

Bird–nest puzzle: can the study of unisexual flowers such as cucumber solve the problem of plant sex determination?

Shu-Nong Bai · Zhi-Hong Xu

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Abstract Unisexual flower development has long been used as a model system to understand the mechanism of plant sex determination. However, based on our investigation of the mechanisms regulating the development of unisexual cucumber flowers, we have realized that understanding how organ development is inhibited may not necessarily reveal how an organ is formed. We refer to this problem as a “bird–nest puzzle,” meaning one cannot understand how a bird lays and hatches its eggs by understanding how its nest is ruined. To understand the biological significance of unisexual flowers, we reexamine the original meaning of sex and its application in plants. Additionally, we propose that the fundamental biological advantage for the selection and maintenance of unisexual flowers during evolution is to promote cross pollination.

Keywords Unisexual flowers · Cucumber · Sex · Sex differentiation · Cross-pollination

Sex is an evolutionarily ancient phenomenon. It is well accepted that sexual differentiation evolved in unicellular eukaryote organisms, and the hallmark events of the sex-related

life cycle, or sexual reproduction cycle, are meiosis and fertilization. Although there is no consensus yet on the origin of meiosis during evolution, the origin of sexual differentiation is considered to relate to heterogamy, and the advantage of different sexes for the creation of genetic variability, which provides opportunities for natural selection and increased fitness, is well recognized. According to its Latin root “*sexus*,” sex originally means “to divide” (*secare*). The word sex is used to describe gender probably because that any organisms of any kind, especially in the animal kingdom, can be divided into two groups: male and female.

Sex is closely related to the daily life of human beings; therefore, the process of sex determination has been a long-standing question in biology since Aristotle. After more than 2,000 years’ belief that sex was determined only by environmental conditions, sex chromosomes were discovered in animals in the early twentieth century (Gilbert 2000). Currently, it is well accepted that both external environmental and internal genetic mechanisms of sex determination can operate in different animal species (Bull 1983).

Although cross-pollination in agronomy can be traced back to 1,000 BC (Stanley and Linskens 1974), Charles Alston, a keeper of the Royal Physick Garden in the seventeenth to the eighteenth centuries, considered sex in flowers to be an insult to his profession (Robbins and Pearson 1933). According to Robbins and Pearson (1933), sex in plants was originally defined by the morphological characteristics associated with gamete generation, just as secondary sex traits are used for the classification of mammals. For example, a flower or a plant was male if it only bore stamens. Therefore, the vast majority of flowering plants were considered to be hermaphrodites or bisexual as perfect flowers bear both stamens and carpels. Following this viewpoint, studies of sex determination in angiosperms essentially investigated how unisexual plants (dioecious) or unisexual

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S.-N. Bai (✉) · Z.-H. Xu
PKU–Yale Joint Research Center of Agricultural and Plant
Molecular Biology, State Key Laboratory of Protein and Plant
Gene Research, College of Life Sciences, Peking University,
5 Yiheyuan Road,
Beijing 100871, People’s Republic of China
e-mail: shunongb@pku.edu.cn

S.-N. Bai · Z.-H. Xu
The National Center of Plant Gene Research (Beijing),
Beijing, China

flowers borne on the same plant (monoecious) were regulated. Since then, this conceptual framework has been continuously applied, according to representative review articles and books on the topic (Irish and Nelson 1989; Dellaporta and Calderon-Urrea 1993; Tanurdzic and Banks 2004; Ainsworth 1999).

Several model systems have been used for the study of unisexual flower development, including dioecious *Silene latifolia*, *Romex acetosa*, and *Carica papaya*, and monoecious maize, cucumber, and melon. Great efforts have been made in the analysis of dioecious plant sex chromosomes (Ainsworth 1999; Charlesworth 2002; Ming et al. 2007a). So far, the most successful case is the mapping and sequencing of the sex loci in *C. papaya* (Ming et al. 2007b). However, the genes identified in the sex locus have provided no clues regarding their functional relationship with the dioecy (Yu et al. 2008). The first breakthrough in monoecious plants was made in the early 1990s when *TASSEL-SEED2* (*TS2*) was cloned and identified as a factor required for stage-specific floral organ abortion (DeLong et al. 1993). Since then, more genes involved in unisexual flower development have been cloned, such as *TS4* and *TS1* in maize (Chuck et al. 2007; Acosta et al. 2009), *F* and *M* genes in cucumber (Mibus and Tatlioglu 2004; Boualem et al. 2009; Li et al. 2009), and *A* gene in melon (Boualem et al. 2008). To some extent, the molecular mechanisms of the aforementioned genes have been revealed in these plants. For example, *TS2* suppresses pistil development in maize by mediating cell death. This process is required for *TS1* and prevented by *SK* (Calderon-Urrea and Dellaporta 1999). While *TS1* plays a role in sex determination by affecting JA signaling (Acosta et al. 2009), *TS4* affects sex determination by targeting *IDS1* in maize (Chuck et al. 2007). It is known that the cucumber *M* gene is specifically expressed in carpels (Saito et al. 2007) and that the melon *A* gene expression is indirectly repressed by the transcription factor *CmWIP1* (Martin et al. 2009). However, additional details of unisexual flower development in these plants remain elusive.

Instead of adopting a genetic approach to clone the genes responsible for unisexual flower development, we have studied unisexual flower development in cucumber by investigating when the inappropriate organs stop growing, what occurs in the inappropriate organs, and how gaseous ethylene selectively promotes female flower development. We observed that morphological divergence leading to male and female flowers occurs in stage 6 floral buds (Bai et al. 2004). While we confirmed that both inappropriate stamens in female flowers and carpels in male flowers are alive (Yang et al. 2000; Hao et al. 2003), we detected primordial anther-specific DNA damage in female flowers (Hao et al. 2003). DNA damage was not detected in the inappropriate carpels of male flowers (Bai et al. 2004); however, we

observed a correlation between the low expression levels of several types of pre-microRNA (miRNA) and arrested carpel development in male flowers (Sun et al. 2010). Furthermore, we demonstrated that the expression of the ethylene receptor *CsETR1* gene is organ-specifically downregulated in the stamens of female flowers compared to the stamens of male flowers and carpels of female flowers (Wang et al. 2010). Organ-specific downregulation of ethylene receptor gene expression or upregulation of ethylene synthesis gene expression in transgenic *Arabidopsis* plants can mimic female flowers (Wang et al. 2010; Duan et al. 2008). We have also identified an ethylene-inducible nuclease, which may be responsible for primordial anther-specific DNA damage in female flowers (Gu et al. 2011). These findings have helped explain, in part, how gaseous ethylene selectively promotes female flowers by inhibiting stamen development (Bai and Xu 2010).

Our observations, together with the fact that both the *F* and *M* genes encode different members of the ACC synthase (*ACS*) gene family, have triggered further questions regarding why ethylene was selected to inhibit stamen development in female cucumber flowers. Taking advantage of the availability of the cucumber genome (Huang et al. 2009), we analyzed the phylogenetic relationship of the *F* and *M* genes. We found that the *M* gene probably diverged and became involved in stamen development prior to the *F* gene (Sun et al. 2010). This result is consistent with the high frequency of *M* gene mutations in cucumber, as well as the equivalent *A* gene in melon (Boualem et al. 2008, 2009; Li et al. 2009), and the proposed recent duplication of the *F* gene (Knopf and Trebitsh 2006). As the *M* gene is preferentially expressed in carpels (Saito et al. 2007), the role of ethylene in the inhibition of stamen development can be narrowed down to how the expression pattern of the *M* gene evolved. Three lines of recent evidence are worth noting with regard to this question. Firstly, organ size can be regulated by the *TCP* genes in the leaf and petal (Efroni et al. 2008; Nag et al. 2009). Secondly, miRNAs such as miR171 are involved in organ size regulation via targeting *TCP* genes (Palatnik et al. 2003; Efroni et al. 2008; Nag et al. 2009). Thirdly, the expression levels of some miRNAs, including miR 171, miR 396, and miR319, are stress-regulated (Sunkar and Zhu 2004; Liu et al. 2008). Therefore, we proposed a “miR initiation” hypothesis to explain the origin of unisexual flowers in cucumber (Sun et al. 2010). This hypothesis suggests that environmental changes trigger changes in the expression of miRs, which alters the expression of *TCP* and leads to the arrest of ovary development. The *M* gene was co-opted to correct miR expression levels in order to rescue the fatal defects in ovary development which prevented seed production. While the *M* gene rescued ovary development, a high level of ethylene production

inhibited stamen development as a side effect. This mechanism was eventually selected during evolution as it introduced the advantages of cross-pollination.

Our initial purpose of studying cucumber unisexual flower development was to understand the mechanisms of sex determination, in particular, how phytohormones play a role in this process. However, our findings on unisexual cucumber flower development, both empirical and theoretical, have revealed a rather accidental series of chain reactions in response to environmental challenges. The relationship between heterospore generation and sexual organ development in cucumber is similar to the relationship between a bird and its nest. In general, a bird needs a nest to lay and hatch its eggs, and in most cases, the nest is built by the bird. Now the question is that can we properly understand how a bird lays and hatches its eggs by investigating how it builds its nest? If the answer is no, how can we expect to understand the sex determination mechanism in cucumber by studying the inhibition of either male or female organ development, which seems comparable to the ruining of a nest rather than building one? We refer to this problem as the “bird–nest puzzle.”

According to Robbins and Pearson (1933), a flower or a plant can only be identified as a separate sex if it bears only stamens or carpels. However, all plants with perfect flowers, which include approximately 90% of angiosperms (Ainsworth 1999), can generate heterogametes as well as heterospores, yet these plants definitely have a sex. The definition of plant sex based on whether the plant or flower bears only stamens or carpels actually introduced difficulties to the application of the organ-based sex definition in plants with perfect flowers. Can we find a proper way to define sex determination in plants? Since the Latin root of “sex” is “dividing,” it is clear that in an evolutionary perspective, the essence of “sex” should be an event or process leading to cells generated from zygotes diverged to either male or female gametes, e.g., heterogametes. In other words, sex determination should be a mechanism which ensures the completion of the divergence. In this sense, the first divergence point during the developmental process of an angiosperm with perfect flowers should be when a primordium initiated from the shoot apical meristem (or more specifically the floral meristem) adapts its developmental path to either a stamen or a carpel. Although Ainsworth’s (1999) opinion suggests that the study of unisexual flowers provides opportunities to understand the developmental regulation of perfect or hermaphrodite flowers, understanding how primordia such as inappropriate stamens in female cucumber flowers are inhibited helps little to understand how the developmental path to stamen is established, just like knowing how a nest is ruined helps little to understand how the nest was built. In contrast, identification

of ABC genes that determine floral organ identities (Coen and Meyerowitz 1991) was actually the most fundamental breakthrough in the effort to understand the mechanism of plant sex determination, or in a better term “sex differentiation,” in the last several decades. Together with recent understanding of the mechanisms which regulate flower development in unisexual plants such as cucumber, these analyses have inspired a reexamination of the traditional concepts of plant sex and will inspire further investigation from new perspectives to address the long-standing question of plant sex differentiation in the future.

What is the role of unisexual flowers in plants then? Based on our investigation of unisexual cucumber flowers, we propose that the main function of unisexual flower development is to promote cross-pollination. As plants evolved a sessile morphogenetic strategy and cannot move to positively select mating partners, cross-pollination provides tremendous advantages to introduce genetic variation in order to increase fitness. Compared with self-incompatibility, a recognition mechanism to avoid self-pollination with normal floral morphology (Rea and Nasrallah 2008), unisexual flowers provide a sacrifice mechanism to avoid self-pollination, or, as this can alternatively be viewed, greatly increase the opportunity for cross-pollination by functionally disabling one of their sexual organs. Regardless of the method adopted, inhibition of male or female organ development will always result in cross-pollination. Moreover, if our “miR hypothesis” is true, unisexual cucumber flowers have originated as a response to randomly occurring environmental challenges. This provides a simpler mechanism for fulfilling cross-pollination compared to the self-incompatibility that requires complicated genetic interactions for recognition. The viewpoint that the main function of unisexual flower is to promote cross-pollination explains the extremely diversified mechanisms of unisexual flower development and the widespread unisexual flower traits across the phylogenetic tree (Charlesworth 2002).

The bird–nest puzzle, derived from our investigation of unisexual cucumber flowers, has inspired a reexamination of the traditional concept of sex determination in plants. This reexamination does not underestimate the significance or contribution of previous efforts, which have improved the understanding of unisexual flower development, but rather opens new opportunities, based on historical contributions, to pinpoint the actual events of plant sex differentiation in a broader perspective.

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References

- Acosta IF, Laparra H, Romero SP, Schmelz E, Hamberg M, Mottinger JP, Moreno MA, Dellaporta SL (2009) tasselseed1 is a lipoxygenase affecting jasmonic acid signaling in sex determination of maize. *Science* 323(5911):262–265
- Ainsworth CC (1999) Sex determination in plants, vol 1, Environmental stress and gene regulation. Sex determination in plants. BIOS Scientific Publishers, Oxford
- Bai SN, Xu ZH (2010) From ethylene promotion of female flowers to ethylene inhibition of stamen development: a review on the study of developmental fate of inappropriate organs in suissexual cucumber flowers. *Sci Sin Vitae* 40(6):469–475
- Bai SL, Peng YB, Cui JX, Gu HT, Xu LY, Li YQ, Xu ZH, Bai SN (2004) Developmental analyses reveal early arrests of the spore-bearing parts of reproductive organs in unisexual flowers of cucumber (*Cucumis sativus* L.). *Planta* 220(2):230–240
- Boualem A, Fergany M, Fernandez R, Troadec C, Martin A, Morin H, Sari MA, Collin F, Flowers JM, Pitrat M, Purugganan MD, Dogimont C, Bendahmane A (2008) A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons. *Science* 321(5890):836–838
- Boualem A, Troadec C, Kovalski I, Sari MA, Perl-Treves R, Bendahmane A (2009) A conserved ethylene biosynthesis enzyme leads to andromonoecy in two cucumis species. *PLoS One* 4(7):e6144
- Bull JJ (1983) Evolution of sex determining mechanism, vol 1. Benjamin/Cummings Publishing, Berkeley. ISBN 0-8053-0400-2
- Calderon-Urrea A, Dellaporta SL (1999) Cell death and cell protection genes determine the fate of pistils in maize. *Development* 126(3):435–441
- Charlesworth D (2002) Plant sex determination and sex chromosomes. *Heredity* (Edinb) 88(2):94–101
- Chuck G, Meeley R, Irish E, Sakai H, Hake S (2007) The maize tasselseed4 microRNA controls sex determination and meristem cell fate by targeting Tasselseed6/indeterminate spikelet1. *Nat Genet* 39(12):1517–1521
- Coen ES, Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* 353(6339):31–37
- Dellaporta SL, Calderon-Urrea A (1993) Sex determination in flowering plants. *Plant Cell* 5(10):1241–1251
- DeLong A, Calderon-Urrea A, Dellaporta SL (1993) Sex determination gene TASSELSEED2 of maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion. *Cell* 74(4):757–768
- Duan QH, Wang DH, Xu ZH, Bai SN (2008) Stamen development in *Arabidopsis* is arrested by organ-specific overexpression of a cucumber ethylene synthesis gene CsACO2. *Planta* 228(4):537–543
- Efroni I, Blum E, Goldshmidt A, Eshed Y (2008) A protracted and dynamic maturation schedule underlies *Arabidopsis* leaf development. *Plant Cell* 20(9):2293–2306
- Gilbert SF (2000) Developmental biology, 6th edn. Sinauer Associates, Sunderland
- Gu HT, Wang DH, Li X, He CX, Xu ZH, Bai SN (2011) Characterization of an ethylene-inducible, calcium-dependent nuclease that is differentially expressed in cucumber flower development. *New Phytol* 192(3):590–600
- Hao YJ, Wang DH, Peng YB, Bai SL, Xu LY, Li YQ, Xu ZH, Bai SN (2003) DNA damage in the early primordial anther is closely correlated with stamen arrest in the female flower of cucumber (*Cucumis sativus* L.). *Planta* 217(6):888–895
- Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B, Ni P, Ren Y, Zhu H, Li J, Lin K, Jin W, Fei Z, Li G, Staub J, Kilian A, van der Vossen EA, Wu Y, Guo J, He J, Jia Z, Tian G, Lu Y, Ruan J, Qian W, Wang M, Huang Q, Li B, Xuan Z, Cao J, Asan WuZ, Zhang J, Cai Q, Bai Y, Zhao B, Han Y, Li Y, Li X, Wang S, Shi Q, Liu S, Cho WK, Kim JY, Xu Y, Heller-Uszynska K, Miao H, Cheng Z, Zhang S, Wu J, Yang Y, Kang H, Li M, Liang H, Ren X, Shi Z, Wen M, Jian M, Yang H, Zhang G, Yang Z, Chen R, Ma L, Liu H, Zhou Y, Zhao J, Fang X, Fang L, Liu D, Zheng H, Zhang Y, Qin N, Li Z, Yang G, Yang S, Bolund L, Kristiansen K, Li S, Zhang X, Wang J, Sun R, Zhang B, Jiang S, Du Y (2009) The genome of the cucumber, *Cucumis sativus* L. *Nat Genet* 41(12):1275–1281
- Irish EE, Nelson T (1989) Sex determination in monoecious and dioecious plants. *Plant Cell* 1(8):737–744
- Knopf RR, Trebitsh T (2006) The female-specific Cs-ACS1G gene of cucumber. A case of gene duplication and recombination between the non-sex-specific 1-aminocyclopropane-1-carboxylate synthase gene and a branched-chain amino acid transaminase gene. *Plant Cell Physiol* 47(9):1217–1228
- Li Z, Liu S, Pan J, Zhang Z, Tao Q, Shi Q, Jia Z, Zhang W, Chen H, Si L, Zhu L, Huang S, Cai R (2009) Molecular isolation of the *M* gene suggests that a conserved-residue conversion induces the formation of bisexual flowers in cucumber plants. *Genetics* 182:1381–1385
- Liu HH, Tian X, Li YJ, Wu CA, Zheng CC (2008) Microarray-based analysis of stress-regulated microRNAs in *Arabidopsis thaliana*. *RNA* 14(5):836–843
- Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, Pitrat M, Dogimont C, Bendahmane A (2009) A transposon-induced epigenetic change leads to sex determination in melon. *Nature* 461(7267):1135–1138
- Mibus H, Tatlioglu T (2004) Molecular characterization and isolation of the *F1f* gene for femaleness in cucumber (*Cucumis sativus* L.). *Theor Appl Genet* 109(8):1669–1676
- Ming R, Wang J, Moore PH, Paterson AH (2007a) Sex chromosomes in flowering plants. *Am J Bot* 94(2):141–150
- Ming R, Yu Q, Moore PH (2007b) Sex determination in papaya. *Semin Cell Dev Biol* 18(3):401–408
- Nag A, King S, Jack T (2009) miR319a targeting of TCP4 is critical for petal growth and development in *Arabidopsis*. *Proc Natl Acad Sci U S A* 106(52):22534–22539
- Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, Weigel D (2003) Control of leaf morphogenesis by microRNAs. *Nature* 425(6955):257–263
- Rea AC, Nasrallah JB (2008) Self-incompatibility systems: barriers to self-fertilization in flowering plants. *Int J Dev Biol* 52(5–6):627–636
- Robbins WW, Pearson HM (1933) Sex in plant world. D. Appleton-Century New York
- Saito S, Fujii N, Miyazawa Y, Yamasaki S, Matsuura S, Mizusawa H, Fujita Y, Takahashi H (2007) Correlation between development of female flower buds and expression of the CS-ACS2 gene in cucumber plants. *J Exp Bot* 58(11):2897–2907
- Stanley RG, Linskens HF (1974) Pollen: biology, biochemistry, management. Springer, Berlin
- Sun JJ, Li F, Li X, Liu XC, Rao GY, Luo JC, Wang DH, Xu ZH, Bai SN (2010) Why is ethylene involved in selective promotion of female flower development in cucumber? *Plant Signal Behav* 5(8):1052–1056
- Sunkar R, Zhu JK (2004) Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. *Plant Cell* 16(8):2001–2019

- Tanurdzic M, Banks JA (2004) Sex-determining mechanisms in land plants. *Plant Cell* 16(Suppl):S61–71
- Wang DH, Li F, Duan QH, Han T, Xu ZH, Bai SN (2010) Ethylene perception is involved in female cucumber flower development. *Plant J* 61(5):862–872
- Yang LL, Chen M, Liu FQ, Geng Y, Chen C, Li YQ, Tsao TH, Xu ZH, Bai SN (2000) Carpel of cucumber (*Cucumis sativus* L.) male flowers maintains early primordia characteristics during organ development. *Chin Sci Bull* 45(8):729–733
- Yu Q, Hou S, Feltus FA, Jones MR, Murray JE, Veatch O, Lemke C, Saw JH, Moore RC, Thimmapuram J, Liu L, Moore PH, Alam M, Jiang J, Paterson AH, Ming R (2008) Low X/Y divergence in four pairs of papaya sex-linked genes. *Plant J* 53(1):124–132