henceforth referred to as the nonmucilaginous seed morph (M^-).

The production of seed mucilage, known as myxospermy, occurs from the outer cell walls of the seed coat epidermal cells in response to seed hydration, forming a water-containing, gel-like pectinaceous layer surrounding the seed (Western, 2012). A number of species have cellulosic threads or thick fibers projecting from their mucilage cells. Our detailed analysis of the *Ae. arabicum* seed surface by light microscopy and SEM (Fig. 6) showed a smooth to slightly grooved surface structure of M^- seeds (Fig. 6, A and B). In contrast, the surface of M^+ seeds was densely covered with dome-like structures and crinkles around their base, each corresponding to a mucilage-producing epidermal cell (Fig. 6F). Upon seed imbibition, these structures were irreversibly swelling, expanding, and forming conical mucilage papillae of up to 200 μm with a globe-like tip (Fig. 6, D–H). Upon drying, the papillae shrank in diameter and formed knob-shaped tips (Fig. 6, G and H). Each of the two seed morphs was unambiguously connected with the fruit morphs: M^- seeds were found inside indehiscent fruits, while M^+ seeds were dispersed from dehiscent fruits.

Comparative morphological analysis showed that the surface differences were accompanied by different positions of the radicle in relation to the cotyledons and the internal cell layers (Fig. 7). The M^+ seeds were oblong and biconvex, having a notorhizal embryo (incumbent: radicle lying along the back of one cotyledon). M^- seeds were ovate and planoconvex, and the embryo was either pseudonotorhizal (radicle situated near the margin of the cotyledons) or nearly pleurorhizal (accumbent: radicle applied to margins of both cotyledons; Fig. 7B). Light microscopic analysis highlighted the difference in the abundance of mucilage as well as embryo position between the two seed morphs (Fig. 7). The mature seed coat of both morphs was composed of multiple layers. The outermost epidermal layer formed large Astra Blue-stainable mucilage papillae in M^+ seeds (Fig. 7D), whereas M^- seeds produced only a very thin film of mucilage from their epidermal layer (Fig. 7I). Directly adjacent to these epidermal layers,

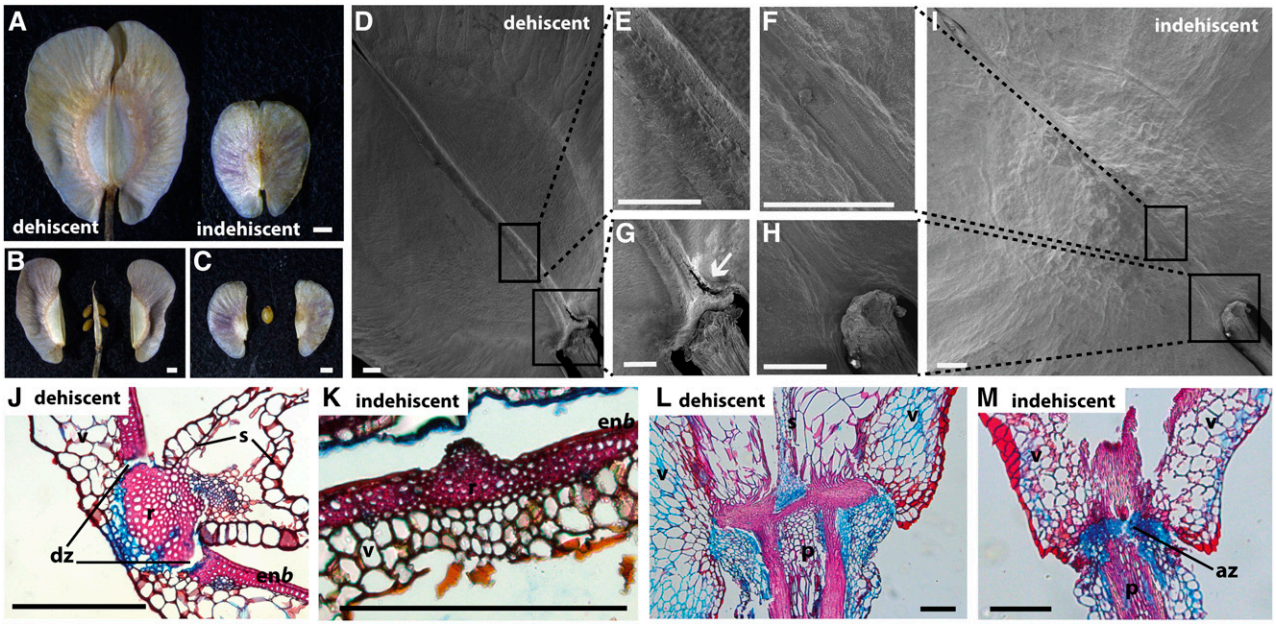

Figure 4. Anatomical comparison between the *Ae. arabicum* fruit morphs. A to C, The two morphs (dehiscent [A, B, D, E, G, J, and L] and indehiscent [A, C, F, H, I, K, and M]) differ in size, seed number, and septum (s) formation. D to I, SEM imaging reveals slight differences at the valve-replum border but not at the fruit-pedicel junction. A site of tissue separation due to fruit dehiscence is indicated by the white arrow in G. J to M, Thin sections stained with safranin and Astra Blue show the presence of a dehiscence zone (dz) in fruits of the dehiscent morph (J). It separates the lignified (red) cells of the replum (r) from those of endocarp layer *b* (en*b*) on the inside of the fruit valves (v). No such structure is found in fruits of the indehiscent morph (K), where the en*b* is fused directly to the lignified cells of the replum. Longitudinal sections of the fruit-pedicel junction reveal the presence of an abscission zone (az) in indehiscent fruits (M). It separates the fruit base from the lignified cells of the pedicel (p). In contrast, there is a solid bridge of lignified cells connecting the fruit base and pedicel in the dehiscent fruit morph (L). Bars = 1 mm (A–C) and 200 μm (D–M).

both seed morphs had an unstained single layer of small palisade cells (Fig. 7, D and I). Inward, this was followed by multiple layers of safranin-stainable crushed palisade cells (Fig. 7, F and K). All these outer tissue layers of the seed coat consisted of dead cells, as indicated by the absence of nuclei (Fig. 8, A–F). Between the embryo and the seed coat, we identified a layer of living cells that completely surrounded the embryo in both seed morphs (Figs. 7, E and L, and 8, A–F). This layer appeared thin around most of the embryo but thicker around the radicle tip, where it could be multilayered (Fig. 7, G and M). In many Brassicaceae species, a living endosperm layer around the embryo plays an important role in the regulation of dormancy and germination of the seeds (Müller et al., 2006; Graeber et al., 2012). To determine if the identified cell layer in *Ae. arabicum* was part of the endosperm, we performed flow cytometric analysis of these layers in both seed morphs. C values of 2.97 $\pm$ 0.05 (M^+) and 2.97 $\pm$ 0.02 (M^-) confirmed triploidy and, therefore, endosperm origin (Fig. 8G). In conclusion, M^+ seeds from dehiscent fruits and M^- seeds from indehiscent fruits differ in several anatomic features, affecting the position of the embryo and the seeds' outermost mucilage-producing epidermal layer.

Germination Physiology of Dimorphic *Ae. arabicum*

The dimorphic syndrome of *Ae. arabicum* has consequences for seed germination. In the case of the indehiscent fruits, in which the whole fruit represents the natural dispersal unit, the M^- seeds need to germinate within the fruit (unless they are released from the fruit coat by external mechanical means). Interestingly, we found that 100% of mature M^+ seeds germinated readily within 3 d under laboratory conditions, whereas mature M^- seeds within indehiscent fruits reached only 50% germination after about 3 weeks under the same conditions (Fig. 9A). To test if these conditions are generally prohibitive for M^- seed germination, we analyzed seeds manually dissected from indehiscent fruits. These isolated M^- seeds were able to germinate faster and to a greater extent (70%) than those within indehiscent fruits, although the overall germination speed and maximum capacity remained lower compared with M^+ seeds (Fig. 9A).

Water uptake is vital for germination and can be controlled by various fruit or seed structures and by mucilage (Weitbrecht et al., 2011; Western, 2012). The distinct coat structures of the two seed morphs and their different germination kinetics prompted us to investigate the water-uptake patterns of mature M^+ seeds, isolated mature M^- seeds, and intact mature indehiscent fruits during germination (Fig. 9B). M^+ seeds showed a classical water-uptake pattern: imbibition was characterized by a very rapid and steep increase in seed moisture content (phase I), followed by a plateau (phase II), which leads to another increase (phase III), coinciding

Table I. *Ae. arabicum orthologs of Arabidopsis fruit developmental genes used in this study*

Gene	Identifier (*Ae. arabicum*)	Percentage Amino Acid Identity with Arabidopsis Ortholog	Identifier (Arabidopsis)
AearALC	AA8G00019	59.35	AT5G67110
AearAP2	AA30G00232	68.39	AT4G36920
AearIND	AA32G00014	68.30	AT4G00120
AearFIL	AA21G00262	87.88	AT2G45190
AearFUL	KX874497	89.96	AT5G60910
AearRPL	AA19G00333	67.99	AT5G02030
AearSHP1	AA61G00296	80.93	AT3G58780
AearSHP2	AA21G00070	89.07	AT2G42830

with embryo elongation and radicle protrusion to complete germination. A three-phase water uptake also was evident for isolated M^- seeds. However, compared with M^+ seeds, their overall moisture content during imbibition remained much lower, and phase II was prolonged, in agreement with the lack of mucilage and the later completion of M^- seed germination (Fig. 9). Interestingly, indehiscent fruits and M^+ seeds took up similar relative amounts of water during phase I, indicating that indehiscent fruit coats aid in water absorption, seemingly compensating for the lack of mucilage of M^- seeds. Although the indehiscent fruits initially imbibed water similarly to M^+ seeds, they then remained in phase II, reflecting the delayed germination of the M^- seeds within the fruits. To investigate if water taken up by the fruit coat was subsequently also taken up by the enclosed seed, we measured the water activity (a_w) during phase II of M^- seeds imbibed within or without fruit coats. Values for a_w related to the amount of freely available water, ranging from 0 (completely dry matter) to 1 (pure water). Dry M^- seeds or indehiscent fruits (a_w approximately 0.4) were imbibed for 18 h. The seeds subsequently dissected from fruit coats showed similar a_w compared with seeds imbibed without fruit coat (a_w approximately 0.8). Thus, the indehiscent fruit coat delayed germination, although the fruit coat was fully permeable for water.

Plasticity of *Ae. arabicum* Fruit Morph Production

Ae. arabicum plants originating from M^+ or M^- seeds, respectively, did not differ significantly in their fruit-morph ratio or total number of fruits, indicating that plants developing from the two different seed morphs are indistinguishable upon maturity (Fig. 10A). Although the succession of dehiscent and indehiscent fruits along an individual infructescence can appear stochastic (Fig. 2B), the two fruit morphs were not distributed randomly on the whole plant: higher order side branches produced a larger fraction of indehiscent fruits than the main branch (Fig. 10B). Other heteromorphic *Aethionema* spp. have been reported to develop more dehiscent fruits after cutting off side branches (Zohary and Fahn, 1950). This prompted us to investigate the potential phenotypic plasticity in *Ae. arabicum* fruit morph production. The constant removal of all side branches significantly increased the production of dehiscent fruits on the main branch to approximately 95% (Fig. 10C). We further observed that sets of plants originating from the same seed batch but grown either in a phytochamber or a greenhouse had different fruit-morph ratios in their progeny. While indehiscent fruits clearly predominated (approximately 70%) in the phytochamber, the majority of fruits (approximately 80%) produced in the greenhouse belonged to the dehiscent morph (Fig. 10D). One of the factors that varied significantly between the nonconditioned greenhouse and the phytochamber was temperature. Thus, we grew plants under two different constant temperatures but otherwise identical conditions and again observed a strong influence on fruit morph production. At a growth temperature of 20°C, the fraction of indehiscent fruits was much higher compared with 25°C (Fig. 10E). This plastic response was not solely an indirect effect of a changed branching pattern, because the shift in fruit morph production could be detected throughout the whole plant (Supplemental Fig. S5). These results indicate that *Ae. arabicum* shows a plastic response of its fruit-morph ratio in response to certain environmental factors.

DISCUSSION

Dimorphic Seeds and Fruits Mediate Alternative Dispersal and Germination Strategies in the Annual Life Cycle of *Ae. arabicum*

Seed and fruit heteromorphism as a bet-hedging strategy plays an important role in the colonization

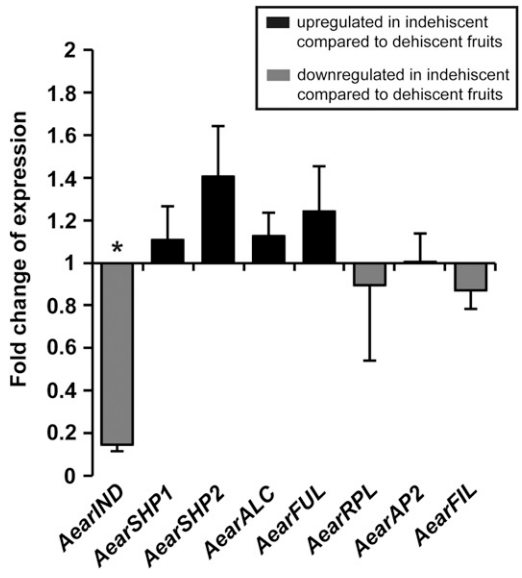

Figure 5. Gene expression analysis of fruit developmental genes via quantitative reverse transcription-PCR. Gene expression levels of *Ae. arabicum* orthologs of *IND*, *SHP1*, *SHP2*, *ALC*, *FUL*, *RPL*, *AP2*, and *FIL* in indehiscent fruits are represented relative to the expression levels in dehiscent fruits (set to 1). Lower expression levels in indehiscent compared with dehiscent fruits are represented by gray bars, and higher expression levels are represented by black bars. A significant difference is indicated by the asterisk ($P \leq 0.05$).

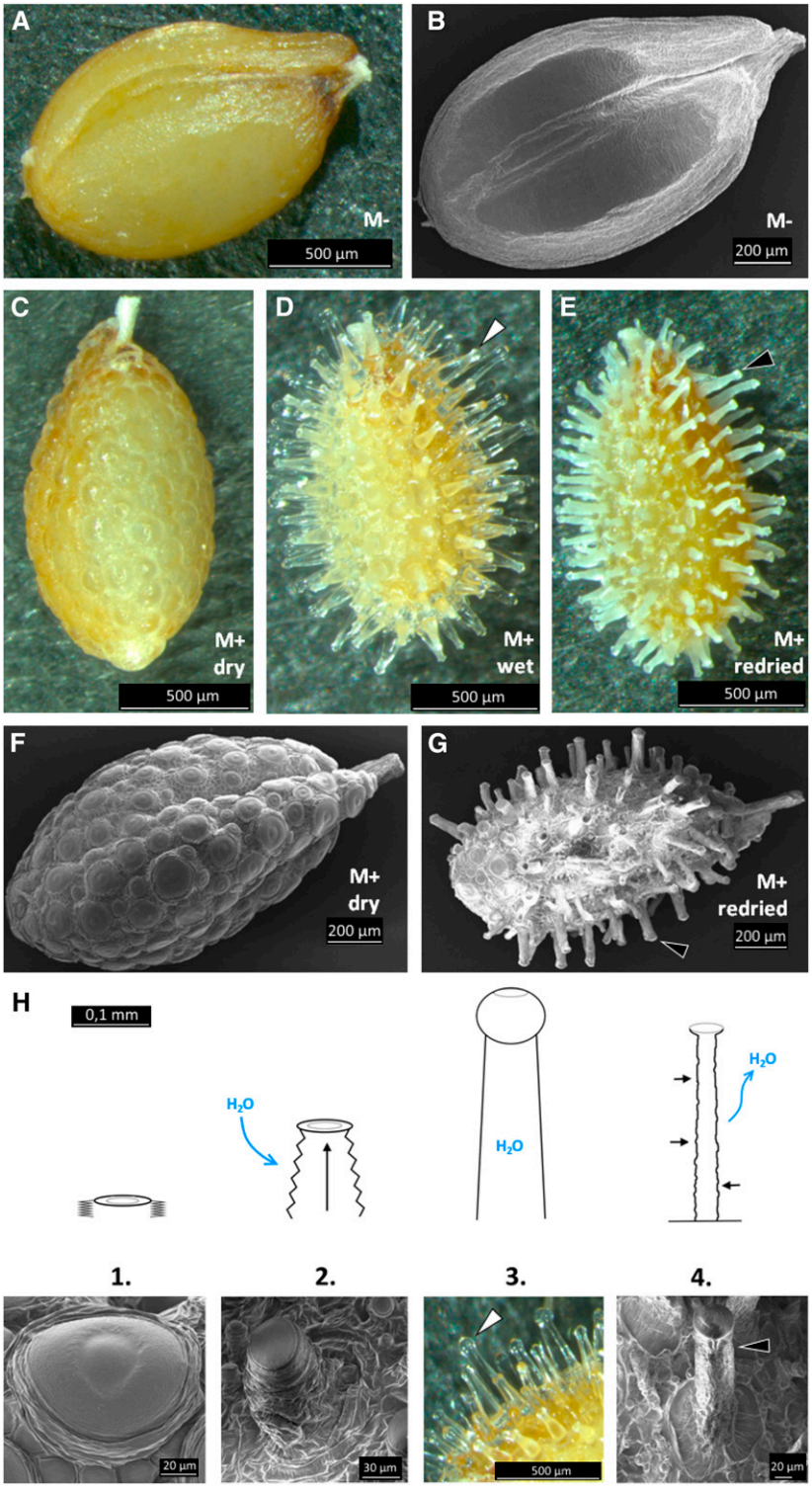

Figure 6. Morphological characterization of dimorphic *Ae. arabicum* seeds. Characteristic images show the two *Ae. arabicum* seed morphs taken with a binocular microscope (A, C, D, E, and H3) or a scanning electron microscope (B, F, G, H1, H2, and H4). Mature seeds from indehiscent fruits (M^- seeds; A and B) have a smooth surface and do not produce mucilage upon imbibition. Mature seeds from dehiscent fruits (M^+ seeds; C–G) are densely covered with dome-like structures and crinkles. Upon imbibition, conical mucilage papillae with a globe-like tip emerge from these structures (D and H). When redried, papillae shrink in diameter and length and finally form dried, knob-shaped tips (E, G, and H). White arrowheads, sticky, ball-shaped tips; black arrowheads, dried, knob-shaped tips.

and survival of several plant species in environmentally unpredictable habitats (Imbert, 2002; Evans and Dennehy, 2005; Lu et al., 2010). Heteromorphic seeds and fruits provide distinct dispersal and germination strategies to aid the distribution of the (mostly annual) species in time and space. We found that *Ae. arabicum*, an annual Brassicaceae plant adapted to arid and semiarid environments, employs a dimorphic dispersal and germination strategy (Fig. 11). On the same infructescence, it produces dehiscent fruits with M^+ seeds and indehiscent fruits harboring M^- seeds. Figure 11 depicts the *Ae. arabicum* dimorphic dispersal and germination strategy

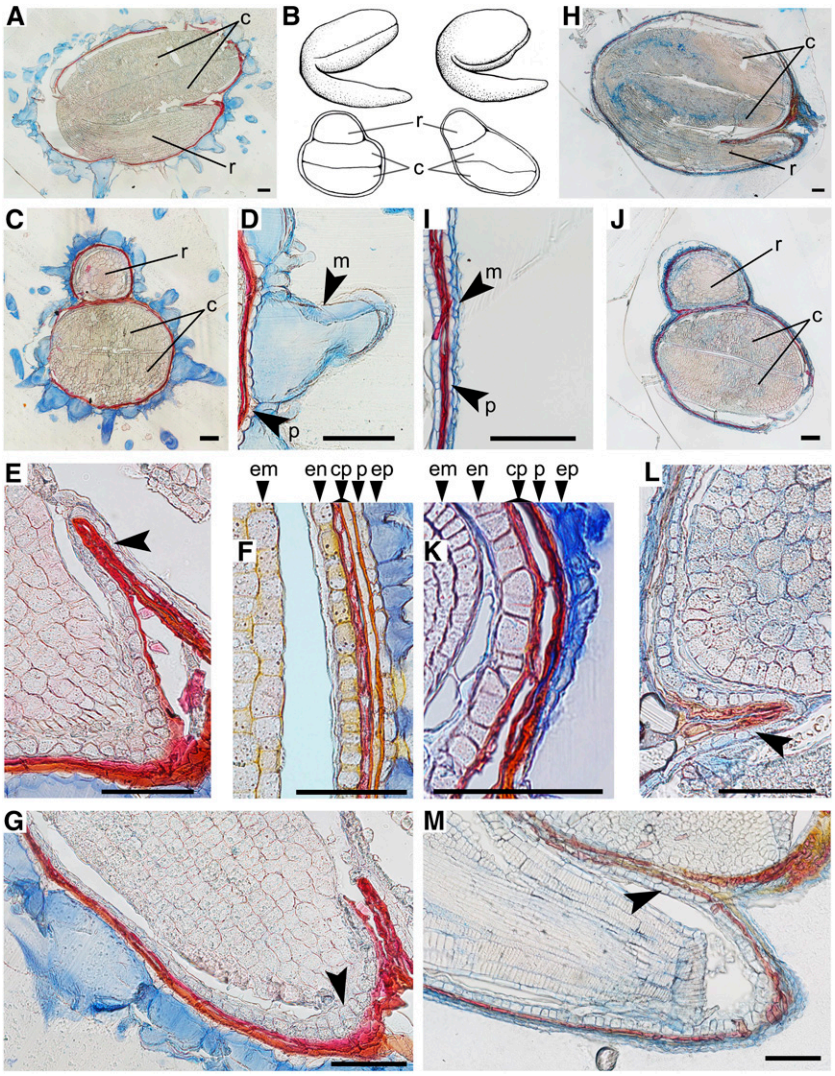

Figure 7. Heteromorphic seeds of *Ae. arabicum* show morphological differences in their outermost mucilage-producing seed coat layers. Mature M^+ (A and C–G) and M^- (H–M) seed morphs show different positioning of radicle (r) and cotyledons (c), as depicted schematically in B (M^+, left; M^-, right). Light microscopic analysis of longitudinal (A and H) and transversal (C and J) whole seed sections highlight differences in the formation of blue-stained mucilage. The seeds' outermost epidermal layer (ep), which is directly adjacent to a single layer of palisade cells (p), forms large mucilage papillae (m) in the case of M^+ seeds (D) but only a very thin film of mucilage in M^- seeds (I). Both seed morphs (F and K) possess multiple red-stained crushed palisade cell layers (cp) in direct contact with the endosperm layer (en). The whole embryo (em) is surrounded by this continuous single-layered endosperm (black arrowheads; E and L), which appears multilayered and thick around the radicle tip (G and M). Cross sections were stained with safranin/Astra Blue. Astra Blue stains unlignified cell walls, cellulose, and mucopolysaccharides blue, and safranin stains lignified, suberized, or cutinized cell walls red. Bars = 100 μm.

as part of the annual life cycle. Two clearly distinct life-history strategies are evident: quickly germinating M^+ seeds dispersing via fruit dehiscence and slowly germinating M^- seeds separating from the mother plant via the abscission of indehiscent fruits (Fig. 11). The formation of morphs with low dispersal ability and delayed seed germination has been interpreted as a low-risk strategy because they stay in the approved habitat near the mother plant, and their fractionated germination increases the chance for at least some of them to encounter favorable environmental conditions (Venable and Lawlor, 1980; Venable and Levin, 1985; Venable et al., 1995; Lu et al., 2012, 2013, 2015). Morphs with high dispersal ability and quick germination, on the other hand, may represent a high-risk strategy that only pays off when the environmental conditions are beneficial. However, the exact opposite combination of the two traits (quickly germinating nondispersing morphs) also has been observed and interpreted as a way to overcome high sibling competition in local populations (Dubois and Cheptou, 2012; Rubio de Casas et al., 2015). The moisture-dependent pedicel movement (Fig. 3, C–E) may aid rain-operated seed dispersal (Gutterman, 1993; Parolin, 2006; Pufal and Garnock-Jones, 2010). Independent of their dispersal behavior (Fig. 11), we interpret the formation of M^+ seeds as a high-risk strategy, because quick and uniform germination in uncertain environmental conditions is always risky, especially for annuals. Thus, the delayed and fractionated germination of M^- seeds would represent a low-risk strategy. However, more research investigating the connection between seed germination, dispersal, and survival under natural growth conditions is needed in order to refine our understanding of the dimorphic life-history strategy of *Ae. arabicum*.

Various forms of seed and fruit heteromorphism have evolved independently, predominantly in the Asteraceae, Amaranthaceae, and Brassicaceae families (Imbert, 2002). Within the genus *Crepis* (Asteraceae), it has evolved independently several times (Imbert, 2002; Dubois and Cheptou, 2012). Within the Brassicaceae, heteroarthrocarpy has evolved in annual *Cakile* spp.

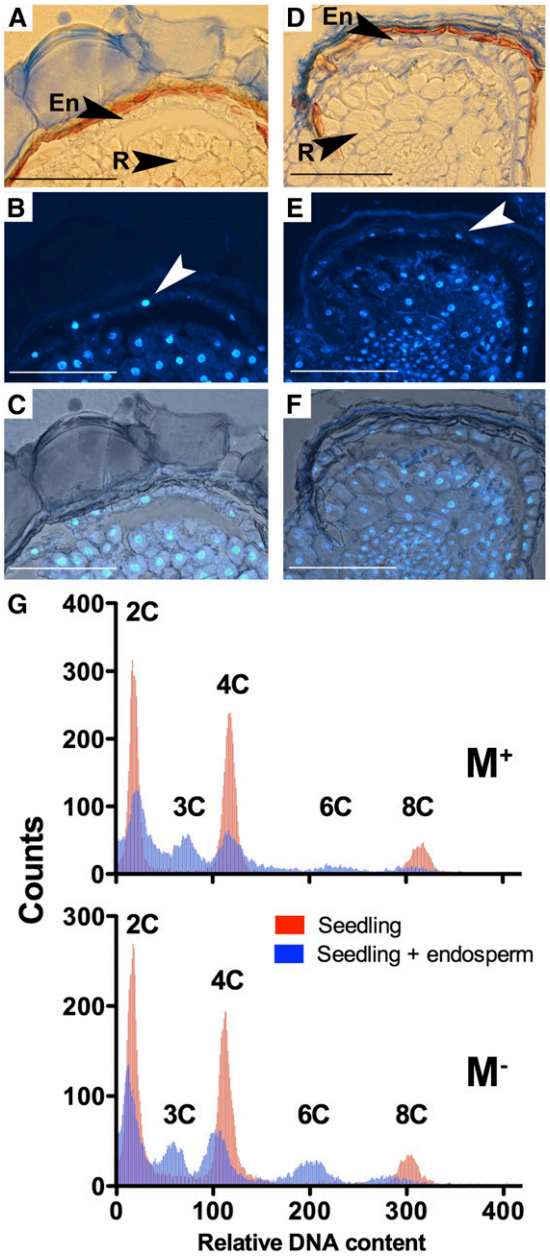

Figure 8. Seed morphs of *Ae. arabicum* possess a living triploid endosperm. The mature M^+ seed morph (A–C) as well as the mature M^- seed morph (D–F) both possess a thin endosperm layer (En) surrounding the radicle (R) containing 4′,6-diamidino-2-phenylindole (DAPI)-stainable nuclei (B and E; white arrowheads). A and D, Bright-field images. B and E, DAPI fluorescence images. C and F, Overlay images. Bars = 100 μm. G, Overlays of representative flow cytometry histograms of seedling tissue and mixtures of seedling with the living tissue layer surrounding the embryo show a specific 3C peak in the latter samples, confirming the presence of triploid endosperm in both seed morphs.

and other annual Brassiceae plants (Hall et al., 2006; Avino et al., 2012), as has a complex fruit and seed heteromorphism in the annual Chorisporeae plant *Diptychocarpus strictus* (Lu et al., 2010, 2012, 2014, 2015). We demonstrate here that heteromorphism has evolved twice within the genus *Aethionema* (38 species analyzed): in the perennial *Ae. saxatile* (Andersson et al., 1983) and independently for the five annual species including *Ae. arabicum* (Fig. 1; Solms-Laubach, 1901; Zohary and Fahn, 1950; Hedge, 1965). In contrast to the Bayesian inference, a maximum likelihood analysis does not support an annual clade (Supplemental Fig. S2). Further phylogenetic research with higher species coverage is needed to address this issue better. We propose that the dimorphism of *Ae. arabicum* has evolved as an adaptation to unpredictable environments such as the arid and semiarid habitats of the Irano-Turanian region (southwest Asia; e.g. Turkey, Syria, Iran, and Iraq) to which this monophyletic group of annual plants is adapted.

Anatomy and Molecular Regulation of Fruit Dehiscence in *Ae. arabicum*

Brassicaceae fruits are typically dehiscent pods. Their opening mechanism depends mainly on the correct

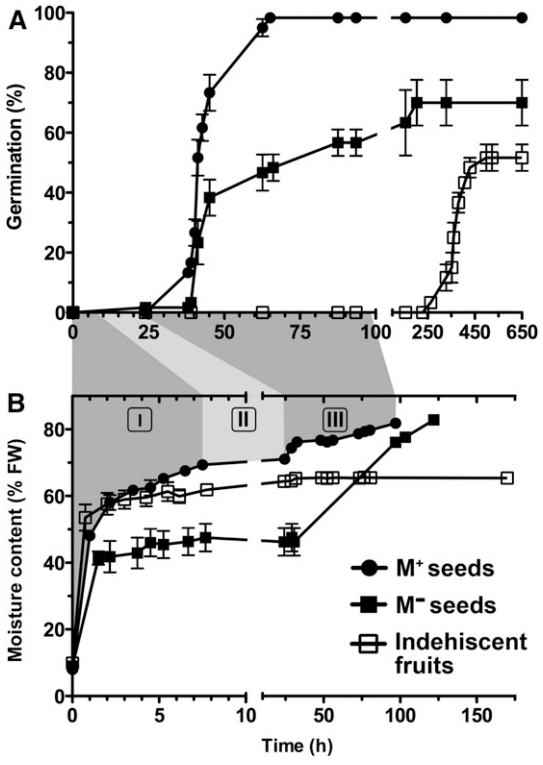

Figure 9. *Ae. arabicum* heteromorphic dispersal units differ in their germination and water uptake patterns. Mature M^+ seeds, M^- seeds extracted from mature indehiscent fruits, and whole intact indehiscent fruits were incubated at 14°C in continuous light. Germination over time (A) is shown in relation to water uptake kinetics (B) expressed as the percentage moisture content of fresh weight (FW). Three phases of water uptake (I–III) are indicated for M^+ and related to their germination kinetics. Note that three phases of water uptake also can be identified for M^-. Phase III generally coincides with the completion of germination by radicle emergence, and, reflecting their delayed germination, seeds in indehiscent fruits remain in the plateau phase II during the investigated interval.

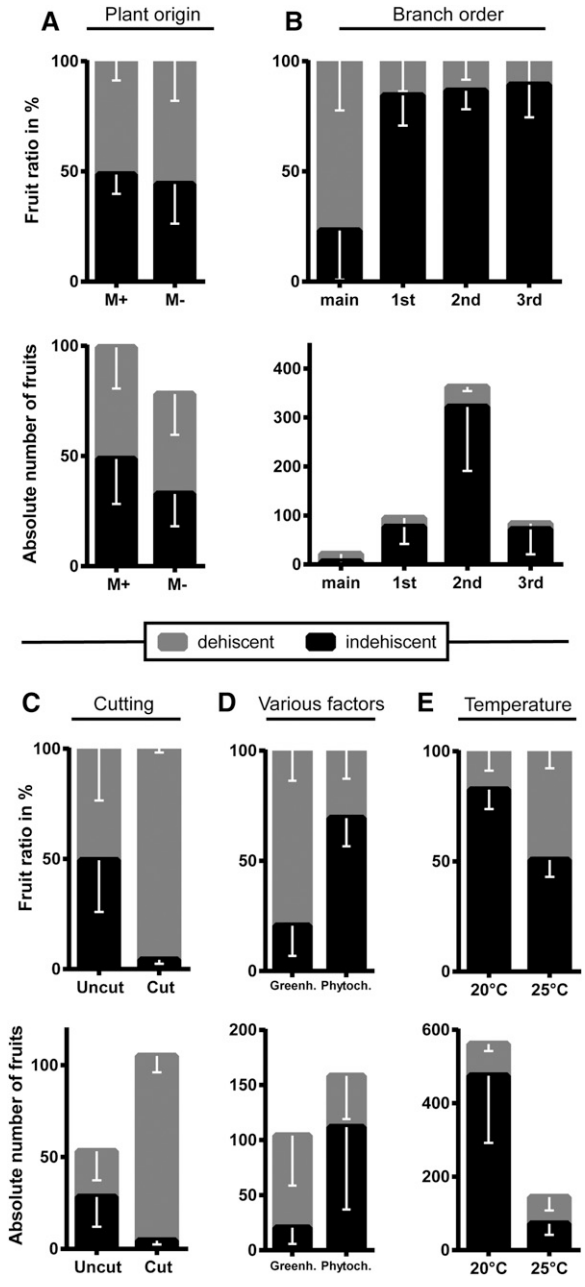

Figure 10. Fruit morph production of *Ae. arabicum* shows phenotypic plasticity in response to environmental triggers. The ratios (top rows) and total numbers (bottom rows) of fruits belonging to the dehiscent (gray bars) or indehiscent (black bars) fruit morph are similar for plants originating from M^+ or M^- seeds (A) but change significantly between main and side branches (B) in response to cutting off all side branches (causing a change in plant architecture; C), upon growth in a controlled phytochamber versus a non-temperature-controlled greenhouse (D), and at different defined growth temperatures (E). Data represent means with SD.

formation of a dehiscence zone at the valve-replum border (Meakin and Roberts, 1990a, 1990b; Spence et al., 1996). This zone typically consists of two directly adjacent and functionally complementary layers of cells: a separation layer and a lignified layer. Studies so far pointed toward a high conservation of the fruit-opening process within the Brassicaceae, and the presence of separation layer cells in the dehiscent fruits of *Ae. arabicum* indicates that fruit opening in this species also may function in a similar way (Fig. 4; Hall et al., 2006; Østergaard et al., 2006; Mühlhausen et al., 2010, 2013; Arnaud et al., 2011). The facts that single orthologs of all investigated fruit developmental genes are present in the genome of *Ae. arabicum* (Table I) and that they are all expressed in the dehiscent fruit morph further support the idea that not only the Brassicaceae-specific opening mechanism, but also its molecular regulation, might be at least partially conserved in the dehiscent *Ae. arabicum* fruits.

A special feature of *Ae. arabicum* dehiscent fruits is the lack of a typical lignified layer present in other Brassicaceae plants, where it forms a lignified bridge connecting the en*b* with the fruit exocarp (Hall et al., 2006; Østergaard et al., 2006; Mühlhausen et al., 2013). In *Ae. arabicum*, the separation layer is located between the lignified cells of the en*b* and the replum, a feature that may by characteristic for the Aethionemeae in general, as it is also observed in dehiscent fruits of *Ae. saxatile* (Mühlhausen et al., 2010). Another peculiar feature of the dehiscent fruits of *Ae. arabicum* is the presence of small cellulose-rich cells in the replum that are connected to the separation layer cells (Fig. 4J). They are anatomically similar to separation layer cells and only present in the dehiscent morph, implying that they may act as a functional extension of the separation layer. This could explain how fruit opening is achieved, although the separation layer itself does not extend toward the fruit exocarp in *Ae. arabicum*.

The anatomy of the valve-replum border of *Ae. arabicum* indehiscent fruits resembles that of indehiscent fruits in other Brassicaceae species (Hall et al., 2006; Mühlhausen et al., 2010, 2013). The dehiscence zone is absent and the lignified cells of the en*b* are connected directly to the lignified part of the replum, thus forming a continuous lignified band around the fruit and preventing fruit opening. In other species, these anatomical changes have been connected with altered expression patterns of genes orthologous to the fruit developmental genes *SHP1*, *SHP2*, *IND*, and *ALC*, which are known to control the formation of the dehiscence zone in Arabidopsis (Liljegren et al., 2000, 2004; Rajani and Sundaresan, 2001; Avino et al., 2012; Mühlhausen et al., 2013). Likewise, our expression analysis revealed a strong down-regulation of *AearIND* in indehiscent compared with dehiscent fruits in *Ae. arabicum* (Fig. 5). This down-regulation alone could be sufficient to induce the anatomical changes underlying the indehiscence phenotype in the respective fruit morph (Fig. 4K), provided that gene functions and regulatory interactions are indeed similar to those in Arabidopsis. There, *IND* is crucial for the formation of the dehiscence zone by directly controlling the lignification of the lignified margin cells and by indirectly controlling the formation of the separation layer by mediating the release of ALC and SPATULA from DELLA repressor proteins (Liljegren

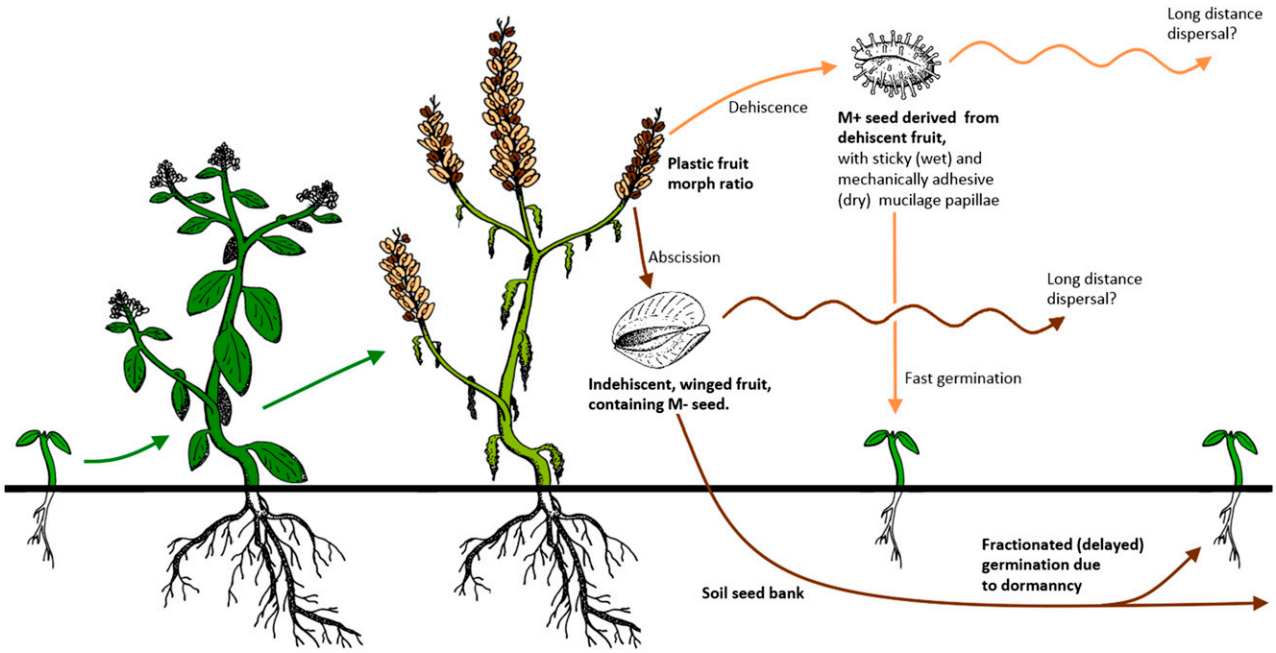

Figure 11. Scenario of different life-history strategies of *Ae. arabicum* seed morphs. After fruit maturation, indehiscent fruits with enclosed M^- seeds abscise from the mother plant, while dehiscent fruits open to release M^+ seeds. These germinate quickly, while M^- seeds remain in the soil seed bank for longer periods, mainly due to pericarp-mediated dormancy. Furthermore, both morphs may be subject to long-distance dispersal due to the presence of adhesive mucilage (M^+) or enclosure in winged indehiscent fruits (harboring M^- seeds).

et al., 2004; Arnaud et al., 2010; Groszmann et al., 2011). Thus, the differential expression of *AearIND* likely represents the first molecular key mechanism that has been identified to cause morph-specific differences during heteromorphic fruit development. It will be interesting to investigate which upstream regulatory mechanisms cause this differential expression and whether it is possible to identify a regulatory connection with other morph-specific differences such as fruit abscission or seed mucilage production.

Morphology and Ecophysiology of Dimorphic Seed Germination Differing in Myxospermy and Fruit Coat Constraint

Our comparative data about the seed morphology of *Ae. arabicum* (Figs. 6–8) allow us to address questions about the differential control of M^+ and M^- seed development. The mature seed coat is of maternal origin, and its development has been characterized genetically in detail in Arabidopsis (Haughn and Chaudhury, 2005). The complex genetic regulation of mucilage secretory cell development from the outer integument in Arabidopsis seeds has been uncovered (Francoz et al., 2015). We show here that M^+ seeds develop mucilage papillae upon wetting, whereas M^- seeds show reduced mucilage production because they lack mucilage secretory cells. In Arabidopsis, defects in the differentiation of the outer integument during seed development have been correlated with a lack of mucilage synthesis (Western, 2012). Several transcription factor mutants affecting outer seed coat differentiation, such as *ap2* (Western et al., 2001) and the *nac-regulated seed morphology1* (*nars1*) *nars2* double mutant (Kunieda et al., 2008), do not produce any mucilage and show altered seed coat surface structure. Especially the shriveled appearance of the Arabidopsis *nars1 nars2* seed coat looks strikingly similar to the *Ae. arabicum* M^- seed surface. It is tempting, therefore, to speculate that *Ae. arabicum* is able to regulate its seed development differentially to produce seeds with or without mucilage by differentially employing conserved developmental regulators.

The adaptive value of seed mucilage also has prompted plant ecologists to propose a role in the long-distance dispersal as well as the local anchorage of seeds (Yang et al., 2012). The myxospermy of *Ae. arabicum* M^+ seeds (Figs. 6 and 11) could assist long-distance dispersal by adherence to animal vectors (Norton et al., 1997; Mummenhoff and Franzke, 2007). However, it also may allow M^+ seeds to adhere to soil particles, a common mechanism for seed retention in dry habitats (Huang et al., 2000; Lu et al., 2010; Gutterman, 2012). Beyond dispersal, seed mucilage may promote seed germination through the attraction and retention of water surrounding the seed (Yang et al., 2012), protect against osmotic stress (Yang et al., 2010), assist the repair of embryo DNA damage (Yang et al., 2011), and promote early seedling growth as an adaptation to harsh desert environments (Yang et al., 2012). Mucilage

produced by the scattered *Ae. arabicum* M^+ seeds was associated with faster germination compared with the nonmucilaginous M^- seeds encased by the indehiscent fruit coat (Fig. 9). A similar behavior has been described for other heteromorphic Brassicaceae plants (Zohary, 1962; Imbert, 2002; Lu et al., 2015). Interestingly, M^- seeds removed from their surrounding fruit coat can germinate quickly, similar to M^+ seeds. Water-impermeable cell layers in fruit coats can prevent imbibition and germination, which is referred to as physical dormancy (Finch-Savage and Leubner-Metzger, 2006). We show here that *Ae. arabicum* indehiscent fruit coats are water permeable. Therefore, the delay in germination is likely caused by a purely mechanical restraint of the fruit coat or by chemical inhibitors. The differential presence of chemical germination inhibitors in heteromorphic seeds and fruits has been described in many species (Matilla et al., 2005). Notably, the presence of larger amounts of the germination-inhibiting plant hormone abscisic acid in the fruit coats of one morph of the heteromorphic species *Salsola komarovii* caused delayed seed germination that could be overcome by removing the fruit coat (Takeno and Yamaguchi, 1991). Furthermore, mechanical restraint was proposed as the cause of the delayed germination of indehiscent siliques of heteromorphic *D. strictus* (Lu et al., 2015). Further biomechanical and biochemical studies of the indehiscent fruit coat of *Ae. arabicum* will shed light on its germination-inhibiting nature.

Phenotypic Plasticity of Fruit and Seed Heteromorphism

Phenotypic plasticity and bet hedging are two evolutionary modes of response to environmental variance (Simons, 2011; Abley et al., 2016). Plasticity describes a concerted change of a given trait over a range of environmental conditions and, thus, critically depends on the availability of cues that allow forecasting of the future state of the environment (Bradford and Roff, 1993; Simons, 2011). Bet hedging, on the other hand, describes a risk-spreading strategy as an adaptation to environmental unpredictability, producing only one fixed phenotypic condition that is suboptimally adapted to any given environment but maximizing the geometric mean fitness across generations (Philippi and Seger, 1989). Heteromorphism in plants has often been considered a mere bet-hedging strategy, emphasizing its independence from environmental conditions (Imbert, 2002). However, our data, in accordance with several other studies, demonstrate that, in some heteromorphic plant species, morph numbers and ratio show plasticity in response to certain environmental stimuli (Mandák and Pyšek, 1999; Imbert and Ronce, 2001; Sadeh et al., 2009; Lu et al., 2013b; Yang et al., 2015). Such a blend of bet hedging and plasticity should be expected to evolve when either the cue that predicts the future environment is weak or fitness is determined by predictable and unpredictable environmental factors alike (Bradford and Roff, 1993; Simons, 2011). Comparative analyses are a powerful approach for unraveling the evolutionary and genetic backgrounds of phenotypic plasticity in heteromorphic fruit and seed development.

The Potential of *Ae. arabicum* for Future Research on Heteromorphism and Plasticity

A fascinating and underexplored aspect of heteromorphism is its genetic and molecular control. Since the differences between the morphs usually are multifaceted, the respective regulatory module(s) must be positioned upstream of several developmental pathways (such as the fruit dehiscence pathway discussed above) and regulate their action in a highly coordinated manner. Moreover, sensory elements are required when morph development is regulated in an environmentally dependent manner. Nevertheless, heteromorphism evolved many times independently (Fernández et al., 2001; Imbert, 2002; Cruz-Mazo et al., 2009), suggesting that its genetic basis is rather simple. A deep understanding of heteromorphism at the phenotypic level is crucial for subsequent molecular studies. Therefore, our data are important prerequisites to understand the developmental and molecular aspects of heteromorphism. Even more challenging will be to unravel the importance of heteromorphism for plant fitness under natural growth conditions. The life-history plasticity revealed by *Ae. arabicum* raises the question of whether it is adaptive. Unfortunately, to assess the adaptive value of plastic responses is not a trivial task (Sultan, 2000). Experimental approaches for that are available, but conclusive investigation of the complex framework of ecological evolutionary developmental biology depends very much on suitable model systems (Sultan, 2000; Gilbert et al., 2015).

Ae. arabicum is a good candidate to become such a model species because it is reliably dimorphic without any intermediate morphs and with striking differences in various anatomical and physiological features and evidence for developmental control and changes in gene expression patterns. In addition, it is easy to grow and has an advantageously short life cycle. With all these features, including a published genome sequence (Haudry et al., 2013), *Ae. arabicum* represents the current best organism in which to investigate and understand the molecular, evolutionary, and ecological aspects of heteromorphism.

MATERIALS AND METHODS

Phylogenetic Inference

We sampled 38 *Aethionema* spp., four *Noccaea* spp. formerly included in *Aethionema* (Al-Shehbaz, 2012), and *Tarenaya hassleriana* as an outgroup. The geographic origins are listed in Supplemental Table S1. DNA was extracted from silica-dried and ground leaf material using the cetyl-trimethyl-ammonium bromide method (Bakker et al., 2016). DNA quality was controlled by agarose gel electrophoresis and Nanodrop 1000 spectrophotometry (Thermo Fisher Scientific). The chloroplast *rbcL-a* gene (forward primers from Huang and Shi [2002] and reverse primers from Fofana et al. [1997]) and the *trnL-F* intergenic spacer (primers from Dumolin-Lapegue et al. [1997]) were used as phylogenetic

markers. Cycle sequencing was performed at Greenomics. Codoncode aligner was used to clean up and align the retrieved sequences. The genes were concatenated to a total alignment of 1,499 bp. For a Bayesian phylogenetic analysis (MrBayes version 3.2.2; nst = mixed, rates = gamma, ngen = 250,000,000, diagnfreq = 5,000, and temp = 0.05), we partitioned the data as follows: *trnL-F*, *rbcL-a* codon positions one and two, *rbcL-a* codon position three. We also ran a maximum likelihood analysis (RaxML version 8; 1,000 bootstraps and GAMMA model of rate heterogeneity) on an unpartitioned data set. The final alignment is available as a nexus file (Supplemental Data S1). Figures were made with FigTree version 1.4.2 and GIMP 2.8.10.

Plant Material and Growth Conditions

Experiments were conducted on *Aethionema arabicum* plants or seeds of accession 0000309 (obtained from Kew's Millennium Seed Bank) or accession ES1020 (obtained from Eric Schranz). Plants were grown on soil under long-day conditions (16 h of light/20°C and 8 h of dark/18°C) in a greenhouse or phytochamber (CambridgeHOK) or in a non-temperature-controlled greenhouse in summer.

Morphometric Analysis

Twenty ripe fruits were harvested from the most basal part of the main inflorescence of seven individual *Ae. arabicum* plants. Fruit length and width were determined using a Leica M205 FA stereomicroscope employing the Interactive Measurement module of the Leica Application Suite software. Subsequently, fruits were opened to assess the presence or absence of a septum and the number of seeds. For one seed per fruit, mucilage production was evaluated 5 min after incubating the seed in a drop of water. In order to identify clustering within our data set, hierarchic cluster analysis followed by two-step cluster analysis was performed using the SPSS 20.0 software package.

Quantification of Abscission, Dehiscence, and Hygrochastic Movement

To quantify fruit dehiscence, a random-impact test was performed on ripe fruits derived from 19 replicate plants. Principally, the test was performed as described previously (Lenser and Theißen, 2014) but using three 5-mm steel balls and an agitation force of 9 Hz.

To quantify fruit detachment force, ripe whole main-branch infructescences were fixed with a metal clamp on a rack with the tip of the infructescence pointing downward. Fruits in direct proximity to the fruit to be analyzed were removed. A hair was folded around the fruit-pedicel junction. Weights were attached to the hair so that a force specified by the attached weight was applied, pulling the fruit away from the infructescence. The force needed to detach the fruit from the stem was recorded.

Analyses to quantify the hygrochastic movement of fruit pedicels were carried out with ripe infructescences in an air-tight glass vessel. All fruits were removed from infructescences, and an equilibrium relative humidity of 53% and 89% at 23°C to 25°C was adjusted by saturated salt solutions as described by Greenspan (1977) and controlled by an electronic thermometer/hygrometer (PCE-313 A; Meschede). Furthermore, individual pedicels were sprayed with 300 μL of water every 10 min. Hygrochastic pedicel movement was documented photographically (Nikon D7100, Sigma 105-mm F2.8 EX DG MACRO OS; one photograph every 1–3 min) and quantified by an angle meter in Adobe Photoshop.

Microscopic Analysis of Fruits and Seeds

Fruits just prior to the onset of ripening-induced yellowing were fixed in 2% formaldehyde, 5% glacial acetic acid, 60% ethanol, and 0.1% Tween 20 at 4°C for 24 h, embedded in Paraplast (Carl Roth), and sectioned. Thin sections were dewaxed and stained for 2 min with safranin/Astra Blue (Sigma-Aldrich; Gerlach, 1984), followed by microscopic analysis using a Leica DM5500 B microscope.

Dry mature seeds were fixed as described (Lee et al., 2012) and embedded in Technovit 7100 (Heraeus Kulzer) according to the manufacturer's instructions. Prior to fixation, seeds were pierced three times with insect pins. Polymerization took place in truncated pyramid-shaped 8-mm-diameter polythene embedding capsules (BEEM). Cuts of 6 to 12 μm thickness were made with a Microm HM355S microtome (Thermo Scientific), using the specimen clamp, knife block N, knife holder C, and Histoblades (Heraeus Kulzer). Embedded samples were placed directly into the specimen clamp without the use of Histoblock. Dry cuts were stained for 5 min in a freshly made mixture (5:1) of Astra Blue (0.5% in 0.5% acetic acid) and safranin (1% in water). Samples were washed once with deionized water and subsequently differentiated with 1% HCl in 96% ethanol. For nuclei analysis, samples were mounted in 20 mL of Vectashield (Vector Laboratories) + 2 mg mL^{-1} DAPI. Microscopic analysis was done using an NiE Upright Microscope (Nikon) and the NIS-Elements Basic Research software.

For SEM analysis, specimens were dried over silica gel for 2 weeks, mounted on specimen stubs using a carbon adhesive disc (Plano), and coated with platinum-iridium with a sputter coater (K575X Turbo; Quorum Technologies). Surfaces were analyzed by SEM (Supra 55VP; Carl Zeiss).

Ortholog Identification

To identify orthologs of Arabidopsis (*Arabidopsis thaliana*) fruit developmental genes in *Ae. arabicum*, Arabidopsis query sequences were searched with BLASTP (Altschul et al., 1990) against a plant-specific, custom-made protein database that included genomes of the species listed in Supplemental Table S2. Results were filtered for having at least 80% query coverage and according to Rost (1999) to detect clearly homologous sequences only. Resulting sequences were aligned using MAFFT version 7.037b (Katoh and Standley, 2013) in automatic mode, and alignments were inspected manually and trimmed using Jalview version 2.8 (Clamp et al., 2004). Duplicated sequences were removed after inspection of initial trees. Final neighbor-joining phylogenies were constructed using Quicktree-SD (Howe et al., 2002; Frickenhaus and Beszteri, 2008) with 1,000 bootstrap samples and displayed and midpoint rooted with FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Quantitative Reverse Transcription-PCR

Fully outgrown green fruits of the dehiscent and indehiscent morph were collected separately from the same plants in three biological replicates. For each replicate, four dehiscent (four developing seeds in each fruit) or 16 indehiscent (one developing seed in each fruit) fruits were pooled into one sample, resulting in equal numbers of dehiscent and indehiscent seeds, respectively. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen). Three micrograms of total RNA was treated with DNase I (Thermo Scientific) and precipitated with a one-tenth volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of ethanol. Two micrograms of DNase I-treated RNA was used for cDNA synthesis with random hexamer primers using the RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Scientific). Quantitative reverse transcription-PCR using the primer pairs listed in Supplemental Table S3 was performed in duplicate using FastStart Essential DNA Green Master Mix (Roche) in the LightCycler 96 system (Roche) with the following parameters: 95°C for 10 min, 45 cycles at 95°C for 10 s and 60°C for 30 s, and one cycle at 95°C for 10 s, 60°C for 30 s, and 97°C for 1 s to obtain the melting curve for each reaction. Cycle threshold values were calculated using LightCycler 96 software (Roche). The geometric means of *Ae. arabicum* orthologs of *ACTIN2* (AA26G00546), *POLYUBIQUITIN10* (AA6G00219), and *ANAPHASE-PROMOTING COMPLEX2* (AA61G00327) were used for data normalization. For each gene, the expression level in indehiscent fruits is presented as fold change relative to dehiscent fruits, for which the average expression level was set to 1.

Flow Cytometry

Two 7-d-old seedlings grown from seeds of each morph were used per sample. Endosperm tissue was prepared from 100 mature seeds per sample after imbibition for 12 h. Seedlings were chopped using a razor blade, either on their own or mixed with endosperm tissue, in 100 μL of Cystain UV Precise P extraction buffer (Partec). Samples were stained with 1 mL of Cystain UV Precise P DAPI fluorescent buffer (Partec) and filtered through a 30-μm filter. Three independent biological replicates consisting of 10,000 nuclei were analyzed using a Partec PAS flow cytometer. Histograms were analyzed using Flowing Software 2.5.1 (www.flowingsoftware.com), and the mean C values of endosperm nuclei with particular DNA contents were calculated relative to the mean 2C values of seedling nuclei.

Seed Germination, Moisture Content, and Water Activity Analysis

Dry mature seeds or fruits were placed in 3-cm petri dishes containing two layers of filter paper, 3 mL of distilled water, and 0.1% Plant Preservative Mixture

(Plant Cell Technology). Plates were incubated in an MLR-350 Versatile Environmental Test Chamber (Sanyo-Panasonic) at 14°C and 100 μmol s^{-1} m^{-2} constant white light. Seed germination, scored as radicle emergence, of three biological replicates of 20 seeds or fruits each was analyzed. Dry seed moisture content was determined by drying four replicates of approximately 100 mg of air-dried fruits or seeds using the Mettler-Toledo moisture analyzer HB43-S for 4 h at 120°C. Water uptake during germination was analyzed by repeatedly weighing four replicates of approximately 100 seeds or 60 fruits imbibing under the same conditions as for the seed germination assay described above. Water activity was determined on four biological replicates of 20 M^- seeds extracted from indehiscent fruits either before or after imbibition for 18 h under the conditions described above using a Labmaster-aw apparatus (Novasina).

Accession Numbers

Sequence data from this article can be found at Comperative Genomics (CoGe, https://genomevolution.org/) under accession numbers AA8G00019, AA30G00232, AA32G00014, AA21G00262, AA19G00333, AA61G00296, AA21G00070, and in the GenBank/EMBL data libraries under accession number KX874497.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. MrBayes tree of the Aethionemeae.

Supplemental Figure S2. RaxML tree of the Aethionemeae.

Supplemental Figure S3. Fruits of *Ae. arabicum* fall into two discrete clusters.

Supplemental Figure S4. Phylogenies of *Ae. arabicum* orthologs of Arabidopsis fruit developmental genes.

Supplemental Figure S5. The temperature-induced shift in fruit-morph ratio is brought about by changes throughout the whole plant.

Supplemental Table S1. Geographic origins of Aethionemeae species used for the phylogeny.

Supplemental Table S2. List of species and respective sequences used for gene phylogeny reconstruction.

Supplemental Table S3. List of primers used for quantitative reverse transcription-PCR analysis.

Supplemental Data S1. Sequence alignment underlying species phylogeny.

Supplemental Video S1. Moisture-induced pedicel movement of an *Ae. arabicum* infructescence.

ACKNOWLEDGMENTS

We thank Nicholas Bowman and Rizwana Mahmood for help with imbibition, germination, and fruit abscission analysis, Christin Grossmann for support with cluster analysis and the random-impact test, Sandrina Lerch for excellent fruit-sketching skills, the Botanical Garden Osnabrück for help with plant cultivation and seed propagation, and Dr. Lorna Ravenhill for consortium management support.

Received May 27, 2016; accepted September 29, 2016; published October 4, 2016.

LITERATURE CITED

Abley K, Locke JCW, Leyser HMO (2016) Developmental mechanisms underlying variable, invariant and plastic phenotypes. Ann Bot (Lond) **117:** 733–748

Al-Shehbaz IA (2012) A generic and tribal synopsis of the Brassicaceae (Cruciferae). Taxon **61:** 931–954

Al-Shehbaz IA, Beilstein MA, Kellogg EA (2006) Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. Plant Syst Evol **259:** 89–120

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol **215:** 403–410

Andersson I, Carlström A, Franzén R, Karlén T, Nybom H (1983) A revision of the *Aethionema saxatile* complex (Brassicaceae). Willdenowia **13:** 3–42

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

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

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

Bakker FT, Lei D, Yu J, Mohammadin S, Wei Z, Kerke S, Gravendeel B, Nieuwenhuis M, Staats M, Alquezar-Planas DE (2016) Herbarium genomics: plastome sequence assembly from a range of herbarium specimens using an Iterative Organelle Genome Assembly pipeline. Biol J Linn Soc Lond **117:** 33–43

Baskin JM, Lu JJ, Baskin CC, Tan DY, Wang L (2014) Diaspore dispersal ability and degree of dormancy in heteromorphic species of cold deserts of northwest China: a review. Perspect Plant Ecol Evol Syst **16:** 93–99

Beilstein MA, Nagalingum NS, Clements MD, Manchester SR, Mathews S (2010) Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. Proc Natl Acad Sci USA **107:** 18724–18728

Bradford MJ, Roff DA (1993) Bet hedging and the diapause strategies of the cricket *Allonemobius fasciatus*. Ecology **74:** 1129–1135

Clamp M, Cuff J, Searle SM, Barton GJ (2004) The Jalview Java alignment editor. Bioinformatics **20:** 426–427

Cruz-Mazo G, Buide ML, Samuel R, Narbona E (2009) Molecular phylogeny of *Scorzoneroides* (Asteraceae): evolution of heterocarpy and annual habit in unpredictable environments. Mol Phylogenet Evol **53:** 835–847

Dinneny JR, Weigel D, Yanofsky MF (2005) A genetic framework for fruit patterning in *Arabidopsis thaliana*. Development **132:** 4687–4696

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG (2010) Germination, postgermination adaptation, and species ecological ranges. Annu Rev Ecol Evol Syst **41:** 293–319

Dubois J, Cheptou PO (2012) Competition/colonization syndrome mediated by early germination in non-dispersing achenes in the heteromorphic species *Crepis sancta*. Ann Bot (Lond) **110:** 1245–1251

Dumolin-Lapegue S, Pemonge MH, Petit RJ (1997) An enlarged set of consensus primers for the study of organelle DNA in plants. Mol Ecol **6:** 393–397

Estornell LH, Agustí J, Merelo P, Talón M, Tadeo FR (2013) Elucidating mechanisms underlying organ abscission. Plant Sci **199-200:** 48–60

Evans MEK, Dennehy JJ (2005) Germ banking: bet-hedging and variable release from egg and seed dormancy. Q Rev Biol **80:** 431–451

Fernández IA, Aguilar JF, Panero JL, Feliner GN (2001) A phylogenetic analysis of *Doronicum* (Asteraceae, Senecioneae) based on morphological, nuclear ribosomal (ITS), and chloroplast (trnL-F) evidence. Mol Phylogenet Evol **20:** 41–64

Ferrandiz C (2011) Fruit structure and diversity. eLS doi/10.1002/9780470015902.a0002044.pub2

Ferrándiz C, Liljegren SJ, Yanofsky MF (2000) Negative regulation of the *SHATTERPROOF* genes by FRUITFULL during *Arabidopsis* fruit development. Science **289:** 436–438

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol **171:** 501–523

Fofana B, Harvengt L, Baudoin JP, Du Jardin P (1997) New primers for the polymerase chain amplification of cpDNA intergenic spacers in Phaseolus phylogeny. Belg J Bot **129:** 118–122

Francoz E, Ranocha P, Burlat V, Dunand C (2015) Arabidopsis seed mucilage secretory cells: regulation and dynamics. Trends Plant Sci **20:** 515–524

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

Frickenhaus S, Beszteri B (2008) Quicktree-SD. AWI Bioinformatics, Bremerhaven, Germany, http://hdl.handle.net/10013/epic.33164.d001

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

Gilbert SF, Bosch TC, Ledón-Rettig C (2015) Eco-Evo-Devo: developmental symbiosis and developmental plasticity as evolutionary agents. Nat Rev Genet **16:** 611–622

Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJJ (2012) Molecular mechanisms of seed dormancy. Plant Cell Environ **35:** 1769–1786

Greenspan L (1977) Humidity fixed points of binary saturated aqueous solutions. J Res Natl Bur Stand **81:** 89–96

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

Gutterman Y (1993) Seed Germination in Desert Plants: Adaptation of Desert Organisms. Springer-Verlag, Berlin

Gutterman Y (2012) Survival Strategies of Annual Desert Plants. Springer, New York, NY

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

Haudry A, Platts AE, Vello E, Hoen DR, Leclercq M, Williamson RJ, Forczek E, Joly-Lopez Z, Steffen JG, Hazzouri KM, et al (2013) An atlas of over 90,000 conserved noncoding sequences provides insight into crucifer regulatory regions. Nat Genet **45:** 891–898

Haughn G, Chaudhury A (2005) Genetic analysis of seed coat development in Arabidopsis. Trends Plant Sci **10:** 472–477

Hedge IC (1965) Aethionema R. Br. *In* PH Davis, ed, Flora of Turkey and the East Aegean Islands, **Vol 1**. University Press, Edinburgh, UK, pp 314–332

Hofberger JA, Nsibo DL, Govers F, Bouwmeester K, Schranz ME (2015) A complex interplay of tandem- and whole-genome duplication drives expansion of the L-type lectin receptor kinase gene family in the Brassicaceae. Genome Biol Evol **7:** 720–734

Howe K, Bateman A, Durbin R (2002) QuickTree: building huge neighbour-joining trees of protein sequences. Bioinformatics **18:** 1546–1547

Huang Y, Shi S (2002) Phylogenetics of Lythraceae sensu lato: a preliminary analysis based on chloroplast rbcL gene, psaA-ycf3 spacer, and nuclear rDNA internal transcribed spacer (ITS) sequences. Int J Plant Sci **163:** 215–225

Huang Z, Zhenghai H, Zhenghai H, Huang Z, Gutterman Y (2000) Structure and function of mucilaginous achenes of *Artemisia monosperma* inhabiting the Negev Desert of Israel. Isr J Plant Sci **48:** 255–266

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

Imbert E, Ronce O (2001) Phenotypic plasticity for dispersal ability in the seed heteromorphic *Crepissancta* (Asteraceae). Oikos **93:** 126–134

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol **30:** 772–780

Khosravi AR, Jacquemoud F, Mohsenzadeh S, Menke M, Mummenhoff K (2009) Phylogenetic position and taxonomic classification of *Aethionema trinervium* (Brassicaceae): a morphologically variable subshrub from southwestern Asia 1. Ann Mo Bot Gard **96:** 564–574

Kunieda T, Mitsuda N, Ohme-Takagi M, Takeda S, Aida M, Tasaka M, Kondo M, Nishimura M, Hara-Nishimura I (2008) NAC family proteins NARS1/NAC2 and NARS2/NAM in the outer integument regulate embryogenesis in *Arabidopsis*. Plant Cell **20:** 2631–2642

Lee KJ, Dekkers BJ, Steinbrecher T, Walsh CT, Bacic A, Bentsink L, Leubner-Metzger G, Knox JP (2012) Distinct cell wall architectures in seed endosperms in representatives of the Brassicaceae and Solanaceae. Plant Physiol **160:** 1551–1566

Lenser T, Theißen G (2014) Quantifying fruit dehiscence using the random impact test (RIT). Bio Protoc **4:** e1200

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

Liljegren SJ, Ditta GS, Eshed Y, Savidge B, Bowman JL, Yanofsky MF (2000) *SHATTERPROOF* MADS-box genes control seed dispersal in *Arabidopsis*. Nature **404:** 766–770

Liljegren SJ, Roeder AHK, Kempin SA, Gremski K, Østergaard L, Guimil S, Reyes DK, Yanofsky MF (2004) Control of fruit patterning in *Arabidopsis* by INDEHISCENT. Cell **116:** 843–853

Linkies A, Graeber K, Knight C, Leubner-Metzger G (2010) The evolution of seeds. New Phytol **186:** 817–831

Lu J, Tan D, Baskin JM, Baskin CC (2010) Fruit and seed heteromorphism in the cold desert annual ephemeral *Diptychocarpus strictus* (Brassicaceae) and possible adaptive significance. Ann Bot (Lond) **105:** 999–1014

Lu JJ, Ma WB, Tan DY, Baskin JM, Baskin CC (2013a) Effects of environmental stress and nutlet morph on proportion and within-flower number-combination of morphs produced by the fruit-dimorphic species *Lappula duplicicarpa* (Boraginaceae). Plant Ecol **214:** 351–362

Lu JJ, Tan DY, Baskin JM, Baskin CC (2012) Phenotypic plasticity and bet-hedging in a heterocarpic winter annual/spring ephemeral cold desert species of Brassicaceae. Oikos **121:** 357–366

Lu JJ, Tan DY, Baskin JM, Baskin CC (2013b) Trade-offs between seed dispersal and dormancy in an amphi-basicarpic cold desert annual. Ann Bot (Lond) **112:** 1815–1827

Lu JJ, Tan DY, Baskin JM, Baskin CC (2014) Germination season and watering regime, but not seed morph, affect life history traits in a cold desert diaspore-heteromorphic annual. PLoS ONE **9:** e102018

Lu JJ, Tan DY, Baskin JM, Baskin CC (2015) Post-release fates of seeds in dehiscent and indehiscent siliques of the diaspore heteromorphic species *Diptychocarpus strictus* (Brassicaceae). Perspect Plant Ecol Evol Syst **17:** 255–262

Mandák B, Pyšek P (1999) Effects of plant density and nutrient levels on fruit polymorphism in *Atriplex sagittata*. Oecologia **119:** 63–72

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

Matilla A, Gallardo M, Puga-Hermida MI (2005) Structural, physiological and molecular aspects of heterogeneity in seeds: a review. Seed Sci Res **15:** 63–76

Meakin PJ, Roberts JA (1990a) Dehiscence of fruit in oilseed rape (*Brassica napus* L.). 1. Anatomy of pod dehiscence. J Exp Bot **41:** 995–1002

Meakin PJ, Roberts JA (1990b) 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

Mohammadin S, Edger PP, Pires JC, Schranz ME (2015) Positionally-conserved but sequence-diverged: identification of long non-coding RNAs in the Brassicaceae and Cleomaceae. BMC Plant Biol **15:** 217

Mühlhausen A, Lenser T, Mummenhoff K, 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

Mühlhausen A, Polster A, Theißen G, Mummenhoff K (2010) Evolution of fruit dehiscence in *Brassicaceae*: examples from *Aethionema* and *Lepidium*. Acta Hortic **867:** 207–219

Müller K, Tintelnot S, Leubner-Metzger G (2006) Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. Plant Cell Physiol **47:** 864–877

Mummenhoff K, Franzke A (2007) Gone with the bird: late tertiary and quaternary intercontinental long-distance dispersal and allopolyploidization in plants. Syst Biodivers **5:** 255–260

Norton DA, Delange PJ, Garnock-Jones PJ, Given DR (1997) The role of seabirds and seals in the survival of coastal plants: lessons from New Zealand *Lepidium* (Brassicaceae). Biodivers Conserv **6:** 765–785

Østergaard L, Kempin SA, Bies D, Klee HJ, Yanofsky MF (2006) Pod shatter-resistant *Brassica* fruit produced by ectopic expression of the *FRUITFULL* gene. Plant Biotechnol J **4:** 45–51

Parolin P (2006) Ombrohydrochory: rain-operated seed dispersal in plants—with special regard to jet-action dispersal in Aizoaceae. Flora **201:** 511–518

Philippi T, Seger J (1989) Hedging one's evolutionary bets, revisited. Trends Ecol Evol **4:** 41–44

Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. J Exp Biol **209:** 2362–2367

Pufal G, Garnock-Jones P (2010) Hygrochastic capsule dehiscence supports safe site strategies in New Zealand alpine *Veronica* (Plantaginaceae). Ann Bot (Lond) **106:** 405–412

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

Ripoll JJ, Roeder AH, Ditta GS, Yanofsky MF (2011) A novel role for the floral homeotic gene *APETALA2* during *Arabidopsis* fruit development. Development **138:** 5167–5176

Rost B (1999) Twilight zone of protein sequence alignments. Protein Eng **12:** 85–94

Rubio de Casas R, Donohue K, Venable DL, Cheptou PO (2015) Gene-flow through space and time: dispersal, dormancy and adaptation to changing environments. Evol Ecol **29:** 813–831

Sadeh A, Guterman H, Gersani M, Ovadia O (2009) Plastic bet-hedging in an amphicarpic annual: an integrated strategy under variable conditions. Evol Ecol **23:** 373–388

Schranz ME, Mohammadin S, Edger PP (2012) Ancient whole genome duplications, novelty and diversification: the WGD radiation lag-time model. Curr Opin Plant Biol **15:** 147–153

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

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

Simon S, Rühl M, de Montaigu A, Wötzel S, Coupland G (2015) Evolution of *CONSTANS* regulation and function after gene duplication produced a photoperiodic flowering switch in the Brassicaceae. Mol Biol Evol **32:** 2284–2301

Simons AM (2011) Modes of response to environmental change and the elusive empirical evidence for bet hedging. Proc Biol Sci **278:** 1601–1609

Solms-Laubach HG (1901) Über die Arten des Genus *Aethionema*, die Schließfrüchte hervorbringen. *In* Botanische Zeitung. Verlag von Arthur Felix, Leipzig, Germany, pp 61–78

Sorensen AE (1986) Seed dispersal by adhesion. Annu Rev Ecol Syst **17:** 443–463

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

Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. Trends Plant Sci **5:** 537–542

Takeno K, Yamaguchi H (1991) Diversity in seed germination behavior in relation to heterocarpy in *Salsola komarovii* Iljin. Bot Mag Tokyo **104:** 207–215

Venable DL (2007) Bet hedging in a guild of desert annuals. Ecology **88:** 1086–1090

Venable DL, Dyreson E, Morlaes E (1995) Population dynamic consequences and evolution of seed traits of *Heterosperma pinnatum* (Asteraceae). Am J Bot **82:** 410–420

Venable DL, Lawlor L (1980) Delayed germination and dispersal in desert annuals: escape in space and time. Oecologia **46:** 272–282

Venable DL, Levin DA (1985) Ecology of achene dimorphism in *Heterotheca latifolia*. 1. Achene structure, germination and dispersal. J Ecol **73:** 133–145

Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH (1995) Adaptive phenotypic plasticity: consensus and controversy. Trends Ecol Evol **10:** 212–217

Weitbrecht K, Müller K, Leubner-Metzger G (2011) First off the mark: early seed germination. J Exp Bot **62:** 3289–3309

Western TL (2012) The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal. Seed Sci Res **22:** 1–25

Western TL, Burn J, Tan WL, Skinner DJ, Martin-McCaffrey L, Moffatt BA, Haughn GW (2001) Isolation and characterization of mutants defective in seed coat mucilage secretory cell development in Arabidopsis. Plant Physiol **127:** 998–1011

Yang F, Baskin JM, Baskin CC, Yang X, Cao D, Huang Z (2015) Effects of germination time on seed morph ratio in a seed-dimorphic species and possible ecological significance. Ann Bot (Lond) **115:** 137–145

Yang X, Baskin JM, Baskin CC, Huang Z (2012) More than just a coating: ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage. Perspect Plant Ecol Evol Syst **14:** 434–442

Yang X, Dong M, Huang Z (2010) Role of mucilage in the germination of *Artemisia sphaerocephala* (Asteraceae) achenes exposed to osmotic stress and salinity. Plant Physiol Biochem **48:** 131–135

Yang X, Zhang W, Dong M, Boubriak I, Huang Z (2011) The achene mucilage hydrated in desert dew assists seed cells in maintaining DNA integrity: adaptive strategy of desert plant *Artemisia sphaerocephala*. PLoS ONE **6:** e24346

Zohary M (1962) Plant Life of Palestine: Israel and Jordan. Ronald Press Company, New York, NY

Zohary M, Fahn A (1950) On the heterocarpy of *Aethionema*. Palestine J Bot **5:** 28–31