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Genetic change and cryopreserved seed

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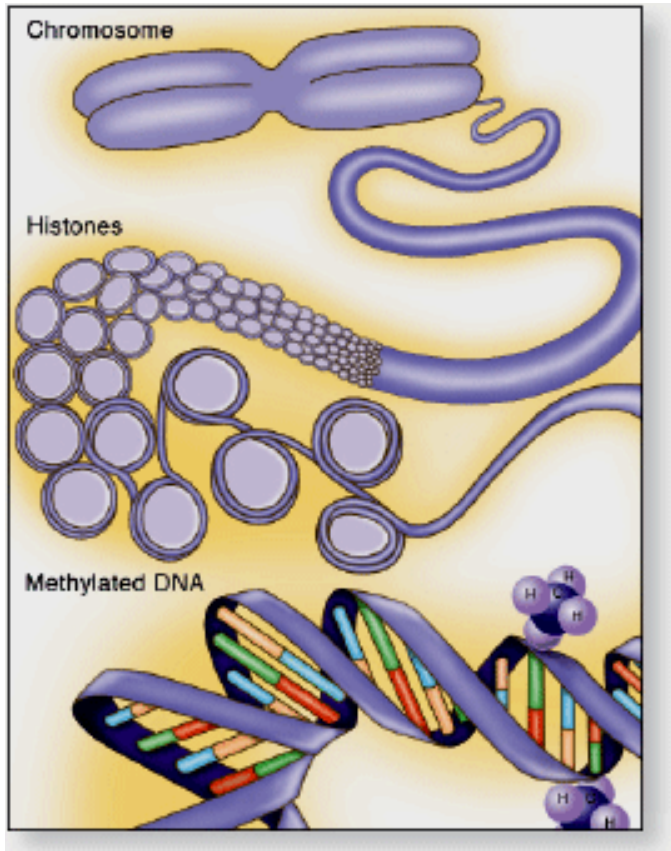
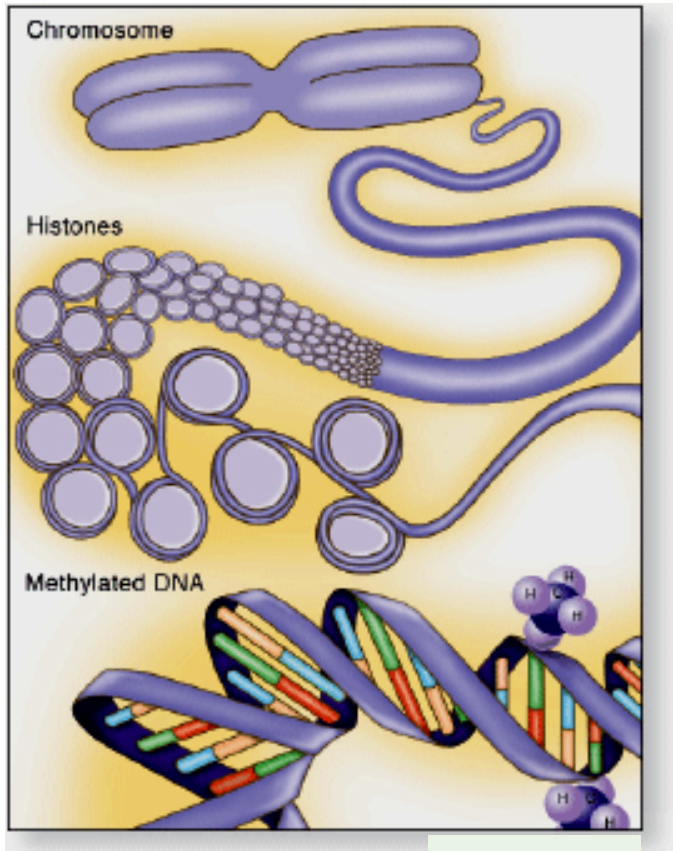
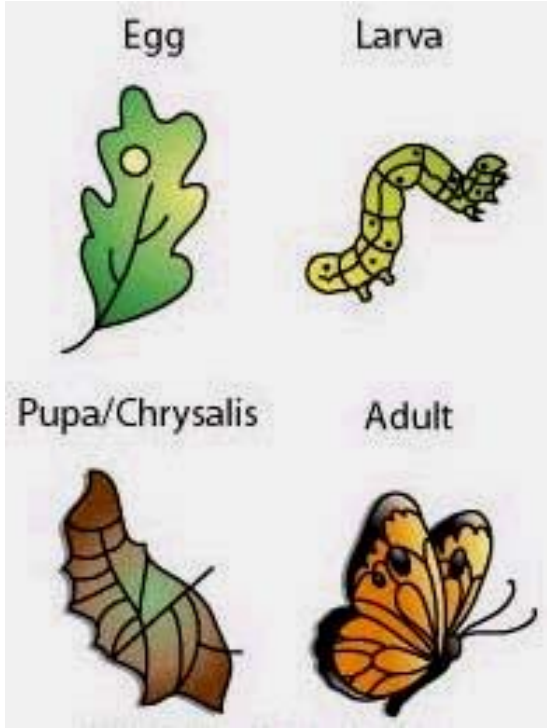
Frozen storage and genetic stability

- Liquid nitrogen storage reduces water, and other molecular activity, to the practical minimum,-20^{0}C less so
- If viability is preserved then change to the genome (gross damage or mutation) during storage is essentially eliminated in most cases
- Other molecular events, including mobility may compromise viability in some circumstances
- New research is looking towards gene expression & the epigenome



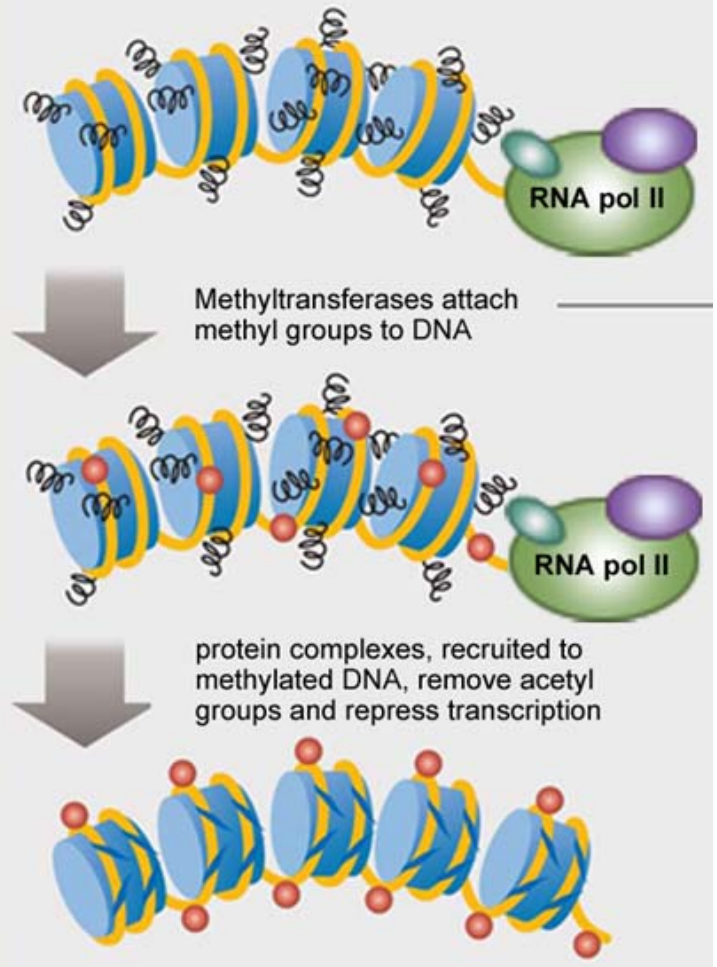
The action of the epigenome – on/off switches



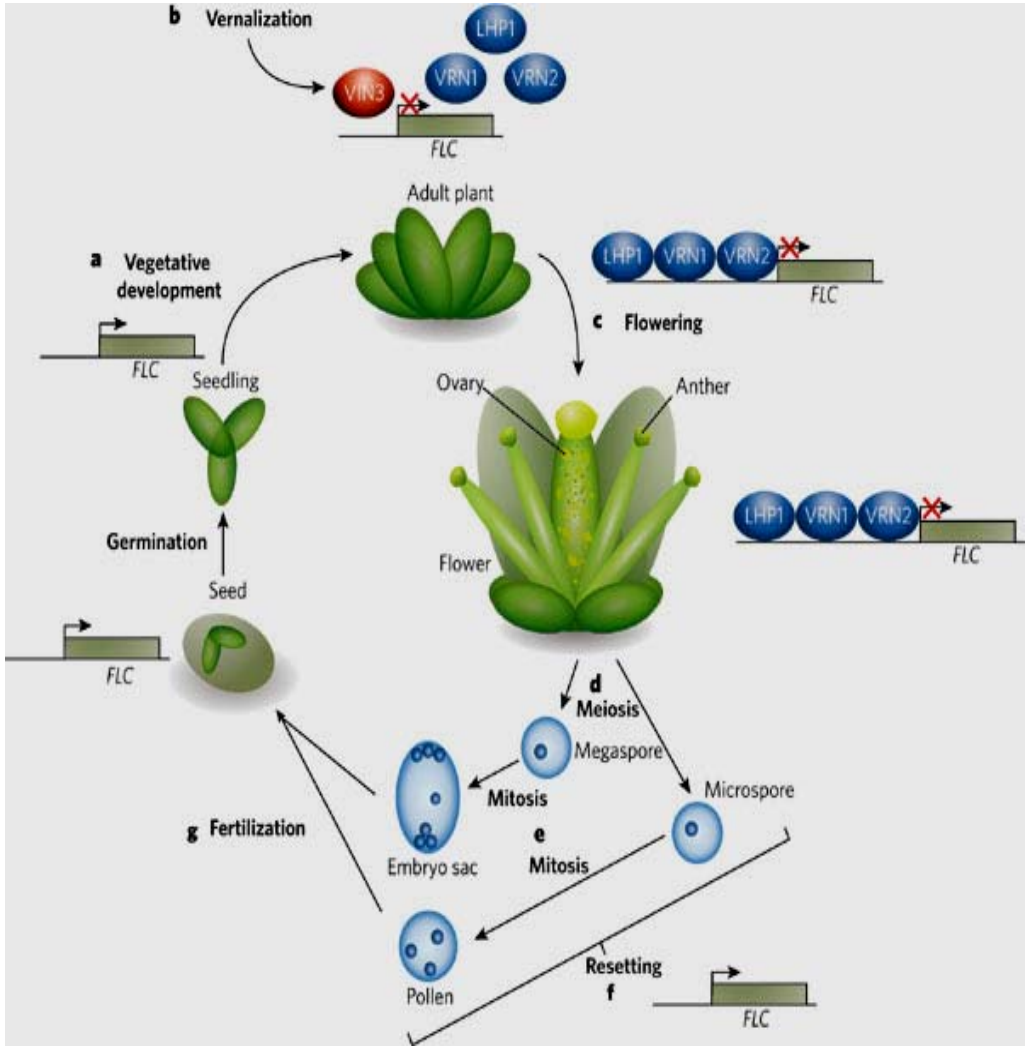


Precise developmental patterns

Blocking gene expression



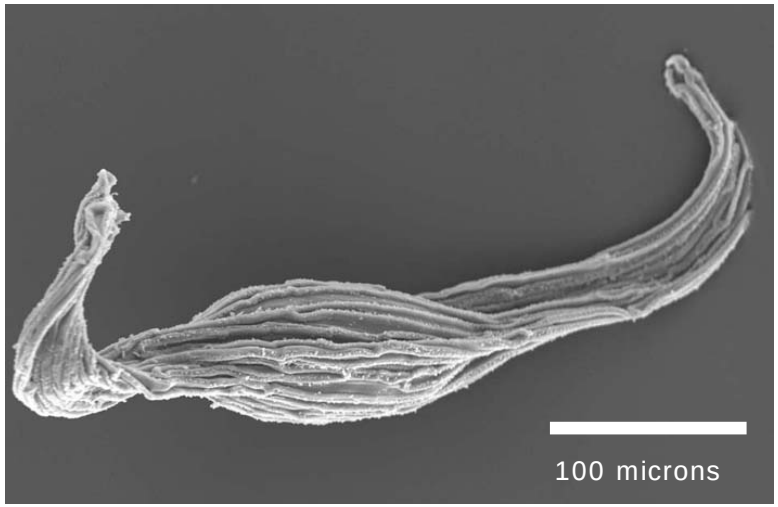
- A common epigenetic control mechanism
  - methyl groups and proteins block the transcription mechanism so that the information in the gene cannot be used
- Epigenetic action is important in plant development



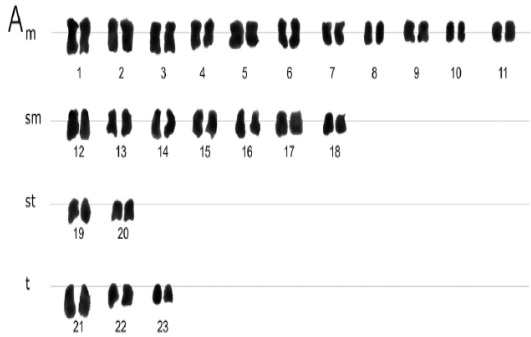
- An environmental stress e.g. low temperature can result in changes in methylation and protein binding to DNA
- This switches the gene off and alters the pattern of development
- During seed formation the situation resets itself, but there are exceptions
- The significance for seed storage?

Epigenetic inheritance in plants
I. R. Henderson & S. E. Jacobsen
Nature 447, 418-424. 2007

Cryopreserving the epigenome in orchid seed

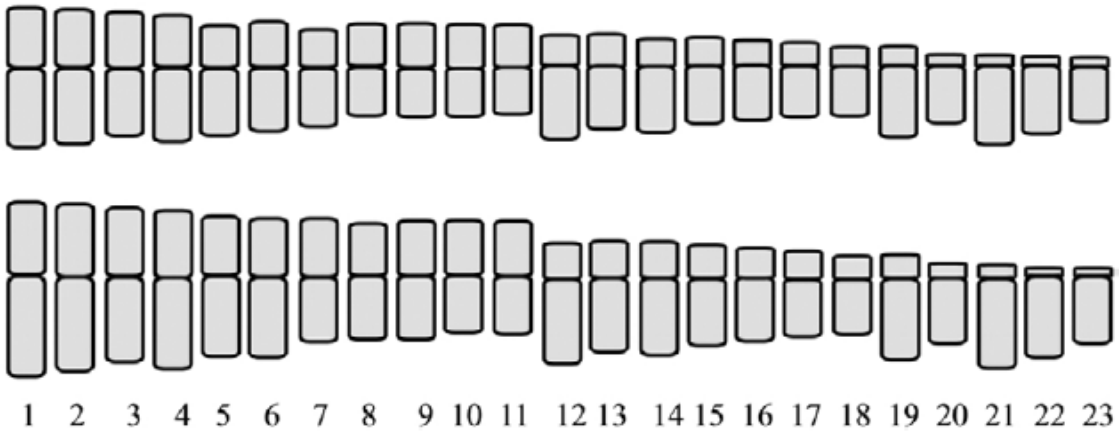


The epigenome, cryopreservation and development



TL: 1.62 ± 0.14a
p: 0.51 ± 0.02a
CI: 31.41 ± 1.62a

TL: 1.86 ± 0.13b
p: 0.66 ± 0.02b
CI: 35.48 ± 1.67b



Surenciski, M. R., Dematteis, M. & Flachsland, E. A. 2007: Chromosome stability in cryopreserved germplasm of *Cyrtopodium hatschbachii* (Orchidaceae). — *Ann. Bot. Fennici* 44: 287–292.

Cyopreservation & methylation in A. thaliana

Table 3. MSAP band type changes during the treatments

Band type	Controls→plants treated without freeze-thawing	Controls→ plants after cryopre-servation	Plants treated with-out freeze-thaw ing→plants after cryopreservation
I → –	2	0	2
II → –	1	0	15
III → –	5	4	0
– → I	5	5	5
– → II	15	1	2
– → III	2	3	2
I → II	8	6	1
II → I	2	3	3
I → III	5	6	5
III → I	4	5	5
III → II	1	0	0
Total	50	33	40

I: type I band; II: type II band; III: type III band; –: absence; →: change.

Wang, et al, Effect of cryopreservation on the development and DNA methylation patterns of *Arabidopsis thaliana* Life Science Journal, Vol 6, No 1, 2009

DNA methylation,seed viability & embryo development

Table 1. Effect of Mutations in DNA Methyltransferase Genes on Embryogenesis and Seed Viability

	Genetic Cross		Abnormal F1 Embryos[a]	
Self-Pollinated	Female	Male	%	*n*
Wild type	Arabidopsis		0	967
met1-6/met1-6			33	568
cmt3-7/cmt3-7			3	578
MET1/met1-6			10	562
cmt3-7/cmt3-7			23	816
MET1/met1-6				
	met1-6/met1-6	Wild type	16	550
	Wild type	*met1-6/met1-6*	8	822
	MET1/met1-6	Wild type	8	420
	Wild type	*MET1/met1-6*	5	403

[a] Embryos at 1 to 6 d after pollination were examined by whole-mount seed clearing.

Wenyan Xiao, Kendra D. Custard, Roy C. Brown, Betty E. Lemmon, John J. Harada, Robert B. Goldberg, and Robert L. Fischer **DNA Methylation Is Critical for *Arabidopsis* Embryogenesis and Seed Viability** Plant Cell 18: 805-814, 2006

Implications for seed storage

Conventionally:

- Epigenetic change may affect developmental process e.g. rooting and flowering
- Preserving the epigenetic status is necessary to preserve typical development
- In seeds and tissue culture (meristems), cryopreservation should preserve the epigenetic status

Innovatively

- Epigenetic changes can be inherited via seed and can confer performance advantages on (at least) the next generation
- Epigenetic priming
- Cryopreservation must be able to preserve this epigenetic condition

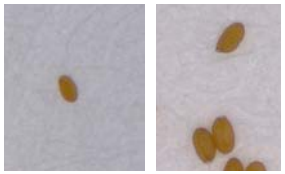
Arabidopsis seed production under water stress

www.sysbio.uzh.ch

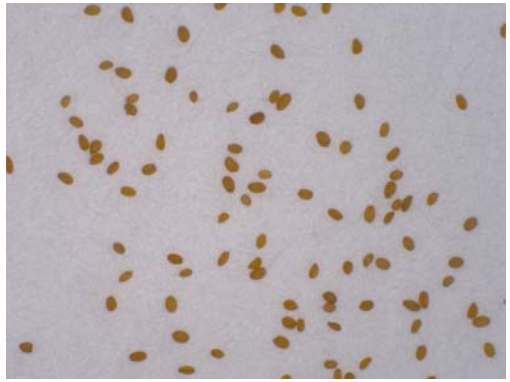
www.mdpi.com

Heritable water stress* responses in A. thaliana

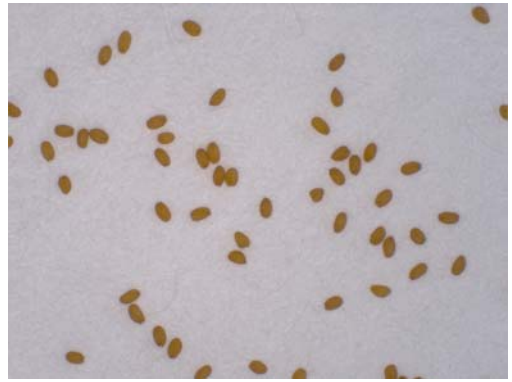
Increased seed size



control



stressed

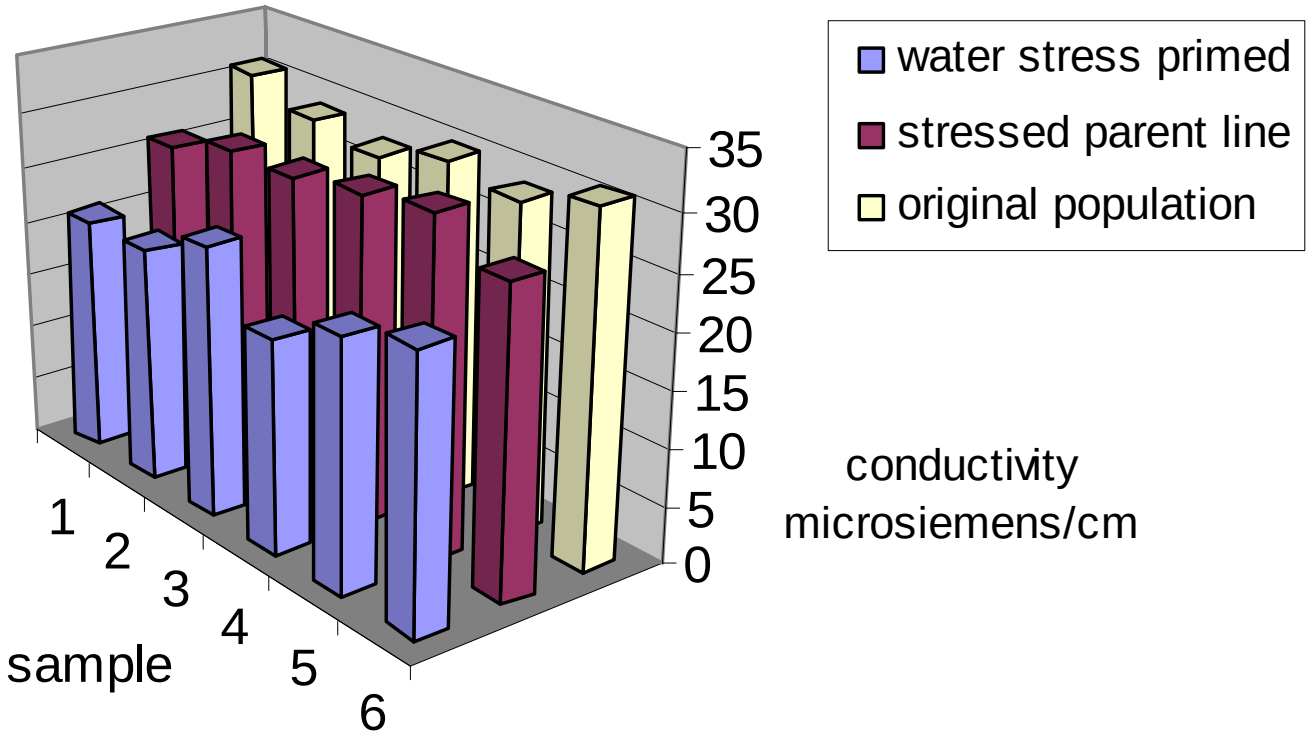


Fewer, smaller leaves with an increased stomatal density –greater control over water relations

No significant reduction in absolute growth rate

(*0.35gH_2O/g dry soil – 50%)

Heritable epigenetic response to water stress in A. thaliana



Electrolyte leakage from osmotically stressed leaf discs

Epigenetics and cryopreservation

- Epigenetic priming may give seed crops a growth, development and/or survival advantage where re-occurring environmental stresses are expected
- The parent line has to be grown under conditions of controlled stress
- Frozen seed storage must not disrupt the pattern of methylation/epigenetic regulation
- Epigenetic stability should become a part of routine analysis to confirm successful frozen seed storage

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