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When the BRANCHED network bears fruit: how carpic dominance causes fruit dimorphism in Aethionema

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Summary

Life in unpredictably changing habitats is a great challenge, especially for sessile organisms like plants. Fruit and seed heteromorphism is one way to cope with such variable environmental conditions. It denotes the production of distinct types of fruits and seeds that often mediate distinct life-history strategies in terms of dispersal, germination and seedling establishment. But although the phenomenon can be found in numerous species and apparently evolved several times independently, its developmental time course or molecular regulation remains largely unknown. Here, we studied fruit development in Aethionema arabicum, a dimorphic member of the Brassicaceae family. We characterized fruit morph differentiation by comparatively analyzing discriminating characters like fruit growth, seed abortion and dehiscence zone development. Our data demonstrate that fruit morph determination is a 'last-minute' decision happening in flowers after anthesis directly before the first morphotypical differences start to occur. Several growth experiments in combination with hormone and gene expression analyses further indicate that an accumulation balance of the plant hormones auxin and cytokinin in open flowers together with the transcript abundance of the Ae. arabicum ortholog of BRANCHED1, encoding a transcription factor known for its conserved function as a branching repressor, may control fruit morph determination. Thus, we hypothesize that the plastic control of fruit morph ratio in Ae. arabicum may have evolved through the modification of a preexisting network known to control correlative dominance between shoot organs.

Significance statement

Although the production of two distinct types of fruits is often found in plants that thrive in unpredictably changing habitats, no work has been reported yet examining the developmental time course or molecular control of fruit dimorphism. Here, we discover the developmental time point of fruit morph determination in dimorphic Aethionema arabicum and identify molecular candidates that may mechanistically link fruit type determination to the developmental program governing primigenic dominance.

Introduction

Heterocarpy describes a phenomenon where at least two different types of fruits are produced on individual plants (Imbert 2002). It is often combined with heterospermy, the development of distinct types of seeds within such fruits, differing in their morphological and/or physiological properties (Baskin et al. 2014). Both phenomena (together referred to as fruit and seed heteromorphism) have evolved several times independently, occur in at least 18 angiosperm families and are particularly common in annual members of the Amaranthaceae, Asteraceae and Brassicaceae (Imbert 2002, Lu et al. 2013b). Due to their different phenotypic traits, the distinct fruit and seed morphs usually feature differential life-history strategies, thus enabling offspring survival at a wide degree of environmental conditions (Lu et al. 2010, Mandák and Pyšek 2001). Consequently, heteromorphism is generally considered as the morphological basis of a bet-hedging strategy to cope with unpredictably changing habitats (Abley et al. 2016, Philippi and Seger 1989, Venable and Lawlor 1980). In addition, heteromorphic fruit and seed morph development often responds plastically in response to certain factors, like plant density, soil moisture content or nutrient availability, resulting in the environmentally-dependent production of different morph ratios (Lu et al. 2013a, Mandák and Pyšek 1999, Sadeh et al. 2009). Although many heteromorphic species have been described and studied with respect to their morph-specific properties and potential adaptive implications (Afonso et al. 2014, Baskin et al. 2014, Dubois and Cheptou 2012, Imbert 2002, Lenser et al. 2016, Lu et al. 2015, Venable et al. 1995, Yamaguchi et al. 1990, Yang et al. 2014), little is known so far about the time course and molecular control of heteromorphic fruit and seed development.

Aethionema arabicum is an annual member of the Aethionemeae, the earliest diverging tribe within the Brassicaceae family (Al-Shehbaz et al. 2006, Franzke et al. 2011). The species is dimorphic, forming two distinct fruit morphs that differ in size, seed number, septum formation, fruit dehiscence and abscission, and two distinct seed morphs with marked differences in surface structure, mucilage production and germination behavior (Lenser et al. 2016, Solms-Laubach 1901). Fruit morphs are not distributed evenly throughout the Ae. arabicum plants but the large, many-seeded, dehiscent morph is predominantly produced on main shoots while an increasing preference for the production of the small, single-seeded, indehiscent morph has been observed on higher-order side-branches (Lenser et al. 2016). In addition, overall fruit morph ratio has been shown to respond to various environmental parameters with a particularly strong shift towards a higher production of the dehiscent morph in response to the removal of shoot branches (Lenser et al. 2016, Zohary and Fahn 1950).

Both findings point towards a possible connection between fruit morph determination and correlative dominance relationships in which the growth of one shoot organ is controlled by another (Bangerth 1989, Snow 1925).

Probably the most prominent and best-studied of these phenomena is apical dominance, in which an actively-growing shoot apex inhibits the outgrowth of axillary buds, such that excision of the apex permits bud activation and formation of branches (Cline 1997, Leyser 2005)...Another example of correlative dominance is the interaction between developing fruits, in which early developing fruits suppress the growth of later developing pollinated ovaries (Bangerth 1989, Smith and Samach 2013). This phenomenon is driven by the seeds, such that pathenocarpic fruit exhibit no dominance, and thus can be considered as 'carpic dominance' (Walker & Bennett, in press). It results in a spectrum of effects from mild growth inhibition through to fruitlet abscission, depending on the species. Bangerth (1989) proposed that the correlative relationships between apices and fruits are facets of the same fundamental phenomenon of 'primigenic dominance' in which early-developing organs inhibit the growth of later-developing ones, and that these processes had a common regulatory mechanism. For instance, in both phenomena, inhibitory effects can be abolished by physical removal of the dominant structures (e.g. shoot tips or early developing fruits), while application of the hormone auxin to the cut site reverses the loss of inhibition (Bangerth 1989, Gruber and Bangerth 1990, McCallum 1905a, McCallum 1905b, Quinlan and Preston 1971, Snow 1925, Thimann and Skoog 1933, Thomas et al. 2003). The growth of an organ is also tightly correlated with its ability to export of auxin (Bangerth, 1989). Collectively, these data suggest that auxin export, and its subsequent rootward transport, is the key signal mediating dominance relationships between shoot organs (Bangerth 1989, Domagalska and Leyser 2011, Smith and Samach 2013)

Beyond the involvement of auxin, little is known regarding the molecular mechanisms that mediate carpic dominance. Conversely, much research has been directed at understanding the mechanism by which auxin regulates shoot branching. Apical dominance forms part of a wider shoot branching regulatory network, in which internal developmental cues and environmental factors such as light intensity and quality, nutrient availability, and planting density are integrated together through hormonal signalling networks (Domagalska and Leyser 2011, Rameau et al. 2015)(Ferguson and Beveridge 2009, Ongaro et al. 2008, Thomas and Hay 2011). Strigolactones and cytokinins are root-derived hormonal signals that play

central roles in the regulation of branching, respectively repressing and promoting bud outgrowth (Gomez-Roldan et al. 2008, Müller et al. 2015, Pillay and Railton 1983, Teichmann and Muhr 2015, Umehara et al. 2008, Wickson and Thimann 1958). Recently, feeding and defoliation experiments in pea have identified sucrose as an additional promoter of branching that seems to be especially important during the early stages of axillary bud release (Mason et al. 2014). Members of BRANCHED1 (BRC1) family of TB1 CYCLOIDEA PCF (TCP)-type transcription factors have been proposed to act central integrators of branching control (Aguilar-Martínez et al. 2007). In several species, BRC1 expression correlates with bud inhibition (Aguilar-Martínez et al. 2007, Braun et al, 2012) and strigolactones promote BRC1 expression, while cytokinin and sugar inhibit BRC1 expression (Dun et al, 2012; Mason et al, 2014). However, recent data indicate that BRC1 expression alone is neither necessary nor sufficient for bud outgrowth inhibition and may instead be involved in determining the threshold needed for bud activation. (Seale et al. 2017). The hormone abscisic acid (ABA) occurs in high concentrations in both buds and fruits undergoing inhibition and may contribute to the inhibition of growth in both systems (Bangerth 1989, Chatfield et al. 2000, Emery et al. 1998, Gocal et al. 1991, González-Grandío et al. 2013, Ruttink et al. 2007).

In this study, we investigated dimorphic fruit development in Ae. arabicum with a special focus on the developmental time course and potential determining factors of morph differentiation. Comparative developmental analyses of fruit growth, septum rupture, dehiscence zone differentiation and seed abortion showed that the onset of morph differentiation is in late flowers approximately two days after anthesis. We further demonstrated that fruit morph determination is a ‘last-minute’ decision which takes place in early flowers shortly after anthesis and directly before the first morphotypical differences start to occur. Although this temporally coincides well with pollination, no obvious connection between fruit morph determination and fertilization-related parameters could be detected. Instead, we present evidence based on hormone and gene expression analyses that the regulatory network determining fruit morph identity in Ae. arabicum and other dimorphic Aethionema species might be a modified version of the regulatory network that usually controls carpic dominance.

Results

Fruit morph differentiation first becomes visible in late flowers

To study the time course that underlies the differential development of the two distinct fruit morphs in Ae. arabicum, a thorough morphological analysis of the different stages of fruit development has been performed. No morphotypical differences could be observed in flower buds and early flowers that had just opened (Figure 1 A,B). First signs of morph differentiation became obvious two days after anthesis when inside those flowers that would later produce dehiscent fruits rapid fruit growth became visible (Figure 1 C). In flowers that would later produce indehiscent fruits, however, fruits remained small and completely covered by the outer floral organs (Figure 1 G). From this stage on, differences in size and shape of the two fruit morphs remained clearly pronounced (Figure 1 D-F,H-J). Apart from these differences, however, fruit development proceeded quite simultaneously between the two morphs. Approximately three to four days after anthesis, the floral organs of the outer three whorls abscised (Figure 1 D,H). Afterwards fruit growth continued constantly until approximately ten days after anthesis fruits reached their final size (Figure 1 E,I). It took another 3 weeks until fruits were completely dry, contained ripe seeds and would either open (dehiscent morph) or fall off the plant (indehiscent morph) upon mechanical stimulation (Figure 1 F,J). Taken together, we show that during fruit development of Ae. arabicum, first morphological differences between the two morphs became visible in late flowers two days after anthesis.

Fruit morphs show no differences in fertilization-related traits

Since the appearance of first differences between morphs is temporally close to fertilization, we wanted to investigate if differences in fertilization may accompany or even cause the onset of morph differentiation. However, fertilization already takes place in early flowers where it is not yet possible to morphologically discriminate between the two morphs (Figure 1). To overcome this problem, we drew advantage from the fact that fruit morphs are not distributed evenly throughout the plant. It has been shown previously that under our standard greenhouse conditions and using Ae. arabicum accession ES1020, more than 95% of fruits produced on 2nd-order branches belong to the indehiscent morph while contrarily, the constant removal of all newly developing side branches from a plant induces the formation of more than 95% of dehiscent fruits on the remaining main branch (Figure S1) (Lenser et al. 2016). Thus, in all following comparative analyses, the distinct developmental stages were harvested either from

2nd-order branches and assumed to develop into the indehiscent morph or from plants without side branches and assumed to develop into the dehiscent morph.

One parameter of fertility is the number and integrity of pollen grains produced on anthers. We thus investigated pollen stainability with Alexander's solution, which discriminates developmentally intact pollen (red) from aborted pollen (green) (Alexander 1969), and found no difference between the two flower types with respect to pollen abundance or integrity (Figure 2 A,B). Likewise, no morphotypical difference could be detected with respect to pollen tube growth which has been investigated by aniline blue staining (Figure 2 C,D). In the context of this analysis it was further noticed that gynoecia that will develop into indehiscent fruits not only contain several ovules but that even pollen tubes seem to make contact with more than one of these ovules (Figure 2 E,F). This is notable given that mature indehiscent fruits contain only a single seed (Lenser et al. 2016), and indicates that during the development of indehiscent fruits all but one ovule will be systematically aborted at some stage of development. Indeed, when late flowers of the indehiscent morph were analyzed, only one ovule showed obvious growth indicating seed development (Figure 2 G,H). Since the internal position of this developing seed varied between different flowers, the decision which of the ovules will develop further seems to be positionally independent or even stochastic.

Pollen integrity and pollen tube growth alone do not show that flowers of the two morphs are equally fertile. To directly compare morph-specific fertilization capability, we emasculated flowers on two comparable branches of ten plants and pollinated the isolated pistils with pollen from flowers of the dehiscent or indehiscent morph, respectively. Analyzing the fruits developing from these hand-pollinated pistils did not reveal any difference in terms of fruit number or composition (Figure 3 A). Of all emasculated flowers, only about 25% developed into intact fruits that all belonged to the dehiscent morph. The remaining flowers either got aborted directly after emasculation (~60%) or during later fruit development (~15%) probably due to damage during emasculation or unsuccessful hand-pollination. This data indicates that there is no morph-specific difference in fertilization capability of Ae. arabicum pollen.

In most angiosperm species, fruit growth is initiated by signals derived from the developing seeds and ovaries will become aborted in the absence of fertilization (Ozga and Reinecke 2003, Sotelo-Silveira et al. 2014). However, the development of parthenocarpic (seedless) fruits without fertilization could be induced by removal of the apical shoot tip in pea plants (Carbonell and García-Martínez 1980). We found the same to be true in Ae. arabicum: Emasculated flowers did not show any signs of fruit growth and became aborted on normally

growing control plants (Figure 3 B). However, the removal of all growing shoot tips together with all residual flowers and fruits lead to varying degrees of fruit development in approximately 60% of emasculated flowers (Figure 3 B,C). To assess morph-affiliation of parthenocarpic fruits, lignin staining was performed on fruit cross-sections to check for the presence or absence of a dehiscence zone at the valve-replum border. Parthenocarpic fruits with as well as without a dehiscence zone were detected (Figure 3 D,E), indicating that even in the absence of fertilization, both fruit morphs can be produced and thus, morph determination happens independently of fertilization.

Seed abortion during indehiscent fruit development first becomes visible in late flowers

Prompted by our finding that seeds seem to become systematically aborted during indehiscent fruit development in Ae. arabicum, we studied the time course of this phenomenon in more detail. Pistils of both morphs and of different developmental stages were fixed and cleared and the number of ovules/seeds was determined (Figure 4). Our results show that for the dehiscent morph, gynoecia within buds and early flowers always contained four to six ovules (Figure 4 A,B,C). During later stages, the number of developing seeds decreased gradually and became more variable resulting in a wide distribution of one to six seeds in late fruits with most fruits containing three to four seeds (Figure 4 A,D-F). For the indehiscent morph, however, the great majority of gynoecia within buds and early flowers contained only four ovules (Figure 4 A,G,H). Although this may indicate an early morph-specific difference we consider it as more likely that this difference is just a consequence of our sampling strategy since floral structures developing on main branches are in general much bigger compared to those on 2nd-order branches (compare scale bars of Figure 4 B,C with Figure 4 G,H). As indehiscent development proceeded, there was a sharp drop in seed number starting in late flowers and completed in early fruits which all contained only a single seed (Figure 4 A,I-K). This indicates that at the same time in late flowers when the first external differences between morphs become visible also the developmental program guiding seed development becomes morph specific.

Developmental time course of internal fruit patterning

To study how differences in internal structures become established during Ae. arabicum fruit morph development, cross sections of different developmental stages were stained with a safranin/astra blue solution and examined microscopically (Figures 5, S2). Like in the previous analyses, no morphotypical differences could be detected in buds and early flowers

(Figure 5 A,B,G,H). In contrast to the anatomy of mature indehiscent fruits, their early developmental stages not only resembled the dehiscent morph in containing more than one ovule but also in the presence of a septum. First internal differences occurred, again, in late flowers where the asymmetric growth of one single seed in the indehiscent morph pushed the septum towards the side of the opposing seed chamber, presumably resulting in the rupture of the septum and fusion of the two locules (Figure 5 C,I). At the same time, also first indications of cell division and differentiation marked the onset of dehiscence zone differentiation exclusively in the dehiscent morph (Figure S2 C,I). Dehiscence zone differentiation proceeded gradually throughout fruit development, becoming completed in late fruit stages with the lignification of cells of the endocarp layer b and the lower replum (Figures 5 D-F, S2). At this stage, blue stained separation layer cells framed by red stained lignified cells clearly indicated the predetermined site of tissue separation in the dehiscent morph, while a closed band of lignified cells was found to surround the single locule of indehiscent fruits, thus preventing fruit opening to occur (Figures 5 F,L, S2) (Lenser et al. 2016).

Morph determination happens in early flowers

All our developmental analyses revealed that first signs of morph-specific differentiation during Ae. arabicum fruit development become visible in late flower stages (Figures 1, 4, 5, S2). The question remained, however, when the fate of a flower to produce the one or the other fruit morph becomes determined. To answer this question, we first investigated as to whether the effect that the removal of side branches induces the development of dehiscent fruits on main branches (Lenser et al. 2016) (Figure S1) can also be observed for 2nd-order branches. Indeed we found that the removal of an increasing number of branches can shift the fruit morph ratio on 2nd-order branches from 98% indehiscent fruits in untreated plants to more than 80% dehiscent fruits on plants where all branches except for a single 2nd-order branch had been removed (Figure 6 A). Side-branch removal thus apparently induces dehiscent fruit development throughout the whole plant.

To determine the exact time point of morph determination, we treated plants (n=15) by removing all branches except for four 2nd-order branches. From these remaining branches, we removed all fruits, flowers and buds except for the five biggest flower buds (branch 1), five early flowers (branch 2), five late flowers (branch 3), and five early fruits (branch 4). For five control plants, we marked five structures of the respective developmental stage on four 2nd-order branches without removing any plant parts. As we expected from our previous results,

all marked structures on the control plants developed into indehiscent fruits (Figure 6 E, 'control' charts). However, on plants undergoing side-branch removal, 63 of 64 fruits that were formed from buds belonged to the dehiscent morph, indicating that at this developmental stage morphs had not yet been determined and could thus be influenced by internal or external factors (Figure 6 B,E). On the same plants, still 47 of 67 fruits developing from early flowers belonged to the dehiscent morph, whereas only indehiscent fruits developed from late flowers and early fruits (Figure 6C-E, early fruits not shown). In a previous study, the Ae. arabicum ortholog of the dehiscence zone identity gene INDEHISCENT (AearIND) has been shown to be differentially expressed between the two fruit morphs (Lenser et al. 2016). Investigating AearIND expression levels at different developmental stages via quantitative reverse transcription PCR (qRT-PCR) revealed that no morph-specific differences can be detected in bud stages and differential expression only becomes apparent in late flowers (Figure 6 F). Taken together, this data indicates that morph determination happens in early flowers, just before first morphotypical differences occur. In late flowers, morph determination already happened and thus cannot be influenced by internal or external factors anymore.

Fruit morph ratio reacts to parameters known to influence primigenic dominance

After having established the time point of fruit morph decision, we wanted to identify potential molecular determining factors and thus investigated a possible connection between fruit morph decision and primigenic dominance. Since the dehiscent fruit morph of Ae. arabicum occurs primarily on the main shoot and can be induced by the removal of side branches (Figures 6, S1) (Lenser et al. 2016, Zohary and Fahn 1950), it develops preferentially under conditions which are typical for the formation of dominant plant organs (Bangerth 1989, McCallum 1905a, McCallum 1905b). Auxin can reverse the decapitation effect in branching control and correlative fruit inhibition (Bangerth 1989, Thimann and Skoog 1933), and we thus investigated if a similar reversion can be observed for the debranching-induced development of dehiscent fruits. Indeed, the application of increasing auxin concentrations to the cut surface progressively decreased the portion of dehiscent fruits that were produced on 2nd-order branches in response to a drastic cutting treatment (Figures 7 A, S3). This prompted us to further test the effect of defoliation and shading on fruit morph production, two factors known to inhibit shoot branching (Mason et al. 2014, Smith and Whitelam 1997). Both treatments resulted in a reduced production of dehiscent fruits when compared to untreated control plants (Figures 7 B,C, S3). These findings corroborate the existence of a connection between the control of fruit morph decision and primigenic

dominance with a preferred induction of dehiscent fruits under growth promoting conditions, and of indehiscent fruits under inhibitory conditions.

Molecular determinants of fruit morph differentiation

Several plant hormones are known to play a role in the regulation of primigenic dominance in the context of shoot branching. To investigate if the same hormones may also influence fruit morph differentiation, we determined the levels of bioactive auxin (indole-3-acetic acid (IAA)), different forms of cytokinins, and ABA directly before morph determination (flower buds shortly before anthesis), directly after determination (late flowers) as well as in late fruits when morph differentiation is complete. Strigolactones have not been included in this analysis because it is technically not possible to measure these compounds just yet (Tarkowská et al. 2014). Hormone analysis revealed that IAA and ABA, which are both known as growth suppressors, showed a peak in abundance in late flowers (Figure 8 A). This peak was significantly higher in flowers of the indehiscent compared to the dehiscent morph. Cytokinin, which is known as a growth activator, on the other hand showed the exact opposite pattern with a strong peak of abundance exclusively in flowers of the dehiscent morph. To investigate if these morph-specific differences in hormone levels reflect a functional connection with fruit morph determination, we spray-treated flowering Ae. arabicum plants with a synthetic auxin (2,4-dichlorophenoxyacetic acid: 2,4-D), cytokinin (6-benzylaminopurine: BAP), ABA, and a synthetic strigolactone analogue (GR24). 2,4-D application led to a strong shift towards a higher ratio of indehiscent fruits while BAP treatment significantly enhanced the portion of dehiscent fruits (Figures 8 B, S4), indicating that these hormones indeed directly influence fruit morph determination. However, no significant change in fruit morph ratio could be detected in response to GR24 and ABA application (Figures 8 B, S4) indicating that at least at the applied concentrations, these hormones alone are not sufficient to influence fruit morph decision.

Gene expression analysis applying qRT-PCR revealed that the Ae. arabicum ortholog of the branching suppressor BRC1 (AearBRC1) showed an expression peak in flowers of the indehiscent but not the dehiscent morph (Figures 9 A, S5, Table S1). Further analyses demonstrated the presence of similar expression patterns for two Ae. arabicum genes encoding putative cytokinin oxidase/dehydrogenase (CKX) enzymes as well as the orthologs of MORE AXILLARY GROWTH 1 (MAX1), MAX2, MAX3, and MAX4 (Figures 9 B-G, Figure S5). CKX proteins catalyze the irreversible degradation of cytokinins in a diverse set of plants (Schmülling et al. 2003) and are thus likely responsible for causing the low cytokinin level in

flowers of the indehiscent morph. MAX orthologs promote strigolactone biosynthesis and signaling (Domagalska and Leyser 2011) implying that strigolactone levels may be high around the time of morph determination in the indehiscent but not the dehiscent morph. Taken together, these data suggest that molecular key factors regulating primigenic dominance may also be involved in the control of fruit dimorphism in Ae. arabicum, with factors that promote dominance also promoting the formation of dehiscent fruits and factors that promote inhibition promoting the formation of indehiscent fruits.

Fruit dimorphism likely evolved twice within the Aethionemeae (Lenser et al. 2016). Thus, the question arises whether the regulation of fruit morph determination might be similar between Ae. arabicum and other dimorphic Aethionema species. Therefore, we extended the hormone spraying analysis to Ae. carneum and Ae. heterocarpum (same evolutionary origin of fruit dimorphism as Ae. arabicum), and Ae. saxatile (independent evolutionary origin of fruit dimorphism). Our results show that, like for Ae. arabicum, the ratio of indehiscent fruits within the plants increases towards higher order branches for all three species, although overall proportions of the two fruit types are rather species specific (compare control groups in Figure 10). Furthermore, all three species respond in a similar way to auxin and cytokinin application as Ae. arabicum, that is with higher percentages of indehiscent fruits in response to auxin and higher ratios of dehiscent fruits in response to cytokinin (Figures 10, S6). This indicates that the molecular control of fruit dimorphism is similar in all Aethionema species under study although it likely traces back to two independent evolutionary origins.

Discussion

Fruit morph determination in Aethionema arabicum is a ‘last-minute’ decision

Prominent differences between the two Ae. arabicum fruit types are the presence of a specific fruit opening mechanism exclusively in the dehiscent morph as well as differences in fruit size and shape (Lenser et al. 2016). Fruit opening within the Brassicaceae is mediated by the presence of specialized cells forming a dehiscence zone which induces controlled tissue separation upon maturity (Spence et al. 1996). A recent study in A. thaliana showed that cell differentiation related to dehiscence zone formation is initiated in open flowers after pollination (van Gelderen et al. 2016), which closely corresponds to the developmental stage where first signs of dehiscence zone differentiation were also detected in the dehiscent morph of Ae. arabicum (Figures 5, S2). Likewise, it has been demonstrated that fruit growth and shape determination of Brassicaceae fruits mainly happens during post-fertilization

development (Eldridge et al. 2016, Ferrandiz et al. 1999, Langowski et al. 2016). This shows that fruit morph determination in Ae. arabicum happens directly before the onset of those developmental processes most important for morph differentiation and thus, developmentally, at the latest possible moment. This 'last-minute' decision could be interpreted as a compromise between developmental necessity (last chance to alter important fruit parameters) and the fitness advantage of being able to plastically adjust fruit morph production at the latest possible moment to short-term changes in environmental conditions. Interestingly, we detected morph-specific expression of AearIND only after morph determination in late flowers, while comparative analyses between other Brassicaceae species forming either dehiscent or indehiscent fruits revealed differential gene expression patterns of dehiscence zone identity genes already in flower buds (Avino et al. 2012, Mühlhausen et al. 2013). Also in A. thaliana, valve margin-specific expression of IND can already be detected in flower buds (Sorefan et al. 2009), indicating that gene expression patterns defining dehiscence behavior of Brassicaceae fruits are usually established well before actual tissue differentiation takes place. It will be interesting to study spatial expression patterns of Ae. arabicum dehiscence zone identity genes in stages prior to morph determination to see if the dehiscent character of such early stages also goes along with valve margin-specific expression of respective genes.

Evolutionary aspects of fruit dimorphism and morph plasticity

Dehiscent, two-locular capsules are the typical fruits produced by members of the Brassicaceae (Al-Shehbaz 2011). They are considered to represent the ancestral fruit form within the family, although indehiscent fruits evolved many times independently (Hall et al. 2002, Mühlhausen et al. 2013). Also within the Aethionemeae, phylogenetic data suggest dehiscence as the basal character state while postulating two independent origins of indehiscence (Lenser et al. 2016). However, the Aethionemeae mark a special case in the evolution of indehiscence because in the context of dimorphism, indehiscent fruits are not formed exclusively but rather develop as an additional alternative to dehiscent fruits on the same plants. Our data on Ae. arabicum fruit development shows that in all developmental stages prior to fruit morph determination (buds and early flowers), flowers exhibit typical features of the dehiscent morph, like the presence of four to six ovules and a septum (Figures 2, 4, 5). Only after the final decision, these structures are degraded in order to adopt the identity of an indehiscent fruit. These findings corroborate the hypothesis that dehiscent fruits

are the ancestral fruit form of the Aethionemeae while indehiscent fruits are produced by a derived developmental program.

In some heteromorphic plant species, fruit morph ratio has been reported to respond plastically to changes in certain environmental conditions (Baker and O'Dowd 1982, de Clavijo and Jiménez 1998, Imbert and Ronce 2001, Lu et al. 2013a, Mandák and Pyšek 1999, Sadeh et al. 2009). In contrast to the production of a fixed fruit morph ratio, a classical bet-hedging strategy that significantly decreases the arithmetic mean fitness in favor of a reduced fitness-variation over time (Evans and Dennehy 2005, Philippi and Seger 1989), such a plastic regulation of fruit morph production may be advantageous because the loss in overall fitness is probably less severe. We showed that in the case of Ae. arabicum, the ratio of indehiscent fruits increases under adverse growth conditions (defoliation, shading) (Figure 7 B, C). This supports our previous idea that, with respect to life-history strategy, the multi-seeded dehiscent fruit morph that produces quick and uniformly germinating seeds represents a high-risk strategy that only pays off under beneficial environmental conditions (Lenser et al. 2016). The single-seeded indehiscent fruit morph whose seeds show delayed and fractionated germination, on the other hand, was thought to represent a low-risk strategy to ensure survival under unfavorable conditions (Lenser et al. 2016). In addition, indehiscent fruits exhibit less seed mass as well as overall biomass compared to dehiscent fruits. If outer conditions are hostile and resources are limited, an increased production of indehiscent fruits thus likely represents an adaptive advantage, also from an energetic perspective.

Plastic regulation of a phenotypic trait is only possible if the environmental changes are, at least to some extent, predictable through the presence of certain environmental cues forecasting future conditions (Abley et al. 2016, Simons 2011), and if a complex network including molecular sensors, signal transmission and gene regulatory pathways for the detection of such cues is available. Fruit heteromorphism evolved many times independently (Cruz-Mazo et al. 2009, Fernández et al. 2001, Imbert 2002), raising the question as to how such plasticity could emerge repeatedly. Shoot branching plasticity in response to various environmental factors is well known (Domagalska and Leyser 2011, Rameau et al. 2015) and there is at least some evidence that also the strength of carpic dominance may be modulated by temperature, light, and nutrient availability (Bangerth 2000). Based on our data, we thus propose that in the case of Ae. arabicum, the preexisting network that regulates carpic dominance has been modified to produce fruit dimorphism, with the underlying environmental plasticity carried over into the production of dimorphic fruit (Figures 7-10). Dehiscent fruits are shown to develop preferentially under growth promoting conditions while

indehiscent fruits are primarily produced under growth inhibitory conditions. These parallels in terms of environmental response are consistent with similar patterns of hormone response and gene expression between primigenic dominance and fruit morph determination. Dehiscent fruits would thus be equivalent to normal dominant fruits, and their effect on indehiscent fruit would be equivalent to the growth-inhibition normally observed in dominated fruits. This is consistent with the depressed growth rate and high abscission potential seen in the indehiscent morph, similar to fruits undergoing correlative inhibition (Bangerth, 1989). There are two key differences to standard carpic dominance in Ae. arabicum. Firstly, instead of variable growth inhibition, indehiscent fruits undergo a precise level of growth inhibition that creates a quantitatively distinct morph. Secondly, instead of complete abortion before abscission, the indehiscent fruits retain a single viable seed. Indehiscent fruit therefore undergo a precise and highly-specific developmental program that produces small but viable fruit. Interestingly, our data on Ae. saxatile, an Aethionema species which independently evolved fruit dimorphism, indicates that this specific modulation of carpic dominance probably evolved at least two times independently. More research, also including more distantly related di- or heteromorphic species, is needed to investigate if alteration of the carpic dominance pathway may be commonly found during the evolution of fruit heteromorphism.

The molecular regulation of fruit morph determination

A clear challenge is to understand how changes in the carpic dominance regulatory program might lead to fruit dimorphism. Currently, due to the lack of transgenic technology in Ae. arabicum, functional data about the molecular regulation of fruit morph determination are limited to hormone application experiments (Figures 8, 10). Furthermore, at a mechanistic level, carpic dominance is not well characterized in any species. Nevertheless, we can offer some speculation, based on evidence derived from measuring hormone and gene expression levels (Figures 8, 9) and on the assumption that the regulatory network controlling carpic dominance is closely related to the shoot branching regulatory network (Walker & Bennett, in press). This hypothesis has been previously proposed because of striking similarities between these phenomena in terms of auxin action and transport (Bangerth 1989) and is further supported by the fact that both processes react in similar ways to environmental factors (Bangerth 2000).

Consistent with previous suggestions, we propose that a conserved core pathway could underlie all primigenic dominance phenomena (Bangerth, 1989; Walker & Bennett, in press),

including fruit morph determination in Aethionema species. In this basic regulatory module, high auxin export from developing organs leads to their growth and dominance in the shoot system and conversely low auxin export leads to inhibition (Bangerth, 1989). Low auxin levels in flowers of the dehiscent compared to the indehiscent morph (Figure 8) could be indicative of this high auxin export, By comparison with shoot branching, we propose that low cytokinin and high strigolactone levels in indehiscent fruit might act to inhibit this auxin export, thereby preventing the growth of the fruit. While a direct effect on fruit morph determination of strigolactone was not observed (Figure 8B), our gene expression analysis (Figure 9) clearly points towards a temporally restricted activation of strigolactone signaling during indehiscent morph development. Further experiments, including optimized strigolactone treatments, will be required to understand the functional relevance of this signalling peak.

We propose that the key factor in generating the precise dimorphism of Ae. arabicum fruits may be the high expression of the. BRC1 transcription factor in indehiscent fruits (Figure 9A). Recent data suggest that BRC1 is particularly important for generating the binary 'switching' behaviour of axillary buds, in which buds normally either remain completely inhibited, or become completely active. By contrast, brc1 buds display a continuous spectrum of activation states in response to auxin (Seale et al. 2017). BRC1 expression in fruits might thus be especially important for a producing a binary readout of hormonal signals, allowing robust switching between fruit types, instead of a morphological continuum of fruit phenotypes (Lenser et al. 2016, Seale et al. 2017). As with shoot branching, the effect of cytokinin and strigolactone on fruit development might also be integrated through modulation of BRC1 expression. So far BRC1 has only been reported to play a role in the repression of branching and floral transition, with gene expression being exclusively detected in dormant axillary buds (Aguilar-Martínez et al. 2007, Niwa et al. 2013). An intriguing possibility is therefore that BRC1 has been specifically recruited to regulate fruit development in Aethionema species. Examination of BRC1 expression in fruits of other Brassicaceae species could begin to test this idea, and targeted gene knockout or overexpression analyses of AeBRC1 will be very informative once technically feasible.

The exact role of ABA during the regulation of shoot branching is not very well understood (González-Grandío et al. 2013). It has been shown to act as a branching repressor in several species and has been implicated to play a role during shade response, possibly being

controlled by auxin, BRC1, or a combination of both (Begonia and Aldrich 1990, Chatfield et al. 2000, Eliasson 1975, Emery et al. 1998, Galoch et al. 1998, Gocal et al. 1991, González-Grandío et al. 2013, Knox and Wareing 1984, Ruttink et al. 2007, Tucker 1976). ABA is also found in high concentrations in inhibited fruits (Bangerth 1989), and we observed that in Ae. arabicum, ABA levels specifically increase in flowers of the indehiscent morph (Figure 8 A), but that fruit morph ratio did not change in response to ABA spray-application (Figure 8 B). We thus propose that ABA does not determine fruit morph, but as in shoot branching acts downstream of auxin (and possibly BRC1) to promote growth inhibition in indehiscent fruits.

Several studies indicate that crosstalk between auxin and cytokinin signaling is involved in various aspects of fruit development, like apical/basal patterning of the gynoecium, regulation of inflorescence meristem activity, and dehiscence zone differentiation (Bartrina et al. 2011, Marsch-Martinez et al. 2012, Sehra and Franks 2015, Sorefan et al. 2009). Future research will elucidate if on top of their role in fruit morph decision, auxin and cytokinin may also be directly involved in morph differentiation. Cytokinin, for example, is known as a positive regulator of gynoecium size and ovule development (Bartrina et al. 2011). It is thus tempting to speculate that in Ae. arabicum, higher cytokinin levels during dehiscent fruit development may be responsible for the increased final fruit size while lower cytokinin levels during indehiscent fruit development may be involved in the process of seed abortion.

Experimental procedures

Plant material and growth conditions

Experiments were conducted on Ae. arabicum (L.) A.DC. plants of accession ES1020 (obtained from Eric Schranz, Wageningen), Ae. carneum (Banks & Sol.) B.F. plants of accession KM2496, Ae. heterocarpum J.G. plants of accession KM2491, and Ae. saxatile (L.) R.Br. plants of accession OSBU 95-0245-10-00 (all obtained from Klaus Mummenhoff, Osnabrück). Plants were grown on soil under long-day conditions (16 h light/20°C and 8 h dark/18°C) in a greenhouse. For comparative analyses of the two morphs of Ae. arabicum, buds, flowers, or fruits were harvested either from 2nd-order branches of plants that grew undisturbed (indehiscent morph) or from the main branch of plants where all newly emerging side branches had constantly been removed during plant development (dehiscent morph).

Lignin staining and microscopic analysis

Flower and fruit stages were fixed in FAA (2% formaldehyde, 5% glacial acetic acid, 60% EtOH, 0.1% Tween-20) at 4°C for 24 h, embedded in Paraplast (Carl Roth GmbH + Co. KG) and sectioned at 8 µm thickness on a Leica RM 2145 microtome. Thin-sections were de-waxed and stained for 2 min with safranin/astra blue (Sigma Aldrich Corporation) (Gerlach 1984), followed by microscopic analysis using a Leica DM5500 B microscope (Leica Microsystems GmbH). The imaging process was managed using the Leica Application Suite V 4.4 software. Images of whole flowers and fruits were acquired using a Leica M205 FA stereomicroscope (Leica, Germany) employing the Multifocus module of the Leica Application Suite software.

Pollen and pollen tube staining

To discriminate aborted from non-aborted pollen, mature anthers at the onset of dehiscence were dissected from early flowers, placed in a drop of Alexander staining solution (Alexander 1969) on a microscopic slide, covered with a coverslip and sealed with rubber cement (Fixogum). Slides were incubated at 40°C in the dark for 1h and analyzed with a Leica M205 FA stereomicroscope as described above.

Aniline blue staining of pollen tubes was performed essentially as described (Ishiguro et al. 2001). Flowers were collected and fixed in ethanol/acetic acid (3:1) for 2h at room temperature. After washing three times for 5 min. with deionized H_2O, pistils were dissected from flowers and incubated in 8 M NaOH overnight. Pistils were then washed three times for 1h with deionized H_2O and incubated with aniline blue solution (0.1% aniline blue in 0.1 M K_2HPO_4-KOH buffer, pH 11) for 3h in the dark. The stained pistils were placed in a drop of glycerol on a microscope slide, covered with a coverslip and observed under UV light excitation with a Leica DM5500 B microscope.

Analysis of ovule number

Flower and fruit stages were fixed in FAA (2% formaldehyde, 5% glacial acetic acid, 60% EtOH, 0.1% Tween-20) at 4°C for 24 h and washed three times with 70% EtOH. Afterwards they were cleared by incubating overnight in clearing solution (2.5g chloral hydrate per ml 30% glycerol). For flower stages, outer floral organs were removed under a stereomicroscope. Cleared pistils were placed in a drop of glycerol on a microscope slide, covered with a coverslip and observed with a Leica DM5500 B microscope.

Determination of fruit morph ratio and experimental growth treatments

For calculation of fruit morph ratio, the number of fully outgrown dehiscent and indehiscent fruits on the main branch, on 1st-order branches, and on higher-order branches (2nd or 3rd) was determined separately per plant. In case of Ae. carneum, plant architecture did not allow the clear distinction of a main branch and thus, only 1st- and higher-order branches were categorized. To exclude fruits that got aborted during development, only those containing at least one fully developed seed were included into the analysis. To study the effect of auxin on the "cutting-response", all branches except for one 2nd-order branch were removed from plants (n=9/treatment). Blocks of 1.2% agar containing 0.1M MES (2-(N-morpholino)ethanesulfonic acid) buffer pH 5.8, 0.1% dimethyl sulfoxide (DMSO) and the synthetic auxin analog 2,4-D (Duchefa Biochemie B.V., The Netherlands) at a concentration of 0 µM (control), 10 µM, or 1 mM, respectively, were immediately placed on top of the cut surface of the main branch and covered with aluminium foil. A fresh cut was made and a new agar block was applied daily. To study the effect of defoliation, all except for 10 leaves were removed from the main branch at the onset of flowering and all except for 3 leaves were removed from higher-order branches (1st, 2nd, and 3rd) once they started to flower (n=9 plants/treatment). For the shading experiment, plants (n=15/treatment) were either grown under full greenhouse illumination (~245 µE m^{-2} s^{-1}) or covered under a 2mm Makrolon® slide (Kunststoffhandel Rexin GmbH, Germany) (~220 µE m^{-2} s^{-1}). For hormone application, plants (n=10/treatment) were sprayed three times per week with deionized water containing 0.1% DMSO, 0.01% Silwet L-77, and 10 µM of the synthetic hormones 2,4-D (Duchefa Biochemie B.V., The Netherlands), BAP (TCI Deutschland GmbH, Germany), ABA (Sigma-Aldrich Chemie GmbH, Germany), or strigolactone GR24 (Chiralix B.V., The Netherlands), starting at the onset of flowering. Control plants were sprayed with the same solution but without the addition of any hormone.

Measurement of hormone levels

Levels of IAA, cytokinins and ABA were determined and compared between buds, late flowers and late fruits of both Ae. arabicum morphs. Cytokinins were extracted in modified Bieleski buffer (methanol/ water/formic acid, 15/4/1, v/v/v) using an internal standard of stable isotopically labelled cytokinins (0.5 pmol of cytokinin bases, ribosides, N-glucosides, 1 pmol of O-glucosides and nucleotides) and then purified using two solid phase extraction columns, a C18 octadecylsilica-based column (500 mg of sorbent, Applied Separations) and after that an Oasis MCX column (30 mg of mixed-mode sorbent with reversed-phase/cation-exchange properties, Waters) (Antoniadi et al. 2015, Dobrev and Kamínek 2002). IAA and

ABA were extracted using an aqueous solution of methanol (10% MeOH/H_2O, v/v) containing 10 pmol of [$^2H_6$]-(+)-cis,trans-ABA and [$^{13}C_6$]-IAA and purified using Oasis HLB columns (30 mg mL^{-1}, Waters) (Floková et al. 2014). Levels of the cytokinins, IAA and ABA were determined by isotope dilution method using ultra high performance liquid chromatography tandem electrospray mass spectrometry with stable isotope-labelled internal standards used as a reference (Floková et al. 2014, Svačinová et al. 2012).

Ortholog Identification

To identify orthologs of A. thaliana genes in Ae. arabicum we applied the method described previously (Lenser et al. 2016). In short, A. thaliana query sequences were searched with BLASTP (Altschul et al. 1990) against a plant-specific, custom-made protein database (Lenser et al. 2016). 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). Final neighbor-joining phylogenies were constructed using Quicktree-SD (Frickenhaus and Beszteri 2008, Howe et al. 2002) with 1,000 bootstrap samples and displayed and midpoint rooted with FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Gene expression analysis via quantitative RT-PCR

Total RNA from buds, flowers and fruits of the two morphs was extracted using QIAzol Lysis Reagent (Qiagen, Hilden, Germany). Extracts were digested with recombinant DNase I (Roche, Mannheim, Germany) followed by RNA clean-up using RNeasy Mini spin columns (Qiagen, Hilden, Germany). RNA integrity was analysed using the Plant RNA Nano assay of a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and absence of genomic DNA was tested by PCR using primers designed to amplify the AearIND gene (Table S2). cDNA synthesis was performed on 500 ng of DNase I digested RNA with Transcriptor RT (Roche, Mannheim, Germany) using oligo$(dT)_{20}$ primers. Quantitative RT-PCR reactions were performed in triplicate on an Mx 3005P cycler (Agilent Technologies, Santa Clara, CA, USA) using the Maxima ^{TM} SYBR Green/Rox qPCR Master Mix (2x) (Thermo Fisher Scientific, Waltham, MA, USA) with 1 µl of 1:5 diluted cDNA as template and 0.3 µM of forward and reverse primer (Table S2). The following thermal profile was used: 95°C for 10 min, 40 cycles of 95°C for 15 sec, 63°C for 30 sec, and 72°C for 30 sec. Raw data were analysed using LinRegPCR (Ramakers et al. 2003, Ruijter et al. 2009) to obtain sample C_T

values and PCR efficiencies (E) for each primer pair. C_T values for triplicate reactions were averaged and relative quantities of expression for each gene were calculated as $E^{(C_{TCal}-C_{TSOI})}$, where Cal is the sample with the lowest C_T value i.e. the highest expression level and SOI is the sample of interest. For normalisation, relative quantities of expression were divided by the geometric mean of the relative quantities of expression of three normaliser genes (AA53G00443, AA118G00007, AA75G00044), whose expression stability throughout all relevant tissues had been determined beforehand using geNorm (Vandesompele et al. 2002).

Accession numbers

Sequence data from Ae. arabicum can be found in CoGe database (https://genomevolution.org/coge/) under the following accession numbers: AearBRC1 (AA26G00528); AearCKX5 (AA31G00410); AearCKX7 (AA8G00229); AearIND (AA32G00014); AearMAX1 (AA32G01051); AearMAX2 (AA21G00053); AearMAX3 (AA21G00246); AearMAX4 (AA590G00001); genes used for qRT-PCR normalization (AA53G00443; AA118G00007; AA75G00044).

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Conflict of Interest

All the authors declare that they have no conflict of interests.

Short legends for Supporting Information

Figure S1: Experimental setup for local separation of fruit morphs.

Figure S2: Comparative analysis of dehiscence zone patterning during fruit development.

Figure S3: Change of fruit morph number in response to auxin treatment, defoliation and shading.

Figure S4: Change of fruit morph number in response to spray-treatment with synthetic hormone solutions.

Figure S5: Phylogenies of Ae. arabicum orthologs of A. thaliana genes.

Figure S6: Change of fruit morph number of different dimorphic Aethionema species in response to spray-treatment with synthetic hormone solutions.

Table S1: Species abbreviations used during phylogeny reconstruction.

Table S2: Overview about primers used for quantitative RT-PCR analysis.

Figure legends

Figure 1: Fruit development in Ae. arabicum. Two distinct fruit morphs develop from Ae. arabicum flowers: a larger, dehiscent (C-F) and a smaller, indehiscent morph (G-J). However, in buds (A) and early flowers (B) phenotypically only a single morph can be recognized. First morphotypic differences only become apparent in late flowers two days after anthesis, when fast fruit growth becomes visible in flowers producing dehiscent fruits (C) while fruits remain concealed by outer floral organs in flowers producing indehiscent fruits (G). Three to four days after anthesis, outer floral organs are shed from early fruits of both morphs (D, H) and fruit growth proceeds for approximately another week until fruits reach their final size (E, I). Afterwards, fruit maturation continues until approximately 30 days after anthesis, fruits are completely dry and readily open (F) or abscise (J) upon mechanical stimulation. Scale bars represent 1 mm.

Figure 2: Comparative analysis of pollen viability and pollen tube growth. Different parameters related to fertility have been comparatively analyzed in early (A-F) and late flowers (G-H) of the dehiscent (A,C,E) and indehiscent morph (B,D,F-H). No morphotypic differences with respect to abundance or stainability of pollen grains could be detected when mature anthers at the onset of dehiscence were treated with Alexander's stain (A,B). Aniline blue staining (C-H) revealed no difference in pollen tube growth (C,D) and in addition showed that pollen tubes in both types of flowers grew towards more than one ovule (indicated by white arrows in E,F), even though indehiscent fruits are known to develop only one ripe seed. In late flowers of the indehiscent morph only one of the ovules showed obvious

growth indicating seed development (indicated by white arrows in G,H). Since the internal position of this developing seed varied between different flowers, the decision which of the ovules will develop further seems to be positionally independent.

Figure 3: Fertilization capacity of pollen and parthenocarpic fruit development. A: Pie chart showing total numbers of dehiscent fruits (dark grey), indehiscent fruits (white), not fully developed fruits (mid grey), and early aborted fruits (light grey) developing from pistils that have been hand-pollinated with pollen derived from flowers of the dehiscent (left) or indehiscent (right) morph, respectively. No morph specific difference in fertilization capacity of pollen can be detected. B: Bar chart depicting the percentage of flowers that show fruit development after emasculation treatment. 15 flowers on three branches were emasculated on five normally growing control plants (■) and five plants where all other branches and floral structures had been removed by cutting (▲). Error bars represent standard deviation. C: Fruit pictures showing a typical fruit of the indehiscent (top left) and dehiscent (top right) morph and a continuum of parthenocarpic fruit phenotypes developing on one exemplary plant undergoing the "cutting treatment" as described in B. D-E: 8 µm cross-sections of parthenocarpic fruits at the valve-replum border stained with safranin and astra blue, which stains lignified cells in red and non-lignified cells in blue. Shown are exemplary pictures indicating that parthenocarpic fruits can belong to both, the dehiscent (D: dehiscence zone present) or the indehiscent (E: dehiscence zone absent) morph.

Figure 4: Timeline of ovule reduction during fruit development. A: Bubble chart depicting the reduction of ovule/seed number during the development of dehiscent (left side) and indehiscent (right side) fruits of Ae. arabicum. The size of each bubble is proportional to the number of biological replicates it represents. Twenty biological replicates have been scored per stage. B-K: Representative pictures of different stages of development towards dehiscent (B-F) and indehiscent (G-K) fruits that have been cleared with chloral hydrate. If present, outer floral organs have been removed. Shown are buds (B,G), early flowers (C,H), late flowers (D,I), early fruits (E,J), and late fruits (F,K). Scale bars represent 500 µm.

Figure 5: Comparative analysis of internal tissue patterning during fruit development. Shown are 8 µm cross-sections of buds (A,G), early flowers (B,H), late flowers (C,I), early fruits (D, J) and late fruits before (E,K) and after (F,L) the onset of lignification. Sections have been treated with safranin and astra blue, which stains lignified cells in red and non-

lignified cells in blue. The distinct stages depict the development towards dehiscent (A-F) or indehiscent (G-L) fruits, respectively. First differences between the morphs become apparent in the late flower stage. Scale bars represent 200 µm.

Figure 6: Fruit morph decision happens in early flowers. A: Bar chart showing that the ratio of dehiscent (dark grey) and indehiscent (light grey) fruits produced on 2nd-order branches is gradually reversed in response to the removal of an increasing number of branches from the plants (a: plants grow undisturbed; b: removal of main branch; c: removal of main branch and all 1st-order branches; d: removal of all branches except for five 2nd-order branches; e: removal of all branches except for one 2nd-order branch). Shown is the fruit ratio in percent ± standard deviation. When high-stringency cutting is applied to different floral stages growing on 2nd-order branches, it is shown that this treatment is able to completely reverse the fate of buds and of approximately 70% of early flowers (B,C,E). This indicates that although the same structures would develop into indehiscent fruits if grown undisturbed (control charts in E), their fate to become the one or other fruit morph is not yet determined and can be influenced by certain factors. At late flower stages, however, the fate already seems determined and only indehiscent fruits are produced despite of the cutting treatment (D,E). Numbers within the pie charts represent the absolute number of dehiscent (dark grey) or indehiscent (light grey) fruits developing from the respective developmental stages in response to high-stringency cutting or on non-cut control plants. qRT-PCR data on AearIND gene expression (F) shows that differential expression between morphs is only detectable after (flowers) but not before (buds) fruit morph determination. Shown is the mean relative expression of four biological replicates ± standard deviation. Significant differences between the two morphs are indicated by asterisks (** $P \leq 0.01$).

Figure 7: Change of fruit morph ratio in response to auxin treatment, defoliation and shading. Bar charts comparing the fruit morph ratio between untreated control plants with A: plants where all branches except for one 2nd-order branch have been removed and different concentrations of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) have been applied to the cut surface; B: plants that underwent a defoliation treatment; C: plants grown under shade conditions. Shown is the ratio of dehiscent (dark grey) and indehiscent (light grey) fruits produced on a single 2nd-order branch (A) or throughout the whole plant (B, C) with individual scoring of fruit numbers on the main branch, 1st-order, and higher order (2nd+3rd) branches, respectively. Error bars represent standard deviation from n=9 (A, B) or

n=15 (C) plants per treatment. Significant differences in comparison to the control group are indicated by asterisks (* $P \leq 0.05$; ** $P \leq 0.01$).

Figure 8: Role of plant hormones in fruit morph formation. The role of plant hormones during fruit morph formation has been analyzed A: by comparatively measuring hormone levels in buds, late flowers and late fruits of the dehiscent (dark grey) and indehiscent (light grey) morph, respectively, and B: by determining the fruit morph ratio of plants spray-treated with 2,4-D (synthetic auxin 2,4-dichlorophenoxyaetic acid), BAP (cytokinin 6-benzylaminopurine), GR24 (synthetic strigolactone analogue), ABA (abscisic acid) and only the plain spraying solution (Control). Error bars represent standard deviation from n=3 biological replicate samples per developmental stage (A) or n=10 plants per treatment (B). Significant differences between morphs (A) or in comparison to the control group (B) are indicated by asterisks (* $P \leq 0.05$; ** $P \leq 0.01$).

Figure 9: Expression of shoot branching regulatory genes during Ae. arabicum fruit development. Comparative gene expression analysis of genes involved in the regulation of shoot branching between different stages (buds, late flowers and fruits) of the dehiscent (dark grey) and indehiscent (light grey) morph by qRT-PCR. Relative expression levels are shown for the ortholog of the central branching repressor of A. thaliana, AearBRC1 (A), two putative cytokinin oxidases/dehydrogenases AearCKX5 (B) and AearCKX7 (C), and four genes whose orthologs are involved in strigolactone synthesis AearMAX1 (D), AearMAX2 (E), AearMAX3 (F), and AearMAX4 (G). Error bars represent standard deviation from four biological replicates per stage. Significant differences between the two morphs are indicated by asterisks (* $P \leq 0.05$; ** $P \leq 0.01$).

Figure 10: Role of plant hormones in fruit morph formation of different dimorphic Aethionema species. The role of plant hormones during fruit morph formation has been analyzed for Aethionema carneum (A), Aethionema heterocarpum (B), and Aethionema saxatile (C). Shown are pictures of the dehiscent (left) and indehiscent (right) fruit morph as well as bar charts depicting the fruit morph ratio of plants spray-treated with 2,4-D (synthetic auxin 2,4-dichlorophenoxyaetic acid), BAP (cytokinin 6-benzylaminopurine) and only the plain spraying solution (Control). The ratio of dehiscent (dark grey) and indehiscent (light grey) fruits has been determined individually on the main branch (only B and C), 1^{st}-order, and higher order (2^{nd}+3^{rd}) branches, respectively. Scale bars represent 1 mm. Error bars

represent standard deviation from n=10 plants per treatment. Significant differences in comparison to the control group are indicated by asterisks (* $P \leq 0.05$; ** $P \leq 0.01$).

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