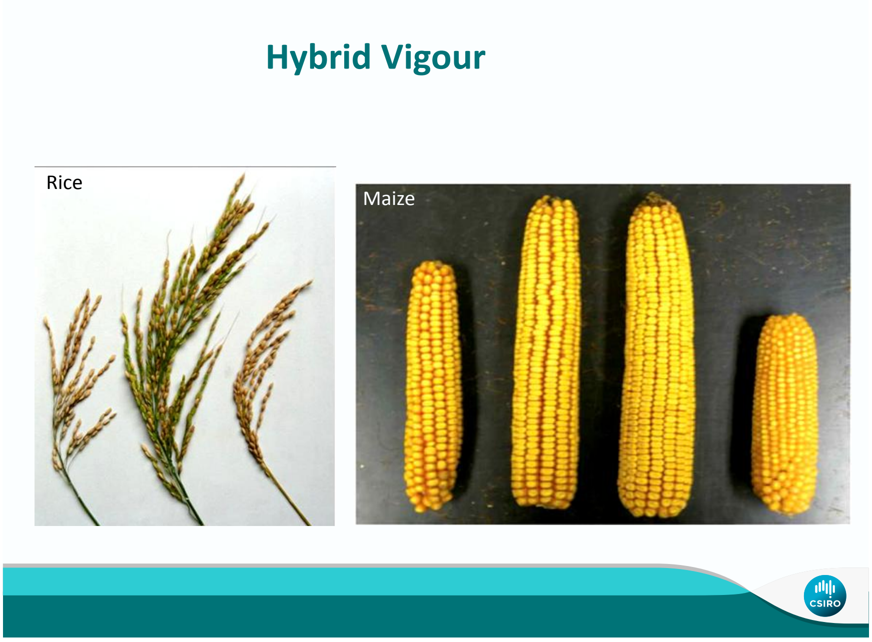
Farrer Oration 2014

Arabidopsis and Agriculture- What can we learn about Hybrid Vigour?

Liz Dennis CSIRO Agriculture

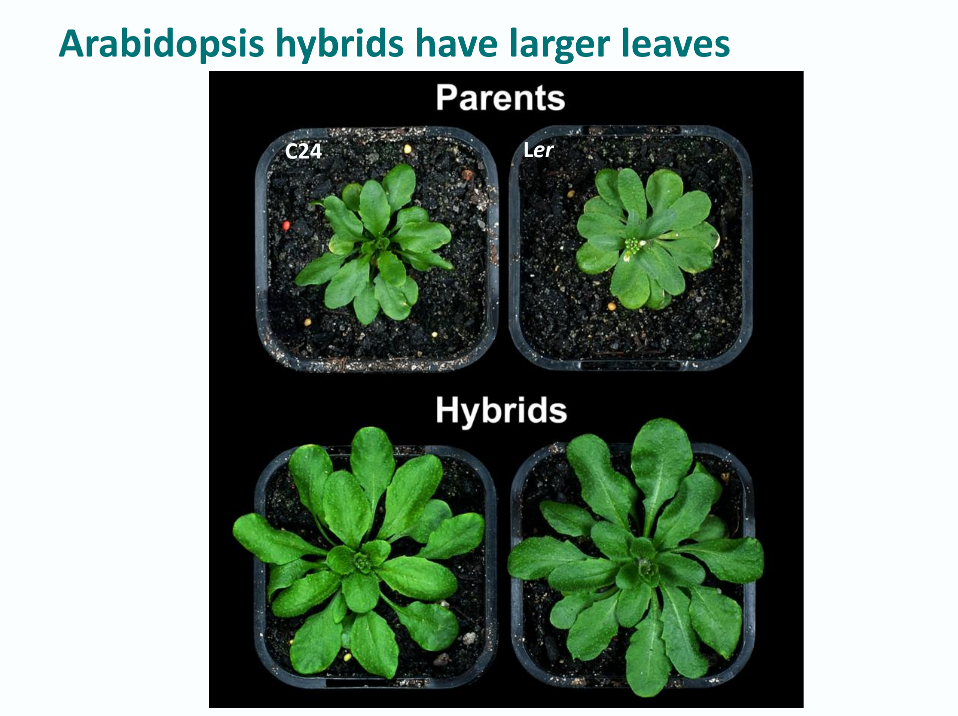
Farrer and Hybrid Vigour

Farrer, known as the father of the Australian wheat industry and after whom this lecture is named was remarkable because he introduced novel technologies into Australian wheat breeding including the idea of crossing wheats with different properties instead of selecting from within a line. His famous wheat "Federation" was a cross between a wheat with good milling and baking qualities and an early maturity Indian wheat that was resistant to drought and disease. He was one of the few breeders in the world doing this sort of breeding at this time and it is worth remembering that this research was done in the early 1900s before the rediscovery of Mendel's work. Following the idea of introducing useful characters by crossbreeding, breeders in maize found that some hybrids gave a much higher yield than did either parent .This led to the concept of hybrid vigour which was first introduced by Schull in 1928. Not all hybrids produce higher yield and breeders chased parents that had good combining ability which when crossed gave high vigour and increased yield. The increased yield is remarkable as the hybrid plants are grown under identical conditions as the parents with the same inputs of water, fertiliser and light.


Hybrid Vigour

Hybrids mean more yield and hence more profits. All the corn produced in the developed world is now hybrid and is the basis of a $9 billion seed industry. Hybrid vigour is used in many other crops such as rice, sorghum and vegetables.

The question of how hybrids produce their greater yield is one of the major questions of plant biology. How does the plant do it? Despite hybrids being used in agriculture for nearly a century we do not understand the molecular basis of hybrid vigour. QTL analysis in maize and rice has indicated that there are hundreds of QTL's involved in producing hybrid vigour so many genes must be involved. We know hybrid vigour must be mediated through changes in expression of genes in the hybrid. But there has been no real idea of what gene expression changes are critical for the production of vigour.



Arabidopsis hybrids have larger leaves

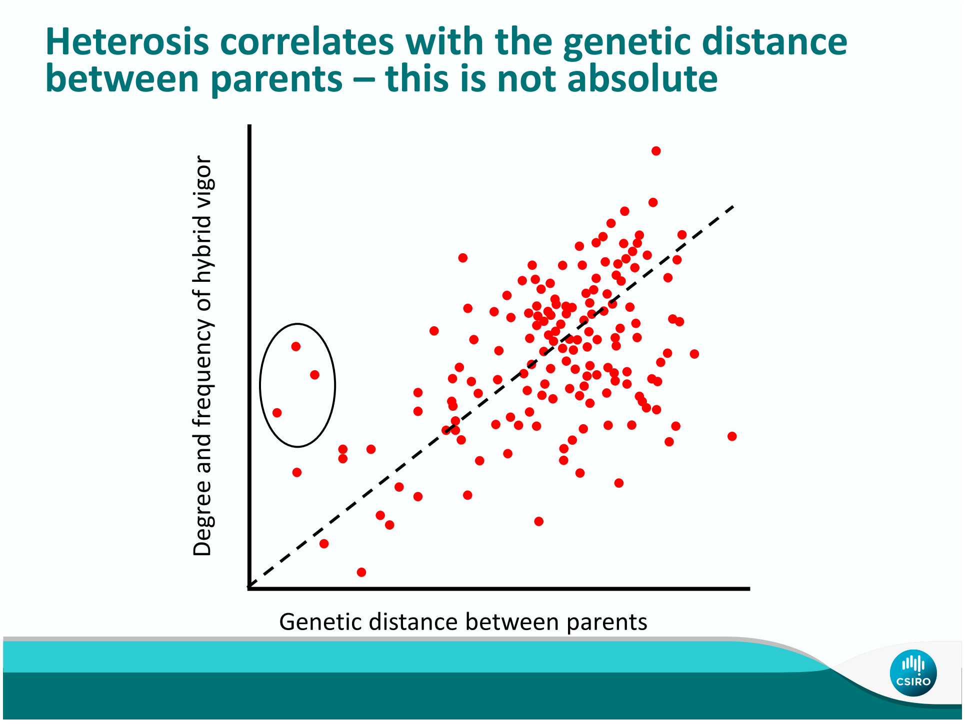
Arabidopsis and Hybrid Vigour

We decided to look for answers in our model plant Arabidopsis. Even though Arabidopsis is a weed it is like the fruit fly of plants. Arabidopsis also shows hybrid vigour quite spectacularly. You see the two parents on the top and the two reciprocal hybrids below. There can be a 200% increase in fresh weight and 90% increase in seed yield in the hybrids.

We have used Arabidopsis to investigate the molecular basis of hybrid vigour and we think the new knowledge in genetics may provide some answer s as to what is the basis of hybrid vigour.

Before I tell you about our work I would like to acknowledge the other workers in our group who have contributed to this study. Firstly Jim Peacock who coleads our group with me and importantly the Postdoctoral Fellows and students who have been critical for the success of the project.

When scientists first investigated the combining activity of maize and other hybrids they found that in general the greater the genetic distance between the parents then the greater the hybrid vigour. This is generally true but there are some examples which are circled which show hybrid vigour with very little genetic distance between the parents. This is where our parents fit.

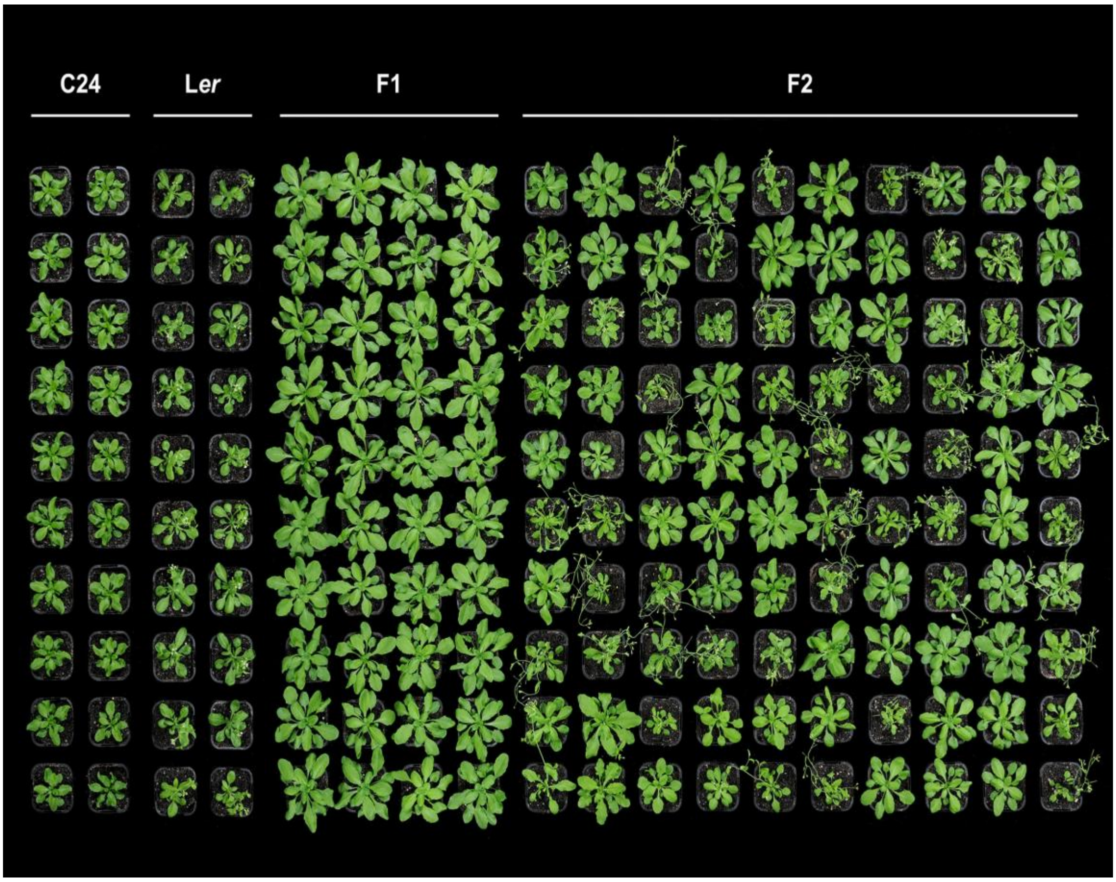


Heterosis correlates with the genetic distance between parents – this is not absolute

Epigenetics plays an important role in controlling gene expression

Now I want to pause to tell you about a recent realisation in genetics. That is that not only do DNA sequences affect the activity of genes but there is another level of control of gene activity called epigenetic control which is more to do with the accessibility of DNA sequences. This occurs in all higher animals and plants. With no change in the DNA sequence the way the DNA is packed can change whether a gene is active or not. For example if chromatin, which is the DNA strand and its associated histone proteins, is packaged tightly by histone proteins or by increased methylation of the cytosines in DNA , then the chromatin is in a repressive state the enzyme that copies the DNA into RNA cannot access the chromatin and the gene is switched off. If the chromatin is in an open state, achieved by losing DNA methylation or modifying the histone proteins then the copying enzyme can gain access and the gene will be active.

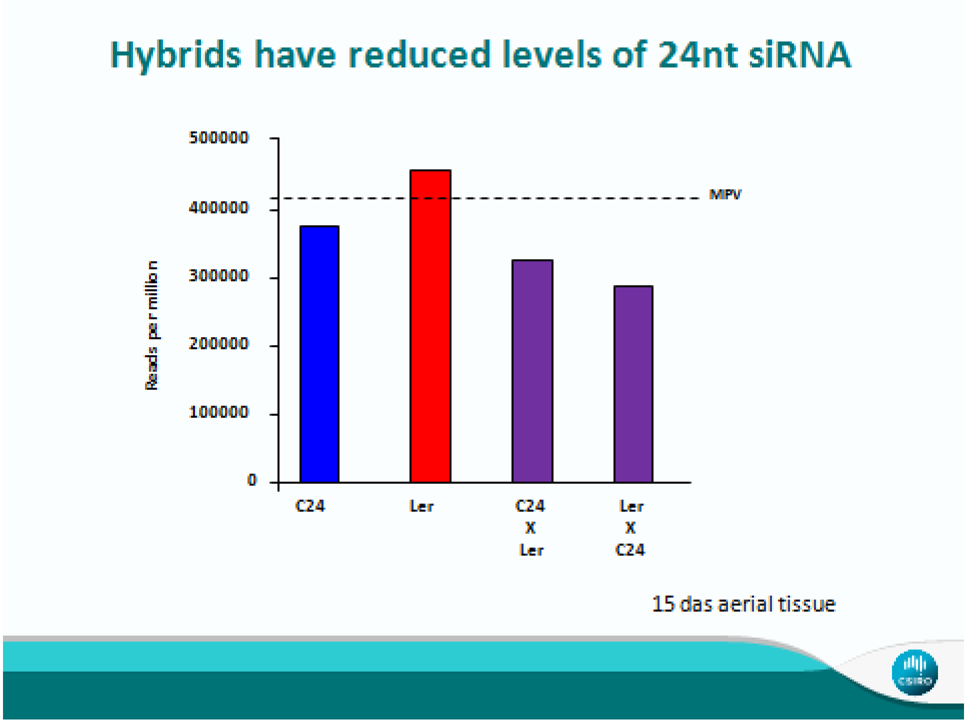
We decided to look for a role of epigenetics in hybrid vigour to see if this was the basis for the examples showing hybrid vigour with little genetic difference between parents. We chose parents with almost identical genomes so in essence we held the genome constant and there was still hybrid vigour.



The parents are two different naturally occurring accessions of Arabidopsis, C24 and Landsberg, and the F1 hybrid has good levels of heterosis. Another feature of hybrid vigour that you can see clearly is that the vigour only holds in the F1generation and decays in the F2 where the sizes are very heterogenous, this clearly wouldn't be a successful crop. . Any molecular explanation of hybrid vigour has to explain this lack of heterosis in subsequent generations and explains why farmers have to buy seed each year.

Epigenetic marks are altered in hybrids

The first epigenetic system we examined was the level of 24 nucleotide small RNAs, which we know are an important for altering DNA methylation in plants. We found a substantial decrease in the levels of small RNA in hybrids relative to the parents indicating that there are epigenetic changes. The reduced frequencies of 24nt sRNAs occur in 15-24% of the genome of the hybrid and only where there are differences in the levels of small RNAs between the parents.



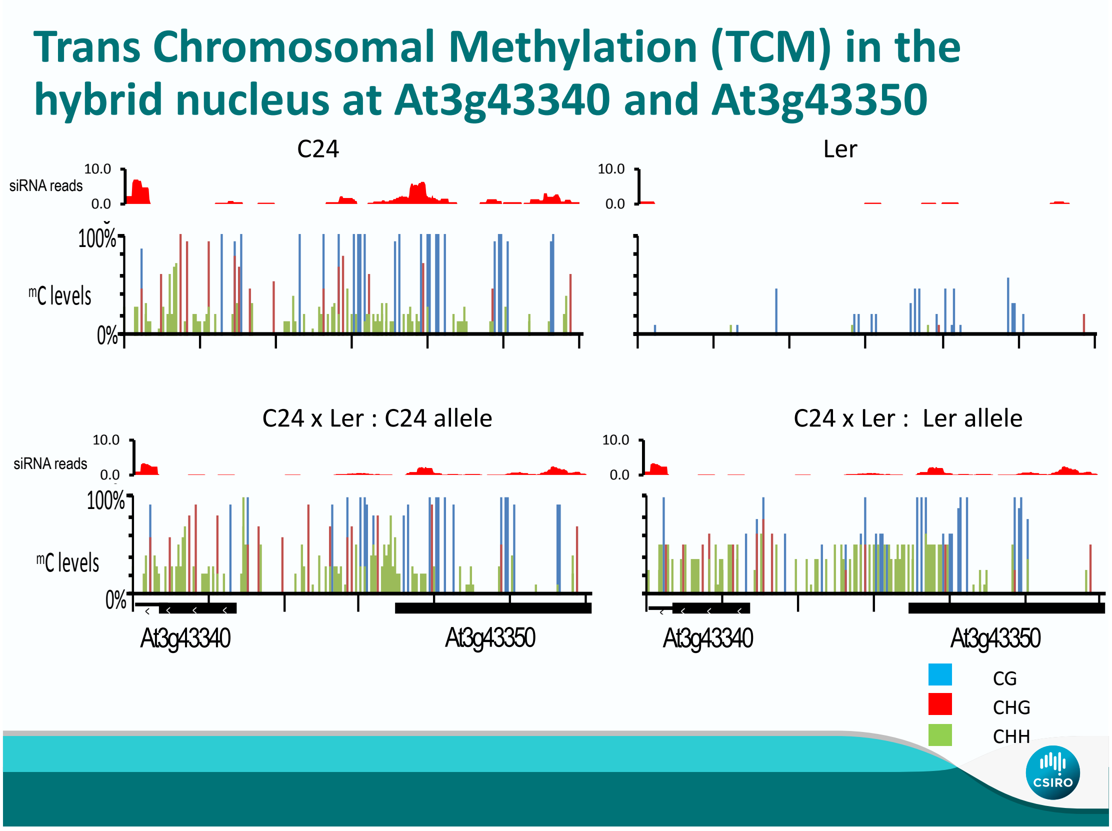
Hybrids have reduced levels of 24nt siRNA

15 das aerial tissue

A new methylation process in hybrids

Given that 24nt siRNAs direct DNA methylation processes we characterised the methylome of parents and hybrids. We found an altered methylation pattern in the hybrid that like the small RNA changes occurred in loci where the two parents had different levels of methylation. The altered methylation levels in the hybrid were generated by a completely new process we have termed Trans Chromosomal Methylation (TCM) and Trans Chromosomal deMethylation (TCdM) in which the methylation pattern of the heavily methylated allele is transferred to the second lightly methylated allele in the nuclei of the F1 hybrids. The transferred pattern involves all three cytosine sequence contexts. We found there are about 800 locations of TCM in the genome in our crosses, enough to alter expression in many genes.

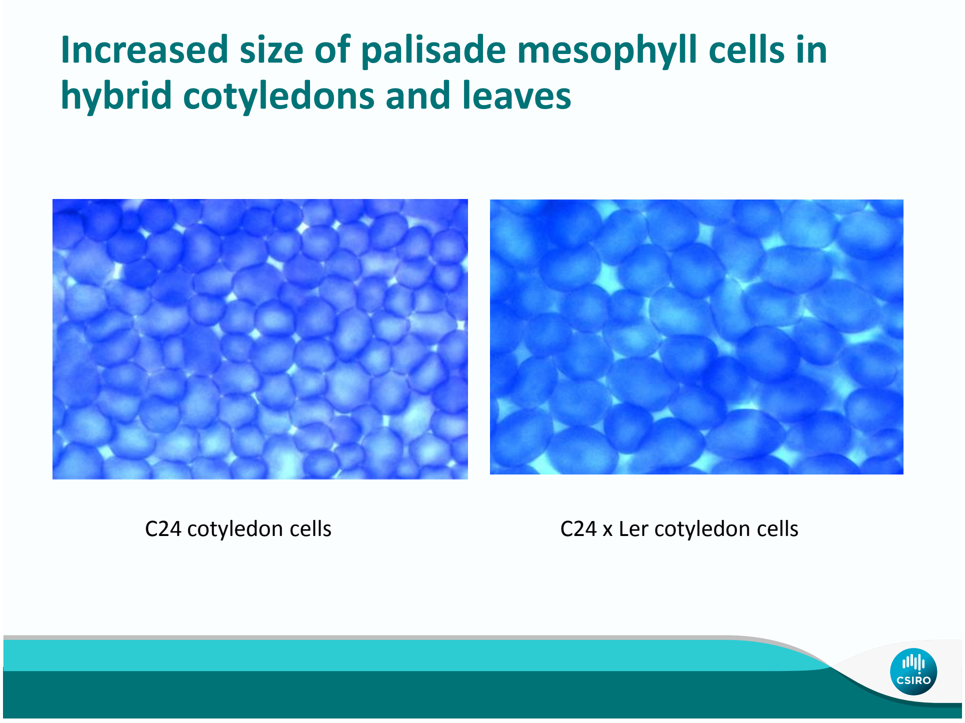
The new TCM patterns are still present in the F2 generation This and other evidence we have, shows that the F1 TCM patterns can be inherited and can be passed to the F2 generation or in backcrosses to plants of the backcross generation.



Trans Chromosomal Methylation (TCM) in the hybrid nucleus at At3g43340 and At3g43350

Photosynthesis is important for hybrid vigour

So which genes are important for controlling heterosis? We know hybrids have larger leaves and these hybrid leaves must be larger because of increased cell size or cell number or some combination of both.. We have determined that in all hybrids the increased leaf size is due to the increased number of palisade mesophyll cells and presumably other cells in the leaves and cotyledons of the hybrids.



Increased size of palisade mesophyll cells in hybrid cotyledons and leaves

C24 cotyledon cells

C24 x Ler cotyledon cells

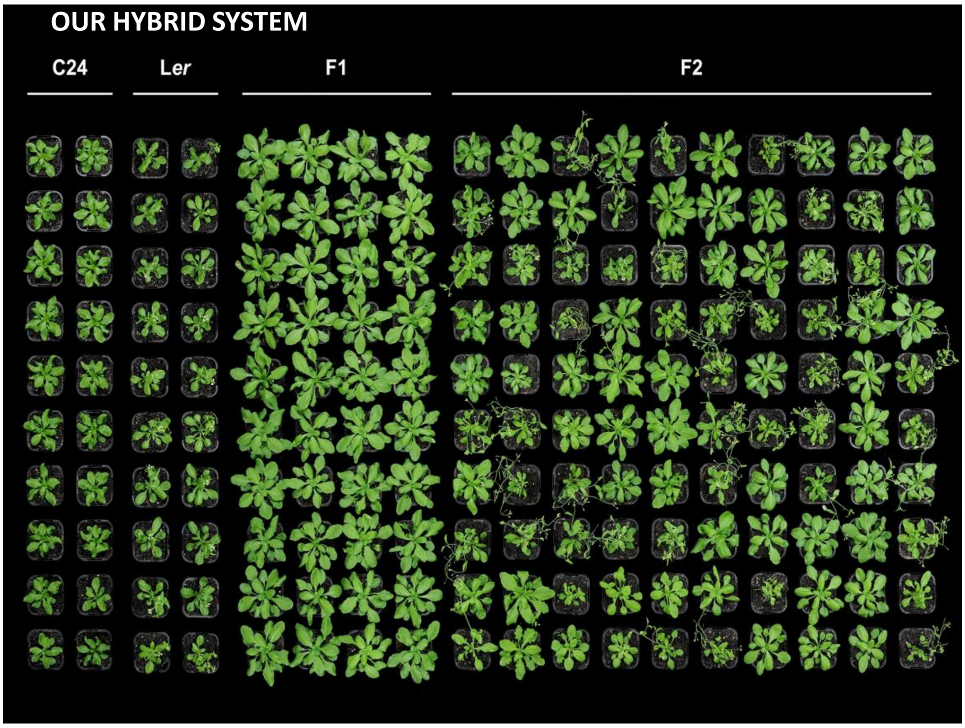
In some of the hybrids there is an additional component of increased size of the mesophyll cells. This will lead to more chloroplasts and chlorophyll in the plant and presumably more photosynthate. We found increases in activities in the early stages of the germinating seedling in 14 out of 16 chlorophyll pathway genes over the period of 3-7 days post germination. There are 187 genes upregulated at this time of development which are targeted to the chloroplast and concerned with chloroplast function or photosynthesis. Thus all these factors are concerned with the structure and operational activities of the chloroplast and are probably associated with the causes of the larger leaf phenotype.

When we look at photosynthesis the parents and the hybrids have equal levels of efficiency of the photosynthetic process ie photosynthesis per unit area, but the total photosynthetic capacity of the hybrid is greater than the parents because of the larger leaf size. The suggestion immediately arises that increased photosynthetic capacity of the hybrids may have a lot to do with the increased vegetative and reproductive yield. We have checked this in one way by providing a photosynthetic inhibitor in early seedlings and found heterosis was completely eliminated and the leaf area of the hybrids is no longer greater than the parents.

Selection for Hybrid Mimics from the large plants of the F2

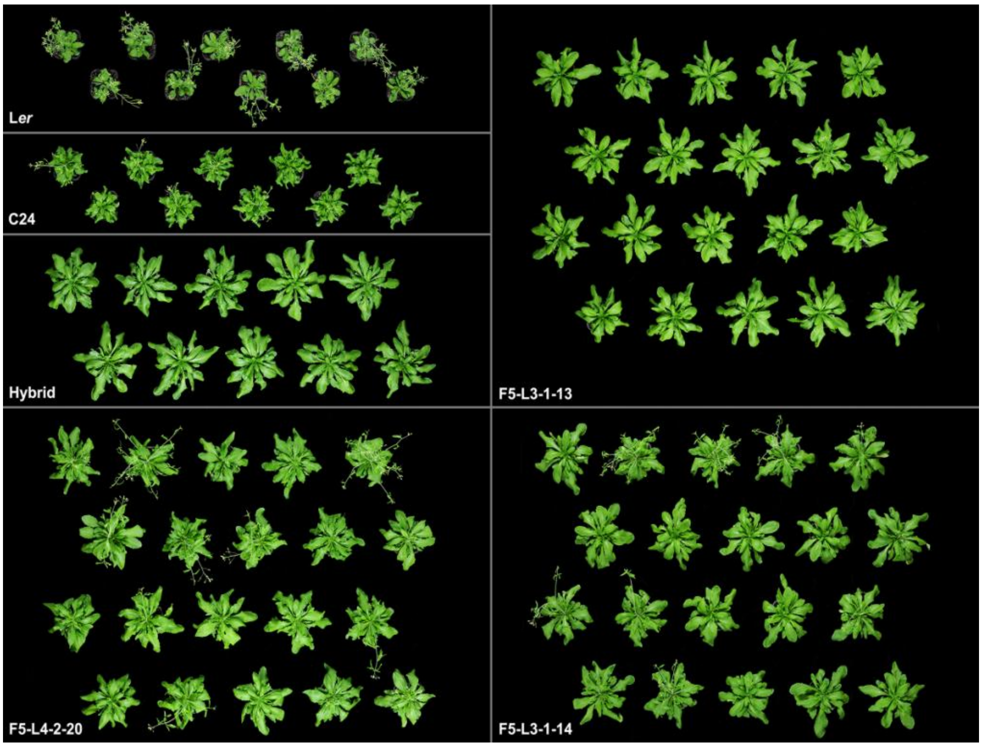
Returning to a major feature of hybrid systems, the size and yield advantage is restricted to the F1 generation. The F2 has heterogeneous plant morphologies, some comparable to F1 plants, being large and non-flowering at this stage, others present a range of biomass sizes and flowering times. Farmers who have explored the prospect of keeping seed from the F1 to generate next generation planting seed, quickly gave up when they were faced with the lack of homogeneity of the F2 and went to the seed store and bought fresh F1 planting seed.

We chose large plants from the F2 which were very similar to the F1 and self fertilised them. We thought the F1-like plants may have retained the chromosomal segments (genes) and associated epigenetic controls which are important in generating a phenotype similar to the F1 plants. We carried out recurrent selection based on large plant morphology and found progressive reduction of morphology heterogeneity and increased size in each successive progeny



This figure shows the parents and the F1. The F2 has much greater heterogeneity but with successive generations of selection for the large plants the heterogeneity was reduced. By the F5 generation these plants approached the F1 in size and seed yield as well as in lack of variance.

By the F5 we had a number of independent lines which we felt we could term hybrid mimics



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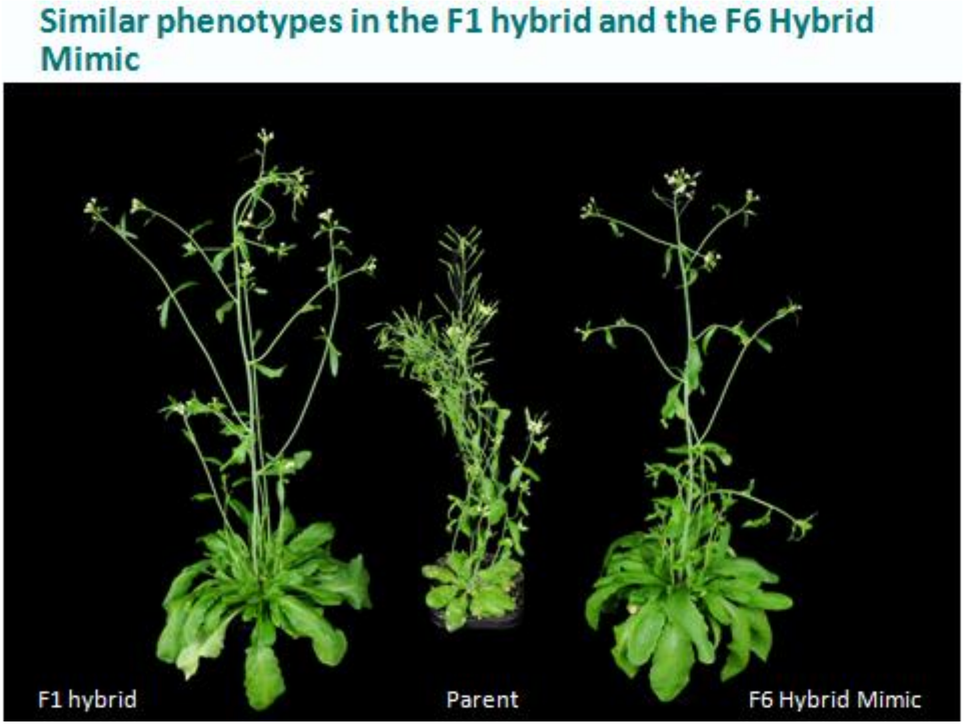
These plants cannot be thought of as hybrids any longer but are near homogeneous pure lines as judged by their morphology. By the F6 it is difficult to tell which is the hybrid and which the Hybrid Mimic. So here we have plants which faithfully mimic the F1 in size and yield and breed true.


Similar phenotypes in the F1 hybrid and the F6 Hybrid Mimic

Pathways important for Hybrid Vigour

We can use our hybrid mimics to define genes of importance in producing hybrid vigour and the hybrid mimic phenotype. As a first step of analysis we have identified the genes with altered expression common to four mimic lines and the F1 and checked their function. This identified a small number of key metabolic pathways and transcription factors, defence response genes were down-regulated, abiotic stress genes upregulated and plant hormone pathway genes were also upregulated. We are investigating these further.

Altered epigenetic states can be maintained in the Hybrid Mimics

The two genes we showed earlier to have TCM events transferring the C24 allele methylation pattern to the Ler alleles in the F1 hybrid retained the methylation pattern in the recurrent selection for the hybrid mimic lines and had similar reductions in transcriptional activity. These two loci and their epigenetic DNA methylation patterns are present as functional units in the F4 hybrid mimic lines, the same as the F1.

We have other examples in other chromosomal segments of TCM induced changes in the F1 also being present in the F4 mimics. These are the first examples of the novel DNA methylation process (TCM) occurring in the F1 and resulting in transcriptional alterations associated with the hybrid formation being retained in the selection process for the large plant phenotype of the hybrid mimics.

The hybrid mimics may provide a useful alternate breeding system for crops which do not have a hybrid system. They may also remove the need for farmers to buy seed each generation and the key genes and pathways may help us identify useful parents for enhanced vigour

Conclusion

I have tried to show you we have made great progress in understanding the basis of hybrid vigour. We have established that epigenetic control of gene activity is a major factor. We have discovered a new process Trans Chromosomal Methylation in one of the major epigenetic controls namely DNA methylation that only occurs in the hybrid

We wondered how this might come about but realised it was through the action of the small RNAs. In the nucleus the RNAs can recognise both copies of the gene and methylate them both ensuring the full pattern is transferred and both alleles have the same methylation pattern.

These processes can change gene activity, and as there are about 800 TCM events in the genome this can affect the activity of many genes.

Now turning to the hybrid plant, hybrids have larger leaves with more cells and in some cases larger cells. The larger leaves mean more photosynthesis which finally must be the basis of the increased yield of hybrids

The hybrid mimics were a big surprise but may be useful in crops.

Now the results we have obtained are in Arabidopsis but we have every reason to believe they will hold in crops. And the previous dogma of genetic distance determining the level of heterosis may actually be dependent on epigenetic distance between the parents.

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