Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in
Plant Biology

Genomics of sex determination

Jisen Zhang[1], Adnane Boualem[2], Abdelhafid Bendahmane[2] and Ray Ming[1,3]

Sex determination is a major switch in the evolutionary history of angiosperm, resulting 11% monoecious and dioecious species. The genomic sequences of papaya sex chromosomes unveiled the molecular basis of recombination suppression in the sex determination region, and candidate genes for sex determination. Identification and analyses of sex determination genes in cucurbits and maize demonstrated conservation of sex determination mechanism in one lineage and divergence between the two systems. Epigenetic control and hormonal influence of sex determination were elucidated in both plants and animals. Intensive investigation of potential sex determination genes in model species will improve our understanding of sex determination gene network. Such network will in turn accelerate the identification of sex determination genes in dioecious species with sex chromosomes, which are burdensome due to no recombination in sex determining regions. The sex determination genes in dioecious species are crucial for understanding the origin of dioecy and sex chromosomes, particularly in their early stage of evolution.

Addresses
[1] FAFU and UIUC-SIB Joint Center for Genomics and Biotechnology, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China
[2] INRA-CNRS-UEVE, UMR1165, ERL8196, Unité de Recherche en Génomique Végétale, 2 rue Gaston Crémieux, Evry F-91057, France
[3] Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Corresponding authors: Bendahmane, Abdelhafid (bendahm@evry.inra.fr) and Ming, Ray (rming@life.uiuc.edu)

Current Opinion in Plant Biology 2014, **18**:110–116

This review comes from a themed issue on **Genome studies and molecular genetics**

Edited by **Kirsten Bomblies** and **Olivier Loudet**

Available online

1369-5266/$ – see front matter, © 2014 Elsevier Ltd. All rights reserved.

http://dx.doi.org/10.1016/j.pbi.2014.02.012

Introduction

Sex determination in flowering plants is a fascinating subject with evolutionary, biological, and economic significance. Identification of sex determination genes and understanding their interacting gene network are challenging because of the difficulty working on monoecious and dioecious species. For monoecious species, there is no sexual dimorphism among individual plants, and identification of sex determination genes relies on sex reversal mutant collections, which are only available for intensively studied monoecious crop plants, such as maize, melon, and cucumber. For dioecious species, sex determination genes often reside on sex chromosomes with a non-recombining sex determination region, which make identification of the sex determination genes rather difficult due to the need to overcome the non-recombining heterogametic chromosome Y or W where the functioning sex determination genes located in.

Male sterile and female sterile mutations could occur at any stage of stamen and carpel development from inception of floral organ primordia to male and female gametophyte genesis. This has been demonstrated by the random distribution of stamen and carpel abortion throughout the developmental process in unisexual flowers [1••]. This random distribution could occur within the same family, which makes the evolution of sex determination in flowering plants appear to be neither conserved nor convergent. However, it should be noted that sex determination genes could be conserved among closely related taxa within a family as demonstrated in cucurbits [2–4].

On the bright side, the rapid advance of genomic technologies expedites the investigation of sex determination genes and regulatory mechanisms of gene network. Sex determination genes have been identified in maize and melon, and epigenetic control of sex determination was shown in both plant and animal systems. Hormonal influence on sex determination were prominent in both maize and melon sex determination networks.

From sex chromosomes to sex determination in dioecious species

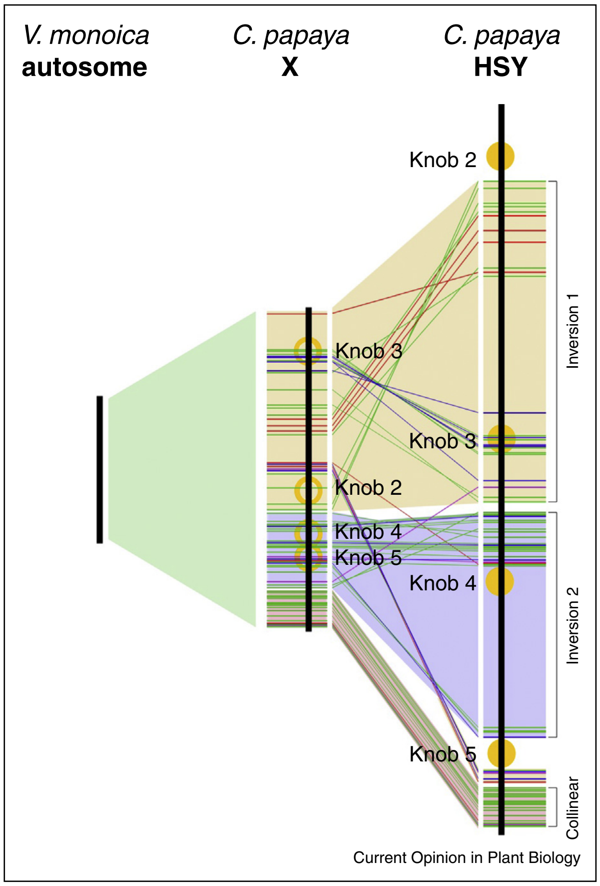
Dioecy is the precondition for the evolution of sex chromosomes [5,6]. The percentage of sex chromosomes in dioecious species in any lineage has not been assessed due to the lack of genetic and molecular data on sex determination for vast majority of dioecious species. Among dioecious species with cytogenetic, genetic, or molecular evidence for sex determination, all appear to have either heteromorphic or homomorphic sex chromosomes. Among the 10 species with evidence of sex chromosomes in hapatics, mosses, and gymnosperm, all have heteromorphic sex chromosomes, supporting their ancient origin. In angiosperm, 19 (51%) of the 37 species with sex chromosomes are homomorphic, likely reflecting their recent origin. Despite the lowest percentage of dioecious species in angiosperm, more species with sex chromosomes were reported than those in other lineages combined. This is due to economic importance of dioecious crops and the overwhelming dominance of angiosperm on earth, which accounts for 90% of all land plant species [7]. It should be noted that homomorphic sex chromosomes could exist in ancient lineage as shown in European tree frogs [8].

The prevalence of sex chromosomes in dioecious species hindered the identification of sex determination genes because map based cloning approach is not suitable in a non-recombining male specific region of the Y chromosome (MSY) or female specific region of the W chromosome (FSW). *Silene latifolia* has been a classical model system for sex chromosome research in plants because the Y and X chromosomes are the largest and second largest in the genome that are readily distinguishable. Although sex reversal deletion lines were generated [9], genomic sequences of the sex chromosomes are still lacking for identification of sex determination genes. Recent advance in genomic technologies expedited the identification of X and Y linked genes and allowed the detection of dosage compensation of X-linked genes merely after 10 million years of sex chromosome evolution [10•,11•,12•], and assessing the synteny between the pseudo-autosomal region (PAR) of the Y chromosome in *S. latifolia* and the orthologous autosomal region in *S. vulgaris* [13].

The trioecious papaya emerged as a model species for sex chromosome research in plants due to its small genome, short generation time, and economic importance as a highly productive tropical fruit crop. Sex determination in papaya is controlled by a pair of recently evolved sex chromosomes with two slightly different Y chromosomes, Y controlling male and Y^h controlling hermaphrodite [14]. The 8.1 Mb hermaphrodite-specific region of the Y^h chromosome (HSY) and its 3.5 Mb X chromosome counterpart were sequenced and annotated, becoming the second sex chromosome pairs to be fully sequenced beside the XY chromosomes in human [15,16••,17,18,19••] (Figure 1). The alignment of paired genes and pseudogenes between X and HSY revealed two evolutionary strata, corresponding to two large scale inversions in the HSY. The first inversion occurred about 7 million years ago (mya) and the second inversion 1.9 mya. The HSY contains 72 annotated genes, whereas the X counterpart contains 84 genes. Comparison of X and Y annotated genes revealed 50 paired, 22 HSY-specific, and 34 X-specific genes. The HSY- and X-specific genes are mostly located in the first inversion, including 20 (91%) HSY-specific and 26 (76%) X-specific genes, reflecting gene gain and loss after the recombination ceased 7 mya. Identification and validation of sex determination genes are ongoing.

Identification of sex determination genes is actively pursued in asparagus and strawberry. An integrated genetic and physical map of the sex determination locus has been constructed in asparagus [20]. Targeted sequence capture from 103 segregating F1 individuals placed the male sterility gene in a 338 kb region of chromosome 4 in gynodioecious diploid *Fragaria vesca* ssp. *Bracteata* [21••].

Figure 1



The genomic expansion of the X and Y chromosomes in papaya in comparison with the orthologous autosomal region in monoecious *V. monoica*. The X chromosome expanded 2.3× than the orthologous autosome, and the HSY expanded 2.3× than the X chromosome and 5.3× than the orthologous autosome.

Plant sex determination genes identified so far are from monoecious species by map based cloning approach since there is no recombination suppression at the sex determination loci. Our understanding of sex determination gene network is mostly from the following two systems: cucurbits and maize.

Sex determination in monoecious species

Sex determination in melon (*Cucumis melo* L.) is controlled by two unlinked genes, *andromonoecious* (*a*) and *gynoecious* (*g*) [22]. Wild type melon is monoecious with the genotype *A-G-*, andromonoecious *aaG-*, gynoecious *AAgg*, and hermaphrodite *aagg*. Map based cloning of the A locus identified *CmACS-7*, a gene encoding the rate limiting enzyme in ethylene biosynthesis, the 1-aminocyclopropane-1-carboxylic acid synthase (*ACS*), which is expressed in carpel primordia, and loss of enzymatic activity leads to stamen development [2]. The *G* locus encodes a C2H2 zinc finger transcription factor *CmWIP1*, and recessive *g* allele has an insertion at the 3′ end 1.3 kb from the stop codon by a DNA transposon of the *hAT* family, termed *Gyno-hAT*, which is required for the initiation and maintenance of DNA methylation of *CmWIP1* [23]. Loss of *CmWIP1* function leads to carpel development.

The interaction between these two genes results in a range of sexual phenotype. *CmACS-7* is expressed in carpel to suppress stamen development, and the *CmWIP1* is epistatic to *CmACS-7* by controlling carpel development where the *CmACS-7* is expressed. Expression of *CmWIP1* gene leads to carpel abortion and hence the lack of *CmACS-7* expression that suppress stamen development, resulting in male flowers. Loss of function mutation in *CmWIP1* leads to carpel development and *CmACS-7* to express in carpels to suppress stamen development, resulting in female flowers. Loss of function mutations in both *CmACS-7* and *CmWIP1*allows carpel and stamen to develop, resulting in hermaphrodite flowers (Figure 2a).

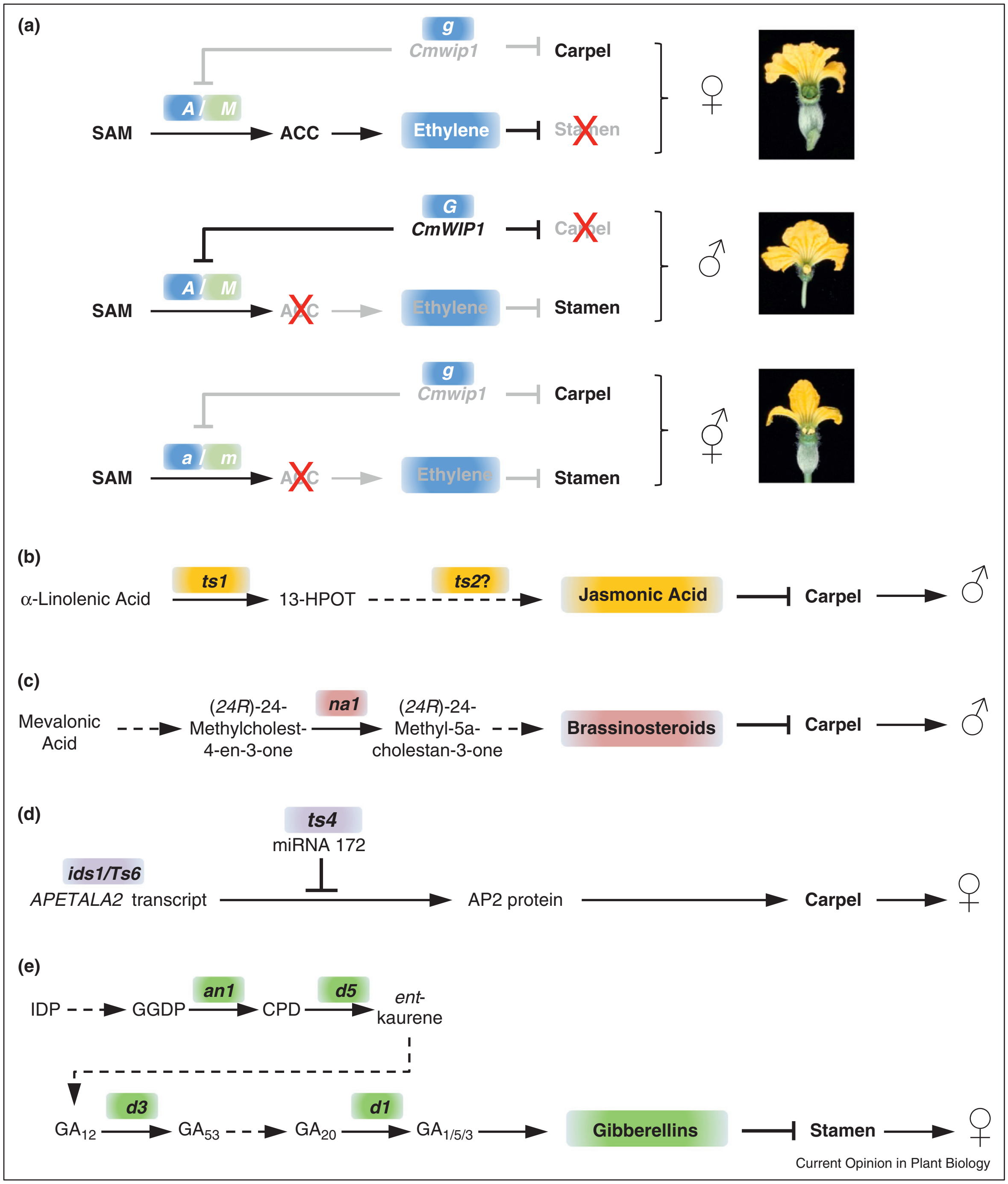
In monoecious cucumber (*Cucumis sativus* L.) sex determination is controlled by three major genes. The *F* gene is partially dominant and controls the degree of femaleness and *FF* genotype is gynoecious. The recessive *a* gene increases maleness, and plants with *aaff* genotype are androecious. The monoecious dominant *M* gene controls selective abortion of stamens [24]. The *M-ff* genotype is monoecious, *M-F-* gynoecious, and *mmF-* hermaphrodite. The *F* locus is linked to an *ACS* gene, *CsACS1G*, which is a duplicated copy in gynoecious lines, not present in monoecious lines [25,26]. The *M* locus encodes *CsACS2*, an ortholog of *CmACS-7*, showing conservation of gene function in these two species of *Cucumis* that were diverged about 3 million years ago [3]. A positive feedback regulation mechanism was proposed for *CsACS2* from a study expressing *CsACS2* in transgenic tobacco, emphasizing the importance of ethylene on sex determination in cucumber [27••]. Ethylene perception is involved in stamen abortion as the ethylene receptor, *CsETR1*, was down regulated in the stamens of stage 6 female flowers and primordial anther-specific DNA damage was observed in stage 7 female flowers [28,29].

The intensive genetic research in maize in the past century produced a large collection of heritable mutants, including many sex reversal mutants. The first plant sex determination gene was cloned in maize [30]. Since then many mutants affecting sex determination have been characterized. There are six *tasselseeds* mutants affecting male flower (tassel) development, plus a dwarf mutant *na1* also showing *tasselseeds* phenotype. These seven mutants could be categorized into three classes. Class I includes recessive mutants *ts1* and *ts2* and dominant mutants *Ts3* and *Ts5*, which cause sex reversal from male to female flowers with more severe phenotype from the two recessive mutants than the two dominant mutants. Class II includes recessive mutant *ts4* and dominant mutant *Ts6*, which cause a lack of female abortion in the male flowers and irregular branching within the inflorescence. Class III includes recessive mutant *na1*, which causes feminized male flowers and severe reduction of plant stature, contrasting to the normal plant height of the other six *tasselseeds* mutants. The *ts1* gene encodes a plastid-targeted lipoxygenase with predicted 13-lipoxygenase specificity in the jasmonic acid biosynthesis pathway, indicating the role of jasmonic acid on promoting male flower development in maize [31]. The *ts2* gene encodes a short chain dehydrogenase/reductase with broad activity [30], and the dehydrogenase/reductase activity may produce a proapoptotic signal or metabolize a substrate for cell viability [32,33]. The class II gene *ts4* encodes a microRNA gene *zma-miR172e* targeting *AP2* floral homeotic transcription factors [34]. The *indeterminate spikelet1* (*ids1*) gene encodes an *AP2* protein [35], and it is a target of *ts4*. The dominant *Ts6* mutant is a G to T transversion mutation of the *ids1* gene near the 5′ end of the *ts4* binding site. The dominant nature of *Ts6* mutant is from preventing silencing of *ids1* gene by the negative regulator *zma-miR172e*. The class III gene *na1* encodes *DE-ETIOLATED2* (*DET2*) enzyme in the Brassinosteroid biosynthesis pathway, indicating the role of steroid hormone promoting male organ development, as in animal system [36••] (Figure 2b–d).

Sex determination genes affecting female flower development in maize have been characterized, all involved in the gibberelic acid (GA) biosynthesis or perception. The *anther ear* (*an1*) gene encodes *ent*-kaurene synthase A (ent-copalyl-diphosphate diphosphate-lyase) in *ent*-kaurene biosynthesis, the first step of GA biosynthesis [37]. The dwarf mutant *d3* gene encodes a cytochrome P450 enzyme, ent-kaurenoic acid oxidase (KAO), which catalyze the last three steps of GA biosynthesis [38,39]. The dominant dwarf mutant *D8* gene encodes a member of the DELLA protein with a conserved N-terminal gibberellin signaling domain, an ortholog of the *Gibberellin Insensitive* (*GAI*) gene in Arabidopsis, involved in GA perception or dose response [40,41]. Metabolic studies of dwarf mutants in maize indicated that dwarf mutants *d1*, *d3*, and *d5* interrupted GA biosynthesis [42]. The *d1* gene controls three biosynthetic steps from GA_{20} to GA_1, GA_{20} to GA_5, and GA_5 to GA_3, where as *d5* controls the step from Copalyl diphosphate (CDP) to *ent*-kaurene [43] (Figure 2e).

Genetic regulation is the primary mode of action for sex determination genes in plants, as shown by these sex determination genes identified in cucurbits and maize. However, plasticity of sex expression has been observed in many monoecious and dioecious species, likely due to

Figure 2



Sex determination pathways in cucurbits and maize. **(a)** Model for sex determination in melon and cucumber based on Boualem *et al*. [2,3] and Martin *et al*. [23], where *CmACS-7/CsACS2 (A/M)* and *CmWIP1 (G)* are repressors of stamen and carpel, respectively. (b–d) Model for gibberellins (GA), jasmonic acid (JA) and brassinosteroids (BR) action in maize florets sex determination. **(b)** TS1 initiates the JA biosynthesis required for stamen development in the tassel. TS2 have been proposed to be involved in the last steps of JA biosynthesis, but this proposal should be taken with caution. The *ts1* and *ts2* mutants block JA biosynthesis and lead to transformation of the male tassel into female flower. **(c)** *nana plant 1* mutant is affected in BR biosynthesis. The reduced BR level in *na1* mutant leads to feminization of the tassel. **(d)** Model for the tassel sex determination based on the negative regulation of *AP2* transcript by the *miRNA 172*. In normal male floret, *AP2* mRNA is not translated because of the presence of the *miRNA 172*. **(e)**The masculinizing dwarf mutants, an1, d1, d3, d5 affect different steps of gibberellins biosynthesis. These GA defective mutants do not undergo stamen abortion in the female inflorescence and produce perfect florets in the ear.

epigenetic regulation of sex determination genes. The molecular mechanisms of epigenetic influence have been elaborated in some plant and animal systems.

Epigenetic influence

Sex reversal under variable environmental conditions in unisexual and hermaphroditic flowers is observed in some plant taxa, including species with sex chromosomes. In woody dioecious species *Ilex integra*, five of 47 trees changed sex from female to male or male to female at different years in a three-year period. The sex reversal is complete for the entire tree and there is no monoecious tree observed [44•]. In the genus *Wurmbea*, there is no sex reversal in male flowers, but frequent sex reversal were observed in female and hermaphrodite flower [45], suggesting a ZW sex chromosome system with loss of function recessive mutations for male flowers.

Direct evidence of epigenetic influence of expression in plants is from the sex reversal of male to androhermaphrodite flowers in *Silene latifolia* after treating the seeds with the nucleotide analog of 5-azacytidine, which induces hypomethylation of DNA [46]. *Silene latifolia* has an XY sex chromosome system, and the sex reversal is likely triggered by inhibiting the carpel suppressing gene in males.

More evidences of epigenetic influence of sex determination were accumulated in animals. In the temperature-dependent sex determination species, sex expression can be modified by the concentration of sex steroid hormones in developing embryos. Cytochrome P450 aromatase, *cyp19a1*, irreversibly catalyses androgen into estrogen, and is overexpressed in differentiating ovaries and suppressed during testis development. In the red-eared slider turtles (*Trachemys scripta*), DNA methylation of the aromatase gene promoter is significantly lower in female-producing than in male-producing temperature. A shift from male-producing to female-producing temperature (not vice versa) changed the level of DNA methylation of *cyp19a1* promoter in gonads [47]. In chicken, demethylation of a 170-nt promoter region of *cyp19a1* was significant but only partially in exogenous estrogen induced sex reversal gonads comparing with normal ZW females, reinforcing the role of epigenetic influence on sex determination and sex expression [48]. In mice, methylation of histone H3 lysine 9 (H3K9) suppress the transcription of the sex determination gene *Sry*, and histone demethylase *Jmjd1a* epigenetically regulate mouse sex determination by demethylating H3K9me2, leading to activation of the *Sry* gene [49••].

Hormonal regulation

Plant hormones regulate diverse developmental processes, including cell elongation, plant growth, flower development, sex determination, and sex differentiation. Ethylene is the primary hormone regulating sex determination in melon and cucumber, promoting female development, as the *a* locus in melon and *M* locus in cucumber are orthologs, *CmACS-7* in melon and *CsACS-2* in cucumber, encoding the rate limiting enzyme in ethylene biosynthesis [2]. The cucumber *F* locus is also a duplicated *ACS* gene, *CsACS1G* [25,26]. The Other hormones affecting sex expression in melon and cucumber such as GA and auxin are likely through crosstalk between each of them with ethylene indirectly [50], and there is no evidence that they are affecting sex determination or differentiation directly.

Three growth hormones are involved in sex determination in maize. GA promotes female flower development as both the anther ear mutant *an1* and the dwarf mutant *d3* are caused by mutation on GA biosynthesis pathway [37,38]. JA promotes male flower development as *ts1* encodes a gene involved in JA biosynthesis [31]. JA is also known for its essential role on stamen filament elongation and pollen maturation in Arabidopsis [51,52]. BR, a plant hormone, shares structural similarities with animal steroids, also promotes male flower development [36••]. It is not clear whether there is crosstalk between JA and BR, and which hormone is the decisive factor for male sex determination in maize.

GA is known to regulate cell elongation and plant growth. Ethylene is a major hormone for fruit maturation. JA and BR are better known as stress response hormones. None of them is a sex specific hormone. In fact, some growth hormones have opposing effect on sex determination in different lineage of flowering plants [53].

Conclusions

The lack of sex determination genes from dioecious species is still a major gap in our understanding of the origin of dioecy and sex chromosomes. Sequencing the sex chromosomes in papaya provides the foundation for identification of sex determination genes. From the sex determination genes identified in cucurbits, conservation of sex determination genes within a family could be prevalent. It appears there is no conservation of sex determination genes or pathways among plant families and no obvious convergent evolution from the data accumulated so far. Evidence of convergent evolution may appear when many more sex determination genes are identified in diverse lineages since the potential sex determination genes and pathways are far less than the approximate 11% or 37 840 monoecious and dioecious species in angiosperm.

Acknowledgements

We thank Jennifer Han for technical assistance. This work was supported by startup funds from Fujian Agriculture and Forestry University and a grant from NSF Plant Genome Research Program to RM (Award No. DBI 0922545), and Program Saclay Plant Sciences (SPS, ANR-10-LABX-40) and ANR MELODY (ANR-11-BSV7-0024) to AB.

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. •• Diggle PK, Di Stilio VS, Gschwend AR, Golenberg EM, Moore RC, Russell JR, Sinclair JP: **Multiple developmental processes underlie sex differentiation in angiosperms**. *Trends Genet* 2011, **27**:368-376.

This review summarized the modes of development in unisexual flowers and reinforced the notion that abortion of stamens and carpels randomly occurred at all stages of their development.

2. Boualem A, Fergany M, Fernandez R, Troadec C, Martin A, Morin H, Sari MA, Collin F, Flowers JM, Pitrat M *et al.*: **A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons**. *Science* 2008, **321**:836-838.

3. Boualem A, Troadec C, Kovalski I, Sari MA, Perl-Treves R, Bendahmane A: **A conserved ethylene biosynthesis enzyme leads to andromonoecy in two *Cucumis* species**. *PLoS ONE* 2009, **4**:e6144.

4. Li Z, Huang S, Liu S, Pan J, Zhang Z, Tao Q, Shi Q, Jia Z, Zhang W, Chen H *et al.*: **Molecular isolation of the M gene suggests that a conserved-residue conversion induces the formation of bisexual flowers in cucumber plants**. *Genetics* 2009, **182**:1381-1385.

5. Ming R, Moore PH: **Genomics of sex chromosomes**. *Curr Opin Plant Biol* 2007, **10**:123-130.

6. Ming R, Bendahmane A, Renner SS: **Sex chromosomes in land plants**. *Annu Rev Plant Biol* 2011, **62**:485-514.

7. Villarreal JC, Renner SS: **Correlates of monoicy and dioicy in hornworts, the apparent sister group to vascular plants**. *BMC Evolut Biol* 2013, **13**:239.

8. Stock M, Horn A, Grossen C, Lindtke D, Sermier R, Betto-Colliard C, Dufresnes C, Bonjour E, Dumas Z, Luquet E *et al.*: **Ever-young sex chromosomes in European tree frogs**. *PLoS Biol* 2011, **9**:e1001062.

9. Farbos I, Veuskens J, Vyskot B, Oliveira M, Hinnisdaels S, Aghmir A, Mouras A, Negrutiu I: **Sexual dimorphism in white campion: deletion on the Y chromosome results in a floral asexual phenotype**. *Genetics* 1999, **151**:1187-1196.

10. • Bergero R, Charlesworth D: **Preservation of the Y transcriptome in a 10-million-year-old plant sex chromosome system**. *Curr Biol* 2011, **21**:1470-1474.

Using RNA-Seq on transcriptomes from a single *S. latifolia* full-sib family several hundreds of X-linked and Y-linked genes were identified. Many X-linked genes remain intact in the Y chromosome with slightly reduced expression, but accumulating non-synonymous mutations.

11. • Chibalina MV, Filatov DA: **Plant Y chromosome degeneration is retarded by haploid purifying selection**. *Curr Biol* 2011, **21**:1475-1479.

Transcriptome sequencing from the parents and F1 male and female plants of two *S. latifolia* crosses led to the identification of more than 400 X-linked and Y-linked. Y-linked genes showed higher rate of non-synonymous mutations and loss of expression, compared to X-linked genes.

12. • Muyle A, Zemp N, Deschamps C, Mousset S, Widmer A, Marais GA: **Rapid de novo evolution of X chromosome dosage compensation in *Silene latifolia*, a plant with young sex chromosomes**. *PLoS Biol* 2012, **10**:e1001308.

Using RNA-Seq data from multiple males and females of an *S. latifolia* inbred line, more than 1700 X-linked and Y-linked contigs were identified. The X-linked allele expression increases, whereas Y-linked allele expression decreases in male plants, showing dosage compensation in this young sex chromosome system evolved 10 million years ago.

13. Blavet N, Blavet H, Cegan R, Zemp N, Zdanska J, Janousek B, Hobza R, Widmer A: **Comparative analysis of a plant pseudoautosomal region (PAR) in *Silene latifolia* with the corresponding *S. vulgaris* autosome**. *BMC Genomics* 2012, **13**:226.

14. Yu Q, Navajas-Pérez R, Tong E, Robertson J, Moore PH, Paterson AH, Ming R: **Recent origin of dioecious and gynodioecious Y chromosomes in papaya**. *Trop Plant Biol* 2008:49-57.

15. Na JK, Wang J, Murray JE, Gschwend AR, Zhang W, Yu Q, Navajas-Perez R, Feltus FA, Chen C, Kubat Z *et al.*: **Construction of physical maps for the sex-specific regions of papaya sex chromosomes**. *BMC Genomics* 2012, **13**:176.

16. •• Wang J, Na JK, Yu Q, Gschwend AR, Han J, Zeng F, Aryal R, VanBuren R, Murray JE, Zhang W *et al.*: **Sequencing papaya X and Y^{h} chromosomes reveals molecular basis of incipient sex chromosome evolution**. *Proc Natl Acad Sci U S A* 2012, **109**:13710-13715.

The sequencing of the HSY and its X counterparts revealed the mechanism of recombination suppression at the sex determining region by two inversions. Gene trafficking from autosome to HSY was documented, but not in the X corresponding region. Gene losses were observed in both X and HSY with higher rate in the HSY.

17. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Pyntikova T, Ali J, Bieri T *et al.*: **The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes**. *Nature* 2003, **423**:825-837.

18. Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, Platzer M, Howell GR, Burrows C, Bird CP *et al.*: **The DNA sequence of the human X chromosome**. *Nature* 2005, **434**:325-337.

19. •• Gschwend AR, Yu Q, Tong EJ, Zeng F, Han J, VanBuren R, Aryal R, Charlesworth D, Moore PH, Paterson AH *et al.*: **Rapid divergence and expansion of the X chromosome in papaya**. *Proc Natl Acad Sci U S A* 2012, **109**:13716-13721.

Comparison between papaya X chromosome and orthologous autosome in monoicious *V. monoica* revealed expression of the X chromosome. Gene content and order are also changed in both lineages. The evolution of X chromosome is more dynamic than previously envisioned.

20. Telgmann-Rauber A, Jamsari A, Kinney MS, Pires JC, Jung C: **Genetic and physical maps around the sex-determining M-locus of the dioecious plant asparagus**. *Mol Genet Genomics* 2007, **278**:221-234.

21. •• Tennessen JA, Govindarajulu R, Liston A, Ashman TL: **Targeted sequence capture provides insight into genome structure and genetics of male sterility in a gynodioecious diploid strawberry, *Fragaria vesca ssp. bracteata* (Rosaceae)**. *G3 (Bethesda)* 2013, **3**:1341-1351.

There is no recombination suppression at the two sex determining loci in strawberry, and map-based cloning approach is suitable to clone the sex determination genes. Using targeted sequence capture, the male sterility locus was mapped in a 338 kb region in Chromosome 4.

22. Poole CF, Grimball PC: **Inheritance of new sex forms in *Cucumis melo* L.** *J Heredity* 1939, **30**:21-25.

23. Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, Pitrat M, Dogimont C, Bendahmane A: **A transposon-induced epigenetic change leads to sex determination in melon**. *Nature* 2009, **461**:1135-1138.

24. Perl-Treves R: **Male to female conversion along the cucumber shoot: approaches to study sex genes and floral development in *Cucumis sativus***. In *Sex Determination in Plants.* Edited by Ainsworth CC. BIOS Scientific Publisher; 1999:189-216.

25. Trebitsh T, Staub JE, O'Neill SD: **Identification of a 1-aminocyclopropane-1-carboxylic acid synthase gene linked to the female (F) locus that enhances female sex expression in cucumber**. *Plant Physiol* 1997, **113**:987-995.

26. Mibus H, Tatlioglu T: **Molecular characterization and isolation of the F/f gene for femaleness in cucumber (*Cucumis sativus* L.)**. *Theor Appl Genet* 2004, **109**:1669-1676.

27. •• Li Z, Wang S, Tao Q, Pan J, Si L, Gong Z, Cai R: **A putative positive feedback regulation mechanism in CsACS2 expression suggests a modified model for sex determination in cucumber (*Cucumis sativus* L.)**. *J Exp Bot* 2012, **63**:4475-4484.

Overexpression of the cucumber ethylene synthase CsACS2 increases ethylene production in transgenic tobacco, and ethylene treatment in cucumber also increases the transcription of CsACS2. These two lines of evidence lead to the conclusion of a positive feedback mechanism in CsACS2 expression and ethylene production in cucumber.

28. Hao YJ, Wang DH, Peng YB, Bai SL, Xu LY, Li YQ, Xu ZH, Bai SN: **DNA damage in the early primordial anther is closely correlated with steman arrest in the female flower of cucumber (*Cucumis sativus* L.)**. *Planta* 2003, **217**: 888-895.

29. Wang DH, Li F, Duan QH, Xu ZH, Bai SN: **Ethylene perception is involved in female cucumber flower development**. *Plant J* 2010, **61**:862-872.

30. DeLong A, Calderon-Urrea A, Dellaporta SL: **Sex determination gene TASSELSEED2 of maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion**. *Cell* 1993, **74**:757-768.

31. Acosta IF, Laparra H, Romero SP, Schmelz E, Hamberg M, Mottinger JP, Moreno MA, Dellaporta SL: **tasselseed1 is a lipoxygenase affecting jasmonic acid signaling in sex determination of maize**. *Science* 2009, **323**:262-265.

32. Calderon-Urrea A, Dellaporta SL: **Cell death and cell protection genes determine the fate of pistils in maize**. *Development* 1999, **126**:435-441.

33. Wu X, Knapp S, Stamp A, Stammers DK, Jornvall H, Dellaporta SL, Oppermann U: **Biochemical characterization of TASSELSEED 2, an essential plant short-chain dehydrogenase/reductase with broad spectrum activities**. *FEBS J* 2007, **274**:1172-1182.

34. Chuck G, Meeley R, Irish E, Sakai H, Hake S: **The maize tasselseed4 microRNA controls sex determination and meristem cell fate by targeting Tasselseed6/indeterminate spikelet1**. *Nat Genet* 2007, **39**:1517-1521.

35. Chuck G, Meeley RB, Hake S: **The control of maize spikelet meristem fate by the APETALA2-like gene indeterminate spikelet1**. *Genes Dev* 1998, **12**:1145-1154.

36. Hartwig T, Chuck GS, Fujioka S, Klempien A, Weizbauer R,
•• Potluri DPV, Choe S, Johal GS, Schulz B: **Brassinosteroid control of sex determination in maize**. *Proc Natl Acad Sci U S A* 2011, **108**:19814-19819.

Plant growth hormone brassinosteroids share structural similarities with animal steroids, and controls sex determination in maize, as demonstrated in the maize dwarf mutant na1.

37. Bensen RJ, Johal GS, Crane VC, Tossberg JT, Schnable PS, Meeley RB, Briggs SP: **Cloning and characterization of the maize An1 gene**. *Plant Cell* 1995, **7**:75-84.

38. Winkler RG, Helentjaris T: **The maize Dwarf3 gene encodes a cytochrome P450-mediated early step in Gibberellin biosynthesis**. *Plant Cell* 1995, **7**:1307-1317.

39. Helliwell CA, Chandler PM, Poole A, Dennis ES, Peacock WJ: **The CYP88A cytochrome P450, ent-kaurenoic acid oxidase, catalyzes three steps of the gibberellin biosynthesis pathway**. *Proc Natl Acad Sci U S A* 2001, **98**:2065-2070.

40. Harberd NP, Freeling M: **Genetics of dominant gibberellin-insensitive dwarfism in maize**. *Genetics* 1989, **121**:827-838.

41. Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F *et al.*: **'Green revolution' genes encode mutant gibberellin response modulators**. *Nature* 1999, **400**:256-261.

42. Fujioka S, Yamane H, Spray CR, Gaskin P, MacMillan J, Phinney BO, Takahashi N: **Qualitative and quantitative analyses of gibberellins in vegetative shoots of normal, dwarf-1, dwarf-2, dwarf-3, and dwarf-5 seedlings of *Zea mays* L**. *Plant Physiol* 1988, **88**:1367-1372.

43. Spray CR, Kobayashi M, Suzuki Y, Phinney BO, Gaskin P, MacMillan J: **The dwarf-1 (d1) mutant of Zea mays blocks three steps in the gibberellin-biosynthetic pathway**. *Proc Natl Acad Sci U S A* 1996, **93**:10515-10518.

44. Tagagi E, Togashi K: **Evidence of sex change in Ilex integra**.
• *Botany* 2012, **90**:75-78.

Sex reversal in dioecious woody tree Ilex integra was observed in different years. The sex reversal occurs for the entire tree and there is no partial reversal leading to monoecy.

45. Vaughton G, Ramsey M: **Gender plasticity and sexual system stability in Wurmbea**. *Ann Bot* 2012, **109**:521-530.

46. Janousek B, Siroky J, Vyskot B: **Epigenetic control of sexual phenotype in a dioecious plant, *Melandrium album***. *Mol Gen Genet* 1996, **250**:483-490.

47. Matsumoto Y, Buemio A, Chu R, Vafaee M, Crews D: **Epigenetic control of gonadal aromatase (cyp19a1) in temperature-dependent sex determination of red-eared slider turtles**. *PLoS ONE* 2013, **8**:e63599.

48. Ellis HL, Shioda K, Rosenthal NF, Coser KR, Shioda T: **Masculine epigenetic sex marks of the CYP19A1/aromatase promoter in genetically male chicken embryonic gonads are resistant to estrogen-induced phenotypic sex conversion**. *Biol Reprod* 2012, **87**:12-21.

49. Kuroki S, Matoba S, Akiyoshi M, Matsumura Y, Miyachi H, Mise N,
•• Abe K, Ogura A, Wilhelm D, Koopman P *et al.*: **Epigenetic regulation of mouse sex determination by the histone demethylase Jmjd1a**. *Science* 2013, **341**:1106-1109.

Male to female sex reversal in mice is caused by lacking the H3K9 demethylase Jmjd1a, which positively controls sex determination gene Sry expression by H3K9me2-specific demethylation.

50. Golenberg EM, West NW: **Hormonal interactions and gene regulation can link monoecy and environmental plasticity to the evolution of dioecy in plants**. *Am J Bot* 2013, **100**:1022-1037.

51. Stintzi A, Browse J: **The Arabidopsis male-sterile mutant, orp3, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis**. *Proc Natl Acad Sci U S A* 2000, **97**:10625-10630.

52. Park JH, Halitschke R, Kim HB, Baldwin IT, Feldmann KA, Feyereisen R: **A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in Arabidopsis due to a block in jasmonic acid biosynthesis**. *Plant J* 2002, **31**:1-12.

53. Yamasaki S, Fujii N, Takahashi H: **Hormonal regulation of sex expression in plants**. *Vitam Horm* 2005, **72**:79-110.