# Future for cytogenetics in plant genomics and breeding

## Invitation

Prof. Dr J.H.S.G.M. de Jong

Farewell address upon retiring as Professor of Plant Cytogenetics at Wageningen University on 1 October 2015

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*Rector Magnificus, dear colleagues, students, friends and family*

*Be welcome to my farewell address on the occasion of my retirement at Wageningen University. It is an honour and pleasure for me to tell you about my career, my work as a scientist and educator, why I loved the work I did and why I still happy to continue doing it.*

In the first part of my presentation I will start with mentioning few details about my early years of chromosome research and the work involved in the research that most of you know me about. First of all: I like images, photos, not only captured with my camera, but also with the microscope. Yes, I am a picture man, and I like to show and explain my knowledge in illustrative presentations and lectures, rather than presenting only text or equations. I am a proud microscopist and feel delighted that my universities (University of Amsterdam and Wageningen University) gave me the opportunity to use the best of their microscopes, starting in the seventies with a bright-field photomicroscope, and later with different electron microscopes, and more recently with the best of advanced fluorescence microscopes. The appreciation and interest for microscopes is typical for Dutch scholars, which is obvious from the list of famous inventors of microscopic optics, with Antonie van Leeuwenhoek, Frits Zernicke from Groningen University and Jan Ploem from Leiden University to mention a few.

My first involvement in chromosome research was in 1970, when I was student in the University of Amsterdam, at the time that Amsterdam Science Park counted only two buildings, that of Animal Physiology and Genetics. It was in the years that the nurse fields behind the lab were full of flowering petunia's. I was there as a master student in the lab of professor Bianchi, together with Frans Smith and Oof Oud, trying to identify individual chromosomes for establishing trisomies as part of a large-scale genetic study on flower colour and shape[1]. I continued this work as a PhD student on chromosome identification in sugar beet hybrids that were selected for nematode resistance[2]. Although chromosome identification technologies and knowledge at that time were pretty limited, I had the change to meet Dr Ramanna in Wageningen who

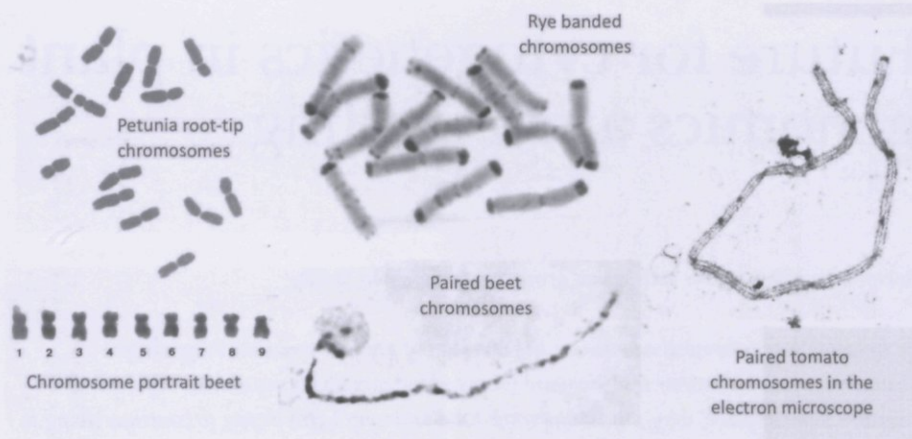



*Figure 1. Traditional chromosome staining in the 70ties.*

encouraged me to study the long meiotic chromosomes[3], given the advantages this type of chromosomes may provide, not realizing at that time how important these so called pachytene chromosomes became in the later years of my career[4]. I also worked with banded chromosomes and with electron microscopic images of paired chromosomes (Figure 1), something I will tell you about later in my lecture.

In this farewell address I have decided to focus on three of my topics during my career; more of these would take far too much of your patience and concentration. The first subject then is about the colourful world of painted chromosomes. For me one of the most influential experimental papers of the last four decades the work of Pardue and Gall[5]. These two researchers were the first to publish successful *in* situ Hybridization (ISH) experiments in which they hybridized satellite DNA of mouse on spread chromosomes fixed on microscopic slides. In one of their seminal papers they clearly showed that the radioactive probe hybridized on the centromere regions of the chromosomes, but what was more interesting at that time: the treatment changed the stainability of the chromosomes by Giemsa and other dyes. Not the ISH was the invention people were waiting for, rather the possibility to mistreat their chromosomes with all kind of chemicals – in this initial report, alkaline solution that was used to open up the DNA structure. This study was a new era an opening to the plethora of chromosome banding technologies that were developed since then. Too many to mention, C-banding, Q banding and R banding[6], but surprisingly, they hardly play any significant role in research anymore.

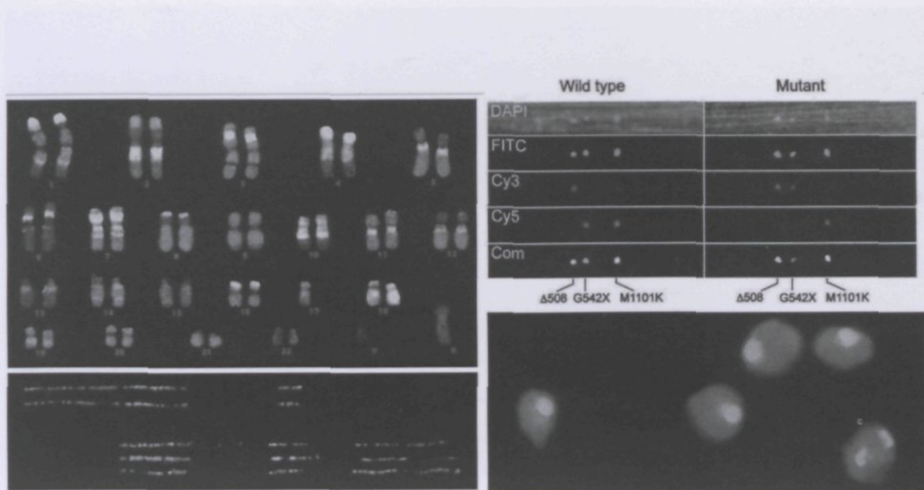



*Figure 2: Different types of chromosome painting in human.*

What we needed for what is known nowadays as Fluorescent *in situ* Hybridisation (FISH) was the development of effective labelling technologies with non-radioactive labels, and the technical innovations for creating very high quality fluorescence microscopes and digital software that we now are so familiar with[7]. The picture that I now show you is an example of all 23 different human chromosomes all with their own colour, or better, pseudo-colour, as image processing was needed for generating sufficient colour for so many chromosomes (Figure 2).

The scientific possibilities of this new technology were countless and unprecedented. Now we are able to distinguish even small areas in the chromosomes and diagnose any variation in chromosome number or structure unequivocally. Initially this methodology was applied to slides containing spread chromosome complements and interphase nuclei, but later on also chromatin fibres and naked DNA spread on microscopic slides were used as target for FISH experiments [8]. Major advantages in FISH technology include 1) simultaneous detection of different DNA probes, by using multiple fluorophores alone or in combination [9], 2) the spatial resolution, which was close to the 1 kb limit on extended DNA fibres[10], and the ultra high sensitivity of less than 1 kb target sequences, and in few cases even of single mutations[11].

The scientific progress in FISH technology of plants was different from that of mammals. First of all, plant geneticists initially focussed on the application of this technology for the distinction between the parental chromosomes in interspecific or intergeneric hybrids. This technique known as genomic *in situ* hybridisation or

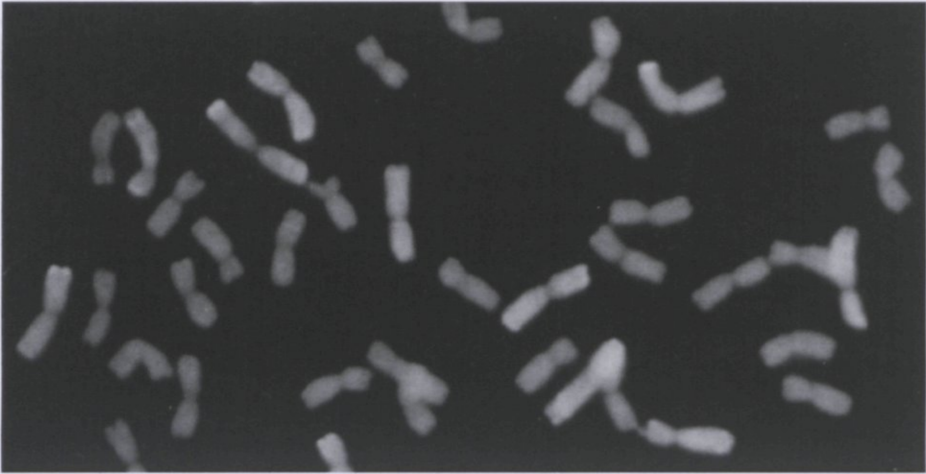


*Figure 3: Genome painting allows distinction between the parental chromosomes in a wheat rye hybrid.*

genome painting was developed by Trude Schwarzacher and colleagues[12] and was based on hybridization of dissimilarly labelled genomic DNAs of parental species thus discriminating the chromosomes from the two parents. Examples in my research are hybrids of rye (Figure 3) and wheat[13], somatic hybrids of tomato and potato[14] etc.

But in other respects FISH in plants lagged behind compared to that in mammals including human. There were several reasons for this retarded development. Firstly, human cytogenetics has great impact in prenatal diagnosis and cancer research with many researchers involved and extensive financial support. Secondly, mammalian chromosomes can relatively easily be sampled by flow sorting and pools of such chromosomes can be used for the production of chromosome-specific DNA, thus enabling probes for each of the 23 chromosome pairs in a multicolour FISH. In plants such chromosome flow sorting is complicated if not impossible at all, whereas FISH with small stretches of plant DNA often paints all chromosomes due to presence of dispersed repeats in the probes. So the method of chromosome painting for plants had to be adopted. This was achieved in IPK Plant Institute in Gatersleben, in the group of Ingo Schubert, together with Paul Fransz in my lab, who developed a chromosome painting technique, based on probes that contains only unique DNA sequences, not the repetitive sequences that in animals give little or no problems, but in plants often produced even staining of all chromosomes [15]. One of the famous chromosome paintings came from the lab of Martin Lysak *c.s.*, who was the first to publish detailed chromosome painting images of the five chromosomes of *Arabidopsis*, the model species that we use for a lot of our experiments [16].

Chromosome painting is great science and the colourful results are equally attractive to look at. It can visualise all kind of chromosome aberrations in the genome, and even rearrangements that genetics analyses were not able to demonstrate.
As a member of international genome consortia of tomato and potato my lab contributed to the ongoing genome assembly programs by localizing DNA sequencing directly on the chromosomes, and provided information about their positions with respect to centromeres, telomeres and repeat-rich heterochromatin domains. In doing so my colleagues used probes with five different fluorescence dyes with emission spectra in blue, green, orange, red and far-red, along with DAPI a special bluish DNA intercalating fluorophore that stains all chromosomes, thus resulting in this picture (Figure 4), a beautiful example of chromosome painting.

One of the goals in these large-scale genome projects was the demonstration of gaps between assembled DNA (contigs) using chromosome painting of plant DNA on their chromosomes. To this end we pooled the sequences of a genome region and painted these on the chromosomes in order to show the gaps between such contigs. In that way we were able to help the genomics people filling the gaps that still remained in the assembling of all chromosomes. Yet there was great scepticism and reservedness as to this kind of chromosome painting work. For good reasons: chromosome painting is time consuming and laborious, and only few labs have the required sophisticated equipment for multicolour fluorescence microscopy.
In a large-scale chromosome painting study, the largest of its kind ever done on a higher plant, my lab cooperated with the chromosome biologists of Steve Stack,

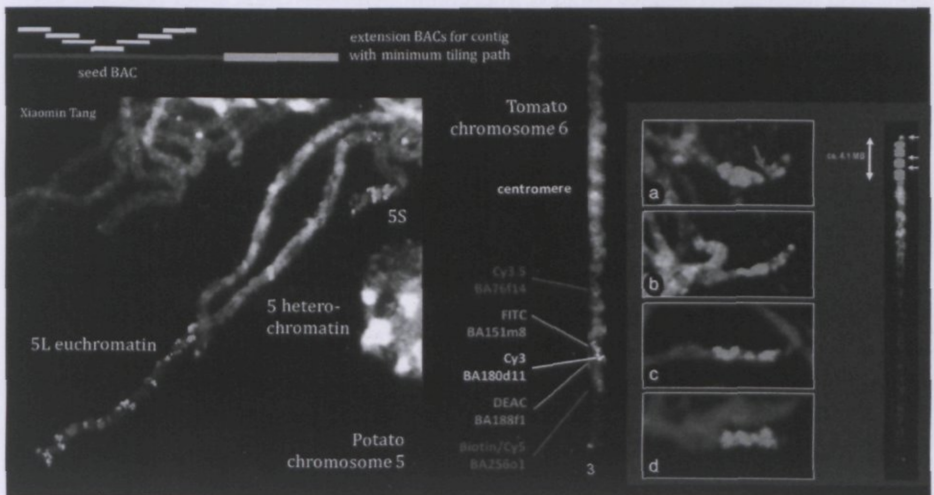



*Figure 4. Different chromosome painting experiments in potato and tomato.*

Colorado State University. Here we painted a huge number of 627 BACs on the 12 chromosomes of tomato, demonstrating that 1/3 of all contigs were wrong in position or orientation[17]. And in general these contigs involve the chromosome parts in and around the centromeres; the litterbin of the genome. Surprisingly, this paper was hard to publish as it proved that quality of genomics needs improvement, and that will fall to deaf ears. But to solace: even this genome assembly correction was not perfect enough and a newer version is on the brink of publication. I will tell you about that later on.

While geneticists and breeders love the DNA of their genes, so do chromosome biologists tend to focus on the remaining part of the genome, the regions of repetitive sequences or what I call the polystyrene foam of the chromosome. These are DNA sequences, often in thousands of copies in a row, and often located around centromeres and chromosome ends. One of the studies in my career I always felt very proud of was the study of Xiaobo Zhong in the labs of Pim Zabel, Paul Fransz and my lab on tandem repeats in tomato[18]. Xiaobo managed with unbeatable patience and accuracy the mapping of these sequences on the ends of the tomato chromosomes, a study since then never done again! We extended this study with a second large group of repetitive elements in tomato, the retrotransposons and showed their position on the chromosomes, along with sequencing data from our colleagues from the lab of Dr. Bouzayen in Toulouse. This study revealed the repeat characteristics on the distribution of these sequences that I never have found since then in any other report[19].

Now you have heard several small stories about repetitive DNA sequences and you may wonder why I like this part of the genome so much. First of al: you can see their position on the chromosomes, in contrast to even the best DNA sequencing and assembly efforts. Why then is such a complete assembly so difficult? Imagine that a genome is compared with a jigsaw puzzle of a tropical beach, with blue sky, white beach and palm trees containing many oddly shaped interlocking and tessellating pieces for assembly. The repeats are the blue pieces of the air; the genes and other single copy sequences are the details in the palm trees and the beach. If you can chop the genome in big pieces without errors – the genome technology that is still far from that – then the completion of the puzzle is relatively easy, as most pieces are large enough to match easily with their neighbours. But sequencing nowadays is often done by Illumina technology, albeit accurate, are difficult for assembly as they produce large number of very small puzzle pieces, which makes the assembly a challenge, especially when you want to put the mostly identical pieces of the blue sky in the right position. So how to avoid the problems of repetitive sequences in the genome? Just ignore them! And that is possible if most of the genome sequences have

been assembled, like the Wageningen bioinformaticists did for tomato. We asked Saulo Aflites, one of my PhD students in the lab of Applied Bioinformatics, to use repeat filtering in search for sequences that are absolutely unique, like exons in most of the genes. Such sequences can thus be selected and amplified by PCR and then used for making probes to paint the chromosomes. The results are promising but there is also still room for improvement. We keep working on it.

It is clear that long arrays of repetitive sequences are the difficult part of the genome to assemble, and the bigger the genome and their chromosomes, like in rye, wheat, onion and lily, the harder the problems in sequencing and genome assembly. That is why model species are chosen for its small genome size, and the model of higher plant species, *Arabidopsis thaliana*, is no exception to this rule. *Arabidopsis* has a genome of only 150 MB and the number of repeats are low and concentrated mostly in big loops captured by specialized regions around the centromeres of the five chromosomes[20]. These domains, which we call chromocentres, are easily visible in interphase nuclei. Some accessions were found to have bigger chromocentres while others have smaller, so Penka Pavlova measured the shape of such chromocentres in a semi-automatic manner and compared nuclear and chromocentre morphology in large number of recombinant inbred lines, plants that have different allele combinations of the same original $F_1$. Based on this large dataset, Martijn van Zanten and Basten Snoek uses sophisticated statistics to find the regions on the chromosomes where the quantitative trait for size and shape of these chromocentres are located on the chromosomes. We are now working on finishing the study on the biological function of those traits.

The type of chromosome research in plant species that I presented so far is the result of joint projects in which each researcher from different disciplines contributed its own outstanding part. I think Wageningen University is a good place for such cooperations. Most of my colleagues like to collaborate with academic institutes and private companies from all over the world merging efforts to get complex studies of plant and animal genomes done. Researchers and students from Wageningen University contributed several papers in high IF journals like Science and Nature, publishing the genomes of tomato, potato, but also pig and chicken, not surprisingly species with agriculture impact. But with the detailed knowledge of such genomes as you can see in this overview exhibiting all kind of features and details of the tomato genome, we also can jump ahead and do much more with this information by comparing the genomes of different species, like here in a comparison of tomato and potato. Both crops have a basic chromosome number of 12 and if we compare the position of all genes on the chromosomes in a MUMmer plot, one would ideally expect a diagonal line if all genes are perfectly what we call collinear. Most is true of

what I said but the more you look into detail the more differences you may descry. Sander Peters and colleagues worked out one of the most conspicuous differences and that can be seen in the comparison of the chromosomes 2 of tomato and potato[21] (Figure 5). The bioinformatics revealed several chromosome rearrangements, inversions and translocations as we call them. The question if we also can see such changes in the chromosome structure with a staining technique? Yes, we can, and very clearly. Not as accurate as Sander showed, but faster and a bit easier. So Dóra Szinay, Erik Wijnker, Yuling Bai and Ronald van den Berg, close colleagues from my own group and from Plant Breeding and Plant Systematics joined efforts to study the rough position of chromosome regions in different crops that are related to tomato and potato, so egg plant, pepper, and several wild relatives. We selected a limited number of chromosome arms of which we expected that chromosome rearrangements had occurred during their evolution. And by the way, if you cross two such relatives which differ in the order of genes on chromosomes strange inversion loops which can give rise to linkage drag, a notorious problem in plant breeding. With the data that we were able to produce a tree showing their evolutionary relationship, which by the way resembles the consensus tree that most taxonomists nowadays are using in their research.

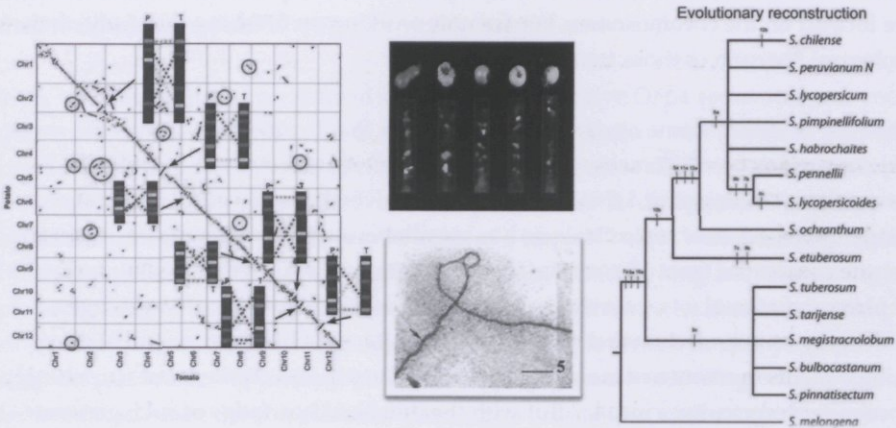



*Figure 5. Comparison of different Solanum crops display large scale chromosome rearrangements. Left: Mummer Plot tomato – potato, middle: chromosome painting of different Solanum crops and an inversion loop as observed in a hybrid with one parent containing an inversion; Right: Phylogenetic tree based on inversion data.*

Let me jump now to the second item in my farewell address and tell you few, yet interesting results that we obtained with the study of meiosis. Meiosis is the process of two special cell divisions that proceed the formation of the gametes. But meiosis is also the motor of genetic recombination generating novel combinations of chromosome segments from mother and father, and so is responsible for most of our genetic variation. That explains why we are so different and why plant breeders love to exploit meiotic recombination to create desirable combinations of traits.
But meiosis is a very difficult and complicated process, and is composed of several consecutive steps of chromosomes pairing, recombination and segregation to the gametes, and so the the progeny. Far too complicated to explain to you now in detail, so let me only give few small examples. If a geneticist wants to study a specific process, then he switches it off by mutation or modern biotechnological processing. In the first case we look at the situation that chromosomes from father and mother fail to pair. Frances Havekes, my first PhD student together with Professor Heyting, studied the mutant with such a mutation in tomato. In the normal situation the identical father and mother chromosomes stay together until the spindle threads in the cell pull them apart to the poles. Without pairing this process goes wrong leading to an erratic segregation of chromosomes at later stages. The resulting spores are unbalanced and so are the gametes that are formed shortly later. In the normal situation one get healthy anthers with uniformly shaped viable sperm cells; in the mutant the gametes are mostly sterile with empty, shrunken inviable sperms. Is this the end of the story or not? After all, I said almost always. It is true that very few cells are formed with the balanced number of chromosomes making the cell complete and balanced. But no recombination between father and mother chromosomes, and that gives new opportunities.

The formation of non-recombinant chromosomes is of great interest that only very few researchers recognised. But the people in the Research & Development and Biotechnology department of Rijk Zwaan Breeding Company did, and they worked out the conceptual part of an induced suppression of crossovers for regenerating non-recombinant plants from any hybrid, plants that look like inbred completely homozygotes. By selecting genetically complementary chromosome substitution pairs of such plants in a way that crossing them will recruit the original hybrid[22] (figure 6). A complicated concept indeed. And a shock in the plant breeding world! My lab got the honour to work out the ideas of Rijk Zwaan using our favourite model plant *Arabidopsis*. Rob Dirks and Erik Wijnker the leading actors in this scientific game told earlier this afternoon about this technology in the symposium.

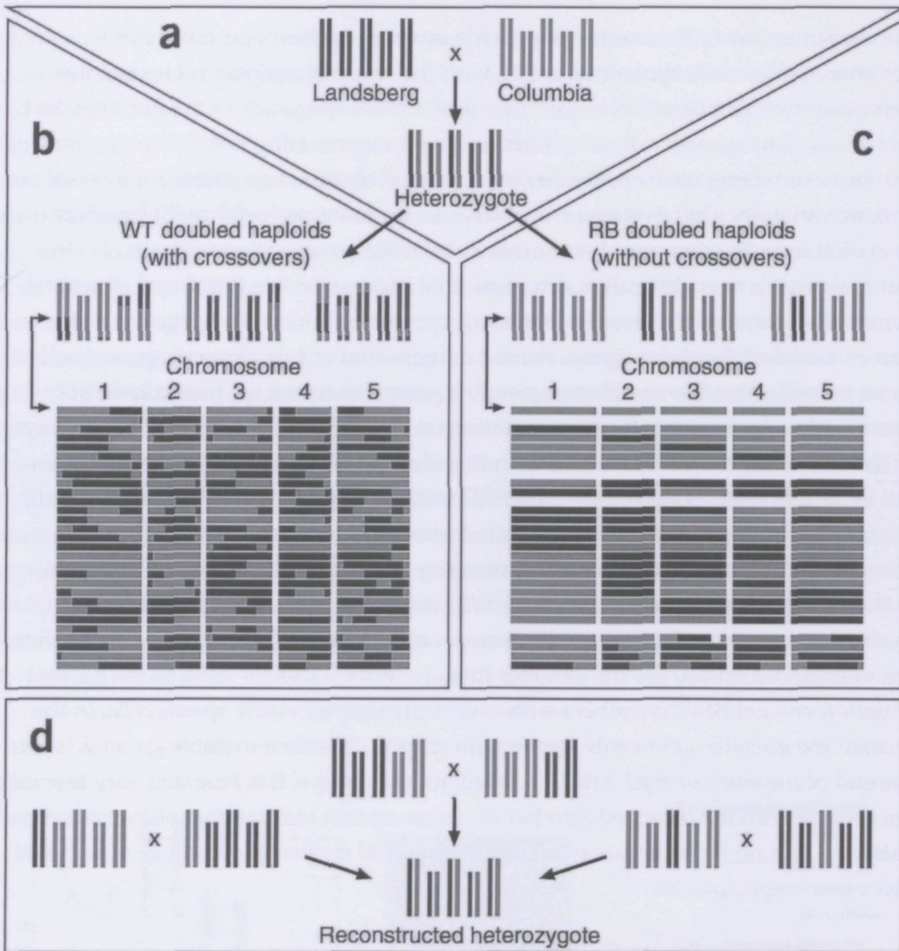
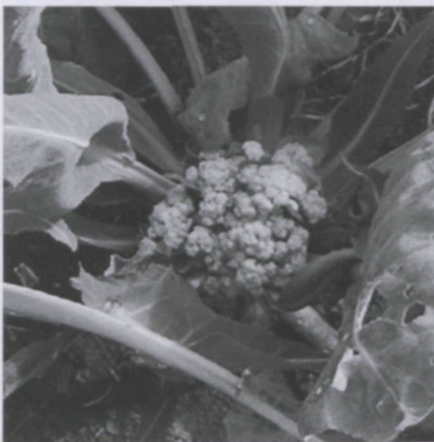



*Figure 6. How non-recombinant plants can be used to recruit identical F1 plants through seed.*

The second example is less complicated. The breeding and growing of *Brassica oleracea* crops, including cauliflower, broccoli and Brussel's sprouts can be notorious difficult as they can produce aberrant offspring, a phenomenon that was not understood for a long time. Now we know the reason: the plant is not so accurate with its meiosis and regularly produces gametes with one or few extra chromosomes, and hence produces offspring plants with extra chromosomes and aberrant phenotype in the curd and leaves. You can see that in this photo. The experiments by Xianwen Ji together with Cilia Lelivelt of Rijk Zwaan clearly revealed the cause of this problem: chromosomes do pair, but they often fail to complete the recombination process, which at later stage lead to unequal segregation and production of unbalanced sperms. We then asked ourselves if all chromosomes display this

abnormal behaviour. The answer is yes. Using one of Saulo's bioinformatics toolkits we were able to make probes with DNA of Arabidopsis and painted these on the cauliflower chromosomes (Figure 7).

My last example of meiosis. Chromosomes divide to different poles. They do so in a way that after two consecutive divisions four daughter cells are produced, each with half the number of chromosomes. However, plants sometimes skip this first or second division resulting in gametes that have double the chromosome number, so called unreduced gametes. It happens once in a while and the progeny can then have double the chromosome number. Think of potato, leek, cotton and wheat, crops that are examples of polyploid plants, which have multiple copies of chromosomes. Breeders are very interested in understanding the conditions under which such disturbances in meiotic divisions may take place. Here again the use of *Arabidopsis* is the model that gives you the tools to tackle this problem. First of all, Greg Copenhaver from Chapel Hill, North Carolina, isolated a mutant that keeps all four spores together, and he inserted artificial genes in such plants that can produce red, green or blue fluorescence in the cells. The advantage of such a genetic material is that you can demonstrate recombination and segregation in a single meiosis. But now about the situation of unreduced gametes. Look at the example that I presented? If the green and red carrying chromosome segregate in the first division but not in the second you see a big green and red spore; if the first meiotic division is skipped and not the second you get two big daughter cells, and each have a mixture of green and red, and so are yellow.



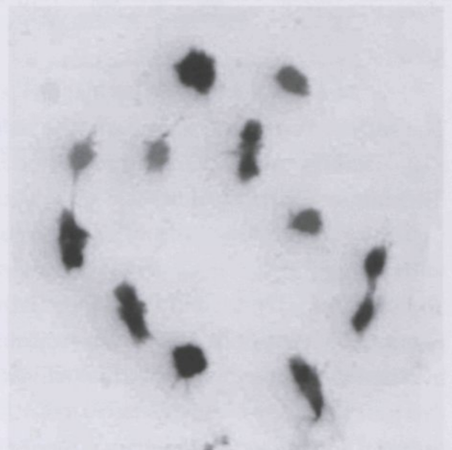


*Figure 7. Aberrant cauliflower curds often have extra chromosomes in their cell complements.*

In the final topic I want to talk about the flow of genes and chromosomes of one plant or animal to the other. We call that **introgression**. As I am eager to teach my students about this phenomenon I first tell them a lovely story in humans using our own genetic information. People can send their DNA to a company in the USA, called 23andme. This company isolate DNA from the epithelial cells in your saliva fluid and analyse thousands of DNA variants in your chromosomes and they look at your mitochondria DNA to see your maternal line of origin and your Y chromosome DNA for your paternal line. And they can show you roughly if you come from North west Europe or that your DNA has more in common with people from Spain.

Let us first look at my DNA: very boring for the geneticist. My DNA resembles mostly that of middle European people. If I compare my DNA with a friend of mine who also sent his DNA to the company that some subtle difference becomes clear: I am more central European and he has more SNPs in common with English – Scandinavian people. But I have one unique feature: I have 3.3% of my DNA with Neanderthal specific SNPs, and this put me in the top 1% of people with Neanderthal DNA. For me a relief, for others an explanation....

Now the really interesting case (Figure 8). Peter is married to Marijke and her genes have seen much more of the world: In her ancestral karyotype we can see that 6% is South east Asian. Is that remarkable? Not when she tells you that her family lived

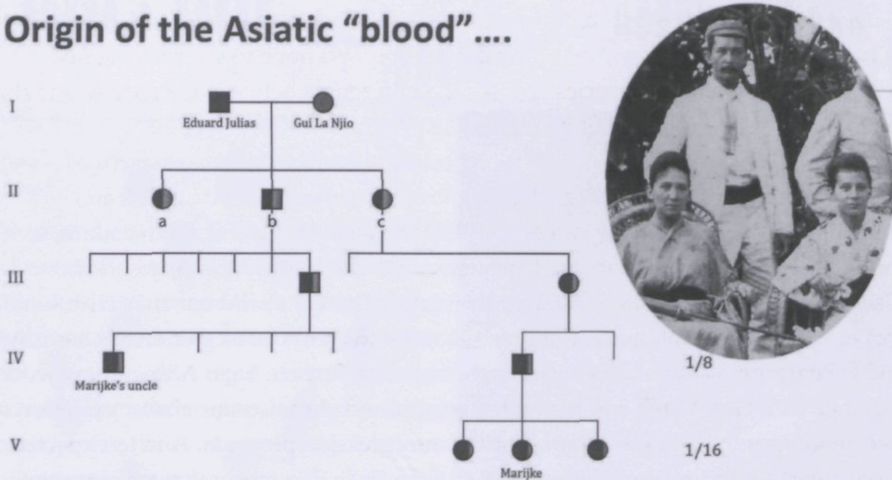



*Figure 8. The origin of 6% Asian SNPs in Marijke's DNA becomes clear if you know that her family came from a tea plantation in Indonesia.*

for generations in the tea plantations in Indonesia. And 6% equals 1/16 part, so let us have a look in her family. Her pedigree gives the answer: her grand grand father married a Chinese lady from which three children were obtained. The pedigree demonstrates introgression of Asian (Indonesian or Chinese) blood into Caucasian persons. And every generation the percentage of Asian blood was halved explaining finally her 6%.

And plants? Do they the same? Yes, they do. Take tomato as an example. A great vegetable, but colour, taste, size, and disease resistances is not always perfect. So we make an introgression breeding plan in a systematic and controlled manner.
I may now suggest that such breeding programs are flawless and straightforward, but nothing could be further from the truth. Every generation we check for viability and select the right traits, but sometimes such genetic tools are not enough. In several cases: tomato (+) potato hybrids and lily hybrids we use genome paintings to establish the mix of parental chromosomes in the hybrids and follow the processes of chromosome segregation and recombination. A beautiful example of modern technologies in support of introgressive hybridisation is now being carried out by my Uruguayan PhD student Paola Gaiero. Paola came to my lab a couple of years ago to learn chromosome staining protocols and meiotic analysis in hybrids between potato, and the wild relatives *Solanum phureja* and *S. commersonnii*. The latter is a species which is almost endemic to Uruguay, and potentially interesting for conferring drought tolerance and disease resistances. Paola was very successful in studying the chromosome pairing in their hybrids using genome and chromosome painting, but I suggested her to include bioinformatics tools to enhance precision and detection of her introgression study. And with success! Soon we hope to see her genomic comparisons of the three species and see the differences between the species and the eventual consequences for her introgression program.

And then banana cytogenetics and genomics. Probably the most difficult and challenging crop I had in my career. Cultivated banana for the western countries, the Chiquita's in Albert Hein, are threatened by Fusarium wild, a very serious disease in banana plantations all over the world. There are wild bananas with some sort of resistance or tolerance, but breeding is almost impossible because of sterility and chromosome aberrations between the crossing parents. Fajar Ahmad now works together with Gert Kema and me on the genetic and chromosome characterization of two genotypes that we want to use for the introgression program. And as expected these banana's and related cultivars, all of them have chromosome rearrangements as you can see in the genetic study at CIRAD, Montpellier, chromosome painting by Guy de Capdeville in my lab several years ago, and in Thailand, together with my wife, Lak.

As explained before numerous chromosome studies can be helpful in tracing the behaviour of wild species chromosomes in introgressive hybridization programs. Here you see the genome paintings of hybrids from the lab of Plant Breeding in which *Solanum nigrum* and *S. brevidens* can be distinguished from the potato parent. Such methods are fast but they do not provide DNA based information of small chromosome regions of plants in the introgression programs. Saulo Aflitos, the Brazilian PhD student, supervised by the staff of Applied Bioinformatics and the lab of Bioinformatics, developed iBrowser, an introgression browser that compared short stretches of chromosomal DNA with one of the parents, here the tomato Heinz 1706, which serves as the reference genome[23]. The two graphs that are shown here (Figure 9) give examples of the iBrowser in hybrids between tomato and the

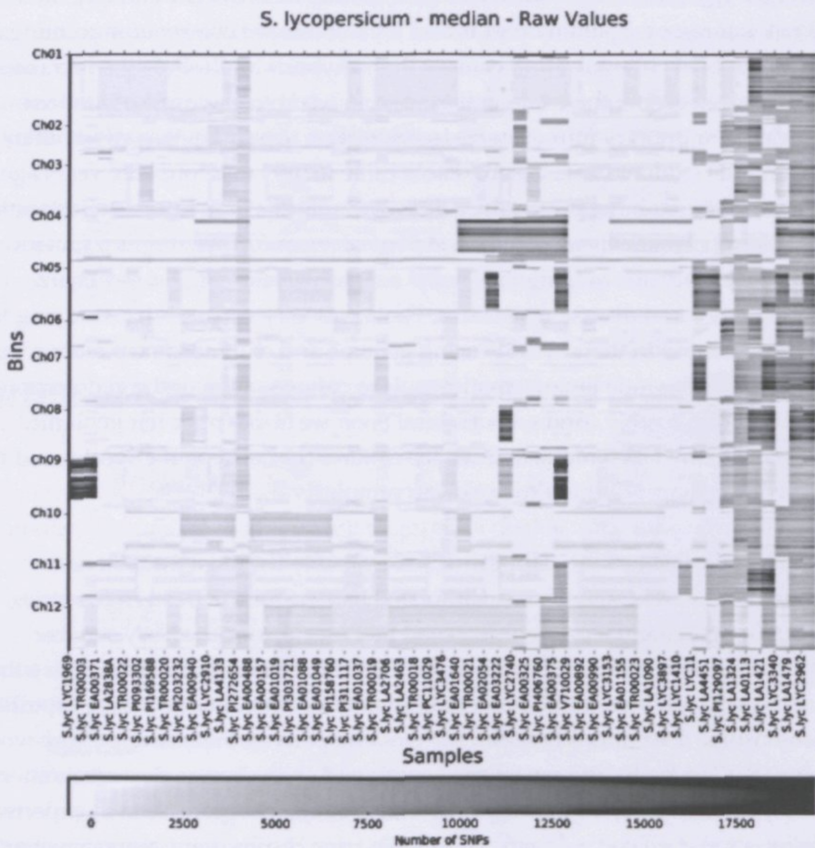



*Figure 9. Introgressions in crop cultivars can be analysed effectively by the iBrowser software. The dark blocks in this heatmap represent the areas of alien DNA.*

wild species *S. pimpinellifolia*. It is a bit the same as what I told you of the ancestral karyotype of Marijke with 6% Asian blood in her DNA. Also here we now can visualize with great precision where the DNA segments of the wild species are located and what genes are involved. A very powerful method indeed.

But we also used the power of the iBrowser in our ongoing studies of Arabidopsis. In this model species we can find DNA sequence information of more than one thousand different accessions. Our interest here was focused on a small inversion that we found in Columbia, the best studied Arabidopsis accession in the world. A long series of chromosome painting experiments elucidated the breakpoints of this inversion in the short arm of chromosome 4. About 10% of all accessions have this inversion. Using the iBrowser tool you can demonstrate the presence of the inversion by a white area in the chromosomal DNA. Why white? Because genetic variation in that region is almost zero due to lack of meiotic crossover. But the most exciting discovery was still to come: when we look closely at the borders of the inversion, we see that the white areas differ between the accessions, because crossovers next to the breakpoints are extremely rare. Extremely rare means that they can serve as evolutionary landmarks and so can tell you which accessions are related and they migrated over the continent of Europe and later also to North America. It is clear that chromosome research now reached the level of population genetics!

In the last part about my research I would like to stress that cytogenetics is more integrated in experimental biology than ever; even more than that: the genomics and bioinformatics created for me a sort of renaissance of this research field. And that brings me to my last remarks. Based on the idea, the concept of DNA fibre chromosome painting, scientists realised that nanotechnology and high-throughput processing of large data sets can help to improve the quality of assembled genomes. I will explain so called optical mapping as follows: take high molecular weight DNA from intact flow sorted plant nuclei and label the DNA with a restriction enzyme and a fluorescent dye every 10 kb on average. The labelled DNA is then unwounded and pulled through a labyrinth of little hubs and channels in a way that single molecules with their labels form sort of beads on a string and can be captured by a ultrasensitive microscopic system. The datasets thus obtained will produce a DNA restriction map *in situ*, revealing repetitive DNA sequences and errors in previous assemblies. They also can establish sizes of adjacent gaps, find insertions and deletions and even correct contig orders. The first results that Gabino Sanchez showed me recently are impressive and promised important breakthroughs in the assembly of the genomes of our model species and wild relatives that we use in our comparative genomics programs.

Now enough about research: I like to spend few words on my teaching activities. I was involved with great pleasure in all kind of teaching activities. And for good reasons: Wageningen University is an outstanding place for students and teachers. I was involved in classes of cytogenetics, later this became GATC, an acronym for *Genetic Analysis, Tools and Concepts*, and more recently I also taught the genetics parts in *Cell Physiology and Genetics*, and *Plant Cell Tissue Culture*. In addition, I organized several international courses on molecular cytogenetics, and was guest speaker in other courses. And above all, I enjoyed very much my involvement as teacher and organiser of the National Biology Olympiad.

*Figure 10. One of my genetics lab classes at work.*

My final slide and probably the most difficult. After working so long in a place that I liked and still like so much, there are many people to thank for the good time we had together. But it will be impossible to mention them all. Allow me to select some groups of people that were very important for me. The people who really inspired me for different reasons: My thanks go to Jaap van der Veen, Jaap Sybenga, Munikote Ramanna, Ab van Kammen, Maarten Koornneef, Piet Stam, Tom Gerats, Boudewijn van Veen, Ton Bisseling, Rob Dirks, Paul Fransz, Eric Schranz, Erik Wijnker, Hans Morélis, Sander Peters, Gert Kema. Among them are the four speakers of this afternoon symposium. Thank you, guys!

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Ladies and gentlemen, thank you for your attention.
It is time for a drink and a chat now.

*Ik heb gezegd.*

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Prof. Dr J.H.S.G.M. de Jong

*'In my long career at Wageningen University, I had the pleasure to study different aspects of plant chromosome biology. I enjoyed both the cooperations with various laboratories in my university as well as my participation in national and international scientific communities. In addition, my projects with breeding compa[illegible] stimulated my interest in developing knowledge on chromos[illegible] for practical applications.'*

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