NOTE

Fracture of the dimorphic fruits of *Aethionema arabicum* (Brassicaceae)[1]

Waheed Arshad, Federica Marone, Margaret E. Collinson, Gerhard Leubner-Metzger, and Tina Steinbrecher

Abstract: Fruits exhibit highly diversified morphology, and are arguably one of the most highly specialised organs to have evolved in higher plants. Fruits range in morphological, biomechanical, and textural properties, often as adaptations for their respective dispersal strategy. While most plant species possess monomorphic (of a single type) fruit and seeds, here we focus on *Aethionema arabicum* (L.) Andrz. ex DC. (Brassicaceae). Its production of two distinct fruit (dehiscent and indehiscent) and seed types on the same individual plant provides a unique model system with which to study structural and functional aspects of dimorphism. Using comparative analyses of fruit fracture biomechanics, fracture surface morphology, and internal fruit anatomy, we reveal that the dimorphic fruits of *A. arabicum* exhibit clear material, morpho-anatomical, and adaptive properties underlying their fracture behaviour. A separation layer along the valve–replum boundary is present in dehiscent fruit, whereas indehiscent fruit have numerous fibres with spiral thickening, linking their winged valves at the adaxial surface. Our study evaluates the biomechanics underlying fruit-opening mechanisms in a heteromorphic plant species. Elucidating dimorphic traits aids our understanding of adaptive biomechanical morphologies that function as a bet-hedging strategy in the context of seed and fruit dispersal within spatially and temporally stochastic environments.

Key words: diaspore dispersal, heteromorphy, pericarp biomechanics, silique anatomy, SRXTM, strain energy.

Résumé : Les fruit présentent une morphologie hautement diversifiée et ils constituent probablement un des organes les plus hautement spécialisés ayant évolué chez les plantes supérieures. Les fruit varient en ce qui concerne leurs propriétés morphologiques, biomécaniques et texturales, souvent comme adaptations de leurs stratégies respectives de dispersion. Alors que la plupart des espèces de plantes possèdent des fruit et graines monomorphes (d'un type unique), les auteurs se concentrent ici sur *Aethionema arabicum* (L.) Andrz. ex DC. (Brassicaceae). Sa production de deux types distincts de fruit (déhiscent et indéhiscent) et de graines chez le même individu fournit un système modèle unique sur lequel étudier les aspects structuraux et fonctionnels du dimorphisme. À l'aide d'analyses comparatives de la biomécanique de la fracture du fruit, de la morphologie à la surface de la fracture et de l'anatomie interne du fruit, les auteurs révèlent que les fruit dimorphes d'*A. arabicum* présentent des propriétés matérielles, morpho-anatomiques et adaptatives claires qui sous-tendent le fonctionnement de la fracture. Une couche de séparation le long de la frontière valve–replum est présente chez les fruit déhiscents, alors que les fruit indéhiscents possèdent de nombreuses fibres avec un épaississement hélicoïdal, liant leurs valves ailées à la surface adaxiale. Cette étude évalue la biomécanique qui sous-tend les mécanismes d'ouverture du fruit chez les espèces végétales hétéromorphes. L'élucidation des traits dimorphes contribue à notre compréhension des morphologies biomécaniques adaptatives qui fonctionnent comme une stratégie de « bet-hedging » dans le contexte de la dispersion des graines et des fruit à l'intérieur d'environnements spatialement et temporellement stochastiques. [Traduit par la Rédaction]

Mots-clés : dispersion des diaspores, hétéromorphie, biomécanique du péricarpe, anatomie de la silique, microscope à rayonnement synchrotronique et tomographie aux rayons X, énergie de déformation.

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Introduction

Across the plant kingdom, fruits are highly diversified in their morphology, representing remarkable botanical architecture and reproductive ingenuity — from the giant pumpkins of *Cucurbita maxima* (Cucurbitaceae), to the microscopic fruit of *Wolffia* (Araceae) duckweeds, which are no larger than 300 μm. The fruit is arguably one of the most highly specialised organs to have evolved in higher plants, mediating the maturation and dispersal of seeds, and representing the end of the reproductive cycle in angiosperms (Ferrándiz et al. 1999; Linkies et al. 2010).

Fruits may range in biomechanical and textural properties from being fleshy and fibrous, to dry and papery. Dry fruits are broadly classified as either indehiscent, in which the pericarp (mature ovary wall) remains closed at maturity, or dehiscent, in which the fruit splits or opens in some manner to release or expose the seed(s) (Spjut 1994). A classic example of the latter category, is in the cabbage family (Brassicaceae). Brassicaceae comprises species with great economic importance for food, fodder, industrial crops, and ornamentals. It also includes important model plants such as *Arabidopsis*, *Brassica*, *Lepidium*, and *Boechera* species (Heywood et al. 2007; Mummenhoff et al. 2008; Hohmann et al. 2015; Christenhusz et al. 2017). Typical fruit morphology within Brassicaceae consists of dehiscent, dry siliques ("capsules"), formed by a pistil composed of two or more carpels with persistent membranous placental tissue (septum). During seed dispersal, the pericarp valves detach from the replum along a separation layer, with varying dehiscence patterns (Fig. 1a) that exemplify evolutionarily labile morphologies.

Although the silique (or silicula) is the dominant fruit "bauplan" within the Brassicaceae, there are many morpho-anatomical variations possessing diagnostic and taxonomic characters (Koch et al. 2003; Hall et al. 2006). Fruit shape diversity in the Brassicaceae is generated by varying patterns of anisotropy, leading to fruit with angustiseptate (compressed at a right angle to the septum), latiseptate (compressed parallel to the septum), or unflattened (terete or angled in cross section) three-dimensional shapes (Koch et al. 2003; Beentje 2010; Eldridge et al. 2016). Computational modelling has provided a simplified framework for this diversity in Brassicaceae fruit shapes (Eldridge et al. 2016). Dehiscent fruits are considered to represent the ancestral fruit type in Brassicaceae species, with indehiscent fruit evolving independently in 20 tribes (Hall et al. 2011; Mühlhausen et al. 2013). For example, most of the species in the large genus *Lepidium* have dehiscent fruit, but indehiscent and didymous *Lepidium* fruit types evolved several times independently within this genus (Mummenhoff et al. 2008; Sperber et al. 2017). Even more specialised fruit morphology is exemplified by heteroarthrocarpic fruit belonging to *Cakile* spp. in the tribe Brassiceae, where joints separate the fruit transversely into distinct proximal and dis-

tal segments (Hall et al. 2011; Willis et al. 2014). Thus, it is clear the morphological diversity within Brassicaceae family provides several fruitful avenues to study biomechanical form and function related to seed dispersal (Dinneny and Yanofsky 2005; Mühlhausen et al. 2013; Sperber et al. 2017).
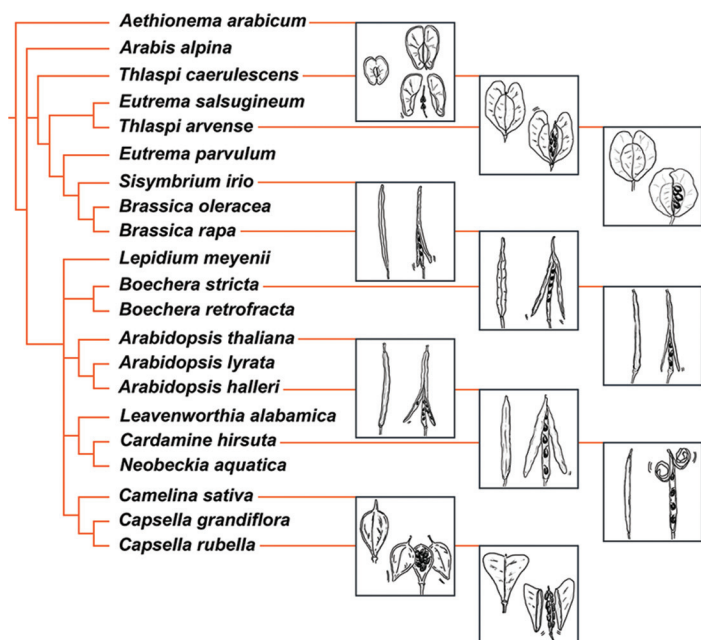
Most plant species possess monomorphic (of a single type) diaspores. However, a number of species exhibit fruit and seed heteromorphism (heterodiaspory), where a given trait exhibits a clear bimodal (dimorphism) or multimodal (heteromorphism) distribution (Imbert 2002; Baskin and Baskin 2014). Such intra-individual variation can often occur within the same fruiting head (infructescence) and be associated with distinct morpho-physiological properties (Baskin et al. 2014; Lenser et al. 2016; Liu et al. 2018; Bhattacharya et al. 2019). The phenomenon of heterodiaspory is of particular importance for relatively short-lived species in spatio-temporally unpredictable environments, and may function as a bet-hedging survival strategy (Venable 1985), particularly in species distributed in desert, saline, and other frequently-disturbed habitats (Imbert 2002; Baskin and Baskin 2014).

In this study, we focus on the dimorphic species *Aethionema arabicum* (L.) Andrz. ex DC., a small, annual, herbaceous species belonging to the earliest diverging sister tribe (Aethionemeae) within the Brassicaceae (Franzke et al. 2011; Hohmann et al. 2015; Mohammadin et al. 2017). The genus *Aethionema* occurs mainly in the western Irano-Turanian region, an often-hypothesised cradle of the Brassicaceae (Hedge 1976; Al-Shehbaz et al. 2006; Beilstein et al. 2006; Mandáková et al. 2017). This divergence is thought to have occurred sometime during the Eocene (ca. 34–56 Ma) (Franzke et al. 2011; Hohmann et al. 2015; Mohammadin et al. 2017). *Aethionema arabicum* is characterised by two types of fruit and seeds produced on the same individual infructescence (Fig. 1b): dehiscent (DEH) fruit with 2–6 mucilaginous (M+) seeds, and indehiscent (IND) fruit each containing a single non-mucilaginous (M−) seed (Lenser et al. 2016). Dehiscence of the DEH fruit morph causes local dispersal of M+ seeds, which adhere to substrates via seed-coat mucilage upon imbibition. M+ seeds possess low dormancy and likely represent an anti-telechorous dispersal mechanism. In comparison, the more dormant IND fruit abscises from the mother plant in its entirety, and has the capacity to disperse longer distances (telechory) via wind and water (Arshad et al. 2019). The production of no intermediate morphs, together with a published genome sequence (Haudry et al. 2013), contributes to the suitability of *A. arabicum* as an excellent model system for fruit and seed dimorphism (Lenser et al. 2018; Mohammadin et al. 2018; Arshad et al. 2019; Wilhelmsson et al. 2019).
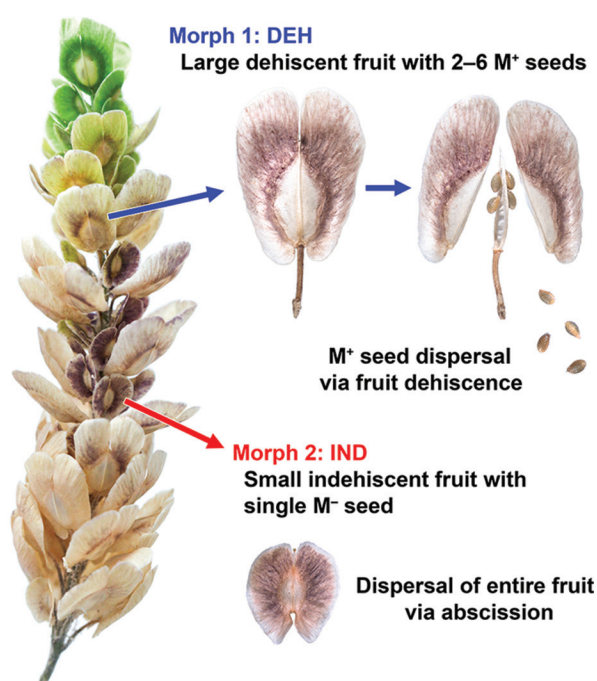
Little is known about the factors influencing fruit valve opening and the fracture behaviour of the two *A. arabicum* morphs. In this study, we elucidate the bio-

Fig. 1. (*a*) The diversity of fruit fracture mechanisms within members of the Brassicaceae. Depicted are the phylogenetic relationships of various "model" species, together with schematic panels illustrating typical mature fruit prior to (left) and during (right) fruit fracture. The mature fruit may be indehiscent (such as the M⁻ single-seeded fruit morph in *Aethionema arabicum*), rarely transversely articulate with indehiscent and dehiscent segments (heteroarthrocarpic, such as *Cakile* spp., not shown), or more typically a two-locular dehiscent capsule with a placental partition (septum) bordered by a replum. Schematic illustrations not to scale. Phylogeny according to Nikolov and Tsiantis (2017). (*b*) The fruit dimorphism in *A. arabicum* is characterised by dehiscent (DEH) fruit with 2–6 mucilaginous (M⁺) seeds, and indehiscent (IND) fruit each containing a single non-mucilaginous (M⁻) seed. Both fruit morphs are produced on the same individual infructescence. While dehiscence of the DEH fruit morph causes local dispersal of M⁺ seeds, the IND fruit abscises from the mother plant in its entirety. [Colour online.]

a Fruit fracture mechanisms in the Brassicaceae

b Fruit dimorphism in *Aethionema arabicum*

mechanical properties during fruit fracture, the fruit valve surface morphology, and the internal fruit anatomy. The morphotype-specific fruit properties are discussed in the context of seed and fruit (diaspore) dispersal mechanisms within the spatially and temporally stochastic environments in which *A. arabicum* survives.

Materials and methods

Fruit valve tensile testing and energy absorption relations

Data from Arshad et al. (2019) were re-analysed to obtain the energy absorption of fruit valve separation. Mature, dry fruit of *Aethionema arabicum* (Turkish accession ES1020, obtained from Eric Schranz, Wageningen University and Research Centre) (Wilhelmsson et al. 2019) were clamped on each side of the fruit wing, leaving a 2 mm gap between the jaws of a single-column tensile testing machine (Zwick Roell ZwickiLine Z0.5, Ulm, Germany) configured with a 200 N load cell. A constant speed for separation was set at 1 mm·min⁻¹. Force-displacement data were obtained using 30 replicates from three mature main branch infructescences. All of the fruit were freshly harvested from plants grown un-

der long-day conditions (16 h, 20 °C : 8 h, 18 °C; light : dark) in a glasshouse, and mechanically tested at room temperature (20 °C) and 31% relative humidity. The total area under the resultant force-displacement curve was calculated as the mechanical energy consumed by the pericarp in straining it to its fracture point. Using a digital camera (Canon EOS 5D Mark II, fitted with a EF 100 mm f/2.8 macro lens) and Fiji (Schindelin et al. 2012), the area representing the pericarp fracture zone was determined for 50 manually separated replicates each of DEH and IND fruit. An unpaired two-sample *t* test was carried out using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

Scanning electron microscopy (SEM) analysis of fractured fruit valve surfaces

Mature, dry fruit pericarps of both *A. arabicum* morphs were mounted on 12.5 mm Cambridge aluminium specimen stubs, using conductive putty (Lennox Educational, Dublin, Ireland) or two-component epoxy (Araldite®; Huntsman Advanced Materials GmbH, Switzerland). Samples were sputter-coated with a 40 nm thickness of

gold or gold–palladium using a Polaron SEM Coating Unit E5100 (Bio-Rad Microscience Division, Watford, UK). Pericarp fracture surfaces were studied using SEM (Hitachi S-3000N, Japan) at an acceleration voltage of 20 kV, with images subsequently contrast adjusted in Adobe Photoshop CC.

Synchrotron-based radiation X-ray tomographic microscopy (SRXTM) of fracture zones

Mature, dry fruit were fixed in 3% glutaraldehyde plus 4% formaldehyde in 0.1 mol/L piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) buffer at pH 7.2, for 3 h. The samples were then rinsed twice for 10 min with 0.1 mol/L PIPES, before dehydration in five changes of ethanol (30%, 50%, 70%, 95%, 100%). Samples were critical-point dried (Balzers CPD-030; Bal-Tec, Germany) using ethanol as the intermediate fluid and CO_2 as the transitional fluid. Dehiscent and indehiscent fruit samples were mounted onto 3 mm diameter brass pin stubs using two-component epoxy (Araldite) and imaged at the TOmographic Microscopy and Coherent rAdiology experimenTs (TOMCAT) beamline of the Swiss Light Source, Paul Scherrer Institute (Stampanoni et al. 2006). Data were acquired using a 10× objective and a sCMOS camera (PCO.edge; PCO, Kelheim, Germany), with an exposure time of 80 ms at 12 keV (isotropic voxel dimensions = 0.65 μm). A total of 1501 projections were acquired equiangularly over 180°, post-processed and reconstructed using a Fourier-based algorithm (Marone and Stampanoni 2012). For verification, three replicates were examined for each fruit morph. Axial tomographic slice data derived from the scans were analysed and manipulated using Avizo™ 9.5.0 (ThermoFisher Scientific, Visualization Science Group Inc., Burlington, Massachusetts, USA) for Windows 10 Pro 64-bit, and contrast adjusted in Adobe Photoshop CC.

Results

Distinct biomechanical events lead to dimorphic fruit failure

To investigate the biomaterial profiles underlying fruit opening mechanisms of the two distinct fruit morphs in *A. arabicum* (DEH and IND), a uniaxial tensile test was performed. Such tests determine the resistance of the component against elongation, and thus enable the derivation of several key material properties and parameters of the tested material (Farquhar and Zhao 2006; Steinbrecher and Leubner-Metzger 2017). We observed two modes of fruit fracture and characterised the force–displacement curves associated with the distinct morphs (Fig. 2). Fruit from the DEH morph were typified by force–displacement curves exhibiting an initial elastic and plastic deformation, followed by a pre-failure event (Fig. 2a). This fracturing event typically initiated at the valve–replum border adjacent to the fruit–pedicel junction, extending along the longitudinal axis of the replum. The crack wake was temporarily held together
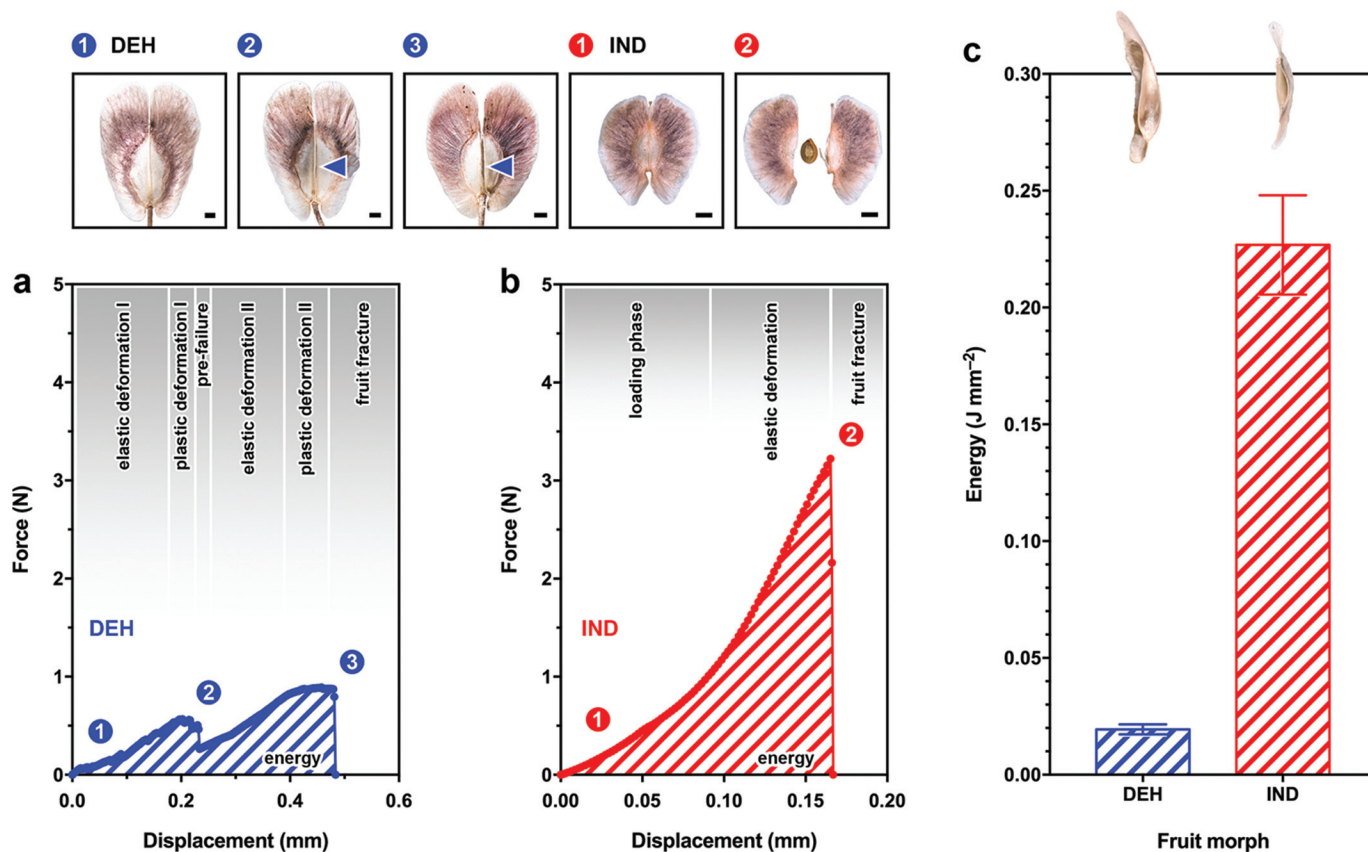
before a second elastic and plastic deformation phase, preceding complete fruit fracture after which no further change in force was detected. In contrast, IND fruit were characterised by a consistent loading phase, comprising non-uniform deformation of the pericarp prior to uniform and linear elastic deformation leading to fruit fracture (Fig. 2b).

The force–displacement curves for DEH fruit show the typical biomechanical response for loading of a benignly "ductile" and elastic material, which can be deformed in multiple stages without causing a complete fracture of the material. The DEH fruit are initially compliant and exhibit a degree of flexibility as the load is increased (Fig. 2a). The more brittle IND fruit, however, failed with less deformation when subjected to loading, with, on average, a 2.6-times higher force. Thus, the dissimilarities in the mechanism and ability of the dimorphic fruit to resist the extension of the initial crack are profound. The comparative biomechanical properties between fruit morphs were also associated with significant differences in the mean mechanical energy consumed by the pericarp in straining it to its failure point ($t_{58} = -9.704$, $P < 0.001$, $d = 2.5$) (Fig. 2c). The energy taken up by each sample is represented by the total area underneath the force–displacement curve up to failure (Hourston et al. 2017; Steinbrecher and Leubner-Metzger 2017). The IND fruit (mean ± SE = 0.227 J·mm^{-2} ± 0.02) had a ca. 12-fold increase compared with DEH fruit (0.019 ± 0.002). Taken together, results show that fruit valve opening in *A. arabicum* has two clear biomaterial and mechanical energy profiles, associated unequivocally with the two morphs.

Comparative fracture surface morphology reveals distinct properties of the fruit endocarp

Because the fruit exhibit clear biomechanical failure patterns, pericarp fracture surface morphology was investigated to determine whether it contributed to the observed fruit failure patterns. At the macroscopic level, experimentally fractured valves revealed the replum and septum typical for dehiscent brassicaceous siliques in the DEH morph (Fig. 3a), while experimentally fractured valves in the IND morph (Fig. 3f) revealed a fruit with a dysfunctional replum and lacking a septum. Scanning electron microscopy (SEM) revealed structural differences at several hierarchical levels of organisation. During the fracturing process, the internal tissues of the two fruit morphs split in two distinct ways; the comparatively even structure of the DEH pericarp fracture surface contrasted with the uneven structure of the IND pericarp fracture surface, which often had protrusions at the valve edge (Fig. 3g). In the DEH fruit, an exocarp layer of thick-walled cells, together with a thin-walled mesocarp and endocarp were visible at the fractured edge (Figs. 3c–3e). Both adaxial and abaxial surfaces were identical in morphology (Figs. 3c and 3d). Furthermore, the region of follicle splitting along the replum during de-

Fig. 2. Biomechanical changes during dehiscent (DEH) and indehiscent (IND) fruit fracture in *Aethionema arabicum*. (*a* and *b*) Typical force–displacement curves of mechanical tests during fruit valve separation in *A. arabicum*, revealing two contrasting fracture patterns. While DEH (*a*) fruit exhibit a multistage slow, gradual failure with distinct deformation events at the valve–replum border (blue arrowheads on panels 2 and 3), IND (*b*) fruit in contrast show a sudden and complete failure preceded by a characteristic loading phase. Numbered panels above force-displacement curves and the corresponding photographs illustrate the process of DEH (1, 2, 3 in blue) and IND (1, 2 in red) fruit fracturing. Scale bars = 1 mm. (*c*) Comparative means of pericarp-specific mechanical energy consumed during the separation of fruit valves from fruit differ significantly (t_{58} = −9.704, P < 0.001, d = 2.5) between DEH and IND morphs. N = 30. Error bars ± 1 standard error of the mean. Data are normalised relative to the mean area of the fracture zone (DEH, 6.94 ± 0.14 mm²; IND, 0.87 ± 0.03). [Colour online.]

hiscence exhibits a concave surface (Fig. 3*e*). The IND fruit pericarp morphology, in contrast, exhibited a different structure (Figs. 3*h*–3*j*). At the abaxial margin (Fig. 3*h*), the valve fracture surface appeared consistently "rough" in texture and comprised cell walls that had been mechanically torn. At the adaxial margin (Figs. 3*i* and 3*j*), the endocarp consisted of a very distinct thick-walled single cell layer, oriented at a perpendicular angle to the longitudinal axis of the fruit. Here, numerous fibres with spiral thickening (Figs. 3*i* and 3*j*) can be seen to run across the adaxial surface, where they were previously connected across the two halves of the pericarp. Thus, there are clear fracture surface morphologies, at various hierarchical levels, which underlie the two observed fruit fracturing behaviours.

Comparative internal anatomy confirms absence of a separation layer in IND fruit

To explore the internal anatomy of the dimorphic fruit, we conducted non-destructive investigations of the internal structure of mature fruit prior to the onset of ripening, with a particular focus at the region of fruit failure. Reconstructed digital sections (orthoslices) obtained by SRXTM revealed high resolution cell and tissue details (Fig. 4) without destruction of the sample or risk of artefacts associated with traditional histology (Betz et al. 2007; Smith et al. 2009). Differences, otherwise determined by tissue and cell wall composition, were highlighted by varying X-ray attenuation. Our schematic interpretation of fruit layers (Figs. 4*c* and 4*d*) indicates that cells of the exocarp, mesocarp, and endocarp layers were all readily distinguishable in the digital sections from both fruit morphs. Two distinct layers of the endocarp were observed; endocarp *a* (en*a*) comprised an inner epidermis of longitudinally elongated, thin-walled cells, while a subepidermal endocarp *b* (en*b*) layer consisted of one to three layers of tightly packed, isodiametric cells. These observations correlate with the thick cell-walled endocarp layer of the IND fruit, as observed by SEM (Fig. 3*j*), which leads to the fibres with spiral thickening after fracturing.

Fig. 3. Morphology of the experimentally fractured valves underlying the observed biomechanical differences between dehiscent (DEH) and indehiscent (IND) fruit of *Aethionema arabicum*. Macroscopic features of separated fruit indicate the presence of a replum and septum in DEH fruit (*a*), while a dysfunctional replum and lack of septum characterise IND fruit (*f* and *g*). SEM images of the abaxial (*c* and *h*) and adaxial (*d*, *e*, *i*, and *j*) edges of fractured pericarps indicate the even structure of DEH pericarp fracture surface, in comparison with the uneven structure of IND pericarp fracture surface. Both abaxial and adaxial fracture surfaces of DEH pericarps are identical in morphology (*c* and *d*). The distinct thick-walled IND fruit endocarp layer (*I* and *j*) possesses numerous fibres with spiral thickening (*j*, arrowhead) across the adaxial fracture surface, where they were previously connected across the two halves of the pericarp. Scale bars = 1 mm (*a*, *f*); 100 μm (*b*, *g*); and 20 μm (*c*–*e*, *h*–*j*). SEM, scanning electron microscopy; M⁺, mucilaginous; M⁻, non-mucilaginous; ex, exocarp; ms, mesocarp; en, endocarp. [Colour online.]
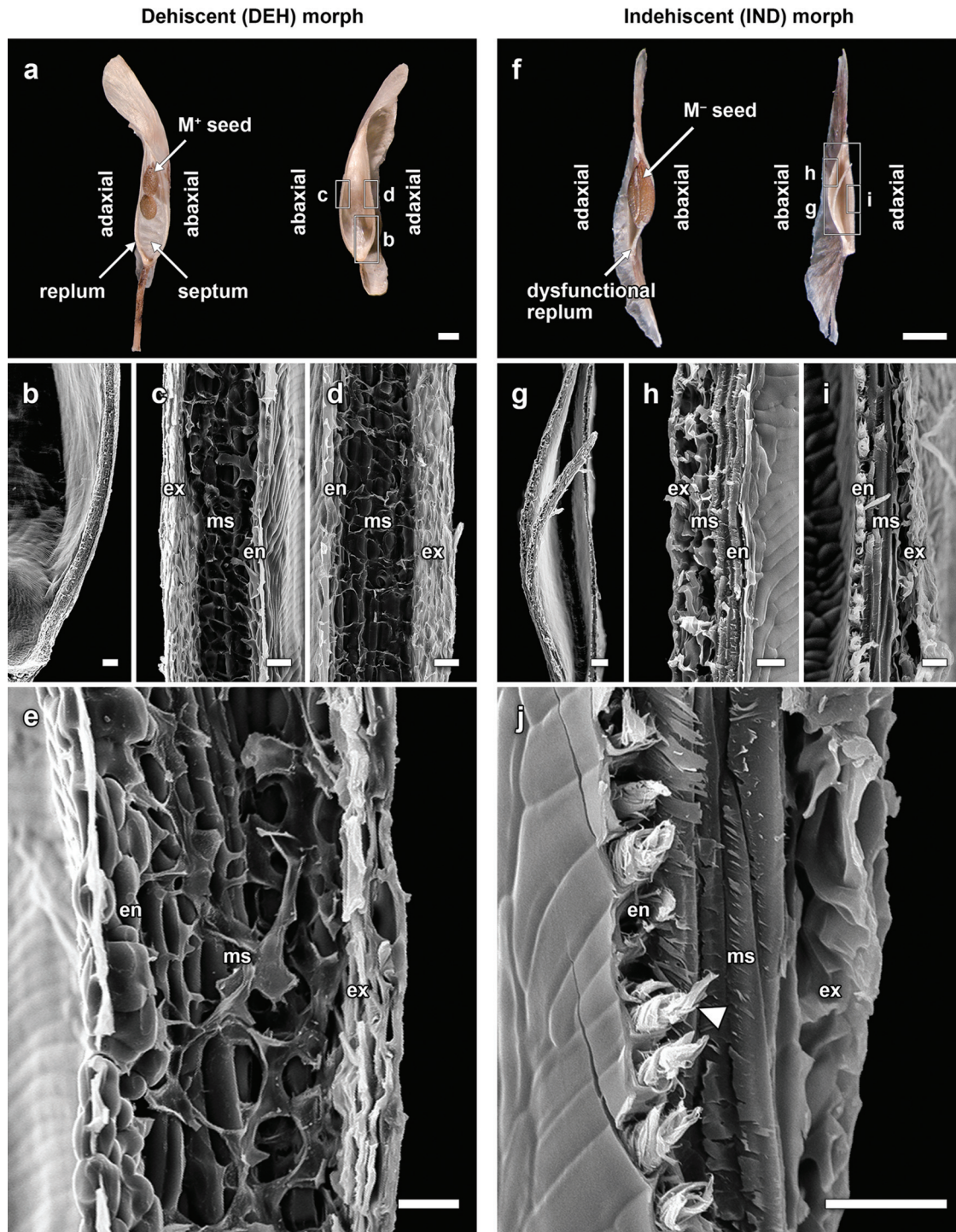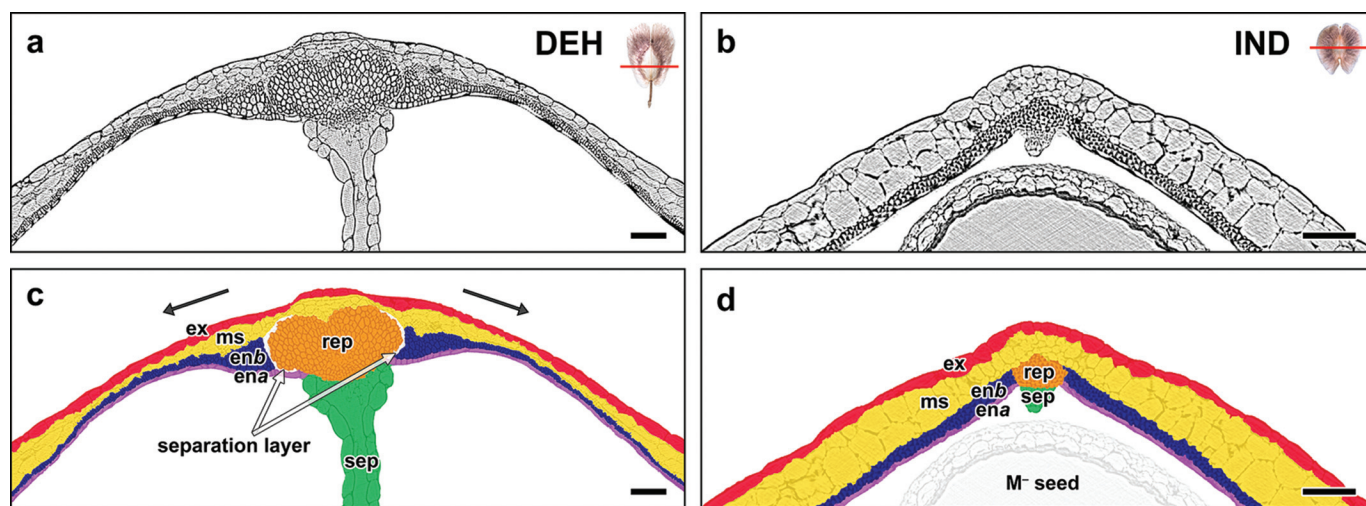
Fig. 4. Comparative SRXTM results obtained from digital transverse sections of mature *Aethionema arabicum* dehiscent (DEH) (*a*) and indehiscent (IND) (*b*) fruit with schematic representations (*c* and *d*) at the valve–replum region. Inset fruit (not to scale) depict the region from which the slice is taken. The exocarp and outer epidermis (ex), two to three cell layers of mesocarp (ms), and two zones within the endocarp plus inner epidermis (en*a* and en*b*) can be distinguished in the fruit valves. A "separation layer" at the valve margin, extending in continuity around the replum when the fruit is further dried, allows DEH fruit valves to detach from the replum. Thus, the endocarp (en*b*), valve margins, and regions of the mesocarp, together with cell-wall degradation in the en*a* layer, contribute to "pod-shattering" biomechanics during DEH fruit dehiscence. Arrows indicate proposed directionality of DEH fruit drying tensions, which contribute to rain-mediated seed dispersal (ombrohydrochory). The IND fruit, characterised by its absence of a separation layer ("dehiscence zone") and septum, does not undergo this highly co-ordinated process, instead retaining a single seed within the pericarp during dispersal. Scale bar = 75 μm. SRXTM, synchrotron radiation X-ray tomographic microscopy; M⁻, non-mucilaginous.

The *A. arabicum* DEH morph is typical for many brassicaceous siliques, in that the margins of the two carpels and the parietal placentae, between which the septum is attached, form a replum. However, of particular significance is the separation between cells of the replum and endocarp layers (en*a* and en*b*) in the DEH fruit morph (Figs. 4*a* and 4*c*). This distinct tissue "separation layer" forms part of the "dehiscence zone", extending along the entire longitudinal axis of the pericarp at the valve–replum boundary. In contrast, tissue organisation within the IND morph pericarp is distinctly different (Figs. 4*b* and 4*d*). The mesocarp contributes to a distinct layer composed of large cells, with more densely-packed cells adjacent to the comparatively smaller, dysfunctional replum. The IND morph appears unilocular and only the remnants of a septum persist; the ovary thus contained a single ovule (M⁻ seed; Figs. 4*b* and 4*d*). Although there is a comparatively reduced replum, cells of the endocarp and the replum were not separated by a dehiscence zone, instead forming a continuous layer. Furthermore, while the en*b* layer becomes multilayered proximal to the replum in the DEH morph, only a few cell-layers are present in the en*b* in the IND morph. Thus, the absence of a "separation layer" or "dehiscence zone", as well as septum absence, are major differences in the internal anatomy of the IND morph pericarp.

Discussion

Distinct fracture biomechanics of dimorphic fruit

The integration of biomechanics and mechanobiology has been a significant methodological advancement to address questions in plant sciences, and has seen a renaissance over recent decades (Read and Stokes 2006; Moulia 2013). Our biomechanical evaluation of fruit opening mechanisms of a heteromorphic plant species links pericarp-specific properties to adaptive seed and fruit dispersal. In contrast to the single fruit fracture mechanism — and associated dispersal strategy — of monomorphic plants, the distinct biomechanical profiles for the *Aethionema arabicum* fruit morphs are correlated with their adaptations for different modes of dispersal (Arshad et al. 2019). Our comparisons of fruit fracture biomechanics, and the morpho-anatomical features that contribute to the observed patterns, show that the behaviours are unequivocally associated with the two fruit morphs. Under natural conditions, it is hypothesised that recurring forces from raindrop impacts and (or) wind on the DEH fruit most likely induce fatigue crack growth along the replum. DEH fruit exhibit a multistaged biomechanical response, with several ranges of linear behaviour during pericarp opening, whereas the IND pericarp provides a more brittle breaking behaviour and prolonged loading phase requiring a significantly higher opening energy (Fig. 2*c*). Torn-out structures (IND fruit, Fig. 3*g*) indicate that friction between cell layers had to be overcome during the fruit fracture process, creating the "rough" fracture surface texture. As previously described by Beismann et al. (2000), shearing cell layers during the tearing process may contribute to the toughness of the material. The winged, dispersal-

enhancing feature of the IND pericarp allows the seed to remain encased during dispersal (Arshad et al. 2019); however, as a rare adaptation for long-range dispersal in desert plants, pericarp "wings" may also serve as a protective measure against the adverse environment during germination and seedling establishment (Ellner and Shmida 1981).

That fruit opening in monomorphic Brassicaceae fruit is dependent on the positioning and formation of the valve margin and its dehiscence zone is well-established (Spence et al. 1996; Avino et al. 2012), but little is known about differences in the opening mechanisms operating in heteromorphic fruit. We found that the IND fruit lacks the distinctive anatomical organisation present in typical dehiscent siliques, and that the cells of the endocarp layers form a continuous band around the replum, thus preventing fruit dehiscence. The IND fruit pericarp, therefore, not only confers enhanced dispersal ability and degree of dormancy (Arshad et al. 2019), but also a mechanism for remaining as a closed disseminule after dispersal. This may suggest that pericarp-mediated dormancy in the *A. arabicum* system may be partly physically and physiologically imposed on M$^-$ seeds. In the indehiscent fruit of *Lepidium didymum* (Brassicaceae), Sperber et al. (2017) found that the thick, hard pericarp imposed a mechanical constraint on the germination of encased seeds by influencing water uptake patterns into seeds inside fruit valves, and that fungi induced selective weakening of pericarp tissue (at distinct predetermined zones), lowering its mechanical resistance to breakage. The mechanisms by which the *A. arabicum* IND pericarp may impose a mechanical constraint to full water uptake by the M$^-$ seed is little investigated. Ongoing ecophysiological, biomechanical, and molecular analyses on the influence of the pericarp tissue during and after IND fruit germination should shed light on its specific role.

The presented fruit fracture biomechanics prompts questions on the contrasting development and molecular regulation underpinning the morph-specific determination of *A. arabicum* fruit. Within the Brassicaceae, the evolutionary transition from dehiscent to indehiscent fruit has been investigated in *Lepidium*, where both dehiscent and indehiscent fruit are produced (Mummenhoff et al. 2008; Mühlhausen et al. 2013). Anatomical changes at the valve–replum border were connected with altered expression patterns of various genes orthologous to the known fruit developmental genes in *Arabidopsis*, including *ALCATRAZ* (*ALC*), *INDEHISCENT* (*IND*), *SHATTERPROOF1* (*SHP1*), and *SHATTERPROOF2* (*SHP2*) (Rajani and Sundaresan 2001; Liljegren et al. 2004; Ballester and Ferrandiz 2017). Orthologues were shown to be expressed in the dehiscence zone-forming fruit valve margin of *Lepidium campestre* dehiscent fruit, whereas in the corresponding tissue of *L. appelianum* indehiscent fruit, expression patterns were down-regulated (Mühlhausen et al. 2013). Indeed, in *A. arabicum*, expression analyses in mature fruit

have previously revealed that the orthologue *IND* (which is involved in the differentiation of the separation layer and acts as the key regulator in controlling valve margin specification) was down-regulated in IND fruit compared with DEH fruit (Lenser et al. 2016). The genetic pathways operating in DEH and IND fruit therefore indicate an avenue for more detailed developmental and molecular time-course characterisations in *A. arabicum*, particularly with reference to valve-margin-specific and dehiscence zone identity genes (Avino et al. 2012; Lenser et al. 2018).

Our microscopy approaches indicated fundamental differences in the structure and organisation of fruit valve layers. Combining SEM with non-invasive and non-destructive methods such as SRXTM provides new possibilities for the visualisation and analysis of the external and internal structure of fossil and extant plant material (Friis et al. 2014; Benedict et al. 2015). Such imaging solves problems associated with cutting or histological sectioning by minimising the introduction of artefacts (e.g., tears, gaps), and allows multiple planes of section through the same specimen to be acquired at high quality resolution (Smith et al. 2009; Adams et al. 2016). In addition to the absence of a dehiscence zone, the identification of valve tissue morphology was of particular significance at the adaxial fracture surface. As structural differences in the valves are associated with the mode of fracturing, the IND fruit tissue organisation could perhaps be interpreted as a remnant of a once active dehiscence apparatus (Fahn and Zohary 1955). Differential timing of anisotropic growth patterns, in turn coordinating the development of fruit growth and maturation leading to dispersal, may also influence the anatomical organisation and material properties observed between DEH and IND fruit. The dimorphic fruit in *A. arabicum* therefore provide an ideal system with which to model post-fertilisation gynoecium growth and shape formation, as has been demonstrated in the monomorphic *Capsella rubella* (Eldridge et al. 2016), to identify tissue-specific activities required to obtain the two distinct morphologies.

Hypothesis for fracturing biomechanics and dispersal in natural conditions

The semi-arid environment in which *A. arabicum* grows is characterised by highly variable rainfall in space and in time, and therefore presents challenging climatic and edaphic conditions for plant growth (Arshad et al. 2019). At the macroscale this may include sporadic rain events, with high precipitation rates over relatively small spatial scales, while at the microscale, topographic factors and soil surfaces are thought to contribute to the variability of water availability (Kigel 1995). The contrasting morpho-anatomical and biomechanical properties of *A. arabicum* fruit contribute to bet-hedging adaptations for successful dispersal in the scree and steppe habitats of Anatolia (Arshad et al. 2019). Prior to dehiscence, the DEH fruit pericarp dries as the fruit approaches matu-

rity. Our working hypothesis is that the highly coordinated events causing tissue separation and endocarp lignification create spring-like tensions in the mature DEH fruit, the elastic energy from which is retained during the dry period until rain-induced (ombrohydrochory) impact events cause "pod-shattering" and M+ seed dispersal to occur. This is consistent with the passive, drying forces acting on microstructures that have been demonstrated during fruit and seed dispersal of other species (Elbaum and Abraham 2014). For example, the shedding of living twigs (in *Salix* spp. and *Populus* spp.) provides a reproductive mechanism (via twig dispersal and subsequent establishment in new habitats) that also relies on fracture mechanics. The relative roughness of the twig fracture surface in the genus *Salix* correlates with the classification of brittle and non-brittle species (Beismann et al. 2000).

Dehiscence upon wetting has generally been associated with plants adapted to arid environments (Gutterman 2002; Pufal et al. 2010), and the work of fracture is typically negatively correlated with moisture content (Farquhar and Zhao 2006). Tensions are created due to the differential drying of the parenchymatic and sclerenchymatic tissues of the pericarp, while degeneration of the middle lamellae of the separation layer cells (via cell wall degrading enzymes) forms a pre-determined breaking zone along the longitudinal axis. This, together with moisture-induced (hygrochastic) movements of fruit pedicels (Lenser et al. 2016), is thought to contribute to the distinct biomechanical fracture mechanism observed in DEH fruit. The mature IND fruit, in contrast, abscises from the mother plant in its entirety and thus has inherently different biotic and abiotic factors influencing its pericarp fracture biomechanics. Post-dispersal time-lapse data obtained during seedling establishment (not shown) suggest that IND fruit valve separation only occurs after completion of germination, as a result of radicle protrusion between two adjoining pericarp valves.

Conclusions

The species richness and divergent fruit shapes in the Brassicaceae provide an invaluable framework to address questions on seed and fruit dispersal. Here, we have shown that the dimorphic fruit fracture patterns in *Aethionema arabicum* are associated with distinct morpho-anatomical features influencing the deformation behaviours of the pericarp during opening. A distinct "separation layer" along the DEH fruit valve–replum boundary contributed to the multistaged fracture events leading to failure. In contrast, IND fruit were shown to possess a distinct endocarp layer with spirally thickened fibres linking its winged valves at the adaxial surface. This, and a lack of a dehiscence zone, mediate the more brittle material properties of IND fruit valves. The *A. arabicum* dimorphic system illustrates how biomechanics approaches can be successfully combined with internal and external imaging techniques, to understand the underlying causes for dispersal-related phenomena. The presented anatomical observations across a range of hierarchical levels contribute to our understanding of dimorphic structures and functions, and further support our knowledge of how these interact as bet-hedging adaptations to the physical environment. Together with recently published (Lenser et al. 2018; Wilhelmsson et al. 2019) and future molecular work into the mechanisms of the diaspore dimorphism in *A. arabicum*, the integration of biomechanics and imaging makes it an exciting time to study fruit- and dispersal-related properties by moving beyond *Arabidopsis* and other monomorphic plants.

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