REVIEW ARTICLE

Dynamics of sex expression and chromosome diversity in Cucurbitaceae: a story in the making

BIPLAB KUMAR BHOWMICK and SUMITA JHA*

Centre of Advanced Study, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700 019, India

Abstract

The family Cucurbitaceae showcases a wide range of sexual phenotypes being variedly regulated by biological and environmental factors. In the present context, we have tried to assemble reports of cytogenetic investigations carried out in cucurbits accompanied by information on sex expression diversities and chromosomal or molecular basis of sex determination in dioecious (or other sexual types, if reported) taxa known so far. Most of the Cucurbitaceae tribes have mixed sexual phenotypes with varying range of chromosome numbers and hence, ancestral conditions become difficult to probe. Occurrence of polyploidy is rare in the family and has no influence on sexual traits. The sex determination mechanisms have been elucidated in some well-studied taxa like *Bryonia*, *Coccinia* and *Cucumis* showing interplay of genic, biochemical, developmental and sometimes chromosomal determinants. Substantial knowledge about genic and molecular sex differentiation has been obtained for genera like *Momordica, Cucurbita* and *Trichosanthes*. The detailed information on sex determination schemes, genomic sequences and molecular phylogenetic relationships facilitate further comprehensive investigations in the tribe Bryonieae. The discovery of organ identity genes and sex-specific sequences regulating sexual behaviour in *Coccinia*, *Cucumis* and *Cucurbita* opens up opportunities of relevant investigations to answer yet unaddressed questions pertaining to floral unisexuality, dioecy and chromosome evolution in the family. The present discussion brings the genera in light, previously recognized under subfamily Nhandiroboideae, where the study of chromosome cytology and sex determination mechanisms can simplify our understanding of sex expression pathways and its phylogenetic impacts.

Introduction

Sexual reproduction evolved as a biological phenomenon to ensure genetic diversity creating allelic combinations that lead to successful selection of fittest survivors ramifying gradually into millions of organisms, belonging to animal or plant kingdom (Burt 2000). There are two basic types of sexual reproduction: hermaphroditism (male and female sex organs in same individual) and dioecism or gonochory (male and female sex organs on separate individuals). While most plants are hermaphroditic, gonochory is a rule in animal kingdom. In spite of this consensus, dioecy has found ways to establish its roots in plant kingdom within a specific confine. To understand how and why dioecy evolved within a limited number of plant species, we must have a quick overview of the evolutionary mechanisms driving the shift from hermaphroditism to dioecy.

There are constant forces in nature for selection of the fittest that can adapt and survive better in changing environments leading to the commencement of speciation process. Sexual reproduction strategies that promote outcrossing and constant flow of genetic variations are favoured in nature to avoid the deleterious effects of inbreeding (Bawa 1980; Holsinger 2000). Dioecy, i.e., the separation of male and female reproductive organs is the ultimate phenomenon that not only ensures avoidance of inbreeding but also facilitates optimal resource allocation between male and female sex organs (Bawa 1980; Ainsworth 2000). Like all biological events, evolution of dioecy is definitely not a sudden consequence but a sequential outcome of a number of processes that has been selectively welcomed in nature and also faithfully nourished in a definite number of species. Dioecy is an outcome of two independent and simultaneous mutations for male and female sterility in the hermaphroditic developmental programme where almost all autosomes carry genes for androecium and gynoecium development, their expression

*For correspondence. E-mail: sumitajha.cu@gmail.com.

being fine-tuned spatially during ontogeny (Kater *et al.* 2001; Honys and Twell 2004; Wellmer *et al.* 2004). For dioecy to be maintained stably among populations, it is essential to achieve strict linkages between the mutant genes to avoid recombination that might re-establish hermaphroditism (Ainsworth 2000). Charlesworth and Charlesworh (1978) had mathematically explored the potency of the intermediate routes for the establishment of dioecy from hermaphroditism. Among the first two ways, namely, androdioecy and gynodioecy, gynodioecy is considered to be the most probable one because of the easy spread of cytoplasmic male sterility (Charlesworth and Charlesworh 1978) and has been experimentally demonstrated in *Sagittaria* (Dorken and Barret 2003), *Silene* (Desfeux *et al.* 1996) and *Rumex* (Navajas-Pérez *et al.* 2005). Monoecy is considered to be the second probable intermediate for the evolution of dioecy and is found to co-occur with dioecy in many systems (Ainsworth 2000). Being a developmental modification, monoecy is followed by several steps to change male : female flower ratio and the resultant dioecious species may also show labile sexual phenotypes. Another route is the polymorphic alteration of positions of anthers and styles to promote out-crossing, the phenomenon being known as distyly which implicates further specializations to achieve dioecy (Ainsworth 2000).

A brief account of evolution of sex chromosomes in dioecious plants

All dioecious species have sex chromosomes undergoing different stages of evolution that determine whether they can be visually discriminated or can be regarded as homomorphic. In the last decade, extensive genomic study of model species has enabled scientists to elucidate six stages for the evolution of sex chromosomes (Charlesworth 2002; Charlesworth *et al.* 2005; Ming *et al.* 2007, 2011; Jamilena *et al.* 2008; Kumar *et al.* 2014). Precisely, the first stage involves complementary dominant mutation causing male and female sterility in two loci and recombination may re-establish hermaphroditism (e.g. *Fragaria verginiana*, Ming *et al.* 2011). In the next step, recombination suppression occurs between the mutant loci and its immediate surroundings (e.g. *Asparagus* and *Ecballium*; Ming *et al.* 2007, 2011). In the third stage, recombination is suppressed in many additional loci increasing the extent of degeneration of Y and male-specific region accumulates transposons and other repetitive elements yet maintaining visual similarity with X (e.g. homomorphic sex chromosomes of papaya; Liu *et al.* 2004; Yu *et al.* 2007). Fourth stage is characterized by considerable expansion of male determining region of Y, accumulation of repetitive sequences, structural rearrangement in Y leading to heteromorphy with large Y and small X (e.g. *Silene latifolia* and *Coccinia grandis*, Delph *et al.* 2010; Bhowmick *et al.* 2012, 2015; Sousa *et al.* 2013). Degeneration of the functional as well as nonfunctional sequences are the next steps of sex chromosome evolution leading to considerable loss of function and size reduction

of Y (e.g. *Cycas revoluta*; Ming *et al.* 2011; no such examples found in angiosperms so far). The ultimate stage of sex chromosome evolution results in complete suppression of recombination and loss of Y chromosome, sex determination system depending on X to autosome ratio (e.g. *Rumex acetosa, Cannabis sativa* and *Humulus japonicas;* Ming *et al.* 2007; Jamilena *et al.* 2008). Considering these aspects, study of the sexual systems of yet unexplored plants acquiring dioecy and chromosomal sex determination would highly benefit the understanding of subtle molecular mechanisms in the path of sex chromosome evolution.

Cucurbitaceae: a target family for studying sex expression dynamics and chromosomal sex determination

The family Cucurbitaceae has been of vital importance in food economies of many tropical countries, being valued as world's second largest vegetable yielding family after Solanaceae. The family comprises about 120 genera and 960 species (Ali *et al.* 2009; Schaefer *et al.* 2009) amongst which, about 94 species occur in India (Renner and Pandey 2013) including many economically important vegetable and fruit crops such as cucumber, melon, watermelon, squash, gourds and pumpkins. This tropical family has members that grow in extreme situations along longitudinal, latitudinal and temperature gradients and therefore variations within and between populations of a species is not difficult to find. Apart from its huge economic importance, the family Cucurbitaceae is an enigmatic one in terms of creation of diversity in sexual forms. It is a well known fact that ~5% of 2,82,000 flowering plant species are dioecious, distributed over 14,600 species in 960 genera and 200 families (Renner and Ricklefs 1995; Dorken and Barret 2003). Cucurbitaceae is among those rare plant groups where we find 67 dioecious species spread over 19 genera (Roy and Saran 1990). Dioecy is considered the ancestral situation from where several shifts in the sexual system probably gave ways to androdioecy or monoecy in the family (Zhang *et al.* 2006). Scientists have focussed their attention towards understanding the genetic architecture, valuable traits and phylogeny of different cucurbits with an aim to improve breeding strategies since a long time. Another discipline of research has included cytogenetic, molecular or biochemical characterization of sexual expression of different taxa which is the principal topic of the present discussion. Indeed, the diverse sexual phenotypes combined with diverse modes of sex determination schemes therein, intrigue many researchers to propagandize Cucurbitaceae as an ideal platform to study evolution of sexual systems.

Contexts of discussion

To gain insights into the genetics of sex determination system in Cucurbitaceae several disciplines of research have been

followed by different researchers in different species. One major aspect of research is chromosome cytology that defines the genetic and chromosomal regulation of sexual characters since chromosome morphology represents structural, numerical, organizational and functional attributes of the genome of a species or an infraspecific taxon. The last reviews on sex expression diversities and cytogenetics of Cucurbitaceae date back to 1990s (Roy and Saran 1990; Singh 1990a; Chattopadhyay and Sharma 1991; Roy *et al.* 1991). The present review assembles contributions made in the field of cytogenetics of Cucurbitaceae with an objective to shed light on the sex determination mechanisms of sexually-interesting taxa and emphasizing the molecular basis of sex determination schemes and related aspects. To arrange these reports which are from different genera in a systematic method, a very recent phylogenetic classification system proposed by Schaefer and Renner (2011) has been followed in this review which is a modification of Jeffrey's system (2005).

Diversity of sexual system and chromosome cytology in 15 tribes

Understudied tribes

Cytological studies in the first seven dioecious tribes (De Wilde *et al.* 2007; Schaefer and Renner 2011) along with Joliffieae, Schizopeponeae and Coniandreae are scanty and remain mostly unknown. Most of the genera now grouped under the first seven tribes were previously known to constitute the subfamily Nhandiroboideae (Jeffrey 2005). The first tribe Gomphogyneae has six genera with varying chromosome numbers of $n = 11$, 14 and 16. The genus *Gomphogyne* is autotetraploid ($2n = 32$) with chromosomes known to be the longest among all cucurbits (Roy and Trivedi 1966; Schaefer and Renner 2011). The genus *Gynostemma* has 10 species of which two are dioecious, having base numbers of $x = 11$ or 12 and reportedly has natural autopolyploids (Ru Juan *et al.* 1989; Gao *et al.* 1995; Chauhan and Pandey 2008). The dioecious member of the genus *Gynostemma* (*G. pedata*) reportedly has no chromosomal heteromorphy (Roy *et al.* 1991). The other dioecious genus *Hemsleya* (Li *et al.* 2007) has 30 species with diploid chromosome number of $2n = 28$ (Samuel *et al.* 1995).

Reports of chromosome counts are known in the four dioecious tribes: Triceratieae ($x = 8$ in *Fevillea*; Roy *et al.* 1991), Actinostemmateae ($n = 8$ in the single genus *Actinostemma*, Schaefer and Renner 2011), Thladiantheae ($n = 9$ in *Thladiantha* and $n = 16$ in *Baijiania,* Roy *et al.* 1991; Schaefer and Renner 2011) and Siraitieae (the single genus *Siraitia* with 3–4 species has $n = 14$ in *S. grosvenorii* having natural triploids and tetraploids, Zhuang *et al.* 1997; Fu *et al.* 2012). It is noteworthy that the genus *Fevillea* (having seven species) of Triceratieae with uncertain sexual expression (Nee *et al.* 2009) represents the ancestral condition of five free stamens with bilocular anthers in Cucurbitaceae and

possibly has interesting cytogenetic features to clarify phylogenetic relations and sexual evolution. There are no reports of cytological study in the other four genera of Triceratieae and the other dioecious tribes Zanonieae and Indofevilleeae.

Sexual expression of the tribe Schizopeponeae ranges from dioecy (*Herpetospermum* including *Edgaria* and *Biswarea*, Anmin and Jeffrey 2011a) to monoecy (*Schizopepon* with monoecious and dioecious species, Anmin and Jeffrey 2011b). The haploid numbers of *Schizopepon* and *Herpetospermum* are known to be $n = 10$ and 11, respectively (Roy *et al.* 1991; Schaefer and Renner 2011) and there are no reports of chromosomal heteromorphy.

The tribe Joliffieae has three dioecious genera of which cytology and sexual behaviour of *Telfairia* has been studied to some extent. Odejimi and Akpan (2006) have tried to investigate the influence of minerals on sex ratio to achieve higher fruit production in *Telfairia* but the concept of regulation of sex expression in this species is completely in dark. *Telfairia occidentalis* has a contradictory diploid count of $2n = 24$ (Akoroda 1990) and $2n = 22$ (Okoli 1987; Uguru and Onovo 2011) and the existence of heteromorphic chromosomes reported by Okoli (1987) has not been substantiated by Uguru and Onovo (2011).

Coniandreae has 19 genera, of which reports of chromosome numbers are from *Apodanthera* (including *Guraniopsis*) with $n = 14$ (Ward 1984), *Corallocarpus* with $n = 13$ (Schaefer and Renner 2011) and $n = 12$ (Singh 1990a), *Kedrostis* with $n = 12/13$ and *Seyrigia* with $n = 13$ (Schaefer and Renner 2011). All these species are monoecious except *Seyrigia* having one dioecious species (Zhang *et al.* 2006; Schaefer and Renner 2011). Sex expression in the flowers of *Apodanthera* is known to be controlled by physiological condition and maturity of the plant (Delesalle 1989, 1992) demanding further investigation.

Among these understudied tribes, detailed study of sexual expression and cytogenetics in the Triceratieae genus, *Fevillea* may reveal interesting background information since it maintains the ancestral floral phenotype. The known genetic linkage map of *Siraitia grosvenorii* of Siraitieae (Liu *et al.* 2011) and molecular phylogeny of *Hemsleya* of Gomphogyneae (Li *et al.* 2010) can provide necessary information for further cytogenetic studies in these and related understudied tribes.

Tribes with sexual polymorphism indiscernible at cytological level

The tribes Momordiceae, Sicyoeae and Cucurbiteae show evidences of environmental influence on various sex forms combined with differentiation at biomolecular level but remarkably lack any discernible sign of cytological differentiation. While varied types of sexual expression is exhibited by different species of only a single genus (*Momordica*) under Momordiceae, polymorphic trend of sex expression is manifested nearly among 200 species belonging to 12 separate genera under Sicyoeae. On the contrary, Cucurbiteae shows reports for monoecious sex expression although sex

determination mechanisms and chromosomal attributes of several genera are still unknown. A detailed discussion covering cytological reports and sexual diversity of each tribe is required to elucidate the present scenario and point out the lacunae to be fulfilled.

Momordiceae: The tribe consists of a single genus *Momordica* with 60 species showing a wide range of sexual expressions although studies involving sexual phenotype regulation have been focussed on some selected species like the monoecious *M. charantia* or the dioecious *M. dioica*. The possible ancestral form of sex expression in this genus was dioecy from which at least seven transition events to monoecy might have followed mainly to overcome low pollination success in dioecious plants (Schaefer and Renner 2010). The genus *M. charantia* receives considerable attention from researchers due to its medicinal and dietary values. In *M. charantia*, sexual differentiation of flowers is regulated at 7th, 10th and 13th day after initial bud formation where researchers got differential protein profiles of hermaphrodite, male and female flowers (Wang and Zeng 1998). Endogenous concentration and foliar application of growth regulators, chemicals, day length and temperature have been found to modulate floral sex determination of *M. charantia* in diverse ways (Behera *et al.* 2011). Preliminary data on zygotic and gametic chromosome numbers of *M. charantia* have confirmed the number of $2n = 22$ (Whitaker 1933; Bhaduri and Bose 1947; Trivedi and Roy 1972; Lombello and Forni-Martins 1998; Kausar *et al.* 2014) along with prevalent occurrence of polyploids. With the advent of molecular cytogenetic approaches, molecular karyotyping with chromomycin A3 (CMA) banding and fluorescence *in situ* hybridization (FISH) with 45S and 5S rDNA sequences has revealed 3/4 telomeric CMA signals, four distal signals for 45S rDNA along with two intercalary signals for 5S rDNA showing no adjacence to 45S signals (Lombello and Pinto-Maglio 2007; Zaman and Alam 2009; Waminal and Kim 2012) in the diploid complement. Crossing experiments in *M. dioica* revealed genic level of sex differentiation with males being heterozygous and females homozygous (Hossain *et al.* 1996). Recent studies have revealed the presence of male-specific molecular markers with RAPD primers OpA15 (Patil *et al.* 2012) and OpC08 (Panigrahi and Mishra 2012), of which OpA 15 has been converted into a SCAR marker (Patil *et al.* 2012). The species also shows a female-gender specific expression of 22-kDa polypeptide along with differential expression of some other proteins (Sinha *et al.* 2001). Somatic chromosomes of male and female plants have been worked out in *M. dioica* showing $n = 14$, asymmetric karyotypes, autopolyploid origin of populations and the absence of distinguishable sex chromosomes (Singh 1990a; Chattopadhyay and Sharma 1991; Baratakke and Patil 2009; Bharathi *et al.* 2011). Analysis of a naturally occurring polyploid form of *M. dioica* having enlarged vegetative structures, revealed abonormal meiotic features resulting in considerably reduced pollen fertility (Agarwal and Roy 1976). The data further

strengthen genic nature of sexual differentiation in *M. dioica* and also gives reasons for rare occurrence of natural polyploid forms in the family. Among the other species, $n = 14$ is reported in the dioecious species *M. cochinchinensis, M. denudata, M. sahyadrica, M. subangulata* (Bharathi *et al.* 2011), $n = 11$ is recorded in *M. rostrata* (Behera *et al.* 2011) and $n = 9$ is reported in monoecious *M. cymbalaria* (Trivedi and Roy 1972; Agarwal and Roy 1976; Roy *et al.* 1991; Bharathi *et al.* 2011). None of the dioecious species have reports of chromosomal heteromorphy. Karyomorphological studies have shown considerable similarities in terms of chromosome number, karyotype symmetry and fertile hybrid production between *M. charantia* and *M. balsamina* (Singh 1990a).

Sicyoeae: Among the 12 genera, cytology of the genus *Luffa* with monoecious and dioecious species has received much attention recently. The genus has chromosome number of $n = 13$ for the monoecious *L. cylindrica*, *L. acutangula* and *L. hermaphrodita* (Whitaker 1933; Bhaduri and Bose 1947; Dutt and Roy 1969; Roy *et al.* 1991; Diaz and Ramirez 1994). Cytological evaluation of cross-compatibility has led researchers to suggest varying degrees of genomic divergence among different species while retaining high chromosomal homology (Dutt and Roy 1969, 1976; Singh 1990b). FISH with 45S and 5S rDNA probes has been performed in *L. cylindrica* showing five pairs of 45S rDNA signals and one pair of non-adjacent 5S rDNA signal (Xu *et al.* 2007; Waminal and Kim 2012). The terminal and interstitial location of 45S and 5S rDNA signals respectively, points to the general tendency of transposition and higher dispersion of the 45S rDNA over the 5S rDNA in the genome as observed in *Momordica charantia* and *Cucumis sativus* (Waminal and Kim 2012). Again, the higher intensity of 45S rDNA signal in one chromosome pair indicates the presence of a high copy number of rDNA genes compared to other chromosomes in *L. cylindrica*. The dioecius species *L. echinata* has genic sexual dimorphism that excludes chromosomal heteromorphy (Roy and Saran 1990).

Within Sicyoeae, *Trichosanthes* deserves special attention because of its highest level of speciation among all cucurbits and varied range of sexual expression. It has about 90–100 species distributed in Asia including one from Hispaniola (Kocyan *et al.* 2007; De Boer and Thulin 2012; De Boer *et al.* 2012). This genus has the basic number $x = 11$ with some known polyploids apart from the prevalent diploids (Sarker *et al.* 1987; Varghese 1972; Singh and Roy 1979b). Cytogenetic study of the dioecious *T. dioica* has confirmed homomorphic sex chromosomes (Whitaker 1933; Bhaduri and Bose 1947; Singh and Roy 1979b; Roy *et al.* 1991; Chattopadhyay and Sharma 1991; Guha *et al.* 2004) in spite of some contradictory observations on chromosomal heteromorphy based on irregularities in meiotic behaviour or somatic chromosome study (Patel 1952; Sarker *et al.* 1987; Chattopadhyay and Sharma 1991). The immense agronomic importance of *T. dioica* in Asian countries has intrigued researchers to focus on intricate biology of flower development and pollination

mechanisms since a long time (Pandey *et al.* 1997, 2003). One urgent requirement for dioecious crop plants is to identify the sex of the plants prior to flowering so as to improve or modify existing breeding strategies. Molecular differentiation between male and female plants is possible by marker sequence linked to male sex as reported by Nanda *et al.* (2013). Initially, male and female plants were screened with ISSR primers of which one primer (ISSR 9, named *TdMSM*) amplified an 800 bp fragment in male plants which was completely absent in female plants. The fragment was sequenced and another set of specific STS primers (*TdSTSM*) were designed from the sequence information which was found to strongly correlate with male sex on further experimental trials. Interestingly, intronic nature of *TdMSM* sequence raises further questions about the role of noncoding sequences in regulating sex determination.

The other dioecious species of *Trichosanthes* (*T. bracteata*, *T. himalensis*, *T. lepiniana* and *T. palmata*) show similar gametic chromosome numbers $n = 11$ (Varghese 1972; Sarker *et al.* 1987; Roy *et al.* 1991). However, heteromorphic sex chromosomes had been reported by Roy and Saran (1990) in *T. kirilowii* var. *japonica* and *T. ovigera.* The three monoecious speices of *Trichosanthes* namely, *T. anguina, T. cucumerina* and *T. lobata* show considerable affinity in terms of karyomorphology (Singh 1990a). There have been reports of cross-compatibility with the formation of fertile hybrids showing normal meiotic cycle and fertile pollens (Singh and Roy 1979a). However, CMA banding patterns in five commercial varieties of *T. anguina* revealed considerable difference at the subspecific level and serve as a diagnostic cytological character (Alam *et al.* 2012). Genic sexual diffferentiaion and reports of heteromorphic sex chromosomes in some species of *Trichosanthes* may reflect the intermediate steps of sex chromosome evolution in the genus.

Among the other genera of Sicyoeae, two monoecious species of *Cyclanthera* (including *Rytidostylis* and *Pseudocyclanthera*) namely, *C. brachystachya* and *C. pedata* are reported to have $n = 8$ (Roy *et al.* 1991) and $n = 16$ (Samuel *et al.* 1995), respectively. The dioecious genus *Hodgsonia* has $n = 9$ chromosomes (Kocyan *et al.* 2007). The monoecious genera *Echinocystis*, *Echinopepon* (including *Apatzingania*, *Brandegea*, *Vaseyanthus*) and *Frantzia* have $n = 16$, $n = 12$ and $n = 12/14$ chromosomes, respectively (Whitaker 1950; Wunderlin 1976; Samuel *et al.* 1995; Monro and Stafford 1998; Kocyan *et al.* 2007; Schaefer and Renner 2011). The type genus *Sicyos* (including *Microsechium*, *Sicyocaulis*, *Sicyosperma*, *Parasicyos*, *Sechium* and *Sechiopsis*) with 75 monoecious species (Sebastian 2012) show $n = 12, 13, 14/15$ for different species (Roy *et al.* 1991; Schaefer and Renner 2011). There are no reports on chromosome analysis for the other four genera under this tribe.

A thorough molecular phylogenetic study in relation to the distinctive evolution of long-fringed petals in *Trichosanthes* has supported the grouping of *Trichosanthes* and *Gymnopetalum* with *Luffa* as the sister clade, while distances have been observed with *Hodgsonia* (De Boer and Thulin 2012;

De Boer *et al.* 2012). Cytogenetic study of the *Trichosanthes*/*Gymnopetalum* clade along with *Hodgsonia* (with $n = 9$, Kocyan *et al.* 2007) and the dioecious species of *Luffa* (e.g. *L. echinata* with no available reports) can help in understanding the phylogenetic relations and also elucidation of chromosomal basis of sexual differentiation in a very broad perspective.

Cucurbiteae: This tribe comprises of mostly monoecious species distributed in 11 genera (Whitaker and Bemis 1975). Studies related to control mechanisms of sex differentiation, cytogenetics and genomic architecture have been focussed in the type genus *Cucurbita* because of its high agricultural values. The switchover mechanisms from monoecy to androdioecy involving floral development stages have been reported very recently in the genus *Cucurbita* (Martínez *et al.* 2014). Sex expression in *C. pepo* is regulated by environmental clues like day length and temperature which in turn strongly regulates intracellular ethylene levels–the major biochemical ground of sexual differentiation in cucurbits (Boualem *et al.* 2008, 2009). The discovery of the gene *CpACS27A,* orthologue of *CmACS7* (*C. melo*) and *CsACS2* (*C. sativus*) (Boualem *et al.* 2008, 2009) elucidates the regulatory roles of ethylene biosynthetic pathway in sexual differentiation by promoting carpel formation in early developmental stages (Martinez *et al.* 2014).

Initial cytological studies in *Cucurbita* have suggested secondary polyploid origin of different species with a basic number $x = 10$ (Weiling 1959) although $x = 20$ has also been reported by Roy *et al.* (1991). Detailed cytological analysis of the 15 species of *Cucurbita* needs to be done to establish the base number and study the evolution of species. However, molecular biologists have explored the genomes of the pumpkin and squash. SSR-based linkage map has been generated for *C. pepo*, followed by much elaborate SNP-based genetic maps (Gong *et al.* 2008; Esteras *et al.* 2012). A comparative analsyis of SSR-based linkage map has been reported in *C. pepo* and *C. moschata* showing macro-synteny, orthologous loci and some chromosome rearrangements (Gong and Lelley 2008). Except for basic chromosome counts (Whitaker 1933; Roy *et al.* 1991; Samuel *et al.* 1995), detailed analyses in *C. pepo* have not been carried out yet which would be useful in clarifying phylogenetic relationships and validating the genetic linkage maps. However, confusing diploid numbers ranging from $2n = 45$–48 and intervarietal variations in karyotypes have been observed in three varieties of *C. maxima* by Jahan *et al.* (2012). Molecular karyotyping in *C. moschata* has revealed proximal 5S rDNA signals in two pairs and distal 45S rDNA signals in five pairs of chromosomes with no evidence for satellites (Xu *et al.* 2007; Waminal *et al.* 2011). This study shows that the copy number of 45S rDNA varies widely between the chromosome pairs and there is no juxtaposition of 5S and 45S signals as found in the tribes Momordiceae and Sicyoeae (Waminal and Kim 2012). Reports of chromosome counts showing $x = 20$ are available for *Cucurbita filicifolia, C. maxima, C. melanosperma* and *C. mixta* (Whitaker 1933; Bhaduri and Bose 1947; Roy *et al.* 1991).

Apart from *Cucurbita*, chromosome count has been reported only for *Sicana* with four species showing $x = 20$ (Schaefer and Renner 2011). Chromosome counts are not reported in nine other genera of Cucurbiteae.

Each of the three tribes namely, Momordiceae, Cucurbiteae and Sicyoeae has a unique story for the evolution of sexual systems. Repeated reversals from dioecy to monoecy in Momordiceae exemplify the ancestral sexual condition and its ongoing evolution in Cucurbitaceae (Schaefer and Renner 2010). The retreats to monoecy in this tribe denote a smart adaptive orchestration of biological processes with the rules of natural selection. The reports of sex-specific molecular markers in Sicyoeae and Momordiceae have contributed substantially to the molecular differentiation of genders in plants (Panigrahi and Mishra 2012; Patil *et al.* 2012; Nanda *et al.* 2013). The well-established phylogenetic relations between the largest genus *Trichosanthes* and *Luffa* (De Boer and Thulin 2012; De Boer *et al.* 2012) can be linked to the evolution of sex determination schemes in Sicyoeae. Within Cucurbiteae, the relation between genic and hormonal suppression of stamen development in *Cucurbita* provides a clear understanding of the networks operating behind unisexual flower development in plants (Martínez *et al.* 2014). However, the molecular, hormonal and genic regulators of sex expression reported in all these three tribes signify the evolution and diversity of biological mechanisms to generate sexually-advanced forms. Although, chromosomal heteromorphy is almost rare in these tribes, it is reported in *Trichosanthes kirilowii* var. *japonica* and *T. ovigera* (Roy and Saran 1990). Studies are required to characterize the genic and chromosomal regulators of sex expression in the understudied species belonging to these tribes.

Tribes with highly evolved sex-determination system and sex chromosomes

In spite of the presence of a large number of taxa, but because of lack of sufficient data, the mechanism of sex determination and its genetic or cytogenetic regulators are being studied of late in few suitable cucurbit models. These reports are from the tribes Bryonieae and Benincaseae where we find the highest order of established mechanism for X–Y sex determination, molecular basis of dioecy–monoecy switchover pathways, origin of nascent sex chromosomes, sex-specific molecular markers and heteromorphic gonosomes. Following is the comprehensive summary of the relevant phenomena reported particularly in these two tribes.

Bryonieae: The tribe Bryonieae has three genera, among which species of *Bryonia* are both dioecious and monoecious (Volz and Renner 2008), *Ecballium* has one species which again has dioecious and monoecious subspecies (Volz and Renner 2008) and *Austrobryonia* has four monoecious species (Schaefer *et al.* 2008; Volz and Renner 2008). Classical study for the establishment of sex-determination mechanisms in plants was initiated in *Bryonia* when Correns (1903) furnished the X–Y

model for sex determination in plant kingdom after performing crossing experiments between monoecious *B. alba* and dioecious *B. dioica*. The genus has $x = 10$ and its dioecious species *B. dioica* is homomorphic (Roy *et al.* 1991; Volz and Renner 2008) like its sister species *Ecballium* with the basic chromosome number of $n = 9$ and 12 (Whitaker 1933; Volz and Renner 2008). Chromosome counts have been reported (Volz and Renner 2008) in *Bryonia alba* ($2n = 20$), *B. aspera* ($2n = 40$, tetraploid), *B. cretica* ($2n = 60$, hexaploid), *B. dioica* ($2n = 20$), *B. marmorata* ($2n = 40$, tetraploid), *B. monoica* ($2n = 20$), *B. multiflora* ($2n = 20$), *B. syriaca* ($2n = 20$) and *B. verrucosa* ($2n = 20$). The significance of *Bryonia* as a model for studying genetic determination of sex in plant kingdom triggered many biologists like Bateson (1909) to work on the subject and strengthen the epistemology of Correns (1903). In spite of an unknown ancestral sexual system for *Bryonia*, molecular phylogeny gained support for at least two transition events between dioecy and monoecy which rules out the scope of inheriting sex chromosomes from a common ancestor (Volz and Renner 2008). The genetics of X–Y sex determination began to take a realistic shape when Oyama *et al.* (2008) provided first molecular insights into the sex-determination scheme. Oyama *et al.* (2008) have not only developed a male-linked molecular marker (*BdY1*) in *Bryonia dioica* that corroborates to the historical consensus of X–Y model but at the same time provided experimental clues for the nascent origin of plant sex chromosomes. The linkage disequilibrium between *BdY1* and male-determining locus as studied by the workers are found to be different in the northern and southern Europe populations. Since suppression of recombination and its gradual spread along the Y chromosome theoretically initiates sex chromosome evolution, it becomes immediately predictable that male-determining region is sufficiently larger in northern Europe population that includes or is close to *BdY1*, resulting in strong linkage to the male-determining region (Oyama *et al.* 2008). On the contrary pseudo-autosomal region (PAR) is suggested to be still larger and encompasses *BdY1* in the southern Europe population of *B. dioica*, therefore, undergoes recombination and exhibits considerable variations and weak linkage with the male sex determining region (Oyama *et al.* 2008). On the whole, recent progress in the genetics of sex determination in *Bryonia* has propagandized it as an ideal platform for unmasking the mysteries of sex chromosome evolution, one of the most argued and awaited topics of present day evolutionary biology.

Benincaseae: The tribe Benincaseae is the largest tribe with 24 genera showing the highest specialization of chromosomal sex determination in one of its components namely *Coccinia grandis*. In spite of the interesting cytogenetic features of *C. grandis*, the genus *Cucumis* has received maximum attention of researchers among all cucurbits mainly because of its higher economic values. Reports of genetic, physical and chromosome maps are from the genus *Cucumis* that will be discussed in the following section. With these special

features, let us focus on the general trends of chromosome numbers and other cytogenetic features of the tribe Benincaseae.

The tribe Benincaseae is dominated by the basic chromosome number of $x = 12$ for most of the genera cytologically investigated so far. These include the type genus *Benincasa*, *Coccinia*, *Cucumis*, *Melothria*, *Ctenolepis* (incl. *Zombitsia)*, *Peponium* and *Solena* (Whitaker 1933; Bhaduri and Bose 1947; Chakravorti 1948; Kumar and Viseveshwaraiah 1952; Yadava *et al.* 1984; Roy *et al.* 1991; Guha *et al.* 2004; Xu *et al.* 2007; Schaefer and Renner 2011; Waminal *et al.* 2011; Bhowmick *et al.* 2012, 2015; Sousa *et al.* 2013). Basic number of $x = 7$ is observed in *Cucumis sativus* and its varieties (Bhaduri and Bose 1947; Roy *et al.* 1991; Kuriachan and Beevy 1992; Waminal and Kim 2012); $x = 11$ is known in species of *Citrullus* and *Lagenaria* (Whitaker 1933; Khoshoo 1955; Roy *et al.* 1991; Waminal *et al.* 2011) and confusing haploid number of $n = 11$ or 12 is reported from dioecious *Zehneria* (Singh 1990a). In the following section, we will discuss the details of cytogenetic investigations carried out in some of the species mentioned above.

Genera with highly specialized sex-determination schemes

Coccinia: The dioecious genus *Coccinia* has about 30 species and is native to Africa except *C. grandis* spreading to tropical Asia, including India (Holstein and Renner 2011; Schaefer and Renner 2011). Chloroplast sequence-based phylogeny shows six major biome shift events within four species assemblages supported by ML tree (Holstein and Renner 2011). Among the large number of African dioecious species, the Indian species of *Coccinia* (*C. grandis*) has been reported to possess chromosomal heteromorphy and X–Y sex-determination system. The significance of cytogenetic investigation in this dioecious model was realized by previous workers who provided background information on diploid number $2n = 24$, X–Y sex determination and chromosomal heteromorphy with larger Y chromosome in males (Whitaker 1933; Bhaduri and Bose 1947; Chakravorti 1948; Kumar and Viseveshwaraiah 1952; D'Cruz *et al.* 1972; Chattopadhyay and Sharma 1991). The early periods of study have been followed by a long gap until researchers continued thorough cytogenetic analyses since the last decade (Guha *et al.* 2004; Bhowmick *et al.* 2012, 2015; Sousa *et al.* 2013). The large size of *Coccinia* Y chromosome indicates the accumulation of tremendous repeats in recently evolved Y chromosomes (Bergero *et al.* 2008; Jamilena *et al.* 2008; Mariotti *et al.* 2009; Heslop-Harrison and Schwarzacher 2011). Again, C-banding experiments reveal heterochromatic nature of *Coccinia* Y chromosome unlike the euchromatic Y chromosome of *Silene* (Sousa *et al.* 2013). Modern cytogenetic tools have aided scientists to get a closer view of the cytogenetic makeup of the chromosomes (Guha *et al.* 2004; Bhowmick *et al.* 2012, 2015; Sousa *et al.* 2013) of late. Sousa *et al.* (2013) made appreciable progress towards the understanding of chromosome ultrastructure and successfully revolutionized our concepts of sex determination in this highly debated

species. The evolution of Y chromosome in this species is not a consequence of rearrangement events like chromosome fusions since hybridization studies did not show any interstitial positioning of telomeric sequences (Sousa *et al.* 2013). Interestingly, FISH with 45S and 5S rDNA showed no difference in signals between the male and female plants (Sousa *et al.* 2013; Bhowmick *et al.* 2015). Two pairs of telomeric 45S signals were localized among which one pair showed the presence of proximally adjacent 5S signal in both male and female plants (Sousa *et al.* 2013), following the trend of Benincaseae (Waminal *et al.* 2011; Waminal and Kim 2012). Interestingly Bhowmick *et al.* (2015) reported a prominent size heteromorphy of 45S rDNA signals between the homologues of one chromosome pair in male and female plants of *C. grandis,* possibly owing to the differential expression of rDNA genes. However, 2C DNA content of male plants was found to be larger than that of female plants by 0.1 to 0.2 pg of DNA (c.a.) suggesting largest divergence between Y and autosomes among all vascular plants (Sousa *et al.* 2013; Bhowmick *et al.* 2015). Differences in the number and types of CMA positive signals, GC rich heterochromatin, 4C DNA content, average packing ratio and isoesterase pattern stand to represent other cytological and biochemical distinctions between the two genders (Guha *et al.* 2005; Bhowmick *et al.* 2012, 2015). The Y chromosome of male plants has a truly enigmatic nature since it lack signals for rDNA sequences and chromomycin A3 (Bhowmick *et al.* 2012, 2015; Sousa *et al.* 2013). Genomic *in situ* hybridization helped to visualize the male-specific region on Y chromosome with increasing concentrations of female blocking DNA (Sousa *et al.* 2013). The Y chromosome has pseudo-autosomal region which is capable of recombining with X and the end-to-end pairing of heteromorphic X–Y chromosomes is documented in meiotic metaphase I images (D'Cruz *et al.* 1972; Bhowmick *et al.* 2012; Sousa *et al.* 2013). This was the only stage where X chromosome could be seen. But none of the banding or *in situ* hybridization studies could identify and characterize morphology of the X chromosome and it was only suspected to be the smallest chromosome in the complement (Sousa *et al.* 2013). Suggestively, X-specific probes can be designed and targeted for *in situ* localization to unambiguously identify the X chromosome. With a concrete understanding of molecular phylogenetic relationships among the 25 species of *Coccinia* (Holstein and Renner 2011), it would be useful to perform cytogenetic analyses in all the related species and reconstruct the steps of sex chromosome evolution in this genus. Since the presence of heteromorphic sex chromosomes is reported in *C. abyssinica* with $2n = 24$ (Roy *et al.* 1991) demanding further validation, similar possibilities cannot be ruled out in the other species.

Recent progress in molecular genetics and developmental biology of *C. grandis* has largely revolutioinized previous concepts of sex expression and Y functions (Shaina and Beevy 2011; Ghadge *et al.* 2014). The Y chromosome is supposed to carry genes for gynoecium suppression, stamen promotion and male fertility. Analysis of a naturally occurring

hermaphrodite *C. grandis* plant confirmed the existence of a third gender (based on *matK* and *trnS^{GCU}–trnG^{UCC}* intergenic spacer phylogeny), which is genotypically a female plant (XX) lacking the male fertility factor but possessing genes for stamen formation (Ghadge *et al*. 2014) and thus, forming morphologically perfect flowers with nonfunctional stamens. The expression of the floral organ identity gene *CgPI* is higher in male flowers (in male plants) than female flowers (in female plants) but contradicted by high expression in female flowers and reduced expression in stamens of male flowers of the gynomonoecious plant (Ghadge *et al.* 2014). Again, there is another report of a nearly 830 bp long male sex-linked SCAR marker having high homology with *APETALA3* MADS-box (AP3) gene from *Medicago truncatula* (Bhowmick *et al*. 2014). Thus, it is understood that organ identity genes (OIGs) have specific roles in controlling sexuality in *Cocinia* flowers and has followed independent pathways in male, female and gynomonoecious plants. Moreover, the chemically inducible (AgNO3) delay in stamen formation suggests the existence of male repressive factor in female plants which can be temporarily modified by external factors (Ghadge *et al*. 2014). Taken together, a cascade of networks involving chromosomal, genetic and developmental as well as biochemical regulators finally lead to the expression of either male or female or 'morphologically' hermaphrodite plants of *Coccinia grandis*.

Cucumis: Because of its huge agricultural importance, the genus *Cucumis* receives highest attention from molecular biologists, cytologists and agriculturists since a long period. Close insights into genome sequences and linkage maps of the related genera *C. sativus* (cucumber) and *C. melo* (melon) have been shown to hold considerable genomic synteny between them (Gonzalo *et al*. 2005; Huang *et al*. 2009; Li *et al*. 2011; Garcia-Mas *et al*. 2012). The preponderance of monoecy in *Cucumis* (Křístková *et al*. 2003) does not completely rule out the occurrence of intermediate types like andromonoecy or gynomonoecy (Roy and Saran 1990). Studies concerning environmental or hormonal regulation of sex expression of cucumbers and melons have been greatly advanced in the last decade; probing for the crucial roles of genic modulation of ethylene-based sexual differentiation in Cucurbitaceae. The phytohormones like GA and ethylene have been shown to promote male and female flower production, respectively (Roy and Saran 1990; Cho *et al*. 2005). In the early stages of flower development, expression of the ethylene biosynthetic enzymes *CmACS7* and *CsACS2* genes result in ethylene production and concomitantly arrest stamen formation (Boualem *et al*. 2008, 2009) in melons and cucumbers, respectively. Expression of the gene *CmWIP1* (suppressing carpel formation) in melons has been shown to epistatically repress *CmACS7* (stamen repressant), leading to the formation of male flowers (Martin *et al*. 2009). Female flowers in melon are formed when a transposon induced hypermethylation results in the suppression of *CmWIP1* and expression of *CmACS7* (Martin *et al*. 2009). A nonfunctional

CmACS7 is responsible for stamen development in hermaphrodite flowers (Martin *et al*. 2009). Thus, an integrated transposon-induced heritable epigenetic model is established in *C. melo* that depicts natural plasticity of an epiallele important for environmental adaptation and evolution of sex determination mechanisms in plants. Again, some cyclin or cyclophilin and most interestingly, one noncoding gene (*CsM10*) with highly conserved region and unknown function were shown to be associated with male sex differentiation (Cho *et al*. 2005) in cucumber. The fact that homeotic genes responsible for male or female sex organ formation are arrested at a position-dependent manner in the primarily hermaphroditic plan, further mystifies the enigmatic and beautifully choreographed genetic interplay behind floral sex expression in cucumbers (Kater *et al*. 2001). The milestone discoveries of genes controlling sex determination have made cucumbers and melons the representative members of Cucurbitaceae.

Beginning in the 1930s (Whitaker 1933), detailed account of base number study in *Cucumis* and its cross-compatibility groups have been worked out (Singh and Roy 1974; Singh and Yadava 1984a). The reports emphasized on derivation of the peculiar base number $x = 7$ of *C. sativus* from $x = 12$ of *C. melo* by fusion or chromosomal translocation events. Waminal and Kim (2012) critically examined the evolution of $x = 7$ in light of hybridization patterns of 45S and 5S rDNA on chromosomes. The pattern of centromeric 45S signals in five chromosome pairs and distal 5S signal in one chromosome pair is a fairly contrasting situation when compared with other cucurbits (e.g. *Luffa, Momordica, Benincasa, Coccinia, Lagenaria, Citrullus*; Waminal *et al*. 2011; Waminal and Kim 2012; Sousa *et al*. 2013). Waminal and Kim (2012) speculated five centromeric 45S rDNA-bearing chromosomes of *C. sativus* to be the fusion products of 10 acrocentric ancestral chromosomes (possibly having distal location of 45S rDNAs) forming a base number $x = 7$ after divergence from $x = 12$ of *Cucumis melo,* as hypothesized previously (Singh and Roy 1974; Singh and Yadava 1984a). The advent of molecular cytogenetic techniques has been a driving power for continuous improvement in chromosome identification and karyotyping in this species (Chen *et al*. 1998; Hoshi *et al*. 1998, 1999) with C-banding patterns. Koo *et al*. (2002) reported the presence of pericentromeric 45S rDNA signals in three chromosomes and 5S rDNA signal in one chromosome of the Korean cultivar, *C.s*. cv. Winter Long; although, later reports showed 45S rDNA sites in five chromosomes (Han *et al*. 2008; Ren *et al*. 2009; Zhao *et al*. 2011; Waminal and Kim 2012). A high resolution cytomolecular map for the Korean cultivar, Winter Long, obtained five signals for repetitive fragment CsRP2 and one signal for CsRP1 and homologues were identified by probing the RAPD markers on mitotic as well as meiotic chromosomes (Koo *et al*. 2005). Apart from reports of chromosome identification with repetitive sequences like type I/II (telomeric), type III (centromeric) or type IV (telomeric) and 45S rDNA (centromeric, Han *et al*. 2008), high density integrated

cytogenetic map was accomplished using 995 SSR loci in seven linkage groups spanning 572.9 cM (Ren *et al.* 2009). FISH mapping with SSR, type III repetitive sequence and 45S rDNA-facilitated chromosomal integration of all seven linkage groups and visual confirmation of euchromatic or gene-rich nature of the SSR markers. Comparative study of sub-specific varieties of cucumber was thoroughly analysed by Zhao *et al.* (2011) with repetitive sequences and 45S–5S rDNA probes suggesting the role of repetitive sequences and transposable elements in *Cucumis* evolution and held support for closer relationship of *C. s.* var. *sativus* (CSS) with *C. s.* var. *xishuangbannesis* (CSX) than with *C. s.* var. *hardwickii* (CSH). Another trial of FISH in the Indian and Japanese cultivars showed centromeric type III signals in six chromosome pairs (Zhang *et al.* 2012) while the previous study reported signals in all seven pairs. Molecular cytogenetic techniques have enabled researchers to publish genetic maps as well as integrated cytogenetic maps for cucumber by probing repetitive sequences on chromosomes with a comparative account with melon (Han *et al.* 2009, 2011; Dawei *et al.* 2011; Yang *et al.* 2012; Sun *et al.* 2013). Despite the high throughput genomic and molecular cytogenetic analysis in *Cucumis sativus*, no cytogenetical differentiation in relation to sex expression has been discerned in this plant.

A stable chromosome number of $2n = 2x = 24$ is known in several varieties of melons including *C. melo* var. *agrestis*, *C. melo* var. Golden Beauty, *C. melo* var. *momordica*, *C. melo* var. muskmelon and *C. melo* var. *utilissimus* (Whitaker 1933; Bhaduri and Bose 1947; Yadava *et al.* 1984). There is no indication of ploidy variation in melons studied so far, although detailed mitotic and meiotic analysis coupled with genome size measurements are yet awaited to assess chromosomal variation which is a common feature of cultivated plants. Among the other species, chromosome number of $2n = 2x = 24$ is known from *C. africanus*, *C. anguria* var. *anguria*, *C. anguria* var. *longipes*, *C. dipsaceus*, *C. ficifolius*, *C. hookeri*, *C. leptodermis*, *C. metuliferus*, *C. myriocarpus*, *C. prophetarum* and *C. sagittatus* (Singh and Yadava 1984a; Yadava *et al.* 1984). Tetraploidy is reported in *C. aculeatus, C. heptadactylis, C. meeusei, C. membranifolius* and *C. pustulatus* ($2n = 4x = 48$) along with one report of hexaploidy in *C. figarei* with $2n = 72$ chromosomes and irregular meiotic behaviour combined with poor pollen fertility (Dane and Tsuchiya 1979; Singh and Yadava 1984b; Yadava *et al.* 1984).

Genera having no reports on sex determination scheme

Benincasa: The type genus *Benincasa* (including *Praecitrullus*, Schaefer and Renner 2011) is represented by two species namely *B. fistulosa* and *B. hispida* (Renner and Pandey 2013), of which *B. hispida* is the popular cash crop on which studies have been performed. After the preliminary information on chromosome counts on the monoecious *B. hispida* ($2n = 2x = 24$, Whitaker 1933), cytological study in this species was fully ignored until Xu *et al.* (2007) and Waminal *et al.* (2011) characterized the chromosomes with respect to the signalling patterns in FISH study with 45S and 5S rDNA probes. There were two signals for 45S rDNA, one on satellite region of one pair and the other on terminal region of another pair with adjacent 5S rDNA signal at proximal side. The localization of rDNA signals although is similar to Momordiceae, Sicyoeae and Cucurbitaeae, the adjacent nature of 45S and 5S signals is a distinguishing feature for Benincaseae (Waminal *et al.* 2011; Waminal and Kim 2012). However, there is an urgent need to investigate the lesser known species of *Benincasa* (*B. fistulosa*) to gain a complete knowledge on cytogenetics of *Benincasa*.

Citrullus: *Citrullus* is an important monoecious vegetable yielding cucurbit with a completely known genome sequence (Guo *et al.* 2013) and the basic number of $x = 11$ (Whitaker 1933; Roy *et al.* 1991). A close phylogenetic relationship between *C. colocynthis* and *C. lanatus* has been suggested by Singh and Yadava (1977) with an indication of structural rearrangement of chromosomes to be the reason for speciation as evidenced by univalent and multivalent formations in their hybrid. Molecular karyotyping with *in situ* hybridization has been carried out recently in *C. lanatus* showing two pairs of distal 45S rDNA signals of which one pair has proximal 5S rDNA signal adjacent to the 45S signal (Waminal *et al.* 2011), maintaining the characteristics of Benincaseae.

Lagenaria: The genus has six species of which *L. siceraria* is a crop of the tropics and has high therapeutic uses. The monoecious *L. siceraria* has a chromosome number of $n = 11$ and there has been fewer reports of chromosome study in the past (Samuel *et al.* 1995). The genus is suggested to be a secondary polyploid having derived its base number from ancestral number of $x = 5$ (Roy 1973). Waminal and Kim (2012) have characterized the karyotype with rDNA binding patterns showing telomeric signals for 45S rDNA on two pairs of chromosomes of which one has and proximally juxtaposed 5S rDNA signal like other Benincaseae genera except *Cucumis*. Interestingly, the other five species of *Lagenaria* are dioecious and occur wild in Africa and Madagascar (Jeffrey 1980) lacking reports of chromosome study.

Melothria: The monoecious genus has 12 species with basic chromosome number $x = 12$ (Whitaker 1933; Roy *et al.* 1991) and is presently known to include *Melancium*, *Cucumeropsis* and *Posadaea* (Schaefer and Renner 2011). Tetraploidy is recorded in *M. assamica*, *M. heterophylla* and *M. perpusilla* ($2n = 48$) along with regular diploids (*M. maderaspatana* and *M. punctata*). The presence of 2–4 B chromosomes is reported in *M. heterophylla* (Roy *et al.* 1991).

Among the other genera of Benincaseae, *Solena* and *Zehneria* have ambiguous sexual phenotypes and chromosome numbers ($n = 12$ or 24) (Singh 1990a, Anmin and Jeffrey 2011c, d; Schaefer and Renner 2011) along with records of polyploidy in *S. amplexicaulis* ($2n = 48$) and *Z. scabra* ($2n = 48$) (Singh 1974). Again, only chromosome

counts are known in *Peponium* and *Ctenolepis* (Schaefer and Renner 2011). Chromosome numbers and sexual phenotypes are unknown in 14 other genera under Benincaseae that are phylogenetically related to the highly advanced models within the tribe (*Cucumis* and *Coccinia*) and hence, studies are required to correlate the status of sexual differentiation and chromosomal evolution in Benincaseae as a group.

Regulatory mechanisms of sexual expression in Bryonieae and Benincaseae range from genic or epigenetic master-players to chromosomal determinants. In one hand, *Bryonia* shows the nascent origin of sex chromosomes with size variations in the male determining region of Y combined with linkage disequilibrium. On the contrary, sex chromosomes of *Coccinia* have stepped further to accommodate repetitive elements in Y, a preliminary phenomenon associated with the degenerative process occuring in Y (Jamilena *et al.* 2008; Ming *et al.* 2011). The sex determination mechanisms in the well-studied models rule out the simplicity of the system and advocate the interplay of OIGs (Ghadge *et al.* 2014), hormone biosynthetic enzymes (Boualem *et al.* 2008, 2009), epigenetic imprinting (Martin *et al.* 2009) and external stimuli (Ghadge *et al.* 2014) besides the impact of male determining region of Y (Oyama *et al.* 2008; Ghadge *et al.* 2014). While interpopulation plasticity in the size of Y chromosome has been dealt with great precision in *Bryonia*, exclusive chromosome characterization in the genus is awaited to unfold the complete story on the origin of sex chromosomes in the genus. On the other hand, a detailed molecular cytogenetic investigation along with identification of OIGs (sequences homologous to OIGs) have contributed greatly to understand the mechanisms controlling sexual evolution in *C. grandis* (Bhowmick *et al.* 2012, 2014, 2015; Sousa *et al.* 2013; Ghadge *et al.* 2014). With an updated knowledge about plastid and nuclear gene-based phylogenetic relations among the species (Holstein and Renner 2011), it is expected that cytogenetic and developmental studies would be extended in future to all species of *Coccinia* to assess the evolutionary status of Y chromosome and sex determination schemes in relation to the different biogeographic species assemblages in its home country, Africa. The information can then be linked to the established concepts of sex determination and genomic sequence of the close relative *Cucumis* to comment on the trend of sexual evolution and its phylogenetic consequence in Benincaseae in a wider view.

occurrence of polyploidy is reported in *Gomphogyne cissiformis* (Roy and Trivedi 1966), *Gynostemma cardiospremum* (Ru-Juan *et al.* 1989; Chauhan and Pandey 2008) and *Siraitia grosvenorii* (Zhuang *et al.* 1997) among which *G. cissiformis* shows rare univalents and trivalents in meiosis, indicating genetic instability (Roy and Trivedi 1966; De Wilde *et al.* 2007). In terms of speciation, polyploidy has given rise to eight species in *Cucumis* (seven tetraploid with $2n = 2x = 48$ and one hexaploid with $2n = 2x = 72$; Singh and Yadava 1984b). Among these, *C. meeusei* and *C. heptadactylis* are autotetraploids; *C. pustulatus*, *C. membranifolius*, *C. aculeatus* and *C. zeyheri* are allotetraploids, whereas *C. figarei* is considered as an autoallopolyploid (Singh and Yadava 1984b). Cases of induced and natural polyploidy have been recorded in the dioecious species of *Coccinia*, *Momordica* and *Trichosanthes*. In *Coccinia*, the induced tetraploid male plants showed the presence of 2Y chromosomes in the somatic complement ($2n = 48$) and abnormal meiotic configurations, although the Y chromosome strongly influenced the male determining features (D'Cruz *et al.* 1972). The naturally occurring autotetraploid *Trichosanthes palmata* showed irregular meiotic behaviour but did not reveal chromosomal heteromorphy like its diploid relative (Varghese 1972). *T. tricuspidata* is also reported to be a natural hexaploid (Singh and Roy 1979a, b). Similarly, no sign of chromosomal heteromorphy could be traced within the natural triploid form of *Momordica dioica* strengthening the concepts of genic sex determination in the plant (Agarwal and Roy 1976). Some previous reports suggested polyploidy in some *Melothria* species also (*M. heterophylla*, M. *purpusilla* and *M. assamica*; Kumar and Vishveshwaraiah 1951).

It becomes evident that the rich genetic diversity in Cucurbitaceae cannot be essentially correlated to polyploidy, unlike the crop families Poaceae, etc. Nevertheless, a series of polyploids have been recorded in *Cucumis* and other taxa where the phenomenon was often associated with an increased size of vegetative and reproductive structures (Varghese 1972; Agarwal and Roy 1976). The sporadic occurrence of polyploidy is sometimes correlated with polysomaty or unreduced gametes in meiosis considering the facultative vegetative reproduction followed in many cucurbit species (Singh 1979). Despite the role of polyploidy in speciation, it has not been found to be associated with sexual differentiation mechanisms in the dioecious or monoecous species of Cucurbitaceae.

Polyploidy in relation to speciation and sexual differentiation in Cucurbitaceae

It is well known that polyploidy has played significant roles in the speciation of plants and has even more implications in crop-yielding plants generating cultivated varieties and ecotypes. Surprisingly, such a norm cannot be crucially applied in Cucurbitaceae, with few examples of polyploid species (Agarwal and Roy 1976). Among the understudied tribes,

Summary and future prospects

It is clear from the above discussion on the ten understudied tribes that further studies are needed to unfold yet unknown aspects of sex-determination system in plants and its cytogenetic modulators. Some genera of Gomphogyneae and Triceratieae represent the ancestral lineage of Cucurbitaceae and therefore chromosome characterization in those taxa can aid in uncovering the primitive condition. The

first seven tribes were previously part of the subfamily Nhandiroboideae with polyphyletic assemblage and ambiguous relationships. Therefore, a detailed study of these tribes is awaited to determine the ancestral sexual condition and its cytogenetic or genic determination. Since chromosomal distribution patterns of rDNA genes distinctly depict the impression of cytogenetic evolution in various tribes, the method can be advanced further for the large number of understudied taxa to improve the concepts of evolution and phylogeny. Recent progress in the field of molecular phylogeny and genetic mapping in some genera (*Hemsleya* and *Siraitia*) provide the scopes for chromosome characterization and comapartive genomic study amongst the related taxa. However, genic, hormonal and environmental factors have been shown to control sexual dimorphism and floral sexuality in the tribes Momordiceae, Cucurbiteae and Sicyoeae. Thorough study in *Momordica* exemplifies the ancestral sexual condition and plasticity of sexual phenotypes in Cucurbitaceae. The extent of speciation, diversity in chromosome numbers and genic sex regulation makes *Momordica* a potent source for further investigation. Phylogenetically related genera *Trichosanthes* and *Luffa* provide a suitable model for elaboration of genic determination of sex in Sicyoeae. The existence of highest number of sexually diverse species and homomorphic or occasionally heteromorphic sex chromosomes within *Trichosanthes* ($x = 11$) provide unique opportunities for unveiling initial steps of sex chromosome evolution. Within Cucurbiteae, the well-established model for sex determination in flowers and detailed genetic map of *Cucurbita* opens avenues for advancing cytogenetic methods within the tribe. Origin and evolution of sex determination system and sex chromosomes have been documented in Bryonieae and Benincaseae although we are in the verge of a solid comprehension of the phenomena. Being the first ever studied model for sex determination system in plants, explicit understanding of nascent origin and behaviour of gonosomes along with sex determination scheme, is acquired in the genus *Bryonia*. Exclusive investigation concerning chromosome ultrastructures and behaviour in the different *Bryonia* species is still awaited to complete the journey started by Correns in 1903. Suggestively, the approach can involve high throughput genetic and cytogenetic mapping along with chromosome banding and *in situ* hybridization strategies to ensure better understanding of the evolution of nascent sex chromosomes introduced in plant kingdom. The evolution of sex-determination system and sex chromosomes have been greatly studied in Benincaseae providing unique clues to the underlying processes and facilitating future scopes of study. An enigmatic interaction between organ identity genes, hormone biosynthetic pathways, epigenetic modulation and chromosomal factors have been documented in the sexually dimorphic *Coccinia grandis* while *Cucumis* displays a model for genic control of floral sex differentiation. There is a need to evaluate the status of chromosomal sex determination in the species of *Coccinia* having undergone repeated biome shifts over a short span of time.

The study can aid in proper assessment of the stability of dioecy and sex chromosomes over a range of species inhabiting different niche. Although chloroplast and nuclear DNA sequence-based phylogeny suggested six biogeographic species assemblages in more or less monophyletic *Coccinia*, cytogenetic investigation can further elucidate genetic relatedness among the species. In future, sex-specific sequences or Y-specific sequences can also be introduced in molecular phylogenetic analysis to obtain evolutionary history of sex chromosomes in the genus and also strengthen species delimitation with respect to sexual isolation. Again, attempts should be made to design X chromosome-specific probes for *C. grandis* to properly identify X chromosome utilizing *in situ* localization technique. Suggestively, the application of whole genome sequencing in *C. grandis* followed by construction of integrated molecular cytogenetic map and comparative analysis with the genome maps of the close relative *Cucumis sativus* would definitely meet the requirements to contribute to the efforts of researchers for unveiling evolutionary pathways and molecular architecture of *C. grandis* sex chromosomes. Another discipline of research can be aimed concerning the cascade of biological networks generating different sexual types of *Coccinia* in particular and unisexual flowers in general. Since floral sex differentiation in *Coccinia* and *Cucumis* has been shown to be under the control of OIGs, they provide exciting scopes to look for a universal model that standardize unisexuality in cucurbit flowers.

Conclusion

Research conducted in past couple of decades has advertised dioecious plant groups as an ideal platform where one can rediscover the evolutionary impacts of biochemical, environmental and chromosomal levels on sexual differentiation. There are plethoras of biological networks interlinked with diverse phenotypic outputs that are beyond the scopes of explanation under one context. Phenotypic gender dimorphism is one of the ultimate results of yet unknown cascades of biological processes orchestrated by various master players acting at different levels of plant biology. The fact stands true for Cucurbitaceae upon which nature has bestowed myriads of varying sexual phenotypes and let them flourish, evolve and stabilize; leading to the origin of 67 dioecious species spread over 19 genera amongst a total count of about 960 species. Thus, the family immediately necessitates discovery of genic and cytogenetic control mechanism of sex differentiation in plants, besides assessing the impact of physical and biochemical conditions. This review is an effort to enlist reports of chromosome cytology in general and to compile some highly informative reports on chromosomal or genic regulation of sex, particularly attempted on selected dioecious species. The variations in sexual phenotypes observed so far in Cucurbitaceae is reciprocated by an even greater variation of cytogenetic characters spread over

the tribes, offering unique opportunity to correlate cytogenetic diversity with that of the sex differentiation schemes in plants. In fact, sexual diversity in this family is manifested at tribal, generic, specific or even subspecific levels including intrapopulation or even individual variation in sexual pattern. Therefore, each and every plant belonging to Cucurbitaceae might possess some kind of uniqueness in its genetic or chromosomal or metabolic framework in order to achieve advanced reproduction strategy and ensure successful survival. A better understanding of these facts have been gained in some models like *Bryonia*, *Coccinia* or *Cucumis* and represent methods for studying a large number of unexplored taxa. Lack of information has been highlighted for tribes which are expected to grab attention of researchers willing to exploit the essence of dioecism combined with evolution of sex chromosomes or sex-determination systems. Indeed the initiatives made in *Coccinia*, *Bryonia* and *Cucumis*, invite one to walk over a yet unexplored boulevard of opportunities awaiting discovery. We are still far from making out the mysteries behind generating such varied modes of sex determination schemes and their phylogenetic or evolutionary consequences which are wonderfully choreographed by the selective forces of nature. On the whole, the attempts made by scientists till date together with the ongoing advancement of technology, are expected to construct a skeleton for a complete understanding of a very huge and complicated network of fundamental life processes that culminates in sexual dimorphism and emergence of specialized chromosomes in future.

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