

REVIEW

Sex and the flower – developmental aspects of sex chromosome evolution

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- **Background** The evolution of dioecious plants is occasionally accompanied by the establishment of sex chromosomes: both XY and ZW systems have been found in plants. Structural studies of sex chromosomes are now being followed up by functional studies that are gradually shedding light on the specific genetic and epigenetic processes that shape the development of separate sexes in plants.
- **Scope** This review describes sex determination diversity in plants and the genetic background of dioecy, summarizes recent progress in the investigation of both classical and emerging model dioecious plants and discusses novel findings. The advantages of interspecies hybrids in studies focused on sex determination and the role of epigenetic processes in sexual development are also overviewed.
- **Conclusions** We integrate the genic, genomic and epigenetic levels of sex determination and stress the impact of sex chromosome evolution on structural and functional aspects of plant sexual development. We also discuss the impact of dioecy and sex chromosomes on genome structure and expression.

Key words: Dioecy, sex determination, sex chromosomes, genome evolution, epigenetics, transposable elements.

INTRODUCTION

While dioecious species represent a minority in angiosperms, there are nevertheless about 15 600 dioecious species (5–6 % of all species) in 987 genera (7 % of all genera) and 175 families (43 % of all families) (Renner, 2014). An important feature of flowering plants is the numerous transitions between breeding systems that have occurred across the phylogeny (Tree of Sex Consortium, 2014). Sex chromosomes have so far been identified in about 40 species and observably different morphology between X (Z) and Y (W) has been confirmed in just 18 species (Ming *et al.*, 2011; Renner, 2014).

The presence of sex chromosomes enables a part of the genome (the non-recombining part of the Y or W) to optimize its function fully for the benefit of one sex and also influences evolution of the genes in the rest of the genome (reviewed by Charlesworth, 2013, 2015). Degeneration of sex chromosomes occurs as a consequence of suppressed recombination, and dosage compensation can be established (Muyle *et al.*, 2017). Degeneration of sex chromosomes has been reported in the following plant species: *Silene latifolia* (Marais *et al.*, 2008; Muyle *et al.*, 2012; Bergero *et al.*, 2015; Papadopulos *et al.*, 2015), *Carica papaya* (Wu and Moore, 2015), *Mercurialis annua* (Ridout, 2017), *Rumex hastatulus* (Hough *et al.*, 2014; Beaudry *et al.*, 2017; Crowson *et al.*, 2017; Hough *et al.*, 2017) and *Rumex rotschildianus* (Beaudry *et al.*, 2017). With the exception of *C. papaya*, the degeneration was found only in the Y-linked regions. In papaya, not only the non-recombining male-specific region of the Y (MSY) but also the corresponding part of the

X chromosome (both located close to the centromere) shows signs of degeneration (Wu and Moore, 2015). The X chromosome in papaya has been influenced by a selective sweep that drastically lowered its diversity (VanBuren *et al.*, 2016). A relatively high percentage of deleted sequences from the Y chromosome (tens of per cent) has been detected in *S. latifolia* (Bergero *et al.*, 2015; Papadopulos *et al.*, 2015) and in *R. rotschildianus* (Beaudry *et al.*, 2017). In contrast, in *M. annua*, only 0.2 % of genes appear to be lost. Pseudogenization of the Y-linked genes is more frequent in *M. annua* (approx. 36 %), suggesting that even in this system there is a tendency to degeneration of the Y-linked alleles. Comparatively, no significant degeneration of the sequences in the non-recombining region has been found in *Salix viminalis* (Pucholt *et al.*, 2017). Albeit the dioecy in the *Salicaceae* is of an ancient origin, this fact suggests that the current sex-determining region (SDR) in *Salix* is relatively young, and switches in sex determination occurred in *Salicaceae*. These switches could have been caused by a new master gene that took over the sex-determining function (reviewed by Beukeboom and Perrin, 2014). An alternative explanation is that a random ZW recombination occurred in sex-reversed individuals. The occasional XY recombination enabled by periodic sex reversal is known to protect the Y chromosome in frogs from degeneration (Perrin, 2009; Rodrigues *et al.*, 2018). Rare cases of hermaphroditism have also been reported in the genus *Salix* (reviewed by Mirski, 2014; Nagamitsu and Futamura, 2014). There are, however, no data concerning the possible influence of sexual phenotype on Z–W recombination in *Salix*. If compared with other taxa, the sex-determining systems in the

genus *Fragaria* (small mobile sex-determining cassette) appear as an efficient method to avoid the spread of W chromosome degeneration (Tennessen et al., 2017). The current data support the view of Bachtrog et al. (2014) that the degeneration of Y and W chromosomes is not inevitable, at least in some systems.

Phenotypic effects of the changes that occur on the sex chromosomes include lowered fitness and fertility, and reduced numbers of one sex in interspecific crosses. The occurrence of these problems in animals and also in plants (Demuth et al., 2014) seems to follow Haldane's rule (Haldane, 1922: 'When in the offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex'). The study of the causes of male rarity and low fertility in male hybrid plants in interspecific crosses (Haldane's rule) between two dioecious species of *Melandrium*, i.e. *S. diclinis* and *S. latifolia*, by Demuth et al. (2014) has also revealed a possible difference in mechanisms behind Haldane's rule in *Silene* and animal models. The dominance theory [supposing that the problems in interspecific hybrids are caused by recessive mutations in X-linked genes as they are only present in one copy in the hybrid of heterogametic sex (reviewed by Turelli and Orr, 1995)] is sufficient to explain male sterility in *Silene* hybrids, whereas male rarity is more likely to involve faster male evolution (Demuth et al., 2014) or faster heterogametic sex evolution (Delph and Demuth, 2016) but not dominance. Faster male evolution means that males are under stronger sexual selection and so the corresponding genes are evolving faster (Wu and Davis, 1993). Faster heterogametic sex evolution results from parts of Y- and W-linked sequences evolving without recombination and then co-segregating with heterogametic sex. This can lead to a species-specific loss of some Y- or W-linked genes or to the species-specific differentiation of the X- and Y-linked alleles as a result of the conflict in optima between sexes, e.g. in the sex ratio (reviewed by Delph and Demuth, 2016). Male rarity in interspecific crosses between *S. diclinis* and *S. latifolia* could also result from the evolution of the mechanisms controlling sex bias in *S. latifolia* and *S. diclinis*. This more severe female bias has also been observed in interspecific crosses between *S. latifolia* and *S. dioica* (Taylor, 1994a, b). In these systems, the female genomes appear to carry alleles that cause a female bias, whilst the Y chromosomes carry alleles tending to restore the 1:1 sex ratio. This restoration is apparently not sufficiently efficient in the interspecific crosses. From this point of view, the rapid evolution of the Y-linked sex ratio-restoring alleles in response to the female bias-generating alleles in the rest of genome could be the cause of male rarity in interspecific crosses between *S. diclinis* and *S. latifolia*. The consequences of the fast evolution of the Y-linked genes are apparent also in interspecific hybrids between dioecious and hermaphrodite species, e.g. in the cross between *S. latifolia* and *S. viscosa* (Zluvova et al., 2005).

The possible role of the X chromosome in speciation (large-X effect) in the genus *Silene* has also been tested. A study by Hu and Filatov (2016) revealed the consequences of the large-X effect for reproductive barriers between *S. latifolia* and *S. dioica*. The interspecific gene flow between these species in X-linked sequences is significantly lower than the gene flow in autosomal sequences, suggesting that the X chromosome could play a role in speciation similar to that in animal models. In these models, the hemizygotic status of X reveals the recessive putatively harmful alleles that accumulated on the X

chromosome and that are compensated for in the original species but cause problems in interspecific hybrids.

The presence of genes with sex-specific expression on sex chromosomes is widespread in plants. Similar to animal species, there are also autosomal sequences which are sex specifically expressed (Zluvova et al., 2010; Zemp et al., 2014, 2016). In contrast to animals though, sexual dimorphism in autosomal gene expression in plants is mostly associated with the presence of sex-specific organs and tissues, and therefore can be mostly detected in flowering plants. However, cases of sex-specific expression of autosomal genes in vegetative plant tissues have been reported (Zluvova et al., 2010; Zemp et al., 2014, 2016). The detailed study by Zemp et al. (2016) compared the gene expression patterns of both sexes in *S. latifolia* with those of a hermaphrodite individual of *S. vulgaris*. The expression patterns in the *S. latifolia* female differed greatly from the expression of the given genes in the *S. vulgaris* hermaphrodite. Both higher and lower expression was observed in an *S. latifolia* female, and sex-biased gene expression was more frequent in sex-linked genes. The results support the possible feminization of the X chromosome and the masculinization of the Y chromosome.

In this review, we summarize the current knowledge concerning the sex-determining mechanism in several model dioecious species including the epigenetic aspects revealed in some species and, subsequently, we review the questions connected with the origin of dioecy and the evolution of the genomes of dioecious plants with a focus on repetitive sequences.

GENETIC MECHANISMS OF SEX DETERMINATION IN FLOWERING PLANTS

Sex-determining systems with largely differentiated sex chromosomes

Species with largely differentiated sex chromosomes show considerable advantages for studies at the cytogenetic level. Heteromorphic sex chromosomes permit the employment of modern techniques such as flow sorting and microdissection to isolate sex-linked sequences directly. On the other hand, it seems to be more complicated to elucidate the sex-determining systems in these species at the molecular genetic level than in species with less differentiated sex chromosomes as too large a number of candidate genes exist in large non-recombining regions.

Genus Silene section Melandrium (Caryophyllaceae). Since the discovery of the first plant XY sex determination system in *Silene latifolia* (Winge, 1923), plant sex chromosomes have attracted considerable scientific attention. The first attempt to study the role of plant sex chromosomes in developmental processes dates back to the 1940s. As a by-product of experiments with polyploids in *S. latifolia*, Westergaard created Y deletion mutants with various sexual phenotypes – hermaphroditic and pollen sterile male flowers (Westergaard, 1946). This and later studies on mutants carrying chromosomal aberrations revealed three important, physically separated Y-linked regions: the gynoeccium-suppressing functional region (GSF), the stamen-promoting functional region (SPF) on the *p* arm, and the male fertility functional (MFF) region on the *q* arm (Westergaard, 1946; Donnison et al., 1996; Farbos et al., 1999) (Fig. 1A–E).

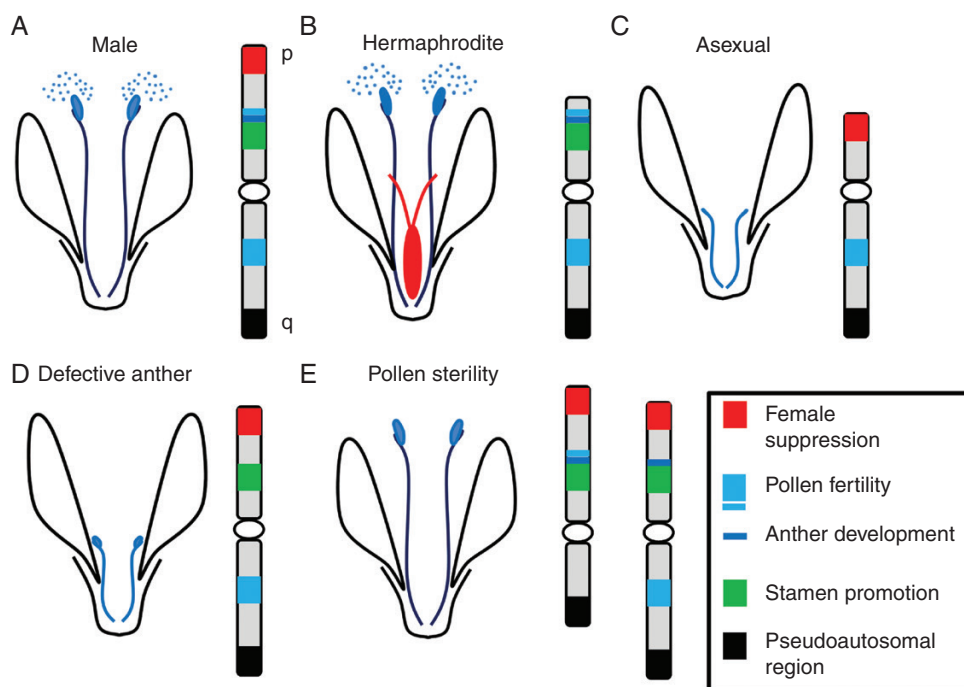


FIG. 1. Schematic functional map of the *S. latifolia* Y chromosome. Study of individuals with chromosomal aberrations revealed three functional regions on the Y chromosome. (A) Wild-type male. (B) Deletion of a distal section (red) of the *p* arm led to a hermaphrodite. Thus, this functions as the gynoecium-suppressing functional (GSF) region. (C) A proximal area of the *p* arm (marked in green) produces an asexual phenotype when deleted. For this reason, it has been designated as the stamen-promoting functional (SPF) region. (D) The region important for further anther development is located near to the SPF region. (E) Deletion of male fertility factors (MFFs) residing mainly on the *q* arm (light blue) but also in proximity to the SPF region on the *p* arm leads to male sterility.

Later, large-scale deletions (disruptions) of the Y chromosome were introduced by means of either gamma or X-ray irradiation (Farbos *et al.*, 1999; Lebel-Hardenack *et al.*, 2002). Radiation-induced mutants, in addition to the deletion mutants already observed by Westergaard (1946) [hermaphrodites (Fig. 1B) and infertile males (Fig. 1D, E)], produced asexual plants (Fig. 1C) (containing a Y deletion), confirming Westergaard's model of the Y chromosome in *S. latifolia* (Donnison *et al.*, 1996; Barbacar *et al.*, 1997; Lardon *et al.*, 1999). The model of Y chromosome organization was later improved by Zluvova *et al.* (2007), who showed (based on deletion mapping and the histological studies of anthers in deletion mutants) the existence of further genes involved in anther development and also one male fertility gene close to the stamen promoter region (Fig. 1D, E). Recently, the integration of deletion mutant data from various resources to study SDRs has been improved using the DelMapper software package (based on the resolution of the travelling salesman problem) and fast neutron-induced mutants (Kazama *et al.*, 2016).

However, despite these efforts, the molecular details of the sex determination in *S. latifolia* remain unknown. In fact, only a few sex-linked genes have been studied in detail in *S. latifolia* so far. The Class B floral MADS-box gene *APETALA3* (*SIAP3* – TM6 lineage) was confirmed to be localized on *S. latifolia* sex chromosomes (Cegan *et al.*, 2010) and, interestingly, expression studies of *SIAP3* revealed a different mRNA pattern in relation to sex, with the Y allele expressed predominantly in the stamens and petals, and the X allele expressed in petals, styles and ovaries (Matsunaga *et al.*, 2003). Therefore, *SIAP3* represents a unique case of a floral developmental gene where an originally identical pair of alleles residing on autosomes underwent sub-functionalization during sex chromosome evolution.

In comparison, in arabidopsis, *APETALA3* controls anther development, and a mutant phenotype involves anther replacement by carpels in the third whorl of the flower (Bowman *et al.*, 1991). It is therefore possible that a homologue of this gene confers sex-specific floral development in *S. latifolia*, even though it is not directly located in one of the functionally described Y-linked regions.

The autosomal genes controlling gynoecium development are probably involved in flower meristem maintenance and in the control of its size. Orthologues of *SHOOTMERISTEMLESS* (*SISTM1* and *SISTM2*) and *CUP-SHAPED COTYLEDON* (*SICUC*) show a male-specific expression pattern in meristematic tissue before any morphological differences between the sexes become apparent (Zluvova *et al.*, 2006). Later, another study showed that *SICLV1* (orthologue of *CLAVATA1*) might also be engaged in carpel suppression (Koizumi *et al.*, 2010).

Another candidate gene with a possible role in sex-specific development in *S. latifolia* is the homologue of *WUSCHEL* (*SIWUS1*) which has also been reported to contribute to meristem maintenance and anther development in arabidopsis (Deyhle *et al.*, 2007). There are two copies of *SIWUS* in *S. latifolia* (*SIWUS1* and *SIWUS2*), one located on the X chromosome with no homologous copy on the Y chromosome (Kazama *et al.*, 2012). Since expression studies have shown no dosage compensation of *SIWUS1* in early floral meristem development, *SIWUS1* could act as a haploinsufficient gene involved in carpel development. Nevertheless, the proposed functions of *SIAP3* and *SIWUS1* in *S. latifolia* are not supported by any direct evidence, since *S. latifolia* was considered recalcitrant to genetic transformation. Recently, a protocol for gene delivery to *S. latifolia* was developed (Hudzieczek *et al.*, 2018), allowing

functional studies of plant sex-linked genes using targeted mutagenesis and overexpression.

Genus Rumex (Polygonaceae). Most widely studied species of this genus show either completely dosage-based (X/A system similar to *Drosophila*; XX/XY1Y2 karyotype) SDRs (e.g. *Rumex acetosa*: Ono, 1935; Yamamoto, 1938) or modified dosage-based (X/A) sex-determining systems (*R. hastatulus*). Two races occurring in *R. hastatulus* differ significantly in their karyotype (North Carolina: XX/XY1Y2 and Texas: XX/XY), and two autosome–heterosome translocations were reported in the North Carolina race (Grabowska-Joachimiak et al., 2015). In spite of these differences, the mechanism of sex determination in both races of *R. hastatulus* appears similar: an X/A balance mechanism modified in the male direction by the Y chromosome. The Y chromosome, or its fragment, can promote anther development in AAXX plants (Y chromosomes are not involved in gynoeceum suppression) and the sex phenotype can be influenced by the environment and/or genetic background (Smith, 1963; Bartkowiak, 1971). Recent efforts in *R. acetosa* have been focused on the isolation of genes with connections to the female sex programme. A set of genes largely expressed in female floral buds was isolated by cDNA subtraction, and the interesting candidate gene *FEM32* underwent molecular and functional analysis (Manzano et al., 2017). This gene encodes a protein with a common architecture in the aerolysin family, acting as β pore-forming toxins. Since genetic analysis of gene function in *R. acetosa* was not feasible, the *FEM32* gene was cloned and studied in transgenic tobacco. The transgenic plants were strongly feminized and their pollen fertility was rather low. The authors concluded that the *FEM32* gene may function in *R. acetosa* by arresting stamen and pollen development probably via programmed cell death (Manzano et al., 2017).

A dominant Y chromosome-based sex-determining system with X and Y chromosomes that appeared indistinguishable when using an optical microscope was found in *R. acetosella* (Singh and Smith, 1971). Later, electron microscopic observations of whole-mount preparations of synaptonemal complexes (SCs) at pachytene made it possible to detect heteromorphic sex chromosomes in *R. acetosella* and in the related species *R. suffruticosus* (Cuñado et al., 2007).

Coccinia grandis (Cucurbitaceae). *Coccinia grandis* possesses the largest Y/autosome divergence in flowering plants (Sousa et al., 2013). Its relatively small genome size (0.943 pg/2C in males), large Y chromosome and phylogenetic proximity to the fully sequenced *Cucumis sativus* (Huang et al., 2009) makes *C. grandis* a promising model to study sex chromosome evolution. Current RNA sequencing (RNA-seq)-based studies (Devani et al., 2017; Mohanty et al., 2017) support the view that the sex-determining mechanism in *Coccinia* could be similar to that of *C. sativus* and *Cucumis melo*, see below.

Sex-determining systems with small sex-specific regions (homomorphic or non-conspicuously differentiated sex chromosomes)

Progress in the identification of genes responsible for sex determination in dioecious species has increased dramatically due to novel sequencing techniques and the employment of

reverse genetic methods. Recently, candidates for sex determination have been discovered in several plant species. For technical reasons, the most significant results have been achieved in species with small SDRs. Here, we are examining several species with non-differentiated sex chromosomes and some with no sex chromosomes.

Genus Diospyros (Ebenaceae). Persimmon (*Diospyros lotus*) possesses a very small SDR despite a long evolutionary history of dioecy (Akagi et al., 2014). In the Caucasian persimmon, *Diospyros lotus*, a dioecious plant with heterogametic males (XY), a male-determining region was identified via genome sequencing and a combination of transcriptomics and phylogenetic analyses. The only sex-determining gene in this region is *OGI* which encodes a small RNA (sRNA) targeting the autosomal *MeGI* gene, a homeodomain transcription factor suppressing anther fertility. As no gynoeceum-suppressing gene has so far been identified in the Y-linked SDR in *D. lotus*, the authors consider an alternative model for sex determination in persimmon based on a single master gene (*OGI*) that could act similarly to the *SRY* gene in the human (Sinclair et al., 1990) (marked as *Sry* in other mammals). The SDR Y protein (transcription factor coded by *SRY*) works as a trigger of male development and so simultaneously indirectly suppresses female development. If the action of the *SRY* gene is absent, the female developmental programme is realized (reviewed by Kashimada and Koopman, 2010). The male-promoting role of *OGI* has also been reported in the polygamous *D. kaki* (Zhang et al., 2016). Taking into account the long evolutionary history of dioecy in the ancestors of *Diospyros*, we can speculate that dioecy could have evolved via the two-mutation-based model (Charlesworth and Charlesworth, 1978) but later the sex-determining system was transformed into a one master gene-based system as a consequence of the sex dimorphism evolution as suggested by Janousek and Mrackova (2010). Last, but not least, the presence of an unknown Y-linked female suppressor was considered possible (Akagi et al., 2014) and therefore the two-gene model could be applied for *D. lotus*.

Genus Fragaria (Rosaceae). This well-studied genus includes gynodioecious, sub-dioecious and dioecious species. Female heterogamety (ZW system) with dominant male sterility alleles was found in sub-dioecious *F. virginiana* (Spigler et al., 2008, 2010) and dioecious *F. chiloensis* (Goldberg et al., 2010). Recent studies (Tennessen et al., 2017; Wei et al., 2017) show that the SDR is located in three different positions in different chromosomes from the same homoeologous group in three different octoploid taxa: *F. virginiana* ssp. *platypetala*, *F. virginiana* ssp. *virginiana* and *F. chiloensis*.

The results obtained by Tennessen et al. (2017) further show that the SDR (i.e. a conserved SDR cassette) repeatedly changed genomic location across octoploid *Fragaria*. This is the first unambiguous evidence of SDR translocation in flowering plants. Only two coding genes, annotated as GDP-mannose 3,5-epimerase 2 and 60S acidic ribosomal protein P0, were identified in this mobile SDR cassette. These two genes are expected to have roles in sex determination, but the mechanism of their action in *Fragaria* has not yet been identified.

Asparagus officinalis (Asparaceae). Garden asparagus (*Asparagus officinalis*), a dioecious species with an XX/XY sex

determination system, possesses homomorphic sex chromosomes with a hypothetical male activator and female suppressor located in the M locus on the Y chromosome (Telgmann-Rauber *et al.*, 2007). RNA-seq analysis of female (XX), male (XY) and supermale (YY) individuals identified male- and female-biased expression and supported the hypothesis that anther abortion in the female flower started during development of the tapetum and early microspore (Harkess *et al.*, 2015). A further study revealed male and female differentially expressed microRNAs (miRNAs) by sRNA and degradome sequencing (Chen *et al.*, 2016a). Finally, genome sequencing of *A. officinalis* revealed 13 genes in approx. 1 Mbp of the non-recombining Y region (Harkess *et al.*, 2017). Two genes in this region have been identified as candidates for sex determination. The function of *SUPPRESSOR OF FEMALE FUNCTION (SOFF)* in the cessation of pistil development has been verified in XY hermaphroditic mutants, while the second candidate gene *Asparagus DEFECTIVE IN TAPETUM DEVELOPMENT AND FUNCTION 1 (AspTDF1)* is exclusively expressed in males and was previously described as *Male Specific Expression 1 (MSE1)* (Harkess *et al.*, 2017; Murase *et al.*, 2017). *AspTDF1* very probably functions as a male activator, considering that a *TDF1* mutant in *Arabidopsis thaliana* exhibited degeneration of tapetal cells similar to *A. officinalis* females (Murase *et al.*, 2017). An orthologous sequence to SOFF has also been found in *A. conchinchensis* (a dioecious species closely related to *A. officinalis*), but it is not present in the hermaphrodite *A. virgatus*, suggesting the ancestral role of SOFF in suppression of female function in dioecious species of the genus *Asparagus*. The role of the *AspTDF1* gene is unclear in related *A. conchinchensis* as it appears pseudoautosomal in this species. However, the fact that SOFF has only a female suppression function suggests that in *A. conchinchensis* there must be some other cause of male organ suppression in females and thus also another mechanism of restoration of anther development in males. Anyway, these findings strongly indicate that the two-gene model represents the most likely scenario for the evolution of sex determination in *Asparagus*.

Carica papaya (Caricaceae). Sex determination in the papaya (*Carica papaya*) has been subjected to detailed genetic studies in the past. Hofmeyr (1938, 1939) and Storey (1938) proposed three alleles; M, M^b and m, to determine plant sex types (M1, M2 and m according to Hofmeyr, 1938), stressing the dominance of maleness (M) and hermaphroditism (M^b) over the female phenotype in heterozygotes. More recent studies showed that sex determination in the papaya is based on the standard XX/XY system which, however, includes the modified Y chromosome (Y^h, corresponding to the M^b allele of Storey, 1938) which is unable to suppress gynoecium development (reviewed by Ming *et al.*, 2007). Plants lacking an X chromosome were absent from the progeny of hermaphrodites, suggesting the presence of a recessive lethal allele on both types of Y chromosome. The exact mechanism of sex determination in the papaya is not yet clear, but it is known that the action of gibberellins is necessary for gynoecium suppression in papaya male plants (Kumar and Jaiswal, 1984). In some plants, the gibberellin inhibitor caused a direct change from male to female. This fact points to a possible difference of the sex-determining mechanism in papaya from the active gynoecium suppressor-based mechanism (also

involving a separate Y-linked anther promoter) suggested by Westergaard (1958). On the other hand, increased concentrations of gibberellins do not cause any change in the sexual phenotype in papaya, suggesting that the level of gibberellins is not itself sufficient to control sex determination (Han *et al.*, 2014). Papaya is intensively studied at both the transcriptomic and genomic level (Liu *et al.*, 2004; Ming *et al.*, 2008; Wang *et al.*, 2012; VanBuren *et al.*, 2015). Most of the miRNAs overexpressed in the male flowers were related to auxin signalling pathways, whereas the miRNAs overexpressed in female flowers were the potential regulators of apical meristem identity genes (Aryal *et al.*, 2014). Results of the sequencing and comparisons of the non-recombining regions between hermaphrodites and males suggest that the difference is not present in any coding region and so implies that hermaphroditism may be controlled by changes in gene regulation. Overall, identification of the gene controlling suppression of carpel development in males is quite a challenging task (VanBuren *et al.*, 2015).

Cucumis melo and *Cucumis sativus* (Cucurbitaceae). No sex chromosomes have so far been identified in *Cucumis melo* (mostly monoecious species). Instead, three genes – each located on a different chromosome – are involved in sex determination (Boualem *et al.*, 2008, 2015; Martin *et al.*, 2009). Monoecy in melon (*Cucumis melo*) is maintained by two dominant alleles of these loci – *Andromonoecious* (A) and *Gynoecious* (G) (Kenigsbuch and Cohen, 1990). First, the A locus was reported as a gene encoding 1-aminocyclopropane-1-carboxylic acid synthase (*CmACS7*), responsible for repressing male organ development (in female flowers) via the ethylene biosynthesis pathway. Therefore, a recessive mutation (genotype *aaG*⁻) in this gene results in individuals carrying male and hermaphroditic flowers (Boualem *et al.*, 2008). Secondly, the G allele was identified as a WIP subfamily C2H2 zinc finger transcription factor (*CmWIP1*) responsible for the arrest of carpel development. In gynoecious individuals (*A-gg*), the G allele is silenced by heritable epigenetic modification – DNA methylation triggered by the insertion of a transposon in the *CmWIP1* promoter region. Furthermore, *CmWIP1* indirectly inhibits the expression of the *CmACS7* (*Andromonoecious*) gene, thus supporting anther development (Martin *et al.*, 2009). Recently, an *androecy* gene *CmACS11* in melon was discovered. *CmACS11* is also involved in catalysing ethylene biosynthesis but it negatively regulates the *CmWIP1* gene and therefore its disruption leads to the formation of males (Boualem *et al.*, 2015). A recent study by Latrasse *et al.* (2017) shows that *CmACS11* represses the expression of the male-promoting gene *CmWIP1* through an epigenetic mechanism (via trimethylation of Lys27 in histone H3). Taken together, the combination of allele variants of *CmACS7*, *CmWIP1* and *CmACS11* is responsible for various sexual phenotypes including hermaphrodite, monoecious, gynoecious and androecious individuals. A similar system of sex determination (also based on the ethylene biosynthesis pathway) has been found in *Cucumis sativus* (Boualem *et al.*, 2009). The melon sex determination system, even without sex chromosomes, illustrates how the evolution of unisexual flowers could take a different evolutionary trail from a ‘two mutations on one chromosome model’.

Genus Populus (Salicaceae). While the sex chromosomes in all studied species of the genus *Populus* are homomorphic,

archeological data suggest that the genus had (together with the genus *Salix*) a dioecious ancestor and that the dioecy in *Salicaceae* is old. In the *Salicaceae*, almost all species are dioecious, suggesting that sex determination may be as old as 45 million years, based on fossils of *Pseudosalix* (leaves and flowers; extinct sister genus to *Salix* and *Populus*) (Boucher et al., 2003) and fossils of two extinct species of *Populus* (Manchester et al., 2006).

Geraldes et al. (2015) identified male heterogamety in several cottonwoods (species of sections *Tacamahaca* and *Aigeiros*): *P. trichocarpa*, *P. balsamifera*, *P. deltoides* and *P. nigra*. Male heterogamety has also been found in aspens (*P. tremula* and *P. tremuloides*, section *Populus*) (Kersten et al., 2014; Pakull et al., 2015). At present, it appears that only one *Populus* species shows female heterogamety (*P. alba*, section *Leuce*; Paolucci et al., 2010). Male heterogamety was, however, also reported in this species (reviewed by Kersten et al., 2017). The study by Geraldes et al. (2015) revealed that the SDRs in *P. trichocarpa*, *P. balsamifera*, *P. deltoides* and *P. nigra* are homologous. In contrast, the SDR in *Populus tremuloides* (aspen) differs in that it resides on the homologous chromosome (LG 19) but in a pericentromeric position, suggesting that there was at least one switch in sex determination among the species showing male heterogamety (Kersten et al., 2014; Pakull et al., 2015).

In addition, two putative sex-determining genes have been identified in *P. trichocarpa* and *P. balsamifera* (Geraldes et al., 2015). The poplar contains sex-linked orthologues of the *A. thaliana* gene *ARABIDOPSIS RESPONSE REGULATOR 17* (*ARR17*) which mediates the output of cytokinins, and of arabisopsis *METHYLTRANSFERASE 1* (*MET1*) which is involved in DNA methylation. Male-specific DNA methylation patterns in PbRR9 (*Populus balsamifera* putative response regulator RR, see above) suggest that the methylated allele could be involved in sex determination in *Populus* (Bräutigam et al., 2017). A more detailed analysis is, however, necessary to confirm this finding and to describe the exact mechanism of the action of PbRR9 in sex determination. One possibility could be that DNA methylation inactivates the Y-linked allele and the gene exhibits haploinsufficiency. Transcription patterns of the X and Y alleles of PbRR9 are, however, so far unknown.

Genus Salix (*Salicaceae*). Female heterogamety has previously been described in three species [*S. purpurea* (Carlson et al., 2017)], *S. viminalis* (Pucholt et al., 2015) and *S. suchowensis* (Chen et al., 2016b)]. Carlson et al. (2017) identified TCP-1 (SapurV1A.1538s0020) as one of the few sex-biased genes that exhibited complete null expression in the male shoot tip. TCP transcription factors play pivotal roles in the control of shoot morphogenesis by negatively regulating the expression of boundary-specific genes. TCP24 negatively modulates secondary wall thickening of the anther endothecium in *Arabidopsis* (Wang et al., 2015). It was suggested that this gene could play a major role in the suppression of the anther endothecium in female *S. purpurea* catkins (Carlson et al., 2017). It was shown that the SDR in *Salix viminalis* evolved independently from the SDR present in most of the studied *Populus* species (Hou et al., 2015; Pucholt et al., 2015). *Salix viminalis* has also been used to evaluate the relative rate of evolution for sex-biased genes. The results showed slower sequence evolution for male-biased genes expressed in the reproductive tissue compared with unbiased and female-biased genes (Darolti et al., 2018).

Mercurialis annua (*Euphorbiaceae*). While initial studies suggested a three-locus model for sex determination in diploid dioecious *Mercurialis annua* (Louis, 1989; Durand and Durand, 1991), more recent research shows that sex determination in natural populations is based on the standard XX/XY sex-determining system (Russell and Pannell, 2015; Ridout et al., 2017). The current data also reveal that *M. annua* possesses a relatively large non-recombining region corresponding to about 40 cM on the X chromosome map (Ridout et al., 2017), and this region also began to accumulate specific repetitive sequences (Veltos et al., 2018). This species is exceptional in variability of breeding systems as the tetraploid races are monoecious and the hexaploid races are androdioecious or monoecious (reviewed by Russell and Pannell, 2015). The hexaploid races are believed to be a result of an interspecific hybridization between the monoecious tetraploid race of *M. annua* and its diploid dioecious relative *M. huetii* (Russell and Pannell, 2015).

Section Otites of the genus Silene (*Caryophyllaceae*). In contrast to the section *Melandrium*, the sex-determining systems of the *Silene* section *Otites* display much higher dynamics and variability. Very small sex-linked non-recombining regions are present in this section (Mrackova et al., 2008; Slancarova et al., 2013; Balounova et al., 2018), as can be deduced from the small number of sex-linked molecular markers. Interestingly, both female and male heterogamety is present in this section (Mrackova et al., 2008; Slancarova et al., 2013) which is a rare phenomenon in plants. The study of Balounova et al. (2018) suggests that the original sex-determining system in *Otites* was female heterogamety and that a switch to male heterogamety occurred in *S. colophylla*. This switch was also connected to the change of the linkage group from which the sex chromosomes were recruited.

MODIFICATION OF SEX EXPRESSION – ENVIRONMENTAL INFLUENCES AND EPIGENETIC PERSPECTIVE

Gynoecium development in genetic males – epigenetics and environmental influences

Sex expression in dioecious plants with differentiated sex chromosomes is usually genetically determined; however, epigenetic processes may play a role in some species and can cause instability of the sexual phenotype. While gynoecium suppression in *S. dioica* shows some level of instability even in natural populations, the sexual phenotype in natural European populations of *S. latifolia* is very stable (van Nigtevecht, 1966). However, modification of sex expression in *S. latifolia* is possible using the epigenetic drug 5-aza-cytidine to inhibit DNA methyltransferase leading to the hypomethylation of DNA (Janousek et al., 1996). Application of this drug to germinating seeds changes some males to androhermaphrodites, while no apparent phenotypic changes are observed in females. The effect can be enhanced with zebularine, another DNA-hypomethylating drug (V. Bacovsky and R. Hobza, unpubl. res.) (Fig. 2A–D). The DNA methylation pattern is transmitted to subsequent generations in crosses of the androhermaphrodite plant as a pollen donor with wild-type females. Androhermaphroditism is, however, not inherited if a hermaphrodite plant is used as

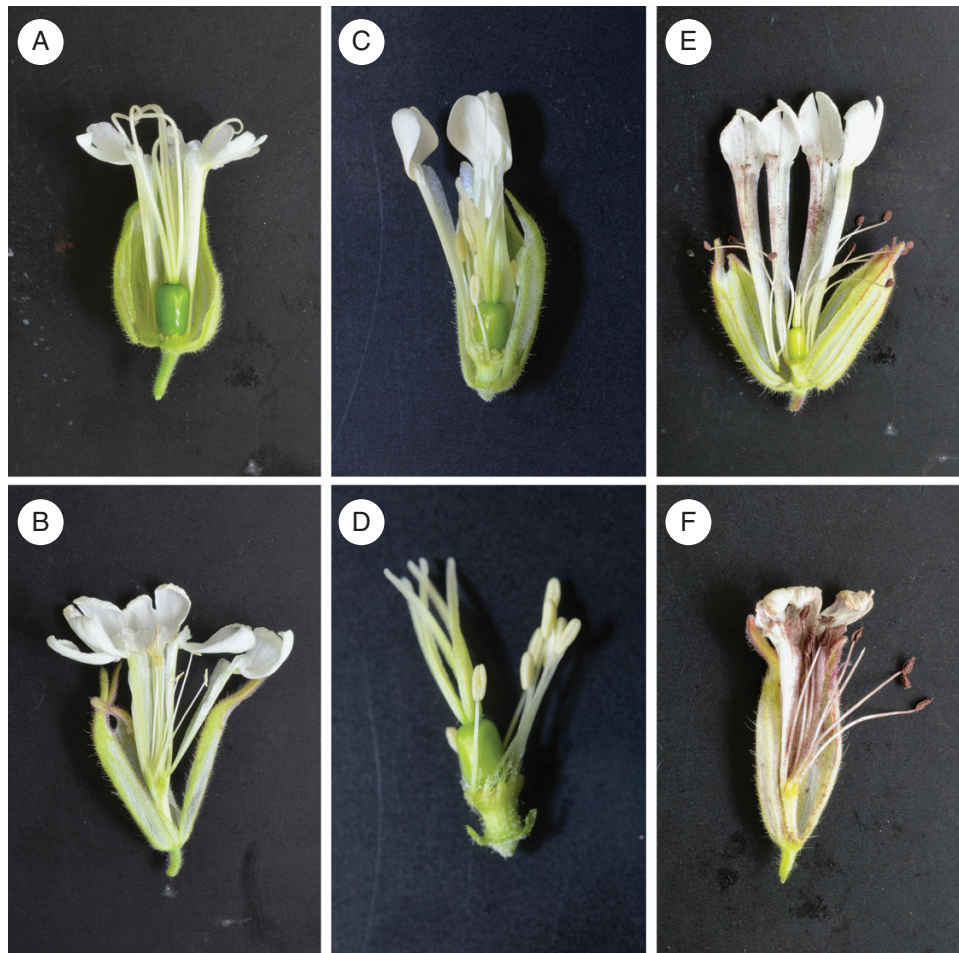


FIG. 2. *Silene latifolia* male and female flower development modification phenomena. Wild-type female (A) and male (B) individual flowers; global view (C) and detail (D) of a flower of a male individual with androhermaphrodite phenotype after zebularine treatment (50 μM). The experimental procedure was otherwise identical to the 5-aza-cytidine experiment by Janousek *et al.* (1996); flower of female (E) and male (F) individuals infected with *M. violaceum*.

a seed parent (Janousek *et al.*, 1996). It was shown (Janousek *et al.*, 1998) that androhermaphroditism is inherited as an allele tightly linked to the Y chromosome and that the Y chromosome is not inherited through the female gametic line in androhermaphrodite plants. These results suggest that the gynoecium-suppressing gene can be epigenetically regulated via DNA methylation. It is possible to speculate that the rare cases of androhermaphroditism observed in the North American populations of *S. latifolia* could be connected to epigenetic changes (Miller and Kesseli, 2011). Indeed, the post-invasion evolution of *S. latifolia* in North America resulted in numerous phenotypic differences between North American and European populations (Blair and Wolfe, 2004), and it is conceivable that at least some of these variations involve epigenetic changes (Blair and Wolfe, 2004). The genetic and/or epigenetic changes in the North American population could also explain the Y chromosome transmissibility through the female gametic line in North American populations.

An analogous example of gene inactivation resulting from the hypomethylation of its surroundings can be found in *A. thaliana*, where global hypomethylation of the genome (achieved using methylation-deficient mutants and transgenic

lines) leads to the hypermethylation and transcriptional inactivation of specific genes [*SUPERMAN* (Jacobsen *et al.*, 1997, 2000); *AGAMOUS* (Jacobsen *et al.*, 2000); *BONSAI* (Saze and Kakutani, 2007)]. Artificial hypomethylation can affect the function of genes in many ways; for example, DNA methylation in introns is important for the correct splicing of genes (To *et al.*, 2015). However, the increase in the penetrance and expressivity of androhermaphroditism in subsequent generations supports the view that the gynoecium suppressor in *S. latifolia* is influenced in a similar way to the *SUPERMAN*, *AGAMOUS* and *BONSAI* genes. *SUPERMAN* probably does not play a key role as a gynoecium suppressor in *S. latifolia* as it is not located on the Y chromosome, but could be involved in stamen suppression during female flower development (Kazama *et al.*, 2009).

Further interesting findings have been obtained in *Populus*. The instability of the sexual phenotype resulting in andromonoecious plants in *P. tomentosa* is probably associated with DNA methylation changes influencing miRNA (miRNA172; Song *et al.*, 2015). Gerald *et al.* (2015) found that there is a gene present in the small SDRs in *Populus balsamifera* and *P. trichocarpa* which codes for DNA methyltransferase. Moreover, recent results indicate that the SDR in *P. balsamifera*

includes a gene with the greatest sex-specific difference in DNA methylation patterns (PbRR9) (Bräutigam *et al.*, 2017). Hypomethylation experiments should be performed to confirm the direct role of correct DNA methylation patterns on the sexual phenotype in *Populus*.

In males of *Carica papaya*, gynoecium development can be promoted by cold treatment (Lin *et al.*, 2016). The comparison of the expression profiles between males and cold-induced hermaphrodites revealed a significant increase in the abundance of transcripts connected with the methylation of Lys9 of histone H3 (H3K9) (Lin *et al.*, 2016). Based on the role of methylation of H3K9 in heterochromatin formation (Rivera *et al.*, 2015), the authors suggested that the probable cause of hermaphroditism is the inactivation of the putative gynoecium suppressor present on the Y chromosome (Lin *et al.*, 2016).

Information about the role of resource (water and/or mineral nutrients) availability on gynoecium formation (and/or investment to seed production) has been obtained for several species. Males from strictly dioecious populations of *Wurmbea dioica* (*Colchicaceae*) never formed gynoecium. In contrast, the response of males from sub-dioecious populations was dependent on the level of resources, with the gynoecium developed more often in high resource conditions (Vaughton and Ramsey, 2012). In gynodioecious *Nemophila menziesii* (*Boraginaceae*), supplemental water increased seed production in hermaphrodites but not in females (Barr, 2004). In sub-dioecious *Fragaria virginiana* (*Rosaceae*), Ashman (1999) showed that the fruit set of hermaphrodites, but not of females, increases significantly with plant size, and plant size increases with greater abundance of soil resources. In the sub-dioecious shrub *Eurya japonica* (*Pentaphylacaceae*), all possible types of sex changes were observed, but there was a tendency for the transformation from the hermaphrodite state to unisexual (male or female) in unfavourable growth conditions (Wang *et al.*, 2017).

Anther development in genetic females – epigenetics and environmental influences

Asymmetry in the ability to develop the organs of the opposite sex has been observed in many dioecious and sub-dioecious species. Male plants are often more prone to the development of fertile gynoecia, occasionally or under specific environmental influences (Delph and Wolf, 2005). In contrast, female plants are mostly not able to form fertile anthers even if anther development is stimulated by environmental factors. Although stamen arrest in *S. latifolia* females is stable and insensitive to the exogenous application of phytohormones (Ruddat *et al.*, 1991), there is a natural mechanism modulating sex expression. When female plants are infected by the parasitic smut *Microbotryum violaceum*, the rudimentary stamens develop into stamens morphologically resembling mature stamens in males (Uchida *et al.*, 2003) (Fig. 2E, F). However, the induced anthers harbour teliospores instead of pollen grains (Antonovics and Alexander, 1992). A recent study showed a strong response of sex-linked genes to pathogen infection (Zemp *et al.*, 2015). This result provides direct biological evidence that genes located on the sex chromosomes are important for male sexual organ development, and their altered expression participates in the defeminization and masculinization of *S. latifolia* female plants.

As in smut infection, the development of stamens in female plants is also promoted by $Ag_2S_2O_3$ and $AgNO_3$ treatment (Law *et al.*, 2002), similarly to several other species [*Cucumis sativa* (Den Nijs and Visser, 1980); *Ricinus communis* (Mohan Ram and Sett, 1980); *Cannabis sativa* (Mohan Ram and Sett, 1982); and *Coccinia grandis* (Devani *et al.*, 2017)]. Although the stamens induced in *Silene* are not as developed as those of male flowers, anther development proceeds further beyond the anthers induced by *M. violaceum* infection, and tapetum and non-viable pollen grains are formed. The extension of the flower life span of plants infected by *M. violaceum* suggests that ethylene inhibition may play a role in the observed morphological changes (Law *et al.*, 2002; Uchida *et al.*, 2003). The role of ethylene signalling in stamen induction in *Silene* is, however, still unclear as two other ethylene inhibitors did not show this effect (Law *et al.*, 2002).

Interestingly, anther promotion by *M. violaceum* has also been described in the gynodioecious *S. dichotoma*, and a related species of *M. violaceum* – *Microbotryum major* (previous name *Ustilago major*) – is able to provoke anther formation in females of *S. otites* (Correns 1928). This comparability of activity is puzzling as the sex determination systems evolved independently in the section *Melandrium* and in the section *Otites* (Mrackova *et al.*, 2009; Slancarova *et al.*, 2013). The parsimonious explanation could be that the actions of these pathogens are able to influence some generally present mechanism controlling gene expression such as ethylene signalling. Similarly, the infection could cause suppression of the nonsense-mediated mRNA decay (NMD) pathway which selectively degrades mRNAs harbouring premature termination codons (PTCs) but also regulates the abundance of a large number of cellular RNAs including coding genes (reviewed by Hug *et al.*, 2016). Suppression of the pathway (i.e. standard reaction of the plant body to the infection) could cause, among other effects, expression of anther-forming genes that would otherwise be silent in the female flower. The fact that other pathogens do not cause stamen formation in *Silene* females could be explained by the fact that only *Microbotryum* is able to penetrate deep inside the flower meristem and avoid the complete destruction of this tissue. A connection has been found between ethylene signalling and the NMD pathway (Merchante *et al.*, 2015) and so both pathways could be involved.

EVOLUTION OF DIOECY AND SEX CHROMOSOMES – FROM HERMAPHRODITISM OR MONOECY TO DIOECY AND SEX CHROMOSOMES

Data based on fossils are still inconclusive as to what original flower type was present in angiosperms – both unisexual and hermaphrodite flowers have been found in very ancient fossils (Friis *et al.*, 2010). Many current models for the evolution of dioecy from hermaphroditism (reviewed by Charlesworth, 2015) include the gynodioecy step, as first suggested by Charlesworth and Charlesworth (1978). This scenario is based on at least two sequential mutations on the same chromosome. The first mutation (most probably recessive) causes male sterility and the second and/or further mutation(s) cause(s) the suppression of gynoecium development in males carrying a proto-Y chromosome (see Fig. 3A). The arrest of recombination between these

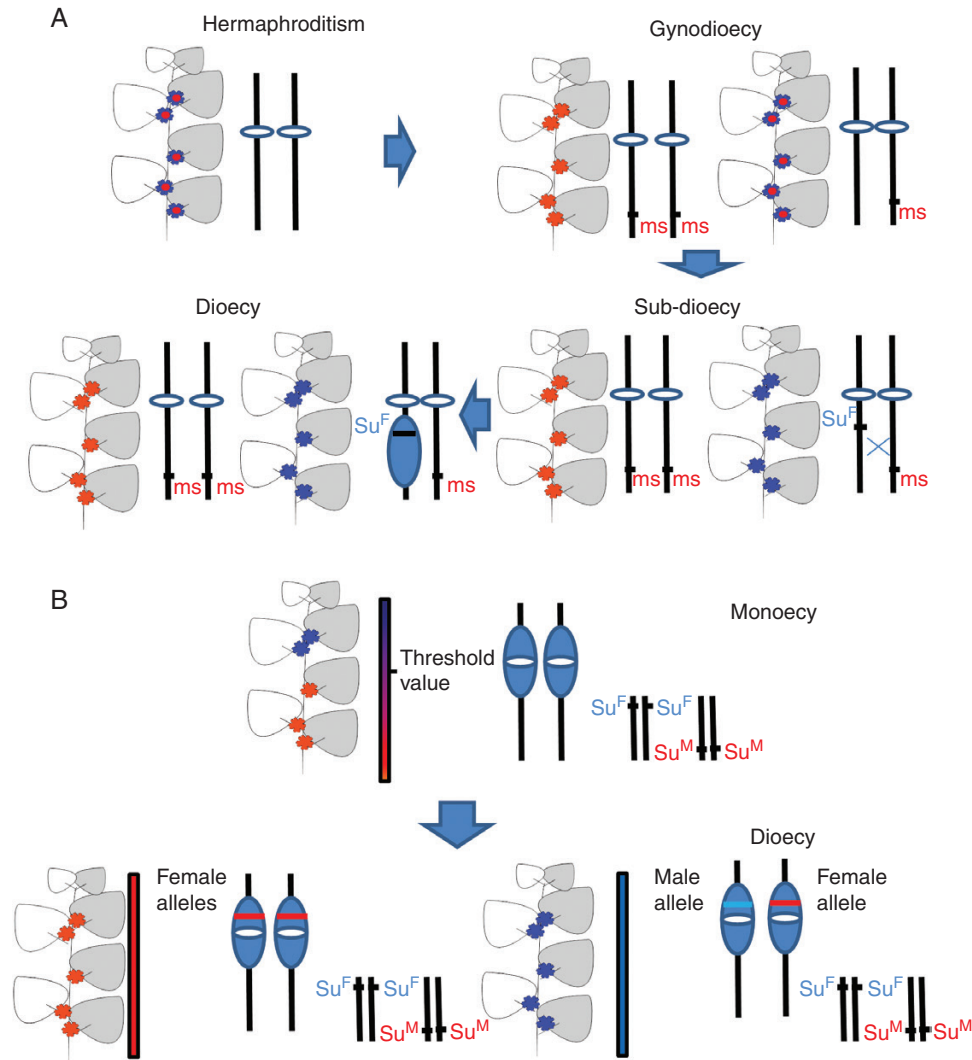


FIG. 3. Comparison of the evolution of dioecy via one-locus- and two-locus-based models. (A) Model of the origin of dioecy from hermaphroditism via gynodioecy – two-locus model. Recessive male sterility alleles (*ms*) can be either represented by nuclear recessive mutations or can reflect the absence of the dominant fertility restorer in the case where the male sterility-causing cytoplasm is present in all plants in the population. In the next step, the dominant female-suppressing allele (*Su^F*) is recruited on the proto-Y chromosome. Occasional creation of asexual or hermaphrodite plants via recombination is possible. The cessation of the gynoeceum is also influenced by genetic background and environment (sub-dioecy). Finally, the recombination between the female suppressor and the male sterility loci is arrested (as a consequence of divergence due to the accumulation of sexually antagonistic mutations and/or chromosomal inversions) and so the generation of asexual or hermaphrodite plants is avoided. This scheme is based on the original model by Charlesworth and Charlesworth (1978). (B) Alternative model of dioecy evolution from the monoecious – one-locus model. According to this model, the suppressors of gynoeceum (*Su^F*) and stamen development (or fertility) (*Su^M*) are already present in the monoecious ancestor and their expression is controlled by intrinsic signals (e.g. hormonal signals connected with apical dominance). Because the strength and direction of the intrinsic signals varies along the plant axis, it results in the upper section carrying male flowers and the bottom part bearing female flowers. In this model, it is thought that there is a threshold value of stimuli separating male and female zones. In this situation, the mutations which are able to modify the effects of internal stimuli can cause an increase or decrease in the size of the male and female zones. Parodioecy (the presence of female flowers in ‘proto-males’ and male flowers in ‘proto-females’) can appear in the intermediate stage (not displayed in this scheme). In the extreme case, one sex can be completely absent in a given plant. Interestingly, if a single master sex-switching locus is recruited near the centromere or other non-recombining region, the sex chromosomes can originate in a single step. This scheme is based on the works by Lloyd (1972a, b, 1975, 1980, 1981), Webb (1999), Renner and Won (2001) and Renner (2016). Note: female flowers are shown in red; male flowers are shown in blue; and hermaphrodite flowers are shown as blue with a red middle part. Sex-linked non-recombining regions are shown as blue ovals.

loci (for the possible mechanisms, see Wright *et al.*, 2016) could subsequently lead to the formation of sex chromosomes. The intermediate stages are characterized by the occurrence of sub-dioecy. Hermaphrodite flowers are present in mostly male individuals, but female plants possess a stable sex phenotype. Charlesworth and Charlesworth (1978) also deduced that the male sterility mutation (if dominant) could subsequently lead to the female heterogamety. This scenario is in accordance

with dominant male sterility alleles in *F. virginiana* (Spigler *et al.*, 2008, 2010) and *F. chiloensis* (Goldberg *et al.*, 2010). The dominant male sterility allele also probably occurs in *Salix purpurea* (Carlson *et al.*, 2017). A further supporting fact is that in at least three plant families where several species were tested concerning heterogamety, only female heterogamety has been found: *Fragaria* (Rosaceae; Spigler *et al.*, 2008, 2010, Goldberg *et al.*, 2010), *Cotula* (Asteraceae; Lloyd, 1975) and

Pistacia (Anacardiaceae; Hormaza et al., 1994; Esfandiary et al., 2012; Kafkas et al., 2015). The model starting with recessive male sterility has been referred to many times as a possible way to dioecy in plants.

Animal biologists often rely on the data and theories obtained in plants (mostly on the gynodioecy-based model) in their theories concerning the earliest phases of sex-determining system evolution as there is a lack of suitable model species in the animal kingdom (e.g. Weeks, 2012; Lorenzi and Sella, 2013; Wright et al., 2016). Interestingly, gynodioecy is extremely rare in animals (nine putative cases but only one confirmed) while androdioecy is more common, with 115 species identified (reviewed by Weeks, 2012). As suggested by Charlesworth and Charlesworth (1978), the route via androdioecy to dioecy is not likely in plants because males would need to produce more than twice as much pollen as hermaphrodites in order to invade the population. Indeed, there are just a handful of androdioecious species (five species from different genera and several species in *Sagittaria*, reviewed by Pannell, 2002). In plants, androdioecy is mostly derived from dioecy, e.g. *Datisca glomerata* (Wolf et al., 2001; Zhang et al., 2006) and the hexaploid *Mercurialis annua* (Russell and Pannell, 2015). The possible mechanism promoting the spread of androdioecy in hermaphrodite populations showing sporophytic self-incompatibility has been suggested in *Phillyrea angustifolia* (Oleaceae) where males are compatible with two reciprocally incompatible types of hermaphrodite (Saumitou-Laprade et al., 2010; Husse et al., 2013).

The original gynodioecy-based model was later slightly modified (Charlesworth, 2015) to reflect the fact that sex-determining loci can evolve in pericentromeric regions with suppressed recombination and that a two-locus-based model can also apply to the evolution of dioecy from monoecy. In this model, the reduction of the proportion of female flowers, i.e. the origin of males, is caused by a dominant mutation (Su^F), which also enables the formation of a larger number of male flowers based on resource allocation. Recessive mutations in other genes could cause the replacement of male flowers with female flowers, i.e. the origin of females (Charlesworth, 2015). There is at least one case suggesting that the evolution of dioecy from monoecious ancestors could proceed via a two-locus model (*Sagittaria latifolia*; Dorken and Barret, 2004).

A less frequently discussed but probably common pathway to dioecy in plants is represented by the so-called monoecy/paradioecy/dioecy pathway (Lloyd, 1980; Webb, 1999; Renner and Won, 2001). In this case, the evolution of dioecy originates from monoecious ancestors. Paradioecy (the presence of male flowers in mostly female plants and vice versa) occurs in the intermediate stage due to the step-wise arrest of the development of the flowers of the opposite sex in both ‘proto-females’ and ‘proto-males’. The fact that there is a pre-existence of pathways for the suppression of both male and female organs in the case of monoecious ancestors has been previously stressed by Renner (2016), based on previous work on the monoecy/paradioecy/dioecy pathway (Lloyd 1972a, b, 1975, 1980, 1981; Webb 1999; Renner and Won, 2001). If this prerequisite is satisfied, mutations in a single gene controlling the expression of the pre-existing sex-determining genes are sufficient to establish dioecy (Renner, 2016).

Two new types of alleles in a developing sex-determining locus can be selected, with each allele shifting the ratio of male

and female flowers in the male or female direction, respectively. Alternatively, the reduction of one type of flower (caused by changes in genetic background or by environmental influences) is compensated by the recruitment of a new sex-determining master gene. In this case, even a single mutation in a single gene can lead to dioecy. This scenario can more easily proceed in long-living and/or vegetatively propagating species than in annual species relying on sexual propagation. There is a greater abundance of dioecious species among woody perennials than in plants with herbal growth (Renner and Ricklefs, 1995; Vamasi et al., 2003; Renner, 2014). The chromosomal localization of the sex-determining master gene is important. If this gene (controlling expression of the genes from previously established sex-determining pathways) is recruited in the pericentromeric or other regions demonstrating reduced recombination, the sex chromosome-based sex determination is established in one step (see Fig. 3B). Interestingly, there are two cases of species possessing SDRs in the proximity of centromeres – in *Coccinia grandis* (Sousa et al., 2016) and in *Carica papaya* (Yu et al., 2007). An increasing body of evidence supports the theory that non-recombining regions have a tendency to spread. This so-called ‘evolutionary strata’ has been observed in several animal model species and also in three plant models – *Silene latifolia* (Nicolas et al., 2005; Bergero et al., 2007; Papadopulos et al., 2015), *Carica papaya* (Wang et al., 2012) and *Populus trichocarpa* (Pandey and Azad, 2016). It is very difficult to establish if in any particular studied species the sex determination has arisen via a one- or two-locus-based model. A one-locus-based scenario can be erroneously identified as two-locus based or, as switches can occur in sex-determining genes, the two-locus-based sex-determining systems can evolve in systems possessing monogenic sex determination (see Janousek and Mrackova, 2010, for a possible scenario).

Diospyros lotus shows indications of a possible one-locus-based scenario of the evolution of dioecy (Akagi et al., 2014). In addition, a system illustrating a possible way to single ‘master gene-based sex determination has been suggested by Boualem et al. (2015) in *C. melo*. In this case, the possible scenario would start with a mostly male population with the prevailing genotype homozygous in the *CmASC11* null allele. In the next step, a recessive allele would be recruited which has lost the ability to suppress female organ development and, concurrently, prevented male organ development (as it is not able to repress the stamen suppressor, *CmACS7* gene).

The only way to affirm the relevance of two- or one-locus-based models in particular taxa is to identify the sex-determining pathways and to perform detailed analysis on the participating genes. Only a limited number of species can be studied to a sufficient extent. The group of chosen species should include both species having hermaphrodite relatives and species having monoecious relatives.

FURTHER EVOLUTION OF SEX CHROMOSOMES AND WHOLE GENOMES OF DIOECIOUS FLOWERING PLANTS – IMPACT OF DIOECY ON GENOME STRUCTURE AND DYNAMICS

The existence of separate sexes and sex chromosomes affects the development of plants. Male and female plants differ in the

presence of respective sexual organs as well as in quantitative traits such as the number and shape of flowers and in qualitative traits such as sex-specific expression of some genes even in vegetative tissues (Zluvova *et al.*, 2010). The unique processes shaping sex chromosomes can also, in parallel, influence the rest of the genome, e.g. its size and the distribution of repeats. Dioecious *Asparagus* species tend to have larger genomes than their hermaphroditic relatives (Kuhl *et al.*, 2005), which is most probably caused by retrotransposon proliferation (Harkess *et al.*, 2016). Similarly, the genome of dioecious *S. latifolia* is larger than the genome of hermaphroditic *S. vulgaris* mainly due to the expansion of the Ty3-gypsy long terminal repeat (LTR) retrotransposon *Ogre* (Cegan *et al.*, 2012). A likely cause of transposable element (TE) proliferation could be the fast diversification of TE families and the subsequent relaxation of epigenetic regulation accompanying the evolution of dioecy. How these processes interact is discussed below.

Besides gene degeneration, the main structural characteristic of plant sex chromosomes is the different distribution of repetitive sequences (satellites and TEs) compared with the rest of the genome (Shibata *et al.*, 2000; Navajas-Pérez *et al.*, 2006, 2009; Kubat *et al.*, 2008; Cermak *et al.*, 2008; Kejnovsky *et al.*, 2009, 2013; Mariotti *et al.*, 2009; Steflava *et al.*, 2013; Kralova *et al.*, 2014; Puterova *et al.*, 2017). As an example, differences in the chromosomal localization of repeats on the sex

chromosomes of *S. latifolia* and *R. acetosa*, both species possessing heteromorphic Y chromosomes, are depicted in Fig. 4A–F. The accumulation of microsatellites on the Y chromosomes in particular (Fig. 4A, D, E) could be a consequence of recombination restriction and may represent an early event shaping the Y chromosome. Microsatellite arrays could then serve as DNA substrates for TE insertions (Kejnovsky *et al.*, 2013), thus accelerating genetic degeneration and size expansion of the Y chromosome (reviewed in Hobza *et al.*, 2015). Moreover, the suppressed recombination between Y chromosomes reduces the rate of concerted evolution and gives rise to the diversification of satellites (Navajas-Pérez *et al.*, 2006) and potentially of TE families. Thus, non-recombining sex chromosomes could serve as a source of new forms of repetitive sequences that could spread into the rest of the genome. The immature epigenetic defence against newly emerged TE families is the most likely cause of the extensive proliferation of TEs and genome expansion in dioecious plants. Simultaneously, an increased outcrossing of dioecious plants can allow TEs to proliferate and spread into the population.

A widely accepted hypothesis suggests that repeats are accumulated in the non-recombining region of the Y chromosome (Charlesworth, 1991). Although this is the case for some TEs, many TEs are under-represented on the Y chromosomes of both *S. latifolia* and *R. acetosa* (Fig. 4C, F). Ty3/gypsy LTR retrotransposons of *Retand*, *Ogre* and *Athila* families have fewer copies on

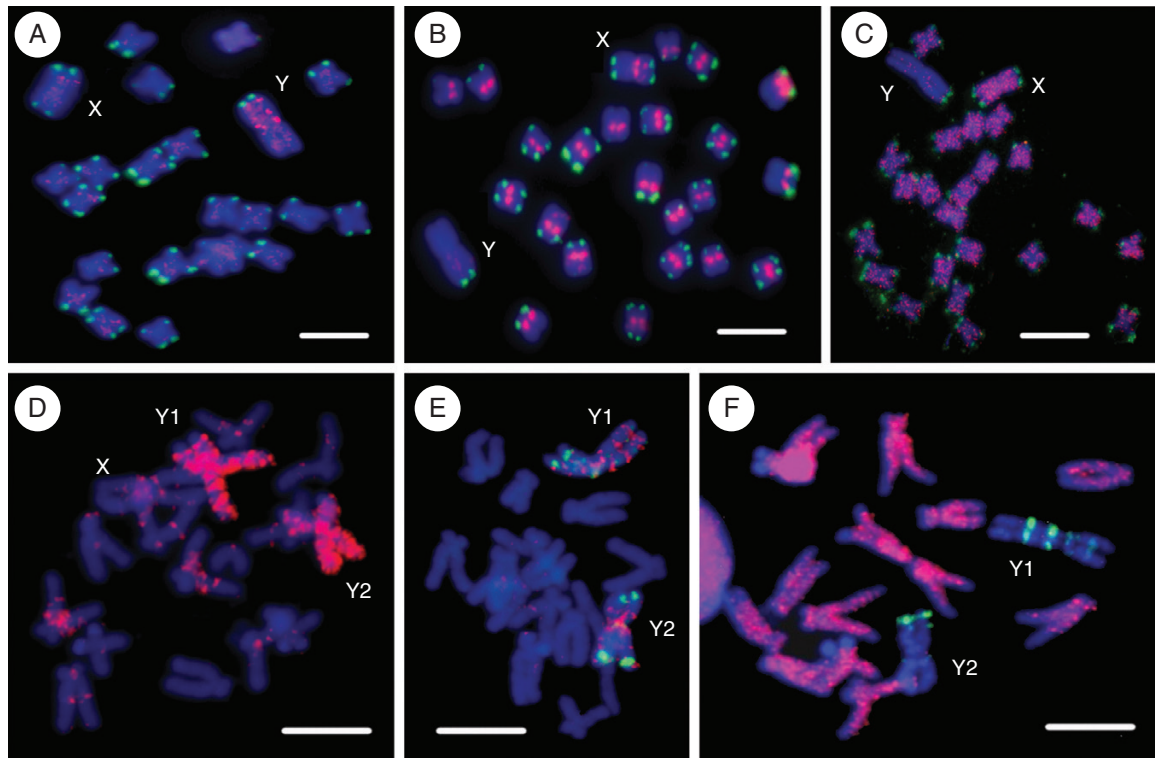


FIG. 4. Chromosomal localization of repetitive DNA in *Silene latifolia* (A–C) and *Rumex acetosa* (D–F) determined by fluorescence *in situ* hybridization. (A) Microsatellite $(CA)_{15}$ is accumulated on the Y chromosome (red signal), (B) satellite STAR (red signal) is present in the centromeres of all autosomes and the X chromosome, and satellite X-43.1 (green signal) is gathered in both sub-telomeres of all chromosomes with the exception of the Y chromosome possessing only one sub-telomeric signal, (C) The *Ogre* LTR retrotransposon (red signal) is ubiquitous on all chromosomes except the Y chromosome. (D) A mixture of all mono-, di- and trinucleotide microsatellites (red signal) shows strong accumulation along both Y chromosomes. (E) The $(TA)_{15}$ microsatellite (red signal) gives a signal at several discrete loci on both Y chromosomes, and the RAYSI satellite (green signal) is present at the distal regions of both Y chromosomes. (F) The Maximus/SIRE LTR retrotransposon (red signal) covers all autosomes and the X chromosome except telomeres/sub-telomeres, but is absent from both Y chromosomes; the RAYSI satellite is localized at several discrete loci on both Y chromosomes (green signal). Scale bars indicate 10 μm .

the Y chromosome than on the X chromosome and on the autosomes of *S. latifolia* (Kejnovsky et al., 2006; Cermak et al., 2008; Filatov et al., 2009; Kralova et al., 2014), whereas only some Ty1-copia retrotransposons are more abundant on the Y chromosome than on autosomes (Cermak et al., 2008). In addition, the absence of TEs on the Y chromosome is often accompanied by a build-up of TEs on the X chromosomes, while TEs accumulated on the Y chromosome usually have fewer copies on the X chromosomes compared with autosomes (Hobza et al., 2017; Puterova et al., 2018). Based on this observation, three TE groups were classified (Puterova et al., 2018). The first group of TEs tend to be preferentially active in females, the second group of TEs in males and TEs from the third group are equally active in both sexes. Hobza et al. (2017) proposed a testable model of preferential TE proliferation either in males or in females and its impact on the chromosomal distribution of TEs. Irregularity in TE distribution on sex chromosomes is indicative of the existence of hidden mechanisms influencing the transposition of TEs sex specifically, i.e. differently in male and female individuals or germlines.

The most likely candidates are epigenetic TE-repressive mechanisms employing specific classes of sRNA molecules that mediate the silencing of all homologous TE copies in the genome, post-transcriptionally or through the deposition of repressive chromatin modifications. Epigenetic mechanisms are known to inhibit TE activity in vegetative tissues, but they also play a critical role in gametogenesis where they can influence sex-specific transgenerational proliferation of TEs as exemplified by the *EVADE* (*EVD*) retrotransposon in *A. thaliana*. *EVD* retrotransposons amplify if transmitted through the paternal germline but are suppressed when maternally inherited due to epigenetic silencing in the maternal sporophytic tissues of the flower (Reinders et al., 2013). Since *A. thaliana* is a hermaphroditic plant, the effect of the silencing on the chromosomal distribution of *EVD* is not directly apparent. This is in contrast to a number of case studies of the dioecious plant *S. latifolia* (Cermak et al., 2008; Filatov et al., 2009; Kubat et al., 2014; Puterova et al., 2018).

The precise mode of TE epigenetic silencing, and how the silencing differs for male and female lineages, is still unknown. It is assumed that the epigenetic reprogramming of plant gametes plays a central role. In plants, a germline is not set aside early in embryogenesis, as in animals, and differentiates later from flower tissues. Thus, the epigenetic marks contributing both to plant development and to TE silencing must be deleted in the germline to re-establish the totipotent state in the zygote. It is presumed that epigenetic marks are gradually lost during germline differentiation due to the downregulation of DNA methyltransferases and heterochromatin remodellers as well as to the active effect of Demeter (*DME*) (Gehring et al., 2009; Hsieh et al., 2009; Calarco et al., 2012). Epigenetic information is then re-established in sperm production and during embryo development. Companion cells of plant gametes, the vegetative cell in pollen and the central cell (*CCN*) in ovuli, retain low levels of heterochromatic marks causing the active transcription of TEs. Current models propose that RNA transcripts from active TEs are converted to mobile small interfering RNAs (*siRNAs*) that can migrate into the sperm and embryo, where they reinforce both transcriptional and post-transcriptional silencing of TEs (Slotkin et al., 2009; Ibarra et al., 2012; Martínez et al., 2016). According to the latest studies in arabidopsis and maize,

it is primarily TEs which are near genes which are subject to epigenetic reprogramming driven by 24 nucleotide *siRNAs*, while TEs in TE islands probably remain heterochromatinized even during germline differentiation (Gent et al., 2013, 2014; West et al., 2014; Li et al., 2015; Sigman and Slotkin, 2016; Vergara and Gutierrez, 2017).

An interesting discovery here is that the male germline adopted unique silencing mechanisms which are not present in the female germline. First, *miRNA* genes have been shown to trigger the production of *easiRNAs* (epigenetically activated *siRNA* molecules) that specifically target TE transcripts in the vegetative nucleus of pollen grains (Creasey et al., 2014). Secondly, pollen-specific tRNA-derived *siRNA* molecules target reactivated TE transcripts. The latter process has been shown to be conserved in plants and may have a role in gamete protection against gametophyte-associated TE reactivation (Martínez et al., 2017). Considering that most of the sex specifically distributed TEs tend to be less abundant on the Y chromosomes of *R. acetosa* and *S. latifolia* (Steflova et al., 2013; Puterova et al., 2018), we can hypothesize that the male germline has a stronger epigenetic defence against TEs than the female germline. This could have evolved due to the existence of a vegetative haploid stage – the pollen tube. TEs (especially the most active ones) have to be under tight control in the male germline to decrease the risk of deleterious changes of the genes required for pollen tube growth. In dioecious species with male-specific Y chromosomes, these mechanisms may slow down irreversible genetic and epigenetic degeneration of Y-linked genes and subsequently contribute to a decrease in the rate of gene loss. Loss of Y-linked genes is slower in *S. latifolia* than in primates, probably due to a combination of several factors such as the minor effect of haploid selection and relatively high effective population size of the former (reviewed in Muyle et al., 2017). Supporting the importance of the male-specific TE silencing mechanisms is the fact that TEs under-represented on the Y chromosome substantially contributed to the recent genome size expansion in *S. latifolia* (Cegan et al., 2012; Puterova et al., 2018).

All in all, parent-of-origin effects, although likely to be widespread in plants, are hidden in traditional hermaphroditic model species but have a significant manifestation in species carrying sex chromosomes. These mechanisms affect the transposition of specific TE families in a sex-specific manner, which influences not only the male haploid stage (pollen tube) but also some aspects of sex chromosome evolution. Male-specific silencing of TEs in particular can decelerate Y chromosome size expansion as well as the genetic and epigenetic degeneration of Y-linked genes and simultaneously increase the size of the X chromosome (X chromosomes are much larger than any autosome in *S. latifolia* and *R. acetosa*). In contrast, female-specific silencing of TEs can contribute to Y chromosome size increase relative to autosomes. Moreover, dioecious plants, when used for studies of sex-specific and sexual organ-specific epigenetic processes in TE–host coevolution, can in some ways be more informative than traditional models due to the existence of separate sexes as well as the presence of unique genomic parts, such as non-recombining Y (*W*) chromosome(s). Such studies may, for example, include the precise quantification of TE families on particular chromosomes as well as sex-specific transgenerational proliferation of selected TE families with known chromosomal distribution.

PERSPECTIVES

Based on the reviewed data, it can be deduced that there are not only differences among studied species concerning sex-determining systems but also common evolutionary trends, e.g. a varying tendency towards the growth and degeneration of non-recombining regions (this feature is shared even with animal models, as reviewed by Schartl *et al.*, 2016 and Wright *et al.*, 2016). It also appears that the evolution of the controlling pathways, which occurs after various sex-determining systems are established, is to some extent convergent. Even though dioecy has originated independently many times, there are similarities in the role of epigenetic factors, e.g. DNA methylation and histone methylation, in their influence on sexual phenotype. Additionally, the role of the ethylene signalling pathway in sex determination is shared by several unrelated species (Den Nijs and Visser, 1980; Mohan Ram and Sett, 1980, 1982; Boualem *et al.*, 2008, 2009; Devani *et al.*, 2017). As hypothesized in this review, the controlling pathways involved in sex determination in plants evolved *de novo* but could also have employed controlling mechanisms that were developed for other purposes. As the number of such pathways is limited, the similarities between unrelated taxa are not so surprising. In comparison with mammals, due to the lack of locomotion, plant genomes evolved mechanisms to achieve more genotypic and phenotypic plasticity to adapt passively to stress (reviewed by Kejnovsky *et al.*, 2009) and some of these mechanisms could also influence the phenotypic plasticity of the sexual phenotype in plants. Due to the huge differences in the depth of knowledge in particular plant models, global comparison of many aspects of sex chromosome evolution is limited. There are an increasing number of studies regarding sex-determining mechanisms, the impact of dioecy on genome structure, epigenetic aspects of sex chromosome evolution as well as the interplay between regulation of TEs and non-recombining region formation, but detailed data on the phenotypic or at the population level are still scarce, especially in new models. Continuing study of many varying model species and a complex view is needed to understand fully the complex aspects of sex chromosome evolution.

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