PLANT DEVELOPMENTAL GENETICS

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We analysed genomic differences between mammals and angiosperms, two groups for which the most extensive genomic data from multiple species exist, and suggests that their genomes are undergoing radically different modes of evolution. The timing of the split between these groups is controversial, but current estimates suggest that it occurred 1000–2000 million years ago. Given their very long period of independent evolution, major differences in genome organization and evolution between the groups are to be expected. Nevertheless, exploring these differences can shed light on factors shaping the genomes of mammals and angiosperms. At the whole genome level (e.g., organization of DNA in the chromosome, diversity in chromosome number and genome size) there are substantial differences between mammals and angiosperms. Recombination plays a role in genome evolution because of its involvement in, for example, genomic rearrangements (chromosomal fusions, inversions and translocations), insertions (including organellar DNA), and repair and deletions of DNA sequences. Much evidence suggests that recombination rates are higher and activity more variable in angiosperms than in mammals, thus leading to differences in genome structure and long-term stability. The higher recombination frequencies are reflected in the greater number of translocations that can occur during species divergence and higher linkage map recombination frequencies reported in angiosperms compared with mammals. Differences in recombination frequencies are also reflected in different frequencies of illegitimate DNA insertions into the genome via recombination.

In both angiosperms and mammals the most significant and abundant mobile elements are retrotransposons, which are major determinants of genome structure and evolution. Angiosperms contain predominantly LTR retrotransposons belonging to the copia and gypsy superfamilies. Within these there is massive diversity, with thousands or tens of thousands of elements contributing up to 80% of the genome in some species. LTR retrotransposons are less abundant, diverse and active in mammals. Instead the non-LTR retrotransposon classes LINEs (long interspersed nuclear elements) and non-autonomous SINEs (short interspersed nuclear elements) predominate. Angiosperms have higher background levels of retrotransposition than mammals, often caused by bursts of activity associated with hybridization, polyploidy. The sequestration of a germ line early in mammalian development means that there are relatively few cell divisions leading to gamete formation, particularly in oogenesis. By contrast, there is no sequestration of the germ line in angiosperms; instead, gametes are formed from somatic cells in the apical meristems. Even ephemeral species such as A. thaliana with short generation times (7 weeks) undergo many hundreds of divisions between the seeds of one generation and those of the next. For the majority of angiosperms this number is likely to be order(s) of magnitude larger. Because the number of mutations and cell divisions are positively correlated, there are many more opportunities for mutations to arise compared with mammals. Furthermore, whereas the mammalian germ line is largely protected from the environment, the angiosperm germ line is vulnerable to environmental stresses that can also stimulate mutations and retrotransposition.

Different life strategies might drive genomic differences between angiosperms and mammals (Fig. 1). Mammals are capable of high levels of mobility, enabling them to find food and mates and escape disease, predation and adverse conditions. Associated with this is a highly complex, yet constrained pattern of development. In contrast, the sessile nature of angiosperms means that they cannot readily escape adverse conditions, herbivores and poor environmental conditions or attract pollinators. Instead their survival depends on being able to respond to

adverse conditions through biochemical complexity and developmental plasticity, the tool kit for plant survival. This is reflected in the large number of genes (perhaps 25% of the total) involved in the production of secondary metabolites.

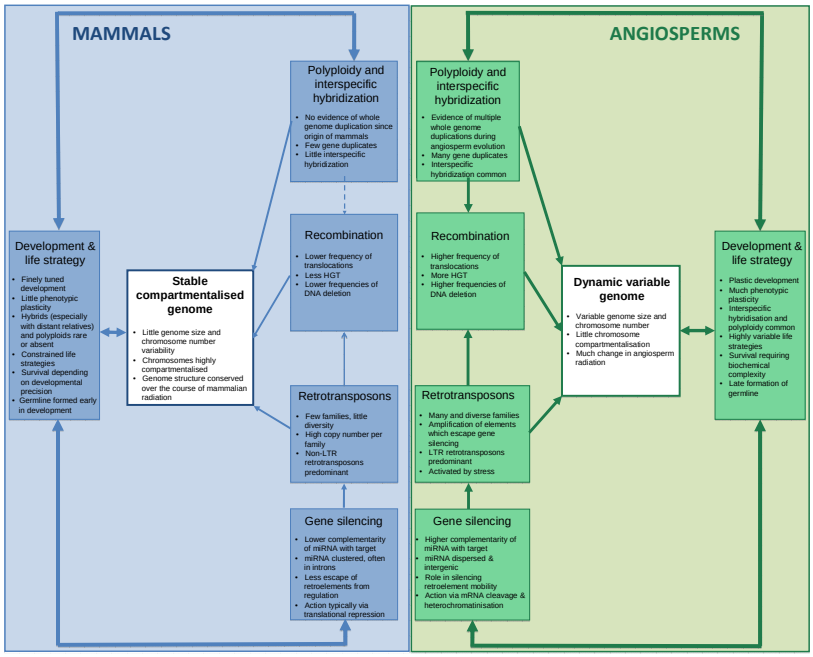

Figure 1: Different interrelationships and their relative strengths, represented by the direction and thickness of the arrows, between mechanisms generating genomic change and the life strategy options and developmental constraints in mammals and angiosperms. HGT refers to Horizontal Gene Transfer, the integration of DNA from sources outside of the nucleus.

The origin and evolution of sex chromosomes have interested evolutionary biologists for a long time. Although sex chromosomes evolve from a pair of autosomes, over time they become different, both from each other and the autosomes, in gene content and structure. While sex chromosomes in most mammals are ancient, sex chromosomes in some fish, insects and dioecious plants are evolutionarily young. Despite the different ages of sex chromosomes in different taxonomic groups, they probably follow similar evolutionary trajectories with discrete identifiable stages. Due to a stepwise loss of recombination between the X and Y chromosomes, some processes of Y degeneration start to occur. One of important processes acting in non-recombining regions is gene degeneration. Degeneration could be a consequence of TE accumulation. The random inactivation model suggests that the process of gene inactivation is triggered by the disruption of promoter regions by TE insertion. TE insertions can lead to an epigenetic phenomenon, or global changes in chromatin status (heterochromatization).

We performed a comprehensive analysis of repetitive DNA distribution on sex chromosomes in Silene latifolia (Fig. 2). Most TEs are distributed uniformly along both the X and Y chromosomes. Two exceptions are Retand elements, which are localized at subtelomeres and Ogre-like elements, which are present on whole X chromosome but restricted to the PAR region of the Y chromosome. Tandem repeats colonize the centromeres (STAR-C) and subtelomeres (X-43.1) of X chromosome, whereas in the Y chromosome STAR-C and STAR-Y are located in the middle of both arms and X-43.1 is at the subtelomere of the q-arm. Telomere-like sequences are present also in centromeres of the X and Y chromosomes. It is evident that the Y chromosome has a different composition and localization of repetitive DNA compared with X chromosome and autosomes. The presence of sex chromosomes and their tendency to accumulate repetitive DNA gives this dioecious species evolutionary potential different from what one might expect in the hermaphroditic species. The content of repetitive DNA may have a role in phenotypic features.

The study of the molecular structure of young heteromorphic sex chromosomes of plants has shed light on the evolutionary forces that control the differentiation of the X and Y during the earlier stages of their evolution. We have used the model plant Rumex acetosa, a dioecious species with multiple sex chromosomes, 2n = 12 +XX female and 2n = 12 + XY1Y2 male, to analyse the significance of repetitive DNA accumulation during the

differentiation of the Y. A bulk segregant analysis (BSA) approach allowed us to identify and isolate random amplified polymorphic DNA (RAPD) markers linked to the sex chromosomes. From a total of 86 RAPD markers in the parents, 6 markers were found to be linked to the Ys and 1 to the X.

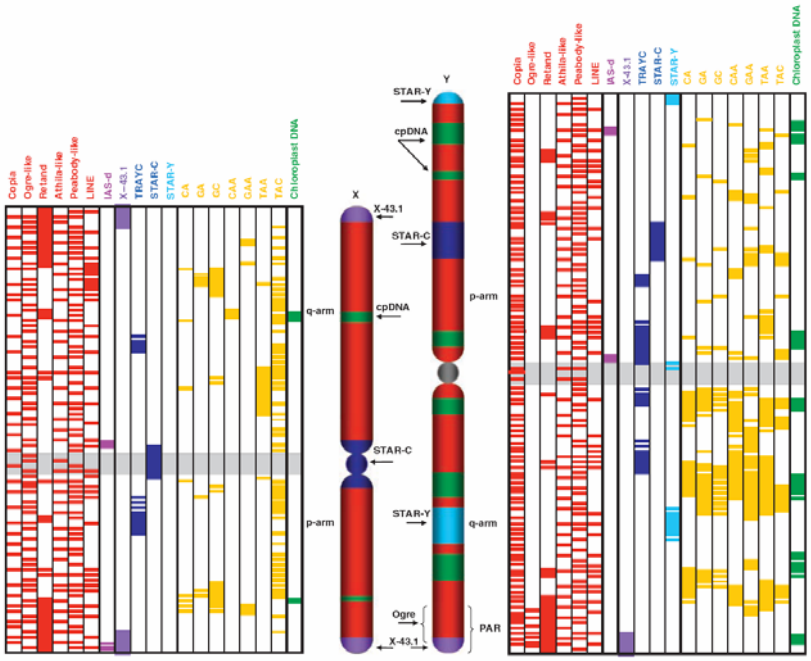

Figure 2: Schematic map of sex chromosomes of Silene latifolia with distribution of various types of repetitive DNA sequences—TEs (red), tandem repeats (blue), microsatellites (yellow) and chloroplast DNA (green). The patterns of elements distribution are derived from FISH data.

Two of the Y-linked markers represent two AT-rich satellite DNAs (satDNAs), named RAYSII and RAYSIII, that share about 80% homology, as well as with RAYSI, another satDNA of R. acetosa. Fluorescent in situ hybridization demonstrated that RAYSII is speciWc for Y1, whilst RAYSIII is located in different clusters along Y1 and Y2 (Fig. 3). The two satDNAs were only detected in the genome of the dioecious species with XX/XY1Y2 multiple sex chromosome systems in the subgenus Acetosa, but were absent from other dioecious species with an XX/XY system of the subgenera Acetosa or Acetosella, as well as in gynodioecious or hermaphrodite species of the subgenera Acetosa, Rumex and Platypodium. Phylogenetic analysis with diVerent cloned monomers of RAYSII and RAYSIII from both R. acetosa and R. papillaris indicate that these two satDNAs are completely separated from each other, and from RAYSI, in both species. The three Y-specific satDNAs, however, evolved from an ancestral satDNA with repeating units of 120 bp, through intermediate satDNAs of 360 bp. The data therefore support the idea that Y-chromosome differentiation and heterochromatinization in the Rumex species having a multiple sex chromosome system have occurred by different amplification events from a common ancestral satDNA. Since dioecious species with multiple XX/XY1Y2 sex chromosome systems of the section Acetosa appear to have evolved from dioecious species with an XX/XY system, the amplification of tandemly repetitive elements in the Ys of the section Acetosa is a recent evolutionary process that has contributed to an increase in the size and differentiation of the already non-recombining Y chromosomes.

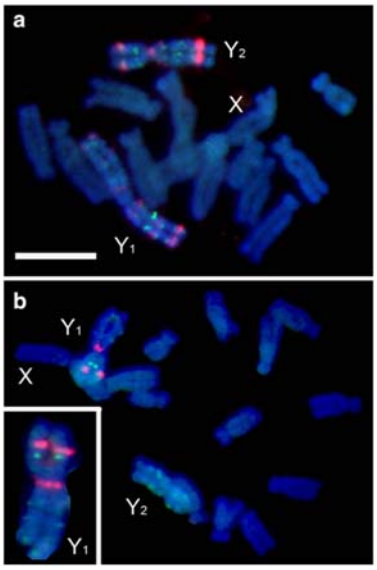

Figure 3: Chromosomal distribution of RAYS tandem repeats in R. acetosa analysed with bicolour FISH. Mitotic metaphase chromosomes of male R. acetosa (counterstained by DAPI, blue) were hybridised with (a) RAYSI (red signals, Cy3 labeled) and RAYSIII (green signals, SpectrumGreen labeled), (b) RAYSII (red signals) and RAYSIII (green signals). The X, Y1 and Y2 chromosomes are indicated, bar = 10 µm.

The chromosomal rearrangements are often considered to be the main mechanism leading to the suppression of recombination between X and Y chromosomes. There are, however, some data indicating that also other mechanisms can play important role in this process. According to our hypothesis, the recombination between X and Y chromosomes can be at the beginning of sex chromosome evolution realized also via epigenetic modification of the non-recombining part of the Y chromosome. It is, therefore, possible to expect that each Y-chromosome has suppressed recombination ability to any of the X chromosomes and moreover to any other Y chromosome. In the case that inversions would be the only mechanism preventing X/Y recombination, some level of recombination between Y chromosomes should be present in X/Y non-recombining region.

In order to test recombination ability of the Y chromosomes, we have prepared Silene latifolia plants possessing one chromosome X and two genetically distinguishable Y chromosomes. Presence of the X chromosome is necessary as the plants carrying Y chromosomes only are non-viable. Recombination frequency was assessed between four molecular markers spread over the Y chromosome and between sexual phenotype and each of these molecular markers. Results showed that no recombination occurred between markers located in the non-recombining region of the Y chromosomes. Surprising results brought the study of the segregation of the pseudoautosomal molecular marker PAR2. Results indicate that there is present a small frequency of recombination between any of the Y chromosomes and the X chromosome but no recombination between the Y chromosomes was found. In this case, the role of inversions can be ruled out as both the Y chromosomes are able to recombine with the X chromosome. In the case of population specific inversion(s) on the Y chromosome (s), the Y chromosomes should significantly differ in recombination frequency with the X. Our study also brought new light in the issue of sex ratio inheritance and in the issue of sex specific expression. Results based on the study of the segregation of the X chromosome coming from XYY plant indicate that generally observed advantage of the pollen tubes carrying X chromosome does not apply to the pollen tubes that apart of the X chromosome carry also Y chromosome. From these results, it is also possible to deduce that sex specific expression (i.e., the Y chromosome caused difference in expression patterns and phenotype) is present already at the stage of pollen tube.

Males and females of animal species often differ in many morphological and behavioral traits. Recent studies have revealed that sexual dimorphism in animals is also common at the level of gene expression. In mammals, this expression difference between sexes is apparent not only at the later stages when the sexual phenotype is controlled by the action of sexual hormones produced by gonads, but also in the pregonadal stage - i.e. before the initiation of gonadal development. In plants with separate sexes, sexual dimorphism seems to be much less pronounced than in animals. Among vascular plants displaying sexual dimorphism, Silene latifolia is the most studied species. So far, all the known S. latifolia sexually dimorphic traits are of quantitative character. The most prominent sexually dimorphic trait is flower number, with males producing several times more flowers than females. On the other hand, females usually produce more biomass, mainly because of formation of heavier

stems, larger leaves and flowers. Many of these traits were confirmed to be genetically determined because either they have been QTL-mapped or display a response to genetic selection experiments.

With the aim to search for genes specifically expressed in male plants, we went through S. latifolia ESTs described as preferentially or specifically expressed in male flowers. As most of these ESTs were isolated before Arabidopsis thaliana genome sequencing and annotation, their homologies to any plant genes were mostly unknown. Using database search combined with phylogenetic analysis, we unambiguously identified A. thaliana orthologues of nine ESTs from S. latifolia. Six of these A. thaliana genes are expressed ubiquitously, which is in a disagreement with the reported expression of their S. latifolia orthologues. Results show that only six of them are expressed exclusively in male flower buds, twelve ESTs are expressed in leaves and flower buds of both sexes, and two ESTs start to be expressed in male flower buds earlier than in female flower buds. Importantly, we have also found one EST (Men470) that is specifically transcribed in male plants and one EST (CCLS79.1) that is transcribed specifically in female plants. RT-PCR performed on samples from bulks of six different male and female individuals did not differ from the results observed on single-plant analysis. These results clearly show that the male and female S. latifolia plants differ in their gene expression even before the initiation of flowering; this situation is similar to the pregonadal stage in mammals. The described ESTs are not only the first qualitative differences between the sexes at the vegetative stage, but also the first described sequences in plants connected with the sexual dimorphism before flowering at all. Our results implicate a possible route of the evolution of the sexual dimorphism in S. latifolia. The initial stages of the sexual dimorphism evolution are driven by the presence of the non-recombining region that attracts sexually-antagonistic genes. Candidate QTLs for these sexually antagonistic genes on the X chromosome have been already found, and the Y chromosome also most likely contains genes that evolved via this mechanism. Genes with sex-preferential or sex-limited expression are expected to evolve later on. These genes should be differentially expressed at the initiation of flowering, when the sex-determining genes are active, as their expression is expected to be controlled by the sex-determination genes.