ELSEVIER

Seminars in Cell & Developmental Biology 18 (2007) 401–408

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

www.elsevier.com/locate/semcdb

Review

Sex determination in papaya

Ray Ming [a,*], Qingyi Yu [b], Paul H. Moore [c]

[a] *Department of Plant Biology, University of Illinois at Urbana-Champaign, 288 ERML, MC-051, 1201 W. Gregory Drive, Urbana, IL 61801, USA*
[b] *Hawaii Agriculture Research Center, Aiea, HI 96701, USA*
[c] *USDA-ARS, Pacific Basin Agricultural Research Center, Hilo, HI 96720, USA*

Available online 24 November 2006

Abstract

Sex determination is an intriguing system in trioecious papaya. Over the past seven decades various hypotheses, based on the knowledge and information available at the time, have been proposed to explain the genetics of the papaya's sex determination. These include a single gene with three alleles, a group of closely linked genes, a genic balance of sex chromosome over autosomes, classical XY chromosomes, and regulatory elements of the flower development pathway. Recent advancements in genomic technology make it possible to characterize the genomic region involved in sex determination at the molecular level. High density linkage mapping validated the hypothesis that predicted recombination suppression at the sex determination locus. Physical mapping and sample sequencing of the non-recombination region led to the conclusion that sex determination is controlled by a pair of primitive sex chromosomes with a small male-specific region (MSY) of the Y chromosome. We now postulate that two sex determination genes control the sex determination pathway. One, a feminizing or stamen suppressor gene, causes stamen abortion before or at flower inception while the other, a masculinizing or carpel suppressor gene, causes carpel abortion at a later flower developmental stage. Detailed physical mapping is beginning to reveal structural details about the sex determination region and sequencing is expected to uncover candidate sex determining genes. Cloning of the sex determination genes and understanding the sex determination process could have profound application in papaya production.

Keywords: *Carica papaya*; Sex determination; Sex chromosomes; Sex reversal; Y chromosome degeneration

Contents

* Corresponding author. Tel.: +1 217 333 1221; fax: +1 217 244 1336.
E-mail address: rming@life.uiuc.edu (R. Ming).

1084-9521/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2006.11.013

1. Introduction

Papaya (*Carica papaya*) belongs to the small family Caricaceae with six genera and 35 species, of which 32 are dioecious, two trioecious, and one monoecious. Papaya is a fast growing, rarely branching, semi-woody tropical fruit tree with a short juvenile phase of 3 to 8 months. Once it starts flowering, it will continue to flower and produce fruit throughout the year. Papaya is cultivated in tropical and subtropical regions worldwide. Papaya fruit is the most nutritious of the 35 commonly consumed fruits based on percentage of US recommended daily allowance for vitamins A and C, folate, potassium, niacin, thiamin, iron, riboflavin, calcium, and fiber [1–3]. Papaya is also cultivated for its milky latex that contains the proteolytic enzyme papain used for hydrolyzing beer peptides (chillproofing), tenderizing meats, and medicinal applications.

Papaya is diploid with 9 pairs of chromosomes. It has a small genome of 372 Mb [4] and a generation time as short as 9 months. Vegetative propagation is possible by inducing rooting from cuttings or by micro-propagating in tissue culture. A genetic transformation system is well established. Transgenic papaya varieties conferring resistance to papaya ringspot virus saved the Hawaii papaya industry from collapse in the mid-1990s [5]. The above favorable properties make papaya an excellent model system for genomic research. Moreover, papaya is in the order Brassicales that includes the well-studied *Brassica* and *Arabidopsis*. Both are in the family Brassicaceae that diverged from a common ancestor with Caricaceae about 72 million years ago (MYA) [6] and can thus serve as an outgroup for comparative and phylogenetic analyses of Brassicaceae genomes.

Papaya is somewhat unusual in that it is trioecious with three basic sex forms: female, male, and hermaphrodite. Cymose inflorescences arise in axils of leaves. The type of inflorescence produced depends on the sex of the tree (Fig. 1). Varieties typically are either dioecious (with unisexual flowers and exclusively male and female plants) or gynodioecious (with bisexual and unisexual flowers and hermaphrodite and female plants). Male trees are characterized by long, pendulous, many-flowered inflorescences bearing slender male flowers lacking a pistil, except for occasional pistil-bearing flowers at the distal terminus. Female trees have short inflorescences with few flowers bearing large functional pistils without stamens. Hermaphroditic trees have short inflorescences bearing bisexual flowers that can be sexually variable.

Not only are the sex forms morphologically distinct, they are inherited in unexpected ratios that are due to a lethal factor associated with male dominant alleles (Table 1). Hermaphrodite papaya trees are primarily self-pollinated. However, seeds from selfed hermaphrodite trees always segregate into hermaphrodites and females at the ratio 2:1. Seeds from female trees segregate at the hermaphrodite to female ratio of 1:1 if they were fertilized by pollen from a hermaphrodite tree, or a ratio of 1:1 male to female when fertilized by pollen from a male tree. Male trees are never produced when hermaphrodites are selfed or when hermaphrodites are used as a pollen source to fertilize female trees. However, male trees occur at a ratio of

Fig. 1. The flowers and fruits of male, female, and hermaphrodite papaya. (A) Female flowers; (B) hermaphrodite flowers; (C) male flowers; (D) female fruit; (E) hermaphrodite fruit; (F) male tree.

Table 1
Sex ratio of crosses between different sex types

Crosses	Sex ratio			
	Female (*mm*)	Male (*Mm*)	Hermaphrodite (M^hm)	Non-viable ($M^{(h)}M^{(h)}$)
Male (*Mm*) selfed	1	2	0	1
Female (*mm*) × male (*Mm*)	1	1	0	0
Hermaphradite (M^hm) selfed	1	0	2	1
Female (*mm*) × hermaphradite (M^hm)	1	0	1	0
Hermaphradite (M^hm) × male (*Mm*)	1	1	1	1

2 male:1 female when the occasional male fruit is selfed, or 1 male:1 hermaphrodite: 1 female when male pollen fertilizes the pistil of hermaphrodite trees. These unexpected sex form ratios have been the subject of extensive studies.

In many regions of the world, hermaphrodites are preferred for their higher productivity since every tree will produce fruit, whereas using female trees for fruit production involves the loss of 6–10% of field space for growing male trees to pollinate the females. However, in subtropical regions with cool winters, female production is preferred because female flowers are stable at low temperature while hermaphrodite flowers tend to fuse anthers to the carpels and produce deformed carpellodic fruit. The lack of true breeding hermaphrodite varieties results in reduced productivity due to sex segregation in seedlings and has been a problem since the beginning of papaya cultivation that persists to this day [7]. Farmers using hermaphrodites for production need to germinate a minimum of five seedlings per hill to assure there are no more than 3% female trees. The five plants in a hill must be grown for 4–6 months until sexes can be determined. Finally, roguing must be practiced to obtain sex ratios conducive to optimal productivity. This process is inefficient of time, labor, water, and nutrients, and also results in delayed production due to competition among the plants in early growth. On the other hand, farmers depending on female tree production need to germinate four seedlings per hill to keep 6 to 10% male trees in the field.

The sex determination system in papaya is particularly intriguing, not only because it has three sex types within the species, but also because it shows frequent sex reversal caused by environmental factors [8,9]. Recent advancements in genomics and molecular biology have provided tools and resources to allow us to characterize the sex determination system in papaya. In this paper we will review the past hypotheses of sex determination in papaya and our current understanding of the sex determination mechanisms learned from recent genomic and molecular evidence.

2. Hypotheses

Sex determination of papaya has attracted the attention of generations of geneticists and breeders because of its intriguing biology and the economic problems caused by segregation of sex types in papaya production. Before the application of molecular techniques in papaya research, there was little evidence to prove or disprove various hypotheses proposed over the years. Nevertheless, some of the hypotheses provided partial revelations into the nature of sex determination in papaya.

2.1. Sex determination in papaya behaves as a single gene with three alleles

Based on the segregation ratios from crosses among three sex types (Table 1), Hofmeyr and Storey independently proposed that sex determination in papaya is controlled by a single gene with three alleles, named as M_1, M_2, and *m* by Hofmeyr and *M*, M^h, and *m* by Storey [8,10,11]. We will follow Storey's designation for its convenience to separate the hermaphrodite allele M^h from male allele *M*. Male individuals (*Mm*) and hermaphrodite individuals (M^hm) are heterozygous, whereas female individuals (*mm*) are homozygous recessive. The dominant combinations of *MM*, M^hM^h, and MM^h are lethal, resulting in a 2:1 segregation of hermaphrodite to female from self-pollinated hermaphrodite seeds and a 1:1 segregation of male to female or hermaphrodite to female from cross-pollinated female seeds.

2.2. Genic balance between sex chromosomes and autosomes

One year after Hofmeyr proposed the one-gene-with-three-allele hypothesis, he published an alternative genic balance hypothesis for sex determination in papaya [9,12]. Although there was no evidence of heteromorphic sex chromosomes, Hofmeyr suggested that the chromosomes bearing the *M*, M^h, and *m* alleles were "sex chromosomes." In this hypothesis, it is assumed that female sex determining factors predominate the "sex chromosomes" while the male sex determining factors are in the autosome. It was further assumed that *M* and M^h represent an inactivated region of the sex chromosomes where vital genes were eliminated, but that the inactivated region represented by *M* is longer than that represented by M^h. The different sex types were the results of genic balance between the sex chromosomes and autosome. Because vital genes were missing in the inactivated regions of *M* and M^h, any combinations of *MM*, MM^h, M^hM^h would be lethal, while *Mm* and M^hm would be viable because an m sex chromosome is present in each genotype.

Hofmeyr went further to assign arbitrary numbers to each sex chromosome and the autosome to come up with a quantitative representation of the genic balance. Although his experiments on induced polyploidy in papaya tended to support the hypothesis, they were not conclusive [13].

2.3. A group of linked genes confined to a small region on the sex chromosome controlling sex determination

Storey [14] revised his hypothesis about a single gene with three alleles to one involving a group of closely linked genes that are confined to a small region on the sex chromosome within which recombination is suppressed. Storey's modified hypothesis is mainly based on the observation that long peduncles are always associated with male flowers but not with hermaphrodite or female flowers and that the lethal factor is associated only with male and hermaphrodite homozygous dominant genotypes. Genes located in the sex determination segment were postulated to include:

Mp, long peduncles of male flowers
l, zygotic lethal factor
sa, suppressor of the androecium
sg, suppressor of the gynoecium
C, hypothetical factor for suppression of recombination at the sex determination region

Based on Storey's model, the genotypes of male, hermaphrodite, and female were given as:

Male = *Mp l C* + *sg*/+ + + *sa* +
Hermaphrodite = + *l C* + *sg*/ + + + *sa* +
Female = + + + *sa* +/+ + + *sa* +

The gene controlling the long peduncle of male flowers, *Mp*, is the only one that distinguishes the genotype of male flowers from that of hermaphrodite flowers. The hypothetical factor for suppression of recombination, *C*, could be a gene, a chromosomal inversion, a deletion, or the proximity of this chromosomal region to the centromere or telomere that would suppress or preclude recombination. The zygotic lethality factor, *l*, could be overlapping chromosomal deletions and these could cause suppression of recombination as well. In this case, *l* and *C* would be the result of a single chromosomal rearrangement rather than two linked genes or factors. The gene *sa* controls the suppression of stamen development when it is homozygous recessive as in female trees. The gene *sg* controls the suppression of carpel development when it is homozygous recessive. Storey suggested that most male and hermaphrodite trees were heterozygous for this gene, because sex reversal from hermaphrodite flowers to male flowers and vice versa were often observed in the field. He also suggested that those constantly fruiting hermaphrodites could be homozygous for the normal allele of the *sg* gene.

2.4. X and Y sex chromosome system with two slightly different Y chromosomes

Based on intergeneric hybridizations between *Carica* and *Vasconcellea* species, Horovitz and Jiménez [15] proposed that sex determination in papaya is of the XX-XY type. The genotypes of male, female, and hermaphrodite were XY, XX, and XY_2, respectively. These researchers suggested that the Y chromosome has a region containing a lethal factor. In their hypothesis, Y_2 is a modified form of the Y chromosome but includes the region of lethality.

2.5. Two sex types of papaya

Hamilton and Izuno [16] reported their discovery of long peduncle female papaya trees bearing predominantly female flowers but occasionally with a few male or hermaphrodite flowers. Based on data from progenies of reciprocal crosses of long peduncle female plants, they hypothesized there are only two sex types in papaya: females and a variable type that ranges from complete male to hermaphrodite with numerous intermediate types in between. Storey [17] subsequently revised his hypothesis to drop the factor controlling the long peduncle of male flowers, *Mp*, from his previous model resulting in the following genotypes of sex:

Male and andromonoecious: (*sa*) *l C* (*SG*)/(*SA*) + + (*sg*)
Female: (*SA*) + + (*sg*)/(*SA*) + + (*sg*)

In this revised model, (*SA*) represents a group of factors that converted the ancestral androecium into the current gynoecium; (*sa*) represents genes controlling normal androecium development; (*SG*) represents the factor or factors controlling carpel abortion in male flowers; (*sg*) allows expression of the (*SA*) factors controlling normal carpel abortion. The function of zygotic lethal factor, *l*, and the factor for suppression of recombination, *C*, remained the same as in his previous hypothesis [14]. Because all but three species of Caricaceae are dioecious, dioecy seemed to Storey to be the evolutionary norm within the family. Storey [17] suggested that the existence of hermaphrodite papaya might be due to human selection.

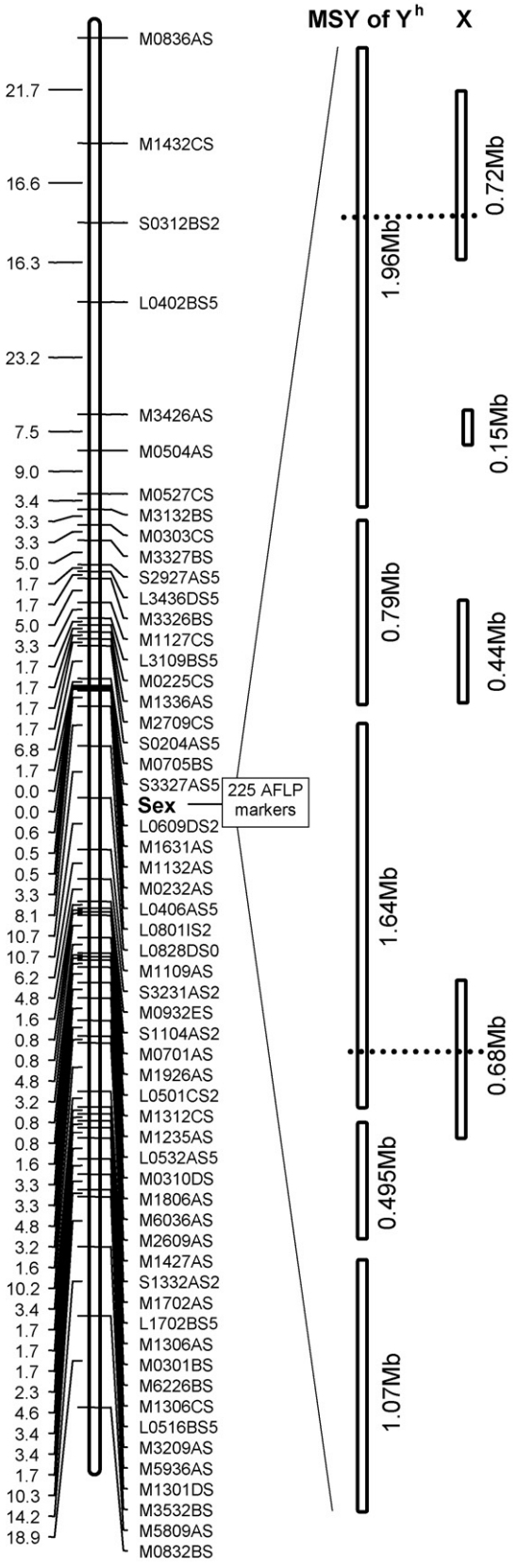
2.6. A trans-regulatory element controlling flower organ development

Recent advances in unraveling the mechanisms of flower development and particularly the ABC (now ABCE) model inspired Sondur et al. [18] to propose a model to explain the regulation of flower organ development in papaya dioecious flowers. In this model, the dominant male allele, *SEX1-M*, is proposed to encode a trans-acting factor that promotes stamen but suppresses carpel development, i.e. a masculinizing factor. The dominant hermaphrodite allele, *SEX1-H*, was suggested as an intermediate with the ability to induce stamens but only reduce carpel size. The recessive female allele, *sex1-f*, was hypothesized as incapable of inducing stamens and could be a null allele. Functional carpels would develop in heterozygous *SEX1-H*/*sex1-f* plants. The lethal factor tightly linked to the *SEX1-M* and *SEX1-H* alleles could result from an additional required function present only in the *sex1-f* allele.

3. Molecular genetics of sex determination in papaya

More recent application of molecular techniques and biotechnology has revolutionized the field of sex determination research

in papaya. Sex-linked DNA markers were developed by several research groups and linkage maps of the papaya genome were constructed. High density genetic mapping, fine mapping, physical mapping, and DNA sequencing of the sex determination locus led to the conclusion that a pair of primitive sex chromosomes controls sex determination in papaya.



3.1. Sex-linked DNA markers

Papaya breeders and researchers have long sought markers, methods, and techniques to identify the sex type at an early seedling stage to improve papaya fruit production. Hofmeyr [9] discovered two morphological markers, flower color and stem color, which were linked to the sex determination locus. These two morphological markers were about 24 and 40 cM away, respectively, and provided very little predictive value. In the 1990s when DNA markers were widely used in plant research, it became a priority to develop sex-linked DNA markers for testing papaya sex types. The first sex-linked marker reported was a microsatellite containing the quadranucleotide repeats, $(GATA)_4$ [19]. Using random amplified polymorphic DNA (RAPD) markers to screen for sex-linked markers, four sequence-characterized amplified region (SCAR) markers were developed by three independent research groups [20–22] to predict the sex types. Although the DNA markers are now available for predicting sex type, it is costly and impractical to test and then transplant millions of seedlings in a short time for commercial production.

3.2. Linkage mapping of sex determination locus

Genetic mapping is the first step to clone an unknown gene. The first genetic map of papaya was constructed with only three morphological markers, sex type, flower color, and stem color [9]. The second genetic map of papaya was constructed with 62 randomly amplified polymorphic DNA (RAPD) markers and the sex determination locus, Sex1, was mapped on linkage group 1 where it was flanked by two markers at 7 cM on each side [18]. These two early linkage maps indicated that the chromosome containing the sex determination locus appears to recombine normally. These two low density maps provided no clue whether there was suppression of recombination at the sex determination locus.

With the goal of cloning the sex determination gene in papaya, a high density linkage map of the papaya genome was constructed using an F_2 population derived from Hawaiian cultivars Kapoho × SunUp and mapped with 1498 amplified fragment length polymorphism (AFLP) markers, the papaya ringspot virus coat protein marker, morphological sex type, and fruit flesh color [23]. The sex determination locus was mapped on linkage group 1 (LG1), which had 342 markers and a total length of 289.7 cM (Fig. 2). Remarkably, a total of 225 markers co-segregated with sex; this number accounts for 66% of the 342

Fig. 2. Molecular marker genetic map of papaya linkage group 1 containing the non-recombinant MSY region that differs between the hermaphrodite Y^h and female X chromosomes. Figures from left to right are the overall genetic map of the primitive Y chromosome with a large number of co-segregating markers around the sex determining locus; the MSY consisting of five BAC contigs totaling 6 Mbp; and the X homolog of the Y chromosome consisting of an incomplete set of 4 BAC contigs.

markers on LG1 and 15% of all markers mapped on the genome. This map made it apparent that severe suppression of recombination occurred at and around a sex determination locus. LG1 was one of the largest linkage groups on the papaya genome and the remaining 117 AFLP markers on LG1 recombined normally. These mapping data also revealed an extraordinary level of DNA polymorphism in the genomic region immediately surrounding the sex locus.

3.3. Fine mapping of the sex determination locus

Fine-mapping of the sex determination locus was initiated simultaneously with a high density mapping project because sex-linked SCAR markers were available, and the first two linkage maps indicated that the chromosome containing the sex determination gene appeared to recombine normally. This large scale fine mapping project was conducted using 2190 female and hermaphrodite plants from three F_2 and one F_3 populations (4380 informative chromosomes), the two SCAR markers (W11 and T12) [22], three cloned *Carica papaya* sex-linked AFLP markers (cpsm), (cpsm10, cpsm31, and cpsm54), and one cloned bacterial artificial chromosome (BAC) end, cpbe55. Despite the large mapping populations screened, not a single recombinant was detected [24].

3.4. Physical mapping of the sex determination locus

Physical mapping of the sex determination locus was conducted concurrently with the high density linkage mapping of the genome and fine mapping of this locus, because sex-linked markers and BAC libraries had been developed [22,25]. Hybridization of the sex-linked SCAR marker W11 to the 13× BAC library identified four positive BACs. A contig map was constructed by cloning the BAC ends and hybridizing the ends to these four BACs. The two most extended ends were used to screen the BAC library to identify and confirm two groups of positive BACs. After 3 years of chromosome walking, a large BAC contig spanning 990 kb was produced. At this time, a large number of sex co-segregating AFLP markers were mapped and those markers exceeding 200 bp in size were excised, re-amplified, and used as probes for physical mapping. Forty-two AFLP-derived SCAR markers hybridized successfully. Among these 42 cpsm markers, 24 (57%) could be assembled on the BAC contig maps. Three cspm markers identified individual BACs and 15 of them contained repetitive sequences so they could not be mapped. Three other previously available sex co-segregating markers, T12, PSDM, and Nafp, were also physically mapped with the BAC contigs. Eighty-two BAC ends (cpbe) were iteratively cloned from contig-terminal BACs and used to close gaps. After exhausting the resources available at that time, a physical map spanning 2.5 Mb and consisting of two major and three smaller contigs containing 4 SCAR, 82 cpbe, and 24 cpsm loci was constructed around the sex determination locus. While gaps still remain in this physical map, at least 57% of the randomly distributed AFLP-derived cpsm markers locate to a small 2.5 Mb region of the chromosome containing the sex determination locus. The physical size of this non-recombining region was estimated to be 4–5 Mbp [24].

After physical mapping of the papaya genome using a hermaphrodite BAC library and continuous chromosome walking to close the gaps, the current physical map of the non-recombining region is about 6 Mbp with four gaps remaining (Q. Yu, P.H. Moore, J. Jiang, A.H. Paterson, R. Ming, unpublished data and Fig. 2). Our current estimate for the size of this targeted region is around 7 Mbp.

3.5. Sample sequencing in the non-recombining region

To assess the sequence features of the non-recombining region, a total of 513 kb random sub-clone sequences (628 reads) from 25 non-redundant BACs in this region were analyzed and compared with 517 kb papaya genome survey sequences [24]. Results showed an increase of transposable elements and inverted repeats but a decrease of gene density in the non-recombining region compared to the rest of the genome. A mosaic arrangement of conserved and diverged sequences was detected between male-specific BAC and female genomic sequences. Duplicated sequences were common in the non-recombining region. Nucleotide insertions, deletions, and substitutions were observed in all duplicated DNA sequences. Sequencing of male-specific DNA fragments from male and hermaphrodite plants indicated a small amount of sequence divergence between dioecious and gynodioecious papaya in this non-recombining region [24].

3.6. A primitive sex chromosome system

The combination of genetic mapping, physical mapping, and DNA sequence data strongly suggests that the sex determination locus is on an incipient sex chromosome. The features of the non-recombining region are consistent with the classical notion that an early stage of sex chromosome evolution involves suppression of recombination around the sex determination locus, leading to gradual degeneration of the Y chromosome [26,27] which in turn leads to new, heteromorphic sex chromosomes [28,29]. Sequencing two pairs of X and Y BACs further demonstrated the characteristics of sex chromosomes. Sequence divergence of X and Y gene pairs allows us to estimate the age of sex chromosomes in papaya and confirm the recent origin of papaya sex chromosomes (Q. Yu, P.H. Moore, J. Jiang, A.H. Paterson, R. Ming, unpublished data).

4. Current understanding of sex determination in papaya

Sex determination in papaya is controlled by a pair of recently evolved sex chromosomes. The Y chromosome has sufficiently degenerated to prevent the survival of the homozygous YY genotype and to reinforce the heterozygosity of the male and hermaphrodite genotypes. There are two slightly different Y chromosomes in papaya. We designate Y for the male-specific sex chromosome in male papaya and Y^h in hermaphrodite papaya because these two Y chromosomes are nearly identical

(Liu et al. [24]; R. Ming, Q. Yu, P. Moore, unpublished data). The previously designated Y and Y_2 might leave the impression that these are two different Y chromosomes. At least two genes differentiate the Y and Y^h chromosomes, one gene controls the long peduncle on male trees and the other masculinizing gene controls the carpel abortion in male flowers. Since embryo abortion of the $Y^{(h)}Y^{(h)}$ genotypes occurs 25 to 50 days after pollination [30], a regulatory gene essential to early embryo development must have resided and degenerated on both Y and Y^h chromosomes. Any combinations of the Y chromosomes YY, Y^hY^h, and YY^h would lead to the same fate of embryo abortion.

It is likely that two genes are involved in sex determination in papaya, one a stamen suppressor or feminizing gene for stamen abortion in female flowers and the other a carpel suppressor or masculinizing gene for carpel abortion in male flowers. The female sex determination gene is possibly an upstream regulator of the B class genes, *AP3* and *PI*, because the lack of a trace of stamens in female flowers suggests the abortion of stamens occurs before initiation of stamen primordia. The male sex determination gene is likely a downstream regulator that aborts the carpels at a later developmental stage since the aborted gynoecium is a prominent feature of the male flower structure and, when growing conditions are optimal, a few male flowers do not undergo carpel abortion and form fruits.

With accumulated genetic and molecular data on papaya sex chromosomes, we can now better explain earlier hypotheses. The male-specific region of the Y chromosome (MSY) behaves like a single genetic unit since there is no recombination. Therefore, if we consider the MSY as a giant locus acting as a single gene with three alleles, the 1938 Hofmeyr and Storey hypothesis makes sense. Of course, the approximately 7 Mbp MSY is not a single gene. To date, seven functional genes have been characterized in this locus (Q. Yu, P.H. Moore, J. Jiang, A.H. Paterson, R. Ming, unpublished data) and likely more remain to be discovered. Also, there are likely two genes, not a single gene with three alleles, controlling sex determination in papaya as discussed above.

The currently most plausible hypothesis with the presently known features of the primitive sex chromosomes in papaya is the one proposed by Storey in 1953 [14] concerning a group of linked genes confined to a small region. The gene controlling long peduncle, *Mp*, exists and should be on the Y chromosome. The lethal factor, *l*, is likely a regulatory gene on both Y and Y^h chromosomes that is critical in early embryo development but which has degenerated to be non-functional and therefore lethal. The recombination suppression factor, *C*, is not likely a gene, but more likely the product of chromosomal location and/or chromosomal inversions that we have recently detected (Q. Yu, P.H. Moore, J. Jiang, A.H. Paterson, R. Ming, unpublished data). The suppressors of androecium and gynoecium, *sa* and *sg*, are likely to be the degenerated versions of the two sex determination genes on Y and/or Y^h chromosomes.

Although the hypothesis of genic balance between sex chromosomes and autosome proved not to be true [9,12], the concept of an inert chromosomal region approximates the degenerated nature of the MSY. However, the extent of sequence degeneration of Y and Y^h might be only slightly different, rather than the more extensive divergent length of the inert region on male and hermaphrodite sex chromosomes envisioned by Hofmeyr [9,12]. The Horovitz and Jiménez hypothesis of X and Y sex chromosomes in general terms is correct [15], although it is not clear whether the authors meant classical heteromorphic sex chromosomes or simply chromosomes bearing sex determination genes. Storey, in his revised concept of sex determination in papaya as consisting of only two sex types, female and the variable male and hermaphrodite, was apparently misled by long peduncle female and hermaphrodite mutants [16]. These mutants are the exceptions not the rule. Another unusual mutation, female lethality, was documented recently [30], but this mutant wouldn't affect the conclusion of a degenerated regulatory element causing embryo abortion on the Y and Y^h chromosomes. The hypothesis of a trans-regulatory element controlling flower organ development remains to be tested.

5. Towards cloning the sex determination genes in papaya

Because there is no recombination in the MSY region, a map-based cloning approach is not an option for identifying the sex determination genes in papaya. Complete sequencing of the MSY and the corresponding region of the X chromosome, as is actively underway, is needed before attempting to clone the sex determination genes. Differential expression of genes on the MSY and genes of the corresponding region of the X chromosome would provide candidate genes for further analyses. Sex reversal Y deletion lines could be valuable for narrowing the list of candidate genes. We have recently produced a few Y deletion lines using γ-ray irradiation of papaya pollen from a male variety AU9 and a deleted region in the MSY was detected in one of the Y deletion lines (Q. Yu, P.H. Moore, R. Ming, unpublished data).

Acknowledgments

This work was supported by a grant from NSF to R.M., Q.Y., P.H.M., J.J., and A.H.P. (Award No. DBI-0553417) and startup funds from the University of Illinois at Urbana-Champaign to R.M.

References

[1] Yamamoto HY. Comparison of the carotenoids in yellow- and red-fleshed *Carica papaya*. Nature 1964;201:1049–50.
[2] Liebman B. Nutritional aspects of fruit. Nut Action Newslett May 1992:10–1.
[3] Chandrika UG, Jansz ER, Wickramasinghe SMDN, Warnasuriya ND. Carotenoids in yellow- and red-fleshed papaya (*Carica papaya* L). J Sci Food Agric 2003;83:1279–82.
[4] Arumuganathan K, Earle ED. Nuclear DNA content of some important plant species. Plant Mol Biol Rep 1991;9:208–18.
[5] Gonsalves D. Control of papaya ringspot virus in papaya. A case study. Annu Rev Phytopathol 1998;36:415–37.
[6] Wikström N, Savolainen V, Chase MW. Evolution of the angiosperm: calibrating the family tree. Proc R Soc Lond B 2001;268:2211–20.
[7] Higgins JE, Holt VS. The papaya in Hawaii. Hawaii Agr Expt Sta Bul 1914;32:6–44.
[8] Hofmeyr JDJ. Genetical studies of Carica papaya L. I. The inheritance and relation of sex and certain plant characteristics. II. Sex reversal and sex forms. S Afr Dept Agri and Sci Bul No 187 1938:64.

[9] Hofmeyr JDJ. Sex reversal in *Carica papaya* L. S Afr J Sci 1939;26: 286–7.
[10] Storey WB. Segregations of sex types in Solo papaya and their application to the selection of seed. Proc Am Soc Hort Sci 1938;35:83–5.
[11] Storey WB. The botany and sex relations of the papaya. Hawaii Agr Exp Sta Bul 1941;87:5–22.
[12] Hofmeyr JDJ. Some genetic breeding aspects of *Carica papaya* L. Agron Trop 1967;17:345–51.
[13] Hofmeyr JDJ, van Elden H. Tetraploidy in *Carica papaya* L. induced by colchicine. S Afr J Sci 1942;38:181–5.
[14] Storey WB. Genetics of papaya. J Hered 1953;44:70–8.
[15] Horovitz S, Jiménez H. Cruzamientos interespecificos e intergenericos en caricaceas y sus implicaciones fitotechicas. Agron Trop 1967;17:323–43.
[16] Hamilton RA, Izuno T. A revised concept of sex inheritance in *Carica papaya*. Agron Trop 1967;17:401–2.
[17] Storey WB. Papaya. In: Simmonds NW, editor. Evolution of crop plants. London and New York: Longman; 1976. p. 21–4.
[18] Sondur SN, Manshardt RM, Stiles JI. A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers. Theor Appl Genet 1996;93:547–53.
[19] Parasnis AS, Ramakrishna W, Chowdar KV, Gupta VS, Ranjekar PK, Microsatellite. (GATA)n reveals sex-specific differences in papaya. Theor Appl Genet 1999;99:1047–52.
[20] Parasnis AS, Gupta VS, Tamhankar SA, Ranjekar PK. A highly reliable sex diagnostic PCR assay for mass screening of papaya seedlings. Mol Breeding 2000;6:337–44.
[21] Urasaki N, Tokumoto M, Tarora K, Ban Y, Kayano T, et al. A male and hermaphrodite specific RAPD marker for papaya (Carica papaya L). Theor Appl Genet 2002;104:281–5.
[22] Deputy JC, Ming R, Ma H, Liu Z, Fitch MMM, et al. Molecular markers for sex determination in papaya (Carica papaya L.). Theor Appl Genet 2002;106:107–11.
[23] Ma H, Moore PH, Liu Z, Kim MS, Yu Q, et al. High-density linkage mapping revealed suppression of recombination at the sex determination locus in papaya. Genetics 2004;166:419–36.
[24] Liu Z, Moore PH, Ma H, Ackerman CM, Ragiba M, et al. A primitive Y chromosome in papaya marks incipient sex chromosome evolution. Nature 2004;427:348–52.
[25] Ming R, Moore PH, Zee F, Abbey CA, Ma H, et al. Construction and characterization of a papaya BAC library as a foundation for molecular dissection of a tree-fruit genome. Theor Appl Genet 2001;102:829–99.
[26] Charlesworth B. The evolution of sex chromosomes. Science 1991;251:1030–3.
[27] Charlesworth B, Charlesworth D. The degeneration of Y chromosomes. Philos Trans R Soc Lond Ser B 2000;355:1563–72.
[28] Charlesworth D, Charlesworth B, Marais G. Steps in the evolution of heteromorphic sex chromosomes. Heredity 2005;95:118–28.
[29] Charlesworth D, Charlesworth B. Sex chromosomes: evolution of the weird and wonderful. Curr Biol 2005;15:R129–31.
[30] Chiu C-T. Study on sex inheritance and horticultural characteristics of hermaphrodite papaya. Master Thesis. National Pingtung University of Science and Technology. Republic of China; 2000. p. 60.