Regulation of apomixis: learning from sexual experience

Daniel Rodriguez-Leal and Jean-Philippe Vielle-Calzada

Apomixis is a natural form of asexual reproduction through seeds that leads to viable offspring genetically identical to the mother plant. New evidence from sexual model species indicates that the regulation of female gametogenesis and seed formation is also directed by epigenetic mechanisms that are crucial to control events that distinguish sexuality from apomixis, with important implications for our understanding of the evolutionary forces that shape structural variation and diversity in plant reproduction.

Address
Group of Reproductive Development and Apomixis, Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV, Irapuato, Guanajuato, Mexico

Corresponding author: Vielle-Calzada, Jean-Philippe (vielle@ira.cinvestav.mx)

Introduction

Although originally defined to include all known forms of vegetative propagation [1], apomixis has been restricted to name a series of inherited developmental mechanisms that give rise to clonal seeds by circumventing meiotically derived chromosome reduction and fertilization of the egg cell in the ovule. In sexual flowering plants, a single generative cell (a female archesporium or a megaspore mother cell) undergoes meiosis to produce four chromosomally reduced cells (the megaspores). In the majority of sexual species, a single functional megaspore gives rise to the female gametophyte [2,3]. After significant cellular enlargement, the nucleus of the functional megaspore usually undergoes three rounds of mitosis before giving rise to a gametophyte composed of seven cells: two companion synergids, the egg cell, a binucleated central cell and three antipodals (Figure 1). Double fertilization of both the egg and central cell is necessary to trigger embryogenesis and endosperm development, respectively. By contrast, apomictic plants can form embryos directly from a somatic cell in the unfertilized ovule (adventitious embryony) or from chromosomally unreduced female gametophytes in which the egg cell develops autonomously into an embryo, by parthenogenesis (gametophytic apomixis). In the latter case, the unreduced female gametophyte can be formed from an aberrant meiotic cycle that prevents reduction and recombination (diplospory) or from direct differentiation of somatic cells in the ovule (apospory). In most cases, the formation of the endosperm is still dependent on fertilization of the central cell (pseudogamy), although rare cases of autonomous development of the endosperm have been also reported [4].

The introduction of apomixis into sexual crops has been perceived as a revolutionary technology that could allow the perpetual self-production of improved hybrids, and the genetic fixation of any desired heterozygous genotype. Apomixis was initially investigated by plant embryologists that characterized the cytological basis of apomictic reproduction in a wide group of species, describing its structural variants and prevalent developmental pathways [4,5]. Their efforts lead those of a few visionary breeders that attempted its transfer by interspecific hybridization between important sexually reproducing crops (maize, rice, pearl millet) and some of their apomictic wild relatives (*Tripsacum dactyloides*, *Elymus rectisetus*, and *Pennisetum squamulatum*, respectively [6]. Although the strategy proved unsuccessful from an applied perspective, resulting interspecific hybrids presently represent a unique resource for understanding the genetic components that control apomixis. Over the past two decades, research has focused on studying the genetic basis and molecular mechanisms that regulate apomictic reproduction through either the elucidation of the genetic control of reproductive methods in natural apomicts in comparison to their sexual counterparts, or through the identification of gene function in sexual model species as a means to elucidate the developmental mechanisms that relate to components of apomixis, such as the formation of unreduced female gametes, or the autonomous development of the embryo or endosperm [7,8].

Recent results in *Arabidopsis* and maize have provided new evidence indicating that genetic basis of female gametogenesis and seed formation are directed by epigenetic mechanisms that are crucial to control events that distinguish sexual from apomictic development. The discovery of these epigenetic components is transforming our current view of the structural variation and diversity that prevails at key steps of plant female gametogenesis, with profound implications for understanding the evolutionary trends that shape innovations in reproductive development and adaptation.

Figure 1
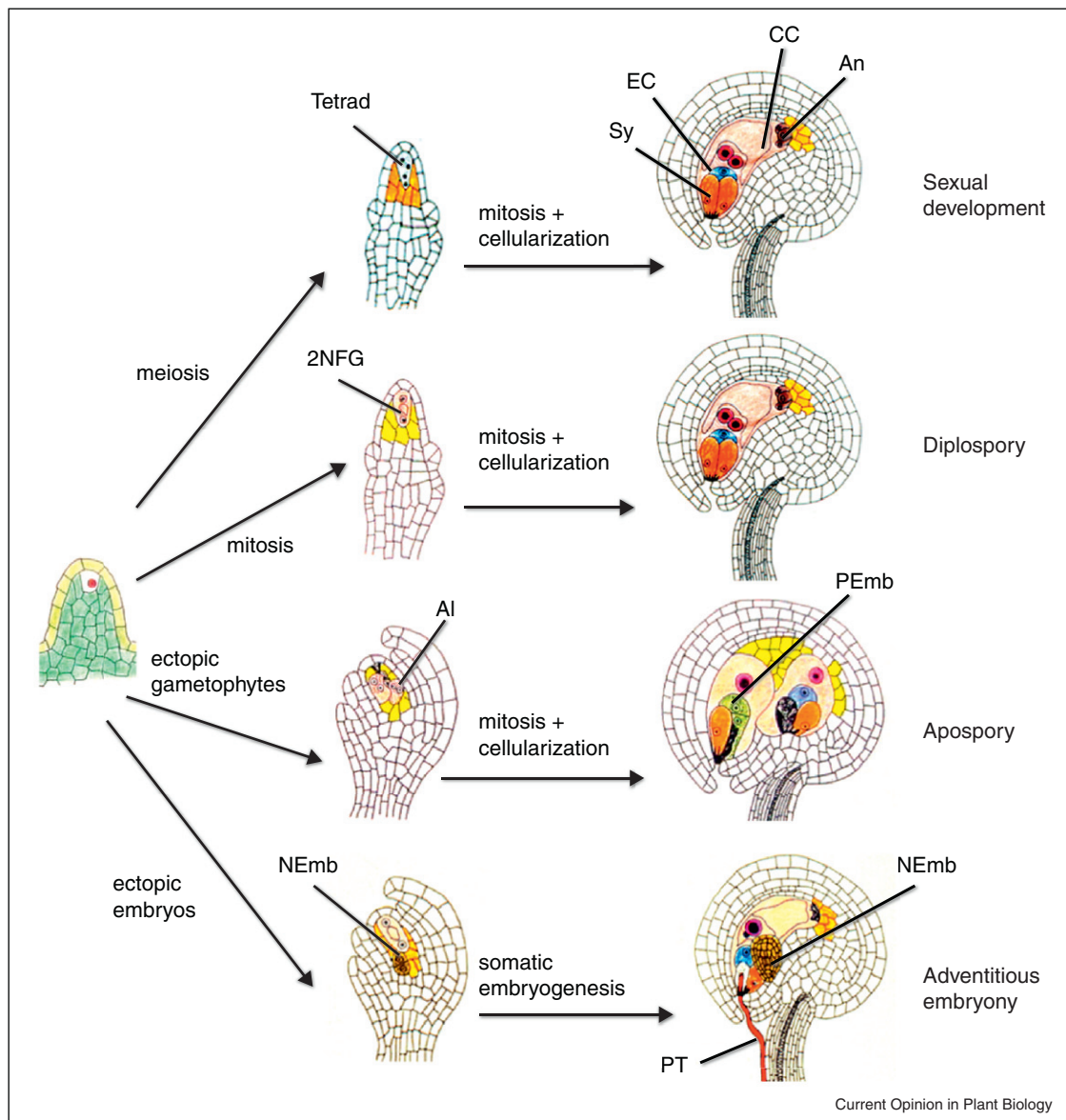
Sexual and apomictic development in flowering plants. During sexual development, a single cell undergoes meiosis to produce a tetrad of chromosomally reduced cells (the megaspores); in most cases a single functional megaspore gives rise to the female gametophyte composed of two synergids (Sy; orange), the egg cell (EC; blue), a binucleated central cell (CC; pink) and three antipodals (brown). By contrast, apomictic plants can form embryos directly from a somatic cell in the developing ovule (nucellar embryos, NEmb; adventitious embryony) or from chromosomally unreduced female gametophytes in which the egg cell develops autonomously into an embryo, by parthenogenesis (PEmb; gametophytic apomixis). In the latter case, the unreduced female gametophyte can be formed from an aberrant meiotic cycle that prevents reduction and recombination (diplospory) or from direct differentiation of somatic cells in the ovule (apospory). Additional legend: Tetrad, tetrad of haploid nuclei; 2NFG, 2-nuclear unreduced female gametophyte; AI, aposporous initial cell.

Genetic control of apomixis

Apomixis is genetically controlled, but the basis of its inheritance remains often contradictory and elusive [6]. Genetic analysis is complicated by the absence of sexually functional female gametes; as a consequence, apomictic genotypes can only be used as male parents and cannot be self-fertilized to generate segregating populations. Whereas the inheritance of adventitious embryony has not been investigated, segregating populations in several apomictic grasses that retain a variable frequency of residual sexuality show that the inheritance of unreduced female gametophyte formation (through either diplospory or apospory) is simple. In most species under study, the basic components of apomixis can be explained by one or two dominant loci that control unreduced gamete formation and parthenogenesis, respectively; however,

polyploidy, segregation distortion [9,10], suppressed recombination [6], epistatic interactions [6], naturally active modifiers [8], and environmental effects [4], complicate their genetic analysis. To this date, no genes associated with these loci have been demonstrated to control components of natural apomixis. In *Hieracium praeltum*, a member of the subgenus *Pilosella*, deletion analysis showed that aposporous apomixis is controlled by the *LOSS OF APOMEIOSIS* (*LOA*) and *LOSS OF PARTHENOGENESIS* (*LOP*) loci. Whereas *LOA* is required for aposporous cell differentiation and suppression of all sexually derived megaspores, the gametophytic activity of *LOP* is necessary for autonomous development of embryo and endosperm [11,12•]. Deletion of *LOA* or *LOP* results in reversion to sexuality, indicating that apomixis is imposed over a default sexual pathway that is interrupted by any of these unrelated loci. Intriguingly, *LOA* function requires the initiation of megaspore formation in the early ovule [12•], suggesting that the specification of sporophytic aposporous initial cells is dependent on the initiation of sexual development.

Current efforts have narrowed down genomic regions harboring presumed genes involved in the initiation of apomixis to a few hundred Kb [6,13]; a few additional approaches have focused on attempting to identify candidate genes on the basis of differential gene expression between sexual and apomictic genotypes [6,13,14,15•,16]. In *Taraxacum* for example, refinement of the genetic linkage map showed a bias towards apomictic plants among recombinants between the two most closely linked molecular markers to the *DIPLOSPOROUS* (*DIP*) locus, suggesting a possible duplication of a presumed DIP gene [17]. In *Pennisetum* sp., although introgression of the Apospory-Specific Genomic Region (ASGR)-carrier chromosome of *P. squamulatum* into sexual genotypes of *P. glaucum* (pearl millet) proved efficient to identify transcripts derived from the chromosome conferring apomixis [18], none were specific to apomixis. The hemizygous chromosomal region containing LOA in *Hieracium* sp. is structurally reminiscent of the hemizygous ASGR region in *Pennisetum* sp., suggesting a convergent evolution that might be necessary for function and maintenance of the trait [15•]. Whereas the vast majority of apomictic genotypes are polyploid, the natural occurrence of diploid populations that retain apomictic reproduction, and the recovery of diploid individuals in several apomictic species, indicates that polyploidy is not an obligatory requirement for functional apomixis [4,19]. In the case of sexual diploid *Erigeron strigosus*, the absence of apomictic development in diploids is supported by evidence suggesting univalent inheritance of the locus bearing diplospory, and recessive-lethal gametophytic selection against the locus controlling parthenogenesis [10]. Recent evidence indicates that discrete genomic regions sufficient for the inheritance of apomixis in the grasses are largely asyntenic, highly heterochromatic,

recombinationally suppressed, and rich in long terminal repeat transposable elements [20].

Apomixis and the epigenetic regulation of sexual development

The initiation of apomixis invariably occurs during early ovule ontogeny; sexual and apomictic development can coexist within the same ovule, or within different ovules of a same individual, suggesting that apomixis could have originated as a modified form of sexual reproduction that has undergone deregulation of key developmental steps during gametogenesis [8]. Because mechanisms such as diplospory are directly dependent on the abnormal division of a meiotic precursor, mutations affecting meiosis are particularly interesting for understanding crucial aspects of apomixis [2]. The combination of meiotic mutants that can generate non-reduced female gametophytes with a haploid inducer resulted in the formation of clonal seeds, a non-recurrent form of synthetic apomixis [21].

While the function of meiotic genes in natural forms of apomixis requires further investigation, recent findings indicate that small RNA (sRNA)-dependent epigenetic mechanisms play an essential role in reproductive cell specification, a discovery with key implications for our understanding of how the gametophytic lineage is established during both sexual and apomictic reproduction. In *Arabidopsis*, dominant mutations in *ARGONAUTE9* (*AGO9*) lead to aposporous-like phenotypes by which somatic sporophytic cells give rise to a female gametophyte without undergoing meiosis [22••]. Additional mutations in genes of the *Arabidopsis* RNA-dependent DNA Methylation (RdDM) pathway result in equivalent defects, suggesting that silencing of heterochromatic repetitive regions is crucial to distinguish sexual from apomictic development [22••]. Similar epigenetic effects were discovered in maize, where mutations in *ARGONAUTE104* (*AGO104*) also give rise to functional unreduced gametes [23••], and mutant phenotypes for DNA methyltransferases *DMT102* and *DMT103* (closely related to Arabidopsis *CMT3* and *DRM2*, respectively) are also reminiscent of apomixis [24•], supporting an essential role for RdDM mechanisms in germ cell specification. Consistent with methylation being involved in reproductive fate and environmental response, significant changes in genomic methylation patterns occur during the formation of triploid diplosporous dandelions produced from diploid sexual mother fertilized by polyploid pollen donors [25,26]. The importance of this type of RNAi-dependent pathways has been also advanced as an important factor in the divergence of flowering plants from Gymnosperms [27,28••].

AGO9 preferentially interacts with 24-nt sRNAs derived from TEs mainly belonging to ancient families of retrotransposons, and its function is necessary for silencing TEs in the female gametes [22••], a mechanism reminiscent of PIWI-dependent pathways in animals [29,30], and

perhaps RNA-dependent genome rearrangements in ciliates [31•,32]. The majority of targeted TEs are located in pericentromeric regions, suggesting a link between ovule reproductive fate and silencing of heterochromatin [33•]. Interestingly, global expression profiling data indicates that AGO104 strongly influences the transcription of centromeric repeats [23••]. The action of an AGO9-dependent pathway has been suggested to represent a defense mechanism against potential transpositionally induced damage during meiosis or female gametogenesis, a mechanism that could ensure the maintenance of genome integrity during the sporophyte-to-gametophyte transition in the ovule [34].

Apomixis in a canalized field of epigenetic possibilities

At least 400 extant genera, including multiple species of *Asteraceae*, *Roseaceae* and *Poaceae*, contain genotypes or entire plant populations that reproduce by apomixis [5], suggesting that asexual reproduction through seeds may have arisen multiple times during plant evolution, perhaps through convergent developmental mechanisms. A classic hypothesis suggests that apomixis could result from the hybridization-derived confrontation of divergent alleles that cause a temporal deregulation of cell specification and fate during early ovule development [35].

While hybridization and polyploidy are strongly correlated with the trait [36,37,38], it is not clear if they are a cause or a consequence of the evolution of asexual reproduction. Recent evidence suggests that apomixis could derive from hybridization, with polyploidy occurring as a possible stabilizing factor providing an evolutionary advantage, either by alleviating certain constraints such as self-incompatibility [39], or by masking the effects of deleterious mutations [10]. In *Boechera*, diploid apomictic genotypes show high levels of heterozygosity that are likely to result from the combination of distinct genomes, suggesting that they are of hybrid origin, and that genomic hybridization might allow for developmental heterochronicity in the ovule [40•], and for the transition from sexuality to gametophytic apomixis [41••]. Interspecific hybridization and allopolyploidy have been advanced as a source of ''genomic shocks'' that may result in phenotypic alterations through new genetic or epigenetic interactions derived from the activity of divergent genomes or dosage changes in factors regulating development [42]. In *Arabidopsis thaliana*, the genomic comparison of phylogenetically distinct ecotypes shows that large genetic [43,44,45] and epigenetic sources of intraspecific variation [46••,47••] can have an impact not only on heterosis [48,49], but also on specific types of genetic and epigenetic incompatibilities [50••,51••,52•].

Figure 2
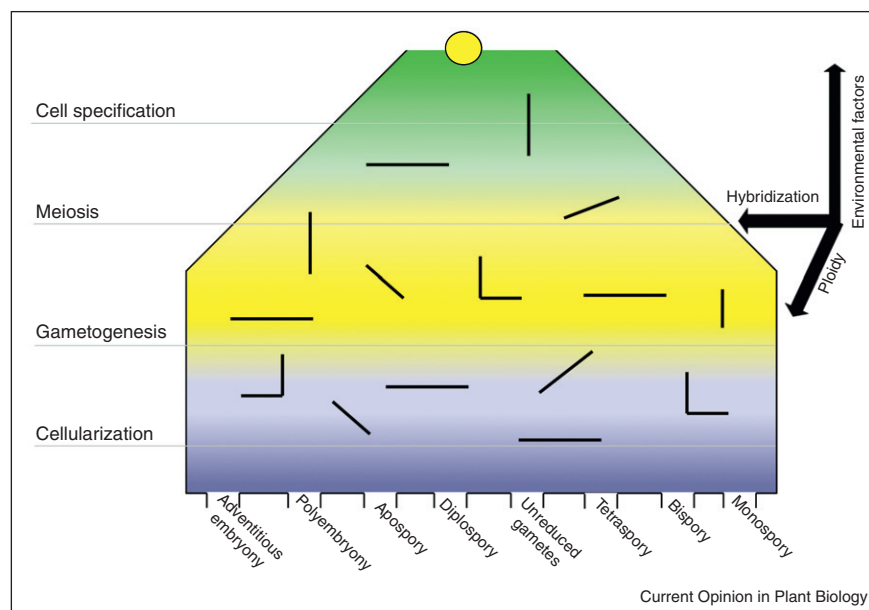
Female gametogenesis in a canalized field of epigenetic possibilities. While sexual reproduction appears strongly canalized in the Angiosperms, natural sources of genetic variation such as hybridization and polyploidy, interacting with environmental factors, could modulate the reproductive outcome by changing the fate of female gametic precursor cells (yellow marble). The shape and slope that defines the developmental pathway followed by a female gametic precursor would depend on the strength and interactions imposed by natural sources of genetic variation. The combination of such interactions might explain the developmental versatility that gave rise to the female gametophytic variants exhibited by flowering plants. Developmental variants leading to the formation of unreduced female gametes could depend on a reproductive source of cryptic genetic variation. Apomixis could represent a canalized alternative in a set of variants of developmental modifications to a general sexual reproductive pathway that includes other structural forms of female gametogenesis such as bispory, tetraspory and polyembryony.

Conrad Waddigton suggested that robustness could stabilize phenotypic variability through ''canalization'', the natural tendency of a trait to resist mutation or environmental modifications [53]. While sexual reproduction appears strongly canalized in the Angiosperms, could natural sources of genetic variation explain the developmental versatility that gave rise to the female gametophytic variants exhibited by flowering plants? In analogy to the role of PIWI-dependent pathways in animals [29,30,54•], sequence recognition involving sRNAs in divergent genotypes could result in epigenetic defects that deregulate female meiosis and cell specification in the ovule. Canalization of sexual development could depend on compatible recognition between sRNAs and their targets; transcriptionally active elements (repetitive elements, genes, or microRNAs, for example) would not be targeted by sRNAs produced by divergent parental genomes, as sequence complementarity would tend to diverge in recently formed hybrids. Following this hypothesis, developmental aberrations leading to the formation of unreduced female gametes could depend on a reproductive source of cryptic genetic variation, a form of standing genetic variation that does not contribute to the normal range of a phenotype, but that contributes to phenotypic modifications after the occurrence of a genetic (hybridization and ploidy, for example) or environmental perturbation [55,56]. Apomixis could represent a canalized alternative in a set of variants of developmental modifications to a general sexual reproductive pathway that includes other structural forms of female gametogenesis such as bispory, tetraspory and polyembryony ([3]; Figure 2). In coming years, the exploration of the potential for natural epigenetic variation to modify gametogenesis will provide new insights into the mechanisms of reproductive innovation and evolution that prevail in flowering plants, a requirement that precedes the possibility of harnessing apomixis for the benefit of sustainable agriculture.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Winkler H: **Über Parthenogenesis und Apogamie im Pflanzenreich**. *Progressus Rei Botanicae* 1908, **2**:293-454.

2. Drews GN, Koltunow AM: **The female gametophyte**. *Arabidopsis Book* 2011, **9**:e0155.

3. Maheshwari P: *An Introduction to the Embryology of Angiosperms*. New York: McGraw-Hill; 1950.

4. Nogler GA: **Gametophytic apomixis**. In *Embryology of Angiosperms,* eBS-V. Edited by Johri BM. Berlin: Springer-Verlag; 1984:475-518.

5. Asker SE, Jerling L: *Apomixis in Plants*. Boca Raton/London: CRC Press; 1992.

6. Ozias-Akins P, van Dijk PJ: **Mendelian genetics of apomixis in plants**. *Annu Rev Genet* 2007, **41**:509-537.

7. Grossniklaus U, Nogler GA, van Dijk PJ: **How to avoid sex: the genetic control of gametophytic apomixis**. *Plant Cell* 2001, **13**:1491-1498.

8. Koltunow AM, Grossniklaus U: **Apomixis: a developmental perspective**. *Annu Rev Plant Biol* 2003, **54**:547-574.

9. Grimanelli D, Leblanc O, Espinosa E, Perotti E, Gonzalez de Leon D, Savidan Y: **Non-Mendelian transmission of apomixis in maize-Tripsacum hybrids caused by a transmission ratio distortion**. *Heredity (Edinb)* 1998, **80(Pt 1)**:40-47.

10. Noyes RD, Rieseberg LH: **Two independent loci control agamospermy (Apomixis) in the triploid flowering plant Erigeron annuus**. *Genetics* 2000, **155**:379-390.

11. Catanach AS, Erasmuson SK, Podivinsky E, Jordan BR, Bicknell R: **Deletion mapping of genetic regions associated with apomixis in Hieracium**. *Proc Natl Acad Sci USA* 2006, **103**:18650-18655.

12. Koltunow AM, Johnson SD, Rodrigues JC, Okada T, Hu Y,
• Tsuchiya T, Wilson S, Fletcher P, Ito K, Suzuki G *et al.*: **Sexual reproduction is the default mode in apomictic Hieracium subgenus Pilosella, in which two dominant loci function to enable apomixis**. *Plant J* 2011, **66**:890-902.
A detailed genetic and molecular analysis of the close developmental link that relates sexuality to apomixis in the ovule, demonstrating that in *Hieracium* sp. sexual reproduction is the default reproductive mode upon which apomixis is superimposed.

13. Akiyama Y, Goel S, Conner JA, Hanna WW, Yamada-Akiyama H, Ozias-Akins P: **Evolution of the apomixis transmitting chromosome in Pennisetum**. *BMC Evol Biol* 2011, **11**:289.

14. Hojsgaard DH, Martinez EJ, Acuna CA, Quarin CL, Pupilli F: **A molecular map of the apomixis-control locus in Paspalum procurrens and its comparative analysis with other species of Paspalum**. *Theor Appl Genet* 2011, **123**:959-971.

15. Okada T, Ito K, Johnson SD, Oelkers K, Suzuki G, Houben A,
• Mukai Y, Koltunow AM: **Chromosomes carrying meiotic avoidance loci in three apomictic eudicot Hieracium subgenus Pilosella species share structural features with two monocot apomicts**. *Plant Physiol* 2011, **157**:1327-1341.
By providing comparative molecular and genomic evidence, the authors show that convergent evolution of repeat-rich hemizygous chromosomal regions may be required for the function and maintenance of apomixis in both eudicot and monocot species.

16. Silveira ED, Guimaraes LA, de Alencar Dusi DM, da Silva FR, Martins NF, do Carmo Costa MM, Alves-Ferreira M, de Campos Carneiro VT: **Expressed sequence-tag analysis of ovaries of Brachiaria brizantha reveals genes associated with the early steps of embryo sac differentiation of apomictic plants**. *Plant Cell Rep* 2012, **31**:403-416.

17. Vijverberg K, Milanovic-Ivanovic S, Bakx-Schotman T, van Dijk PJ: **Genetic fine-mapping of DIPLOSPOROUS in Taraxacum (dandelion; Asteraceae) indicates a duplicated DIP-gene**. *BMC Plant Biol* 2010, **10**:154.

18. Zeng Y, Conner J, Ozias-Akins P: **Identification of ovule transcripts from the Apospory-Specific Genomic Region (ASGR)-carrier chromosome**. *BMC Genomics* 2011, **12**:206.

19. Voigt-Zielinski M-L, Piwczyński M, Sharbel T: **Differential effects of polyploidy and diploidy on fitness of apomictic Boechera**. *Sexual Plant Reproduction* 2012, **25**:97-109.

20. Ochogavia AC, Seijo JG, Gonzalez AM, Podio M, Duarte Silveira E, Machado Lacerda AL, Tavares de Campos Carneiro V, Ortiz JP, Pessino SC: **Characterization of retrotransposon sequences expressed in inflorescences of apomictic and sexual Paspalum notatum plants**. *Sex Plant Reprod* 2011, **24**:231-246.

21. Marimuthu MP, Jolivet S, Ravi M, Pereira L, Davda JN, Cromer L, Wang L, Nogué F, Chan SW, Siddiqi I, Mercier R: **Synthetic clonal reproduction through seeds**. *Science* 2011, **331**:876.

22. Olmedo-Monfil V, Duran-Figueroa N, Arteaga-Vazquez M, Demesa-
•• Arevalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada JP: **Control of female gamete formation by a small RNA pathway in Arabidopsis**. *Nature* 2010, **464**:628-632.
This paper presents the first identification of epigenetic factors that specify female gametogenesis in plants. It demonstrates that an ARGONAUTE-dependent genetic route that involves members of the

RNA-directed DNA methylation and trans-acting siRNA pathways restricts gametogenesis to a single cell, indicating that the genetic basis of female gametogenesis are directed by epigenetic mechanisms that are crucial to control events that distinguish sexual from aposporous development.

23. Singh M, Goel S, Meeley RB, Dantec C, Parrinello H, Michaud C,
•• Leblanc O, Grimanelli D: **Production of viable gametes without meiosis in maize deficient for an ARGONAUTE protein**. *Plant Cell* 2011, **23**:443-458.
This paper demonstrated for the first time a conserved function for ARGONAUTE proteins in controlling early cell specification in the ovule of Arabidopsis and maize, including the formation of viable unreduced female gametes.

24. Garcia-Aguilar M, Michaud C, Leblanc O, Grimanelli D:
• **Inactivation of a DNA methylation pathway in maize reproductive organs results in apomixis-like phenotypes**. *Plant Cell* 2010, **22**:3249-3267.
This paper shows that RNAi-dependent inactivation of some methyltransferases in maize result in mutant phenotypes that are reminiscent of apomixis in the grasses.

25. Verhoeven KJ, Van Dijk PJ, Biere A: **Changes in genomic methylation patterns during the formation of triploid asexual dandelion lineages**. *Mol Ecol* 2010, **19**:315-324.

26. Verhoeven KJF, Jansen JJ, van Dijk PJ, Biere A: **Stress-induced DNA methylation changes and their heritability in asexual dandelions**. *New Phytologist* 2010, **185**:1108-1118.

27. Cibrian-Jaramillo A, De la Torre-Barcena JE, Lee EK, Katari MS, Little DP, Stevenson DW, Martienssen R, Coruzzi GM, DeSalle R: **Using phylogenomic patterns and gene ontology to identify proteins of importance in plant evolution**. *Genome Biol Evol* 2010, **2**:225-239.

28. Lee EK, Cibrian-Jaramillo A, Kolokotronis SO, Katari MS,
•• Stamatakis A, Ott M, Chiu JC, Little DP, Stevenson DW, McCombie WR *et al.*: **A functional phylogenomic view of the seed plants**. *PLoS Genet* 2011, **7**:e1002411.
By using a functional phylogenomic approach to explore the genomic origins of seed plant diversification, this paper provides evidence indicating that RNA interference has played a significant role in the divergence of monocots from other angiosperms.

29. Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, Hannon GJ: **Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila**. *Cell* 2007, **128**:1089-1103.

30. Juliano C, Wang J, Lin H: **Uniting germline and stem cells: the function of Piwi proteins and the piRNA pathway in diverse organisms**. *Annu Rev Genet* 2011, **45**:447-469.

31. Mochizuki K: **Developmentally programmed, RNA-directed**
• **genome rearrangement in Tetrahymena**. *Dev Growth Differ* 2011, **54**:108-119.
A detailed review of what is known about DNA programmed rearrangements in a ciliated protozoan, and how they compare to epigenetic rearrangements in a variety of eukaryotes.

32. Mochizuki K, Fine NA, Fujisawa T, Gorovsky MA: **Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in Tetrahymena**. *Cell* 2002, **110**:689-699.

33. Duran-Figueroa N, Vielle-Calzada JP: **ARGONAUTE9-dependent**
• **silencing of transposable elements in pericentromeric regions of Arabidopsis**. *Plant Signal Behav* 2010, **5**:1476-1479.
An addendum to [21] showing that ARGONAUTE9-interacting small RNAs map to pericentromeric regions of Arabidopsis chromosomes, suggesting a link between the AGO9-dependent sRNA pathway and heterochromatin formation.

34. Armenta-Medina A, Demesa-Arevalo E, Vielle-Calzada JP: **Epigenetic control of cell specification during female gametogenesis**. *Sex Plant Reprod* 2011, **24**: 137-147.

35. Carman JG: **Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony**. *Biol J Linn Soc* 1997, **61**:51-94.

36. Aliyu OM, Schranz ME, Sharbel TF: **Quantitative variation for apomictic reproduction in the genus Boechera (Brassicaceae)**. *Am J Bot* 2010, **97**:1719-1731.

37. Kantama L, Sharbel TF, Schranz ME, Mitchell-Olds T, de Vries S, de Jong H: **Diploid apomicts of the Boechera holboellii complex display large-scale chromosome substitutions and aberrant chromosomes**. *Proc Natl Acad Sci USA* 2007, **104**:14026-14031.

38. Schranz ME, Kantama L, de Jong H, Mitchell-Olds T: **Asexual reproduction in a close relative of Arabidopsis: a genetic investigation of apomixis in Boechera (Brassicaceae)**. *New Phytol* 2006, **171**:425-438.

39. Horandl E: **The evolution of self-fertility in apomictic plants**. *Sex Plant Reprod* 2010, **23**:73-86.

40. Sharbel TF, Voigt ML, Corral JM, Galla G, Kumlehn J, Klukas C,
• Schreiber F, Vogel H, Rotter B: **Apomictic and sexual ovules of Boechera display heterochronic global gene expression patterns**. *Plant Cell* 2010, **22**:655-671.
By comparing transcriptomic profiles of sexual and apomictic ovules, the authors show that most heterochronic and stage-specific tags are significantly downregulated during early apomictic ovule development, whereas most late stage-specific tags are upregulated in the apomictic ovules, providing evidence of transcriptional effects linked to ploidy and the hybrid nature of ancient apomicts.

41. Beck JB, Alexander PJ, Allphin L, Al-Shehbaz IA, Rushworth C,
•• Bailey CD, Windham MD: **Does hybridization drive the transition to asexuality in diploid boechera?** *Evolution* 2012, **66**:985-995.
This paper evaluated the hypothesis that diploid *Boechera* accessions are hybrids, concluding that diploid apomicts exhibited high levels of heterozygosity resulting from the combination of disparate genomes, and that the genomic consequences of hybridization allow for the transition to gametophytic apomixis in this genus.

42. Chen ZJ: **Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids**. *Annu Rev Plant Biol* 2007, **58**:377-406.

43. Cao J, Schneeberger K, Ossowski S, Gunther T, Bender S, Fitz J, Koenig D, Lanz C, Stegle O, Lippert C *et al.*: **Whole-genome sequencing of multiple *Arabidopsis thaliana* populations**. *Nat Genet* 2011, **43**:956-963.

44. Koornneef M, Alonso-Blanco C, Vreugdenhil D: **Naturally occurring genetic variation in *Arabidopsis thaliana***. *Annu Rev Plant Biol* 2004, **55**:141-172.

45. Platt A, Horton M, Huang YS, Li Y, Anastasio AE, Mulyati NW, Agren J, Bossdorf O, Byers D, Donohue K *et al.*: **The scale of population structure in *Arabidopsis thaliana***. *PLoS Genet* 2010, **6**:e1000843.

46. Becker C, Hagmann J, Muller J, Koenig D, Stegle O, Borgwardt K,
•• Weigel D: **Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome**. *Nature* 2011, **480**:245-249.
A genome-wide DNA methylation analysis of 10 Arabidopsis lines derived for 30 generations from a common ancestor. The authors show that equivalent regions were often affected by differential methylation in independent lines, and that differentially methylated sites showed less association with transposable elements and short interfering RNA expression than invariant positions.

47. Schmitz RJ, Schultz MD, Lewsey MG, O'Malley RC, Urich MA,
•• Libiger O, Schork NJ, Ecker JR: **Transgenerational epigenetic instability is a source of novel methylation variants**. *Science* 2011, **334**:369-373.
The authors examined spontaneously occurring variation in DNA methylation in *Arabidopsis thaliana* individuals propagated by single-seed descent for 30 generations. They present an exhaustive genomic atlas of CG single methylation polymorphisms and differentially methylated regions, comparing recent and ancestral states. Their results show that transgenerational epigenetic variation in DNA methylation is able to generate new allelic variants as a source of phenotypic diversity.

48. Greaves IK, Groszmann M, Ying H, Taylor JM, Peacock WJ, Dennis ES: **Trans chromosomal methylation in Arabidopsis hybrids**. *Proc Natl Acad Sci USA* 2012, **109**: 3570-3575.

49. Shen H, He H, Li J, Chen W, Wang X, Guo L, Peng Z, He G, Zhong S, Qi Y *et al.*: **Genome-wide analysis of DNA methylation and gene expression changes in two Arabidopsis ecotypes and their reciprocal hybrids**. *Plant Cell* 2012, **24**:875-892.

50. Durand S, Bouche N, Perez Strand E, Loudet O, Camilleri C: **Rapid**
•• **establishment of genetic incompatibility through natural**
 epigenetic variation. *Curr Biol* 2012, **22**:326-331.
Natural epialleles originating from structural variation can cause the rapid
establishment of reproductive traits such as genetic incompatibility in
Arabidopsis, in this case through rearranged gene copies that cause de
novo DNA methylation and silencing through RNA-directed DNA methy-
lation.

51. Smith LM, Bomblies K, Weigel D: **Complex evolutionary events**
•• **at a tandem cluster of *Arabidopsis thaliana* genes resulting in**
 a single-locus genetic incompatibility. *PLoS Genet* 2011,
 7:e1002164.
This paper describes in detail the genetic basis and molecular mechan-
isms that regulate a case of non-additive interaction between alleles,
providing novel insights into how tandem arrays can produce genetic
novelty in plants.

52. Todesco M, Balasubramanian S, Cao J, Ott F, Sureshkumar S,
• Schneeberger K, Meyer RC, Altmann T, Weigel D:
 Natural variation in biogenesis efficiency of individual
 ***Arabidopsis thaliana* microRNAs**. *Curr Biol* 2012,
 22:166-170.
This paper shows for the first time that phenotypic variation can result
from natural variation in miRNA biogenesis caused by frequent poly-
morphisms that affect processing of miRNA precursors.

53. Waddington CH: **Canalization of development and the**
 inheritance of acquired characters. *Nature* 1942, **150**:563-565.

54. Calarco JP, Martienssen RA: **Genome reprogramming and small**
• **interfering RNA in the Arabidopsis germline**. *Curr Opin Genet*
 Dev 2011, **21**:134-139.
A synthetic review that emphasizes the movement of small RNA signals
important for silencing transposable elements, as well as the control of
meiosis and germ cell fate in plants.

55. Gibson G, Dworkin I: **Uncovering cryptic genetic variation**. *Nat*
 Rev Genet 2004, **5**:681-690.

56. Masel J, Trotter MV: **Robustness and evolvability**. *Trends Genet*
 2010, **26**:406-414.