

SCIENTIFIC REPORTS



OPEN

Aberrant imprinting may underlie evolution of parthenogenesis

Olga Kirioukhova^{1,2}, Jubin N. Shah¹, Danaé S. Larsen¹, Muhammad Tayyab¹, Nora E. Mueller¹, Geetha Govind^{1,10}, Célia Baroux³, Michael Federer³, Jacqueline Gheyselinck³, Philippa J. Barrell^{3,11}, Hong Ma^{3,4,5}, Stefanie Sprunck⁶, Bruno Huettel⁷, Helen Wallace⁸, Ueli Grossniklaus³ & Amal J. Johnston^{1,2,3,9}

Received: 13 December 2017

Accepted: 11 May 2018

Published online: 13 July 2018

Genomic imprinting confers parent-of-origin-specific gene expression, thus non-equivalent and complementary function of parental genomes. As a consequence, genomic imprinting poses an epigenetic barrier to parthenogenesis in sexual organisms. We report aberrant imprinting in *Boecheera*, a genus in which apomicts evolved from sexuals multiple times. Maternal activation of a MADS-box gene, a homolog of which is imprinted and paternally expressed in the sexual relative *Arabidopsis*, is accompanied by locus-specific DNA methylation changes in apomicts where parental imprinting seems to be relaxed.

Genomic imprinting refers to epigenetic gene regulation that leads to the parent-of-origin-specific expression of alleles, and it was proposed to differentially control offspring development^{reviewed in 1}. Because genomic imprinting causes parental genomes to be non-equivalent, it prevents parthenogenetic embryo development by enforcing contribution of both parental genomes². In flowering plants, most imprinted genes are mono-allelically expressed in the embryo-nourishing endosperm tissue, and a few are imprinted in the embryo^{reviewed in 3}. Imprinting mechanisms may also serve as barriers to inter-specific or inter-ploidy hybridization in sexual plants, perhaps as a sensor that detects the correct parental gene dosage^{reviewed in 4}. In contrast, apomicts can tolerate a skewed parental genome constitution, e.g. absence of the paternal genome in the embryo and sometimes an altered parental genome dosage in the endosperm^{5,6}.

DNA and histone methylation play predominant roles in genomic imprinting. An imprinted and paternally expressed MADS-box gene encoding the transcription factor PHERES1 (PHE1) promotes embryo growth and is maternally repressed by a H3K27me3 histone methyltransferase MEDEA (MEA), which restricts growth in the sexual species *Arabidopsis thaliana*^{4,7}. The contrasting imprinting effects between MEA and PHE1 lend support to the parental offspring theory⁶. Since parthenogenetic embryos lack a direct paternal contribution, we hypothesized that a relief of imprinting may have played a role in the evolution of parthenogenesis in plant species that reproduce asexually through seeds via apomixis. The *Boecheera* genus is closely related to *Arabidopsis*, belonging to the same major clade within the Brassicaceae phylogeny⁸, and it consists of both sexual and apomictic (parthenogenetic) populations⁹. The genetic basis of parthenogenesis in *Boecheera* is currently unknown. Here, we asked whether changes in the status of imprinting are involved in parthenogenesis in *Boecheera*. For this, we analysed the spatio-temporal expression pattern and DNA methylation status of the *Boecheera* homolog of PHE1, which is a paternally-expressed imprinted gene in *Arabidopsis*. We examined a diploid sexual *B. stricta* (*Sex-1*) and a triploid

¹University of Heidelberg, Centre for Organismal Studies, Laboratory of Germline Genetics & Evo-Devo, Heidelberg, Germany. ²Jacobs University, Life Sciences & Chemistry, Laboratory of Germline Genetics & Evo-Devo, Bremen, Germany. ³University of Zurich, Department of Plant and Microbial Biology and Zurich-Basel Plant Science Center, Zurich, Switzerland. ⁴The Pennsylvania State University, the Huck Institute of Life Sciences, Department of Biology, The University Park, Pennsylvania, USA. ⁵Fudan University, State Key Laboratory of Genetic Engineering, Institute of Plant Biology, School of Life Sciences, Fudan University, Shanghai, China. ⁶University of Regensburg, Cell Biology and Plant Biochemistry, Regensburg, Germany. ⁷Max-Planck-Institute for Plant Breeding, Cologne, Germany. ⁸University of the Sunshine Coast, Faculty of Science, Health, Education and Engineering, Genecology Research Centre, Maroochydore, Australia. ⁹ETH Zurich, Department of Biology and Zurich-Basel Plant Science Center, Zurich, Switzerland. ¹⁰Present address: University of Agricultural Sciences, College of Agriculture Sciences, Department of crop physiology, Hassan, India. ¹¹Present address: New Zealand Institute for Plant and Food Research, Christchurch, New Zealand. Jubin N. Shah and Danaé S. Larsen contributed equally to this work. Correspondence and requests for materials should be addressed to U.G. (email: grossnik@botinst.uzh.ch) or A.J.J. (email: a.johnston@jacobs-university.de)

apomict *B. gunnisoniana* (*Apo-1*) using cytological and molecular approaches. A closely related diploid apomict, *B. divaricarpa* (*Apo-2*)¹⁰, was included for additional comparisons, in order to eliminate ploidy effects.

Apomictic reproduction requires two major alterations of the sexual pathway: meiosis is avoided to generate unreduced gametes, followed by parthenogenesis enabling embryo development without a paternal contribution⁵. In apomictic *Boechera*, both female and male meioses are equally circumvented (Supplementary Figs 1 and 2 and explanations therein) and the female and male gametogenesis produce unreduced egg and sperm cells, respectively (Supplementary Fig. 3). In *Arabidopsis*, EGG-CELL 1.1 (*EC1.1*) peptides accumulate in the egg cell prior to fertilization and prevent multiple sperm fusions likely through male–female signalling processes¹¹. Abundant transcripts of *EC1.1* were detected in *Boechera* by heterologous mRNA *in situ* hybridization with an *Arabidopsis* probe. The *Boechera EC1.1* was expressed in the egg cell before fertilization in the sexual *Sex-1* line as well as at the onset of parthenogenesis in the apomictic *Apo1* line (Fig. 1a,b). This likely reflects the requirement of egg–sperm signalling in the apomict similarly to the sexual *Arabidopsis*¹¹. Preventing pollination in *Apo-1* did not lead to parthenogenetic embryo development (Fig. 1h), further supporting the view that some aspects of fertilization are necessary for parthenogenesis in *Boechera*. In the sexual *Sex-1* as well as the apomict *Apo-1*, the central cell exhibited weak but detectable signal of *MEA* transcripts (Fig. 1c,d) similar to *Arabidopsis*¹², in which it has been shown to prevent autonomous divisions in the central cell¹³. In both the sexual *Sex-1* and the apomicts, *Apo-1* and *Apo-2*, the pollen tube enters the embryo sac, and the two sperm cells each target the egg and central cell, respectively (Fig. 1i–p). Regardless of the reproductive mode, nuclear fusion occurs between one of the two sperm cells and the central cell, which is followed by primary endosperm divisions (Fig. 1i–n). However, unlike egg–sperm karyogamy leading to zygote formation in the sexual (Fig. 1i,l), the second sperm nucleus in the apomicts persisted in the vicinity of the nucleus of the egg but no fusion occurred even at a stage when mitotic divisions in the endosperm advanced (Fig. 1k,m–p). This observation suggests that presence of the sperm near the egg cell of the apomict might serve as activation source to induce pseudogamous parthenogenesis. The resulting parthenogenetic embryos were indistinguishable from the sexual ones in terms of morphology and expression of the cell-division marker gene *CYCB1;1* (Fig. 1q,r). Furthermore, self-pollination in *Boechera* apomicts seems to be required for maintaining genome integrity in the progeny. The majority of the individuals arising from inter-specific pollination between apomicts displayed a range of genomic alterations and partial breakdown of genome integrity (Supplementary Fig. 4 and discussions therein). Some of the progeny that was of solely maternal genotype (i.e. of parthenogenetic origin) upon inter-specific pollination exhibited an array of vegetative and reproductive defects including self-incompatibility, which was not observed in the apomictic self-progeny. Global epigenetic changes in the maternal genome could possibly account for such morphological aberrations in clonal offspring. Collectively, our observations suggest that male cues from the self-parent are likely essential at fertilization for the initiation of pseudogamous parthenogenesis, and might be necessary for the maintenance of epigenetic states.

To understand the impact of apomictic mode of development on genomic imprinting we analysed the *PHERES1* locus of *Boechera*. In *Arabidopsis*, the maternal allele is silenced by *MEA*, but the paternal allele is expressed during seed development^{14,15}. Following incompatible hybridizations, the maternal *PHE1* allele can be de-repressed^{4,16}. We identified two *Boechera* homologs of *PHE1*: *PHERES-LIKE 1* (*PHEL1*) and *PHEL2*. *PHEL1* has up to 64% amino acid sequence identity with *Arabidopsis* *PHE1* or *PHE2* (Supplementary Fig. 5). *PHEL2* represents a pseudo-gene without detectable expression (Supplementary Fig. 6a). In order to characterize the imprinting status of *PHEL1* in *Boechera* sexuals and distinguish the maternal and paternal alleles, we used another sexual diploid species, *B. perennans* (*Sex-2*) offering a sequence polymorphism in *PHEL1*. In reciprocal crosses between *Sex-1* and *Sex-2*, we analyzed allele-specific expression of *PHEL1* by RT-PCR experiments. Only the maternal allele of *PHEL1* is expressed in the embryo and endosperm tissues of the *Boechera* sexuals (Fig. 2a, Supplementary Fig. 6b,c), unlike its *Arabidopsis* counterpart *PHE1*¹⁴. It is likely that expression of the maternal *PHEL1* allele results from a switch in the state of imprinting within sexual *Boechera* species, which likely arose in response to hybridization-driven speciation and subsequent genome modifications as previously proposed¹⁷.

In sexual *Boechera* species, *PHEL1* is expressed at very low levels both in the female (gynoecia) and male (anthers) reproductive organs, yet expression in the mature female tissues is nearly three-fold greater than in the male (Fig. 2b). *PHEL1* expression could not be detected *in situ* in sexual ovules due to its low abundance, but the levels of the corresponding transcripts were quantified by qRT-PCR. *PHEL1* transcripts were abundant in the gynoecia and siliques of *Apo-1* and *Apo-2*; the asexual gynoecia had up to 40-fold higher *PHEL1* expression levels compared to the sexual ones irrespective of the ploidy level of the apomict (Fig. 2b). In addition, faint but specific *in situ* expression of *PHEL1* was detected in the apomictic embryo sac of *Apo-1* (Fig. 1f,g, compare to *Sex-1* in Fig. 1e and to the sense probe in Supplementary Fig. 6d). *PHEL1* expression in the apomicts showed 250–400-fold higher transcript levels in female than male floral organs and in the embryos compared to the levels of expression in the sexual (Fig. 2b). We propose that these high levels of the maternal *PHEL1* transcript prior and during embryo development may play a role in parthenogenesis.

In *Arabidopsis*, METHYLTRANSFERASE 1 (*MET1*) is pivotal for maintenance of CG DNA methylation, while CHROMOMETHYLASE 3 (*CMT3*) maintains non-CG-methylation; and DOMAINS-REARRANGED METHYLTRANSFERASEs 1 and 2 (*DRM1/2*) control RNA-dependent DNA methylation (*RdDM*) *de-novo* in all contexts¹⁸. Their function is thought to be critical for epigenetic reprogramming and genomic imprinting during gametogenesis and seed development³. When we examined genes expression levels of the corresponding *Boechera* homologs, we noticed a complex situation with respect to common and/or taxon-specific expression patterns of genes coding for DNA methyltransferases (Fig. 2c,d, Supplementary Fig. 7). In apomicts, *MET1* was marginally lower in gynoecia, and significantly down-regulated in anthers, in comparison to the sexual lines (Fig. 2c). This situation persisted after fertilization in *Apo-1*. *DRM2* was significantly upregulated in gynoecia and siliques of both apomicts (Fig. 2d). In *Arabidopsis*, DNA methylation at the *PHE1* locus is regulated by cytosine methylation machinery involving *MET1* and *DRM2*, and influences its parental expression levels¹⁵. Taken together, reduced

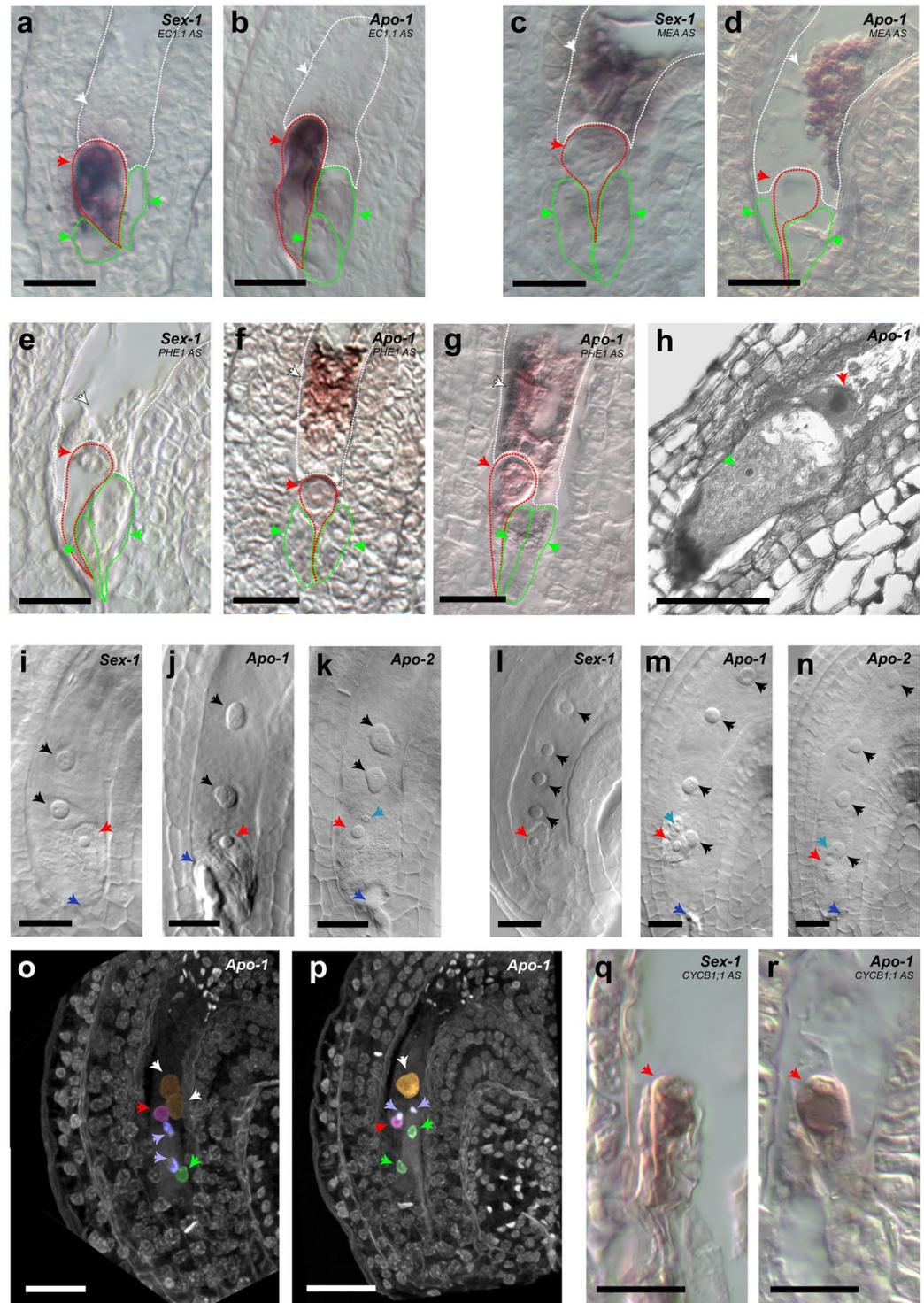


Figure 1. Pseudogamous parthenogenesis in *Boechera* is accompanied by sexual-like gene expression patterns and deregulation of a MADS-box gene. **(a,b)** Heterologous mRNA *in situ* signals of *EC1.1* in sexual *versus* parthenogenetic egg cells. Arrow-heads: red – egg cell, green – synergids, white – central cell nuclei. **(c,d)** *MEA* signals in central cells. **(e–g)** Heterologous signals of *PHE1* in apomictic *versus* sexual embryo sacs. **(h)** An apomictic egg cell of *Apo-1* (red arrow-head) at 3 days after emasculating. **(i–n)** Fertilized ovules (dark-blue arrow-heads – pollen tube entry, black arrow-heads – endosperm). **(m,n)** An unfused sperm nucleus (light-blue arrow-head) is visible proximal to the parthenogenetic egg cell (red arrow-heads). **(o,p)** Confocal micrographs of *Apo-1* ovaries at fertilization. **(o)** Two sperm cells (light-blue arrow-heads) discharged into an apomictic embryo sac. **(p)** Sperm cell arrival coincides with polar nuclei fusion (white arrow-head). **(q,r)** *CYCB1.1* mRNA *in situ* signals at one-celled embryo stage (red arrow-heads). Scale bars in **(a–r)** 20 μ m.

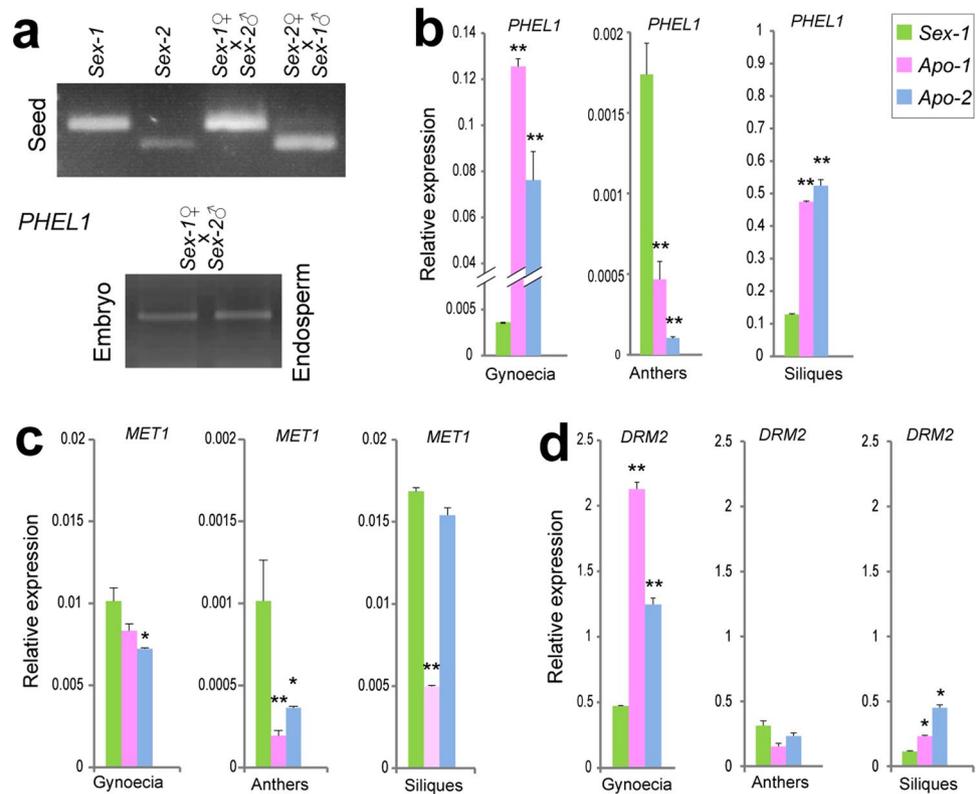


Figure 2. The imprinted *PHEL1* is upregulated in maternal, female gametophytic, and sporophytic tissues of apomictic *Boechera*, correlating with deregulation of DNA methyltransferase genes. **(a)** Maternal and paternal *PHEL1* transcripts assayed by allele-specific RT-PCR in immature seeds, embryo, and endosperm fractions upon reciprocal crosses between two sexual *Boechera* accessions. **(b–d)** Relative transcript levels of *PHEL1*, *MET1*, and *DRM2*. Expression deviation from sexual *Boechera* (t-test significance levels: ** $\alpha \leq 0.01$; * $\alpha \leq 0.05$).

MET1 and increased *DRM2* levels in female floral tissues might provide an explanation for high levels of maternal *PHEL1* in *Boechera* lines. Furthermore, in seedlings of the *Boechera* apomicts, increased *PHEL1* expression also correlated with upregulation of *DRM2* and down-regulation of *MET1* (Supplementary Fig. 7d–f). Although cell-type-specific comparative gene expression and DNA methylation profiling remains to be elucidated (and poses a significant challenge in non-model systems like *Boechera*) we propose that *MET1/DRM2*-mediated DNA methylation changes might be responsible for the elevated expression of *PHEL1* in *Boechera* apomicts.

We tested by bisulfite sequencing whether the active maternal *PHEL1* allele in apomictic *Boechera* exhibits DNA methylation footprints distinct from those in the sexual. We found that the most distal 3' region ca. 2 kb downstream of the *PHEL1* gene showed strong DNA methylation in a non-CG context in both sexual and apomictic gynoecia (3'-#3, Fig. 3b). Intriguingly, we found that a 0.6 kb DNA fragment distal to the *PHEL1* gene (3'-#2) is present only in the *Sex-1* line, and is heavily methylated primarily in CG but also in non-CG contexts (Fig. 3a,b). This methylated region (3'-#2, or 3'MR) consisted of several repeats (Supplementary Fig. 8). The 3'MR was absent in an *Apo-1* *PHEL1* allele; a similar deletion was also found in *Apo-2*. In brief, a heavily methylated distal DNA fragment was absent in two apomict-specific *PHEL1* alleles but present in a sexual, and this deletion positively correlated with elevated *PHEL1* expression in the apomicts.

Apomixis is reported in only about 0.5% of the Brassicaceae genera, which mostly occur in extreme environmental conditions (discussed in¹⁰). In particular, the North-American *Boechera* species are likely to have arisen from millions of years of evolutionary bottle-necks and reticulate evolution. Hybridization between sexual *Boechera* genotypes may have paved the way for genomic imprinting to become relieved from tight control, based on a genomic landscape with two contrasting genomes^{17,19}. Ultimately, some *Boechera* hybrids may have had an epigenetic environment conducive for the evolution of novel apomictic traits, such as parthenogenesis. Existence of multiple independently evolved apomictic *Boechera* population allow us to propose that some convergent (epi) genetic mechanisms may play a prominent role here. Our findings suggest that parent-of-origin expression of *PHEL1* or *PHE1*¹⁵ across genera is highly correlated with DNA methylation pattern of the corresponding loci; however, this regulation is modified in *Boechera* in terms of a) reversion of the imprinting status resulting in expression of the maternal *PHEL1* allele; and b) deletion of the heavily methylated 3'MR in the alleles specific to apomicts, with a concomitant increase in expression. Figure 3c proposes a model where distinct epigenetic regulation of *PHE1/PHEL1* based on DNA methylation may have enabled parthenogenesis to evolve in *Boechera*. Our findings in *Boechera* show similarity to an artificially induced case of parthenogenesis in mice²⁰, where loss of distal DNA methylation causing maternal activation of the paternally expressed *Insulin-like growth factor 2*

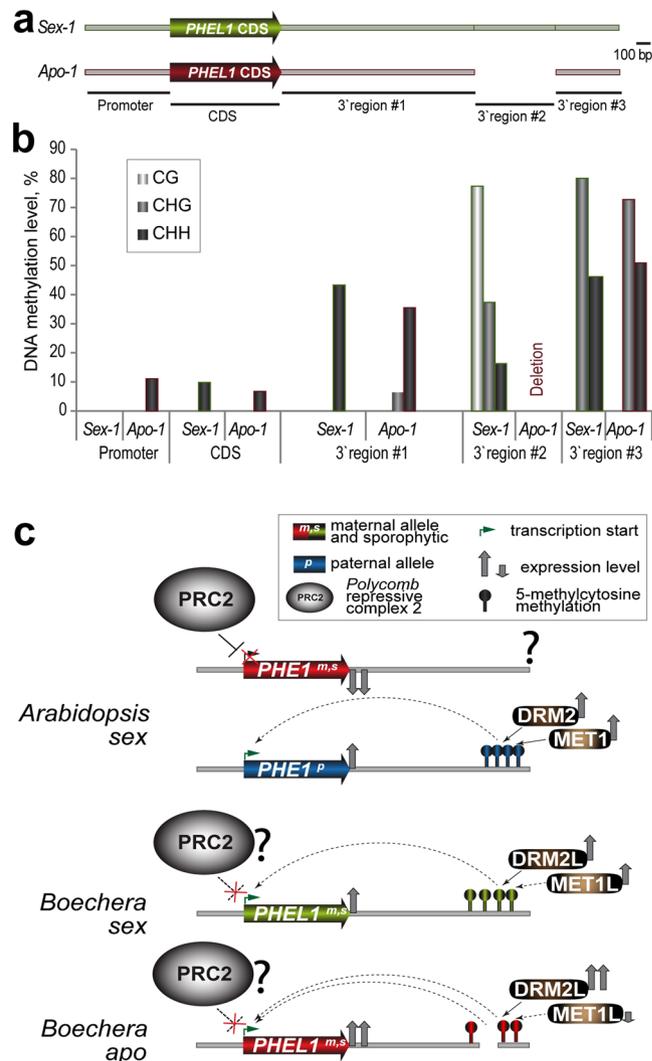


Figure 3. DNA methylation analysis of the *PHEL1* loci in a sexual and an apomictic *Boecheira* line, and a proposed epigenetic model of *PHEL1* regulation in sexual (*sex*) versus apomictic (*apo*) lines. **(a,b)** Scheme of *PHEL1* loci and corresponding percent methylation identified by bisulfite-sequencing. **(c)** An illustration of the proposed epigenetic regulation of *Arabidopsis PHE1* versus *Boecheira PHEL1* by DNA/histone methylation. PRC2, *Polycomb* Repressive Complex 2 containing the MEA histone methyltransferase.

(*Igf2*) gene was sufficient to induce parthenogenesis. We thus propose that alterations in the control of genomic imprinting enable the adjustment of parental gene dosage necessary for parthenogenesis to evolve.

Materials and Methods

Plant material and growth conditions. Diploid sexual and/or triploid asexual *Boecheira* seeds were kind donations from various sources¹⁰. Both triploid *Apo-1* and diploid *Apo-2* were first analyzed for ploidy by bulked seed flow-cytometry¹⁰, which revealed the presence of occasional 6C (hexaploid) and 4C (tetraploid) embryo peaks, respectively. The offspring seedlings were ploidy-analyzed using a FacsCantoII cytometer (BD Biosciences, USA), and rare hexaploid *Apo-1* plants were eliminated from further analyses. Single seed flow-cytometry gave an over-estimate of apomeiosis per plant as only the fertile seeds were taken for analyses; therefore, expressivity of apomeiosis and parthenogenesis was determined by individual seed ploidy analyses by flow-cytometry, and subsequently by ovule clearing and seed counts¹⁰. Plants were grown under long-day conditions at 18–21°C.

RNA extraction, cDNA synthesis, real-time qRT-PCR. RNA isolation and cDNA synthesis were performed as described in¹⁰. Locus-specific fragments across all *Boecheira* strains were PCR-amplified based on conserved *Arabidopsis* and *Boecheira* sequences available from public repositories (NCBI, <http://www.ncbi.nlm.nih.gov>; Phytozome, <http://phytozome.jgi.doe.gov>) and sequenced. We cloned the entire *PHEL1* and *PHEL2* loci in all *Boecheira* species analysed here based on a *Sex-1*-specific template. Allele-specific *PHEL1* transcript fragments were genotyped upon *Bst*UI digestion (New England Biolabs, USA). qRT-PCR SYBR Green assays were performed in a StepOnePlus Real-Time-PCR System (Applied Biosystems, USA) with three biological replicates and normalized as described¹⁰ using *RPS18* gene as a reference. Primer sequences are given in Supplementary Table 1.

DNA methylation analysis by bisulfite sequencing. DNA methylation conversion was performed using EpiTect Bisulfite kit (Qiagen, Germany). For each library, 8–10 egg cell-containing gynoecia at the stage just prior fertilization were processed according to the manufacturer instructions yielding two BS-seq libraries (*Sex-1* 120 ng and *Apo-1* 200 ng). Library was sequenced using standard Illumina 2500 pipeline at the MPIZ Genome Centre, Cologne, Germany. In brief, library quality check was performed with FastQC method. BS-seq was set to 6 Gb for *Sex-1* and 18 Gb for *Apo-1* aiming 30 × genome coverage. Conversion efficiency was evaluated using Bismark alignment and methylation caller²¹. Detected genome-wide methylation levels of cytosines in the CG, and non-CG contexts were 19.6%, and 8.1%, respectively, which was similar to *Arabidopsis*²² and indicated a very good level of bisulfite conversion; the high conversion efficiency was further confirmed by detecting long stretches (ca. 0.5–1 kb) of fully bisulfite converted DNA. Sequence reads were mapped to the corresponding *PHE1* sequences from *Sex-1* and *Apo-1* using Bismark platform.

Heterologous *in situ* mRNA hybridization. *Arabidopsis*-specific heterologous *in situ* probes were prepared from corresponding cDNA clones; and mRNA *in situ* hybridization²³ was modified to include an additional RNase A treatment step (20 µg/ml for 30 min incubation at 37 °C) to remove unspecific background signals. The mRNA *in situ* mRNA hybridization worked efficiently, particularly when the transcripts were abundant. For *MEA* and *PHE1* RNA probes, it was necessary to add hybridization solution on slides during each day for up to three days to enhance its very weak signal; the specificity of probe binding is ensured by RNase A treatment (see above). In the case of probing against *PHERES*-like genes in *Boechera*, although the *in situ* probe used cannot distinguish gene-specific transcripts due to a high degree (~80%) of nucleotide identity between *PHE1* and *PHE2*, we are confident that we indeed detected *PHE1*-specific transcripts *in situ* because *PHE2* signals were barely detectable even in qRT-PCR assays. Ovule and seed clearing, DIC and confocal microscopy upon propidium-iodide staining, and image analyses using Imaris (Bitplane, Switzerland) were performed as described^{24,25}.

References

- Kinoshita, T., Ikeda, Y. & Ishikawa, R. Genomic imprinting: a balance between antagonistic roles of parental chromosomes. *Semin. Cell. Dev. Biol.* **19**, 574–579, <https://doi.org/10.1016/j.semcdb.2008.07.018> (2008).
- Solter, D. Differential imprinting and expression of maternal and paternal genomes. *Annu. Rev. Genet.* **22**, 127–146, <https://doi.org/10.1146/annurev.ge.22.120188.001015> (1988).
- Gehring, M. Genomic imprinting: insights from plants. *Annu. Rev. Genet.* **47**, 187–208, <https://doi.org/10.1146/annurev-genet-110711-155527> (2013).
- Josefsson, C., Dilkes, B. & Comai, L. Parent-dependent loss of gene silencing during interspecies hybridization. *Curr. Biol.* **16**, 1322–1328, <https://doi.org/10.1016/j.cub.2006.05.045> (2006).
- Koltunow, A. M. & Grossniklaus, U. Apomixis: a developmental perspective. *Annu. Rev. Plant Biol.* **54**, 547–574 (2003).
- Haig, D. & Westoby, M. Genomic imprinting in endosperm: its effect on seed development in crosses between species, and between different ploidy levels of the same species, and its implications for the evolution of apomixis. *Philos. Trans. R. Soc. Lond. B* **333**, 1–14 (1991).
- Kohler, C. *et al.* The Polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene. *PHERES1*. *Genes Dev.* **17**, 1540–1553, <https://doi.org/10.1101/gad.257403> (2003).
- Huang, C. H. *et al.* Resolution of Brassicaceae phylogeny using nuclear genes uncovers nested radiations and supports convergent morphological evolution. *Mol. Biol. Evol.* **33**, 394–412, <https://doi.org/10.1093/molbev/msv226> (2016).
- Roy, B. A. The breeding systems of six species of *Arabidopsis* (*Brassicaceae*). *Am. J. Bot.* **82**, 869–877 (1995).
- Shah, J. N. *et al.* Depletion of key meiotic genes and transcriptome-wide abiotic stress reprogramming mark early preparatory events ahead of apomeiotic transition. *Front. Plant Sci.* **7**, 1539, <https://doi.org/10.3389/fpls.2016.01539> (2016).
- Sprunck, S. *et al.* Egg cell-secreted EC1 triggers sperm cell activation during double fertilization. *Science* **338**, 1093–1097, <https://doi.org/10.1126/science.1223944> (2012).
- Spillane, C. *et al.* Positive darwinian selection at the imprinted MEDEA locus in plants. *Nature* **448**, 349–352, <https://doi.org/10.1038/nature05984> (2007).
- Chaudhury, A. M. *et al.* Fertilization-independent seed development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **94**, 4223–4228 (1997).
- Kohler, C., Page, D. R., Gagliardini, V. & Grossniklaus, U. The *Arabidopsis thaliana* MEDEA Polycomb group protein controls expression of *PHERES1* by parental imprinting. *Nat. Genet.* **37**, 28–30 (2005).
- Makarevich, G., Villar, C. B., Erilova, A. & Kohler, C. Mechanism of *PHERES1* imprinting in *Arabidopsis*. *J. Cell Sci.* **121**, 906–912, <https://doi.org/10.1242/jcs.023077> (2008).
- Schatlowski, N. *et al.* Hypomethylated pollen bypasses the interploidy hybridization barrier in *Arabidopsis*. *Plant Cell* **26**, 3556–3568, <https://doi.org/10.1105/tpc.114.130120> (2014).
- Beck, J. B. *et al.* Does hybridization drive the transition to asexuality in diploid *Boechera*? *Evolution* **66**, 985–995, <https://doi.org/10.1111/j.1558-5646.2011.01507.x> (2012).
- Zhang, X. *et al.* Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. *Cell* **126**, 1189–1201, <https://doi.org/10.1016/j.cell.2006.08.003> (2006).
- Carman, J. G. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispority, tetraspority, and polyembryony. *Biol. J. Linn. Soc.* **61**, 51–94 (1997).
- Kono, T. *et al.* Birth of parthenogenetic mice that can develop to adulthood. *Nature* **428**, 860–864, <https://doi.org/10.1038/nature02402> (2004).
- Krueger, F. & Andrews, S. R. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. *Bioinformatics* **27**, 1571–1572, <https://doi.org/10.1093/bioinformatics/btr167> (2011).
- Cokus, S. J. *et al.* Shotgun bisulfite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature* **452**, 215–219, <https://doi.org/10.1038/nature06745> (2008).
- Johnston, A. J. *et al.* Dosage-sensitive function of RETINOBLASTOMA RELATED1 and convergent epigenetic control are required during the *Arabidopsis* life cycle. *PLoS Genet.* **6**, e1000988, <https://doi.org/10.1371/journal.pgen.1000988> (2010).
- Johnston, A. J., Matveeva, E., Kirioukhova, O., Grossniklaus, U. & Grissem, W. A dynamic reciprocal RBR-PRC2 regulatory circuit controls *Arabidopsis* gametophyte development. *Curr. Biol.* **18**, 1680–1686, <https://doi.org/10.1016/j.cub.2008.09.026> (2008).
- She, W. *et al.* Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. *Development* **140**, 4008–4019, <https://doi.org/10.1242/dev.095034> (2013).

Acknowledgements

We would like to acknowledge funding from a) the German Science Foundation and the Baden-Wuerttemberg State (LGFG) to A.J.J.; b) the University of Zurich, the Swiss National Science Foundation, and the EU Framework V (ApoTool) to U.G.; c) the German Science Foundation (SFB924) to S.S.; and d) ETH Zurich in the group of W. Gruissem. We are grateful to B. Roy, K. Boutilier, E. Schranz, A. Schneider, A. Johnston, S. Young for *Boechera* seed donations. C. Frey (University of Zurich) and M. Zimmer (University of Heidelberg) for help with plant cultivation, S. Schmitt (DKFZ Heidelberg) and M. Langlotz (ZMBH) for help with flow cytometry, and C. Koehler (SLU, Uppsala, Sweden) for an mRNA *in situ* probe. A.J.J. thanks W. Gruissem (ETH Zurich) for support, A. Medzihradzky and J. Lohmann (University of Heidelberg) for the use of a tissue-embedding platform, and M. Gehring (Whitehead Institute) and J. Mateo (University of Oviedo) for helpful comments on the manuscript.

Author Contributions

A.J.J. and U.G. conceived the idea, and A.J.J. designed the study. Sequencing of genomic loci, cytology, *in situ* mRNA hybridization (I.S.H.), DNA methylation and real-time qRT-PCR analyses across several *Boechera* strains were performed by O.K., J.N.S., D.S.L., M.T., N.E.M., L.L., G.G., B.H., H.W., and A.J.J., J.G. and M.F. contributed in ISH (for *PHEL1*) and flow cytometry experiments, respectively. H.M., C.B., P.B. and O.K. performed the cytological analyses of meiosis and fertilization events. S.S. provided an unpublished gene probe of *EC1.1*. O.K. and A.J.J. wrote the manuscript, which was further text-edited by H.W., H.M., C.B. and U.G.

Additional Information

Supplementary information accompanies this paper at <https://doi.org/10.1038/s41598-018-27863-7>.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2018