

# The Honey Bee Epigenomes: Differential Methylation of Brain DNA in Queens and Workers

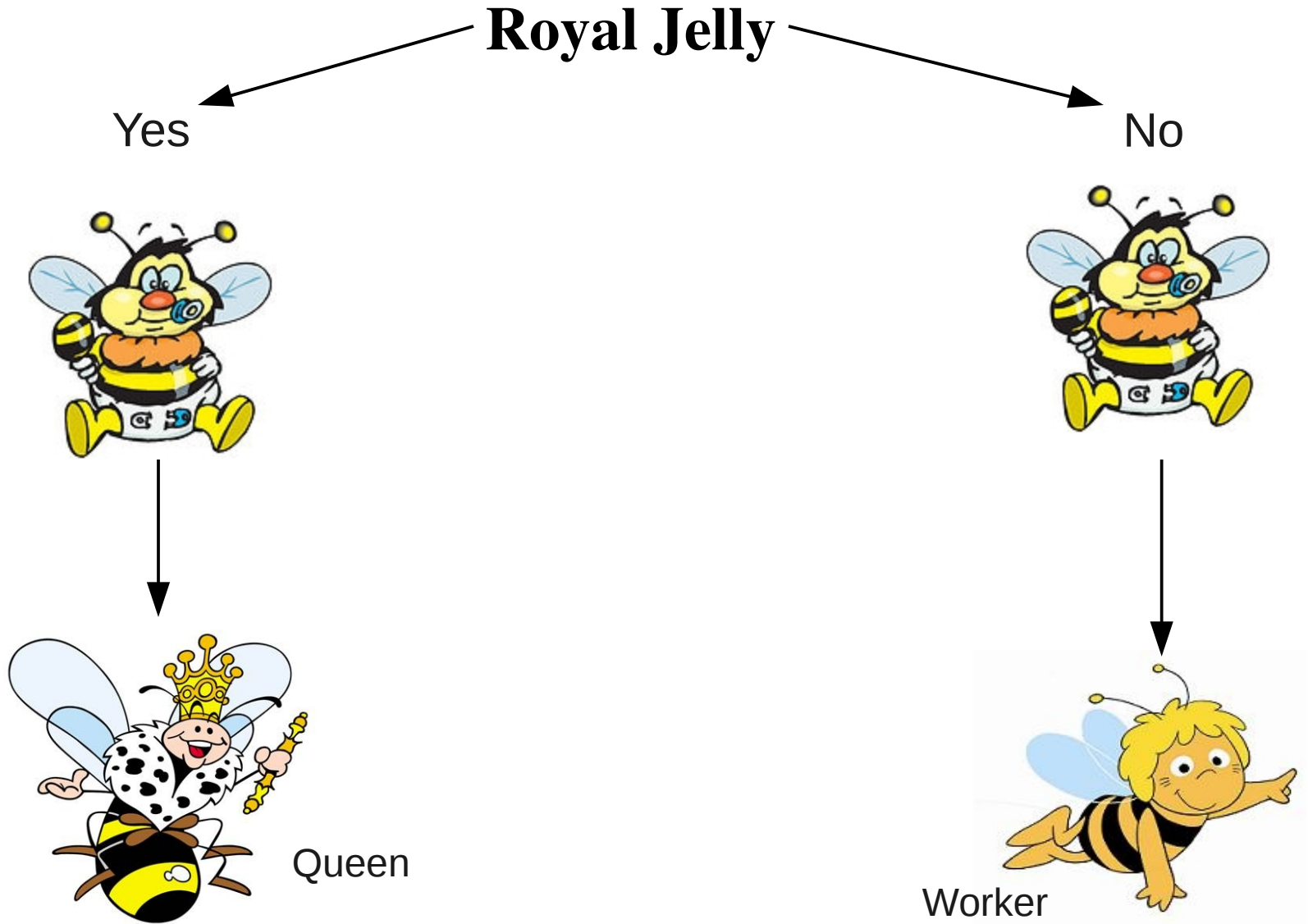
**Frank Lyko, Sylvain Foret, Robert Kucharski, Stephan Wolf,  
Cassandra Falckenhayn, Ryszard  
Maleszka**

# Queens vs Workers

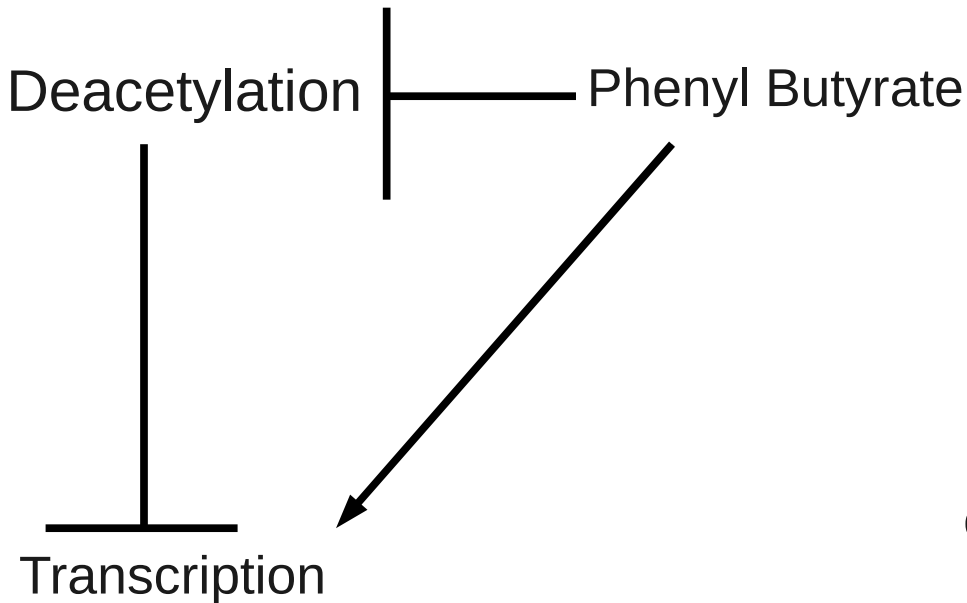


- Size
- Morphology
- Fisiology
- Longevity
- Behaviour

# Different feeding of royal jelly determines the larval development



But different feeding may not be the only determinant factor

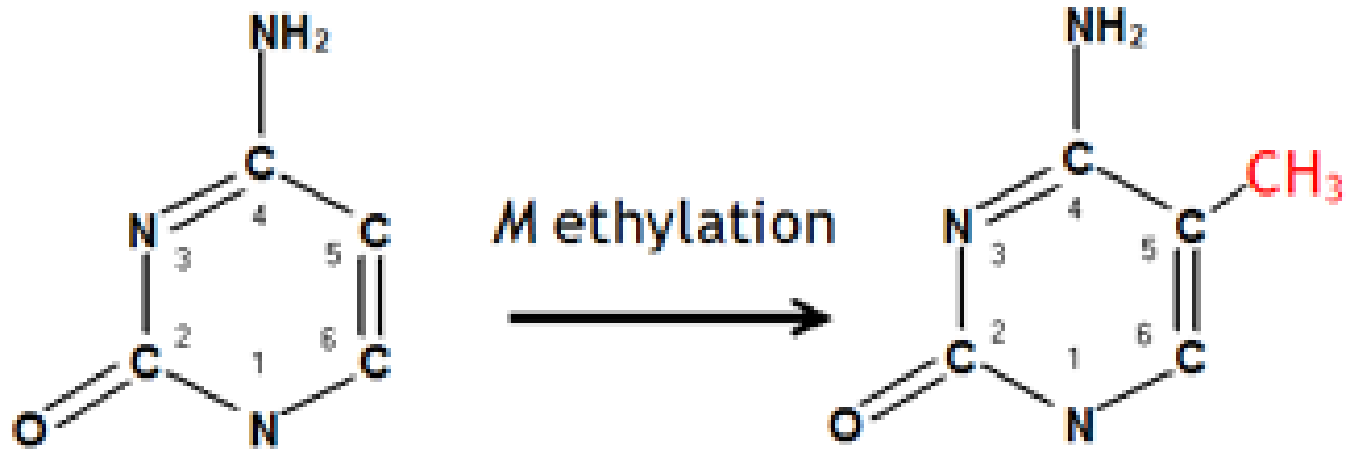


siRNA against DNMT3 originates queen-like phenotypes

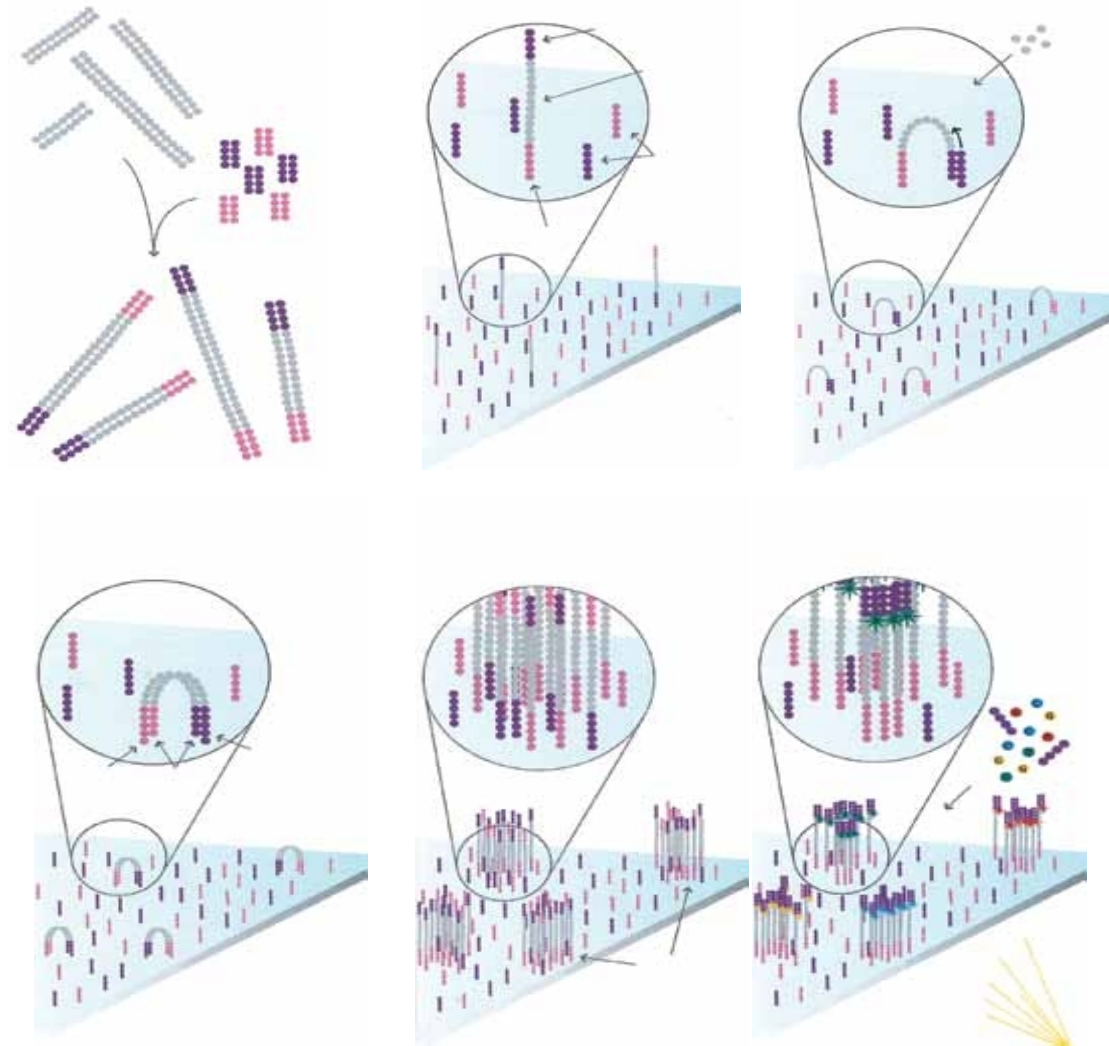
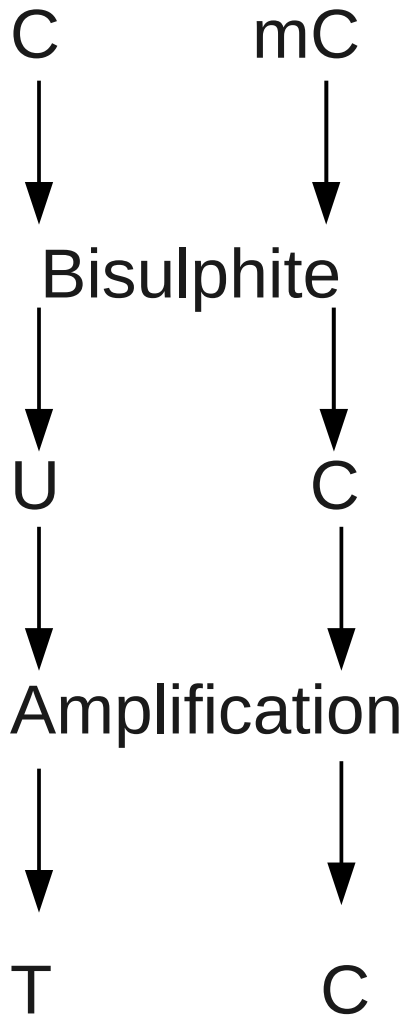


Different methylation may be involved in development of caste-specific phenotype and behaviour

# Cytosine Methylation

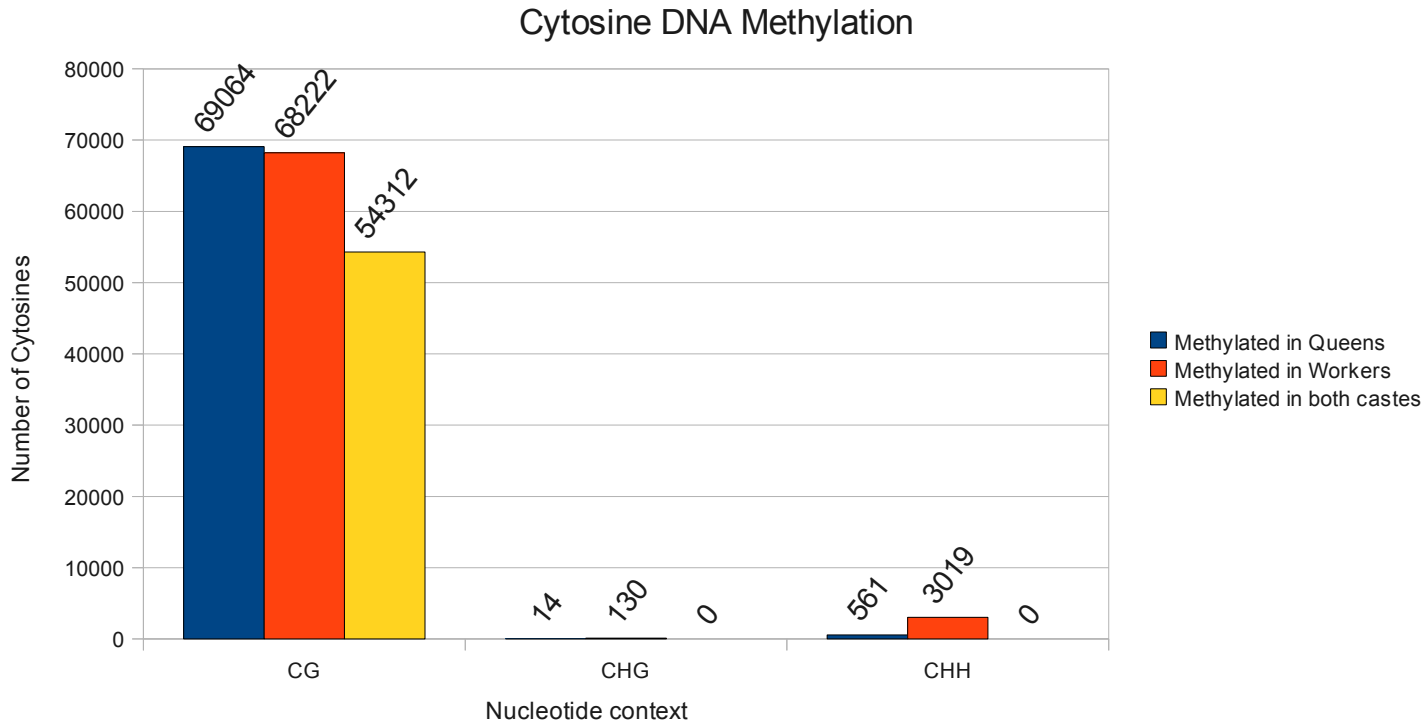


# Methylation assay with shotgun bisulfite sequencing technology



# Results

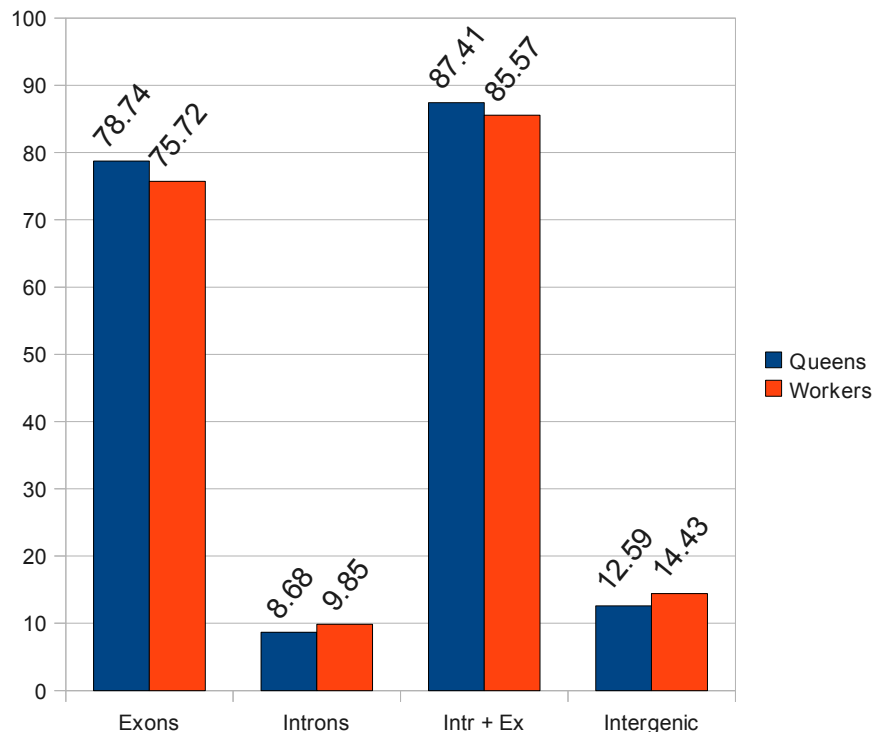
- Methylated cytosines (mCs) are only 70000 against the 60 milion of the entire genome
- mCs are found mainly in the CpG dinucleotide
- The overriding majority of these mCs are in exons



H= A, T or C

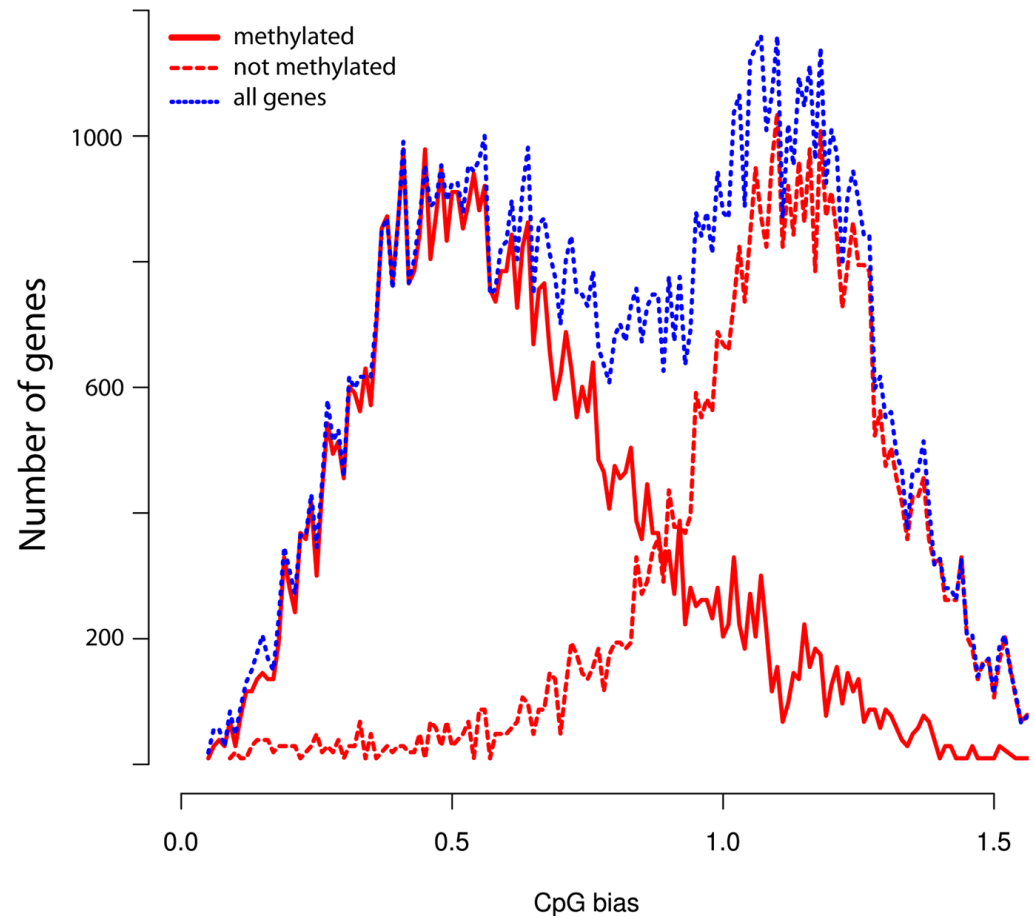
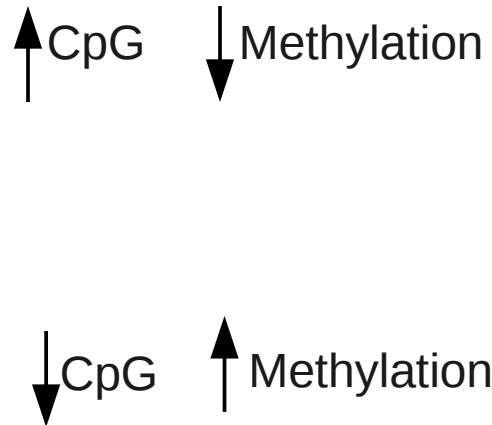
# Results

- Methylated cytosines (mCs) are only 70000 against the 60 million of the entire genome
- mCs are found mainly in the CpG dinucleotide
- The overriding majority of these mCs are in exons

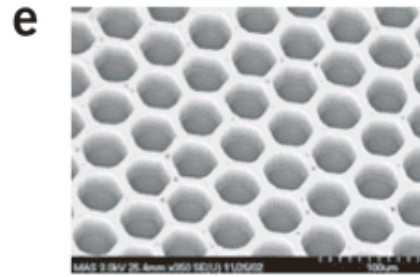




# Low CpG containing exons are more methylated than high CpG containing ones

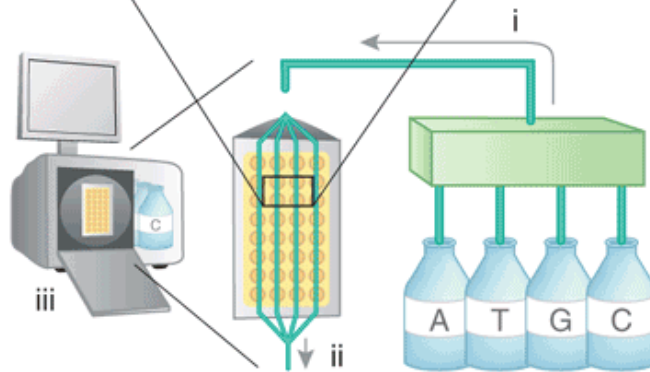
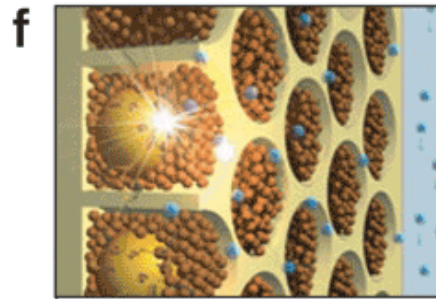
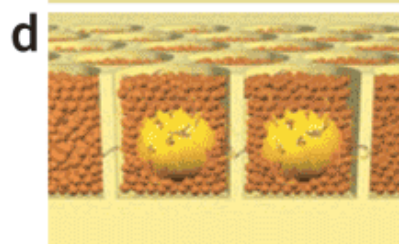
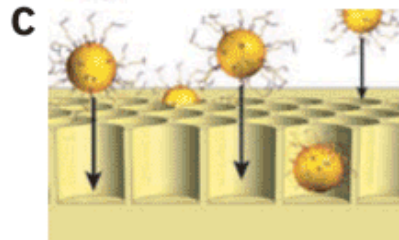
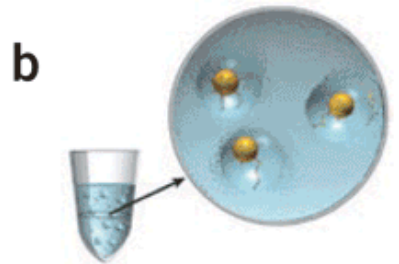


# Amplicons analysis with 454 sequencing technology



Sequence specific primer

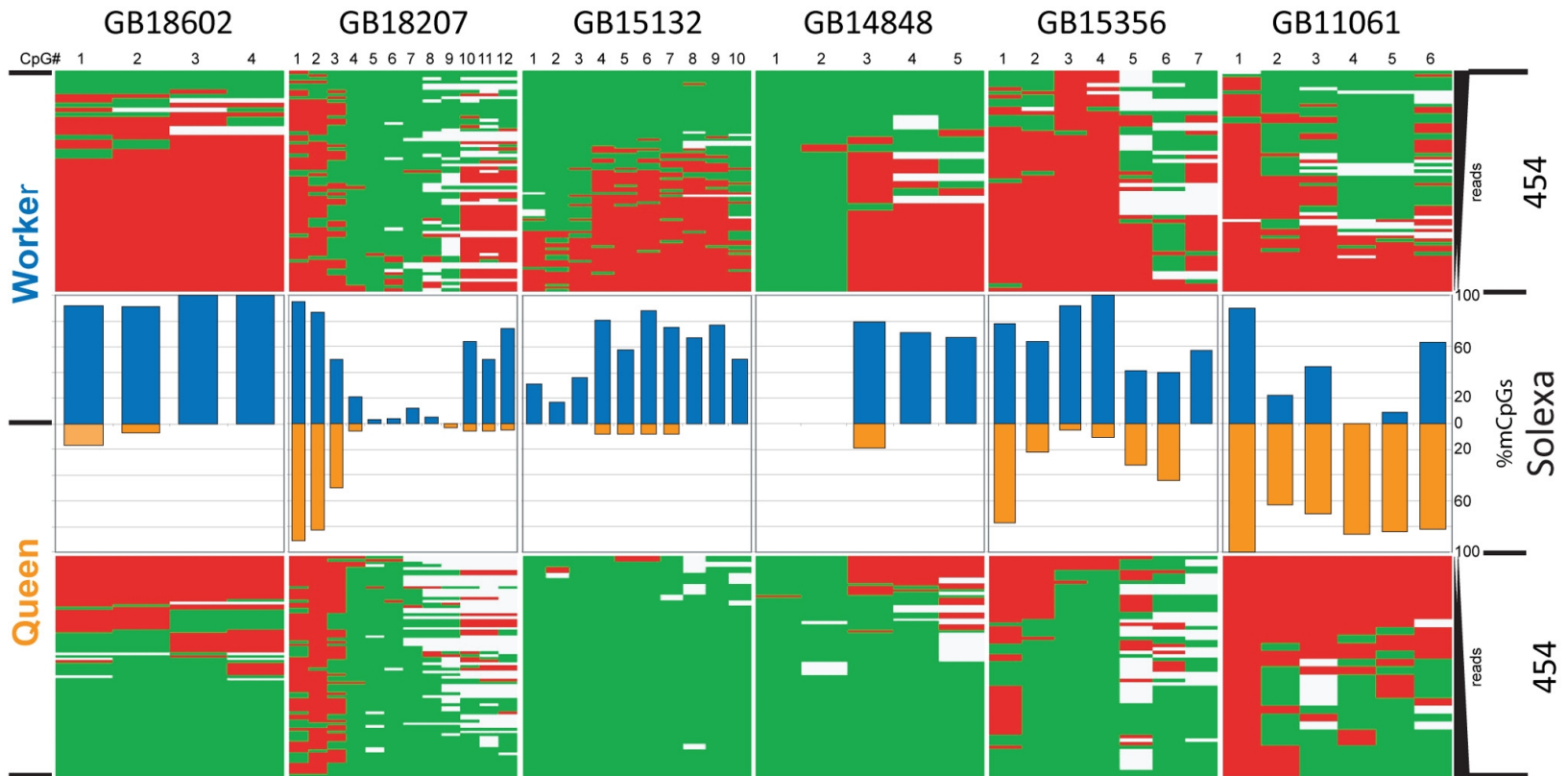
Adapter



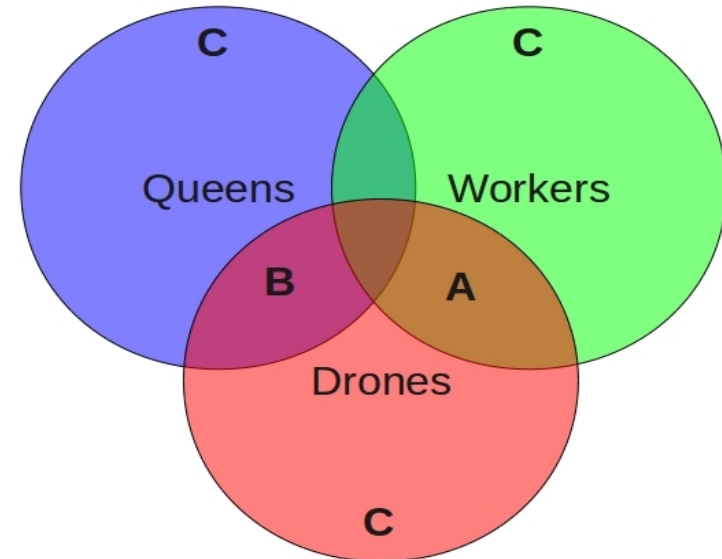
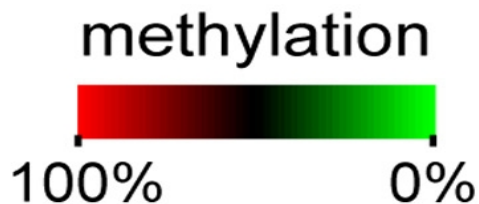
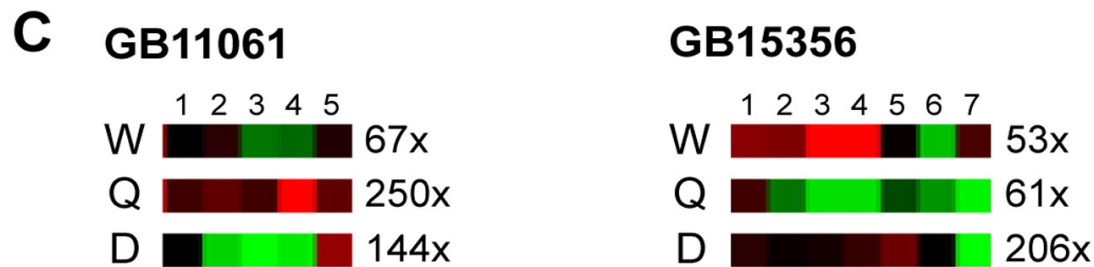
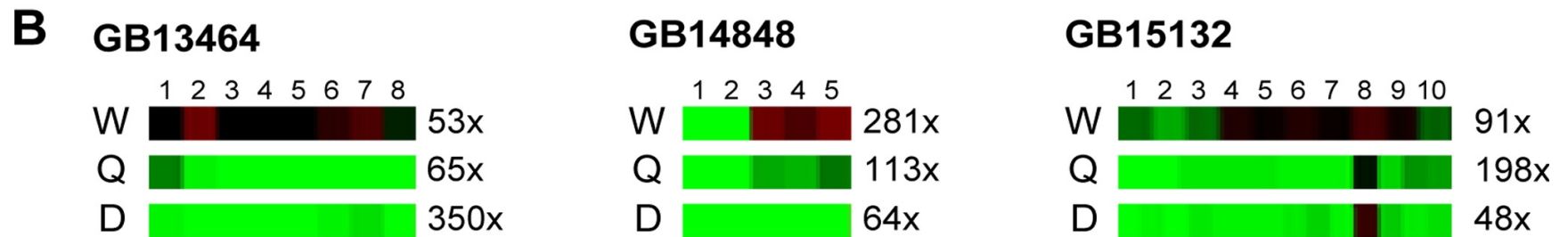
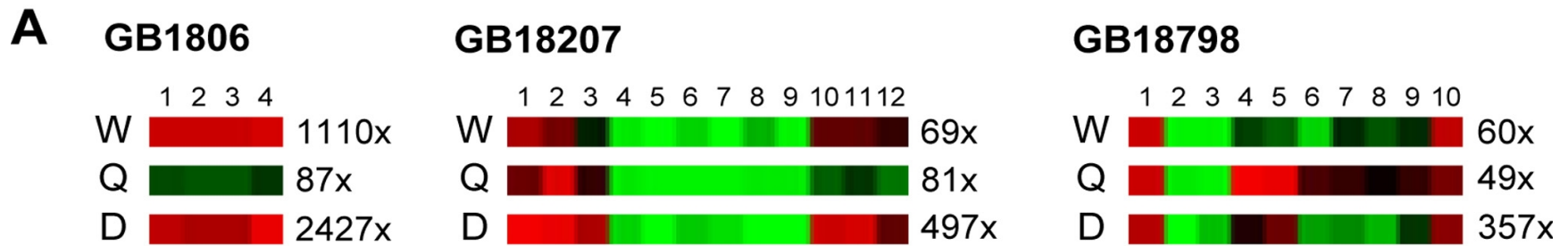
To validate Solexa results, 8 nuclear genes and 4 mitochondrial genes were selected to be sequenced with the 454 sequencing technology

# Methylation analysis results

Results from Solexa sequencing and 454 sequencing are very similar, suggesting that data obtained from Solexa sequencing is trustworthy



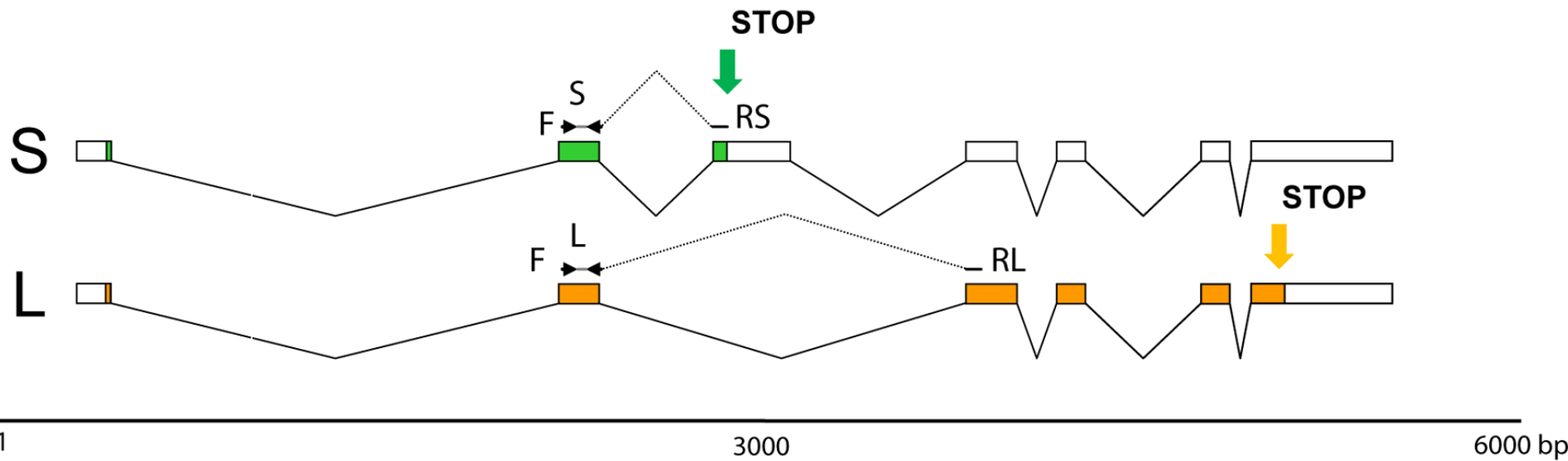
# Analysis of methylated genes in Queens, Workers and Drones



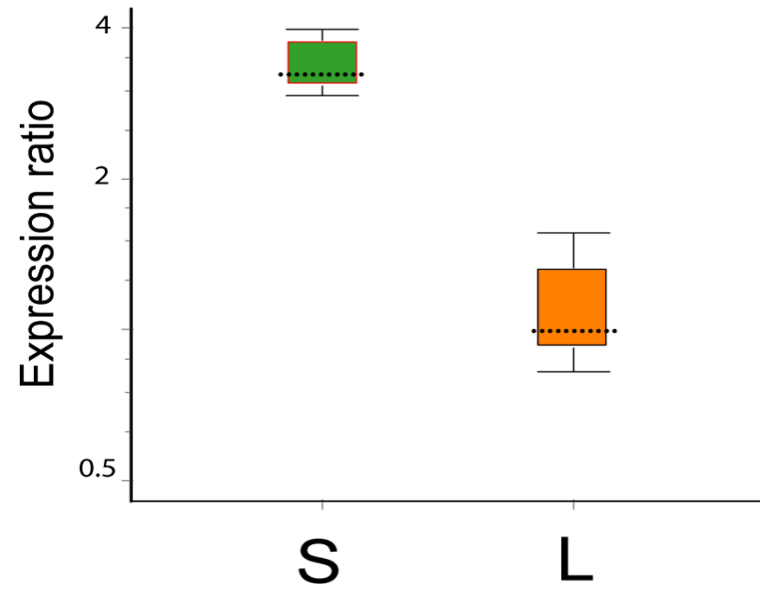
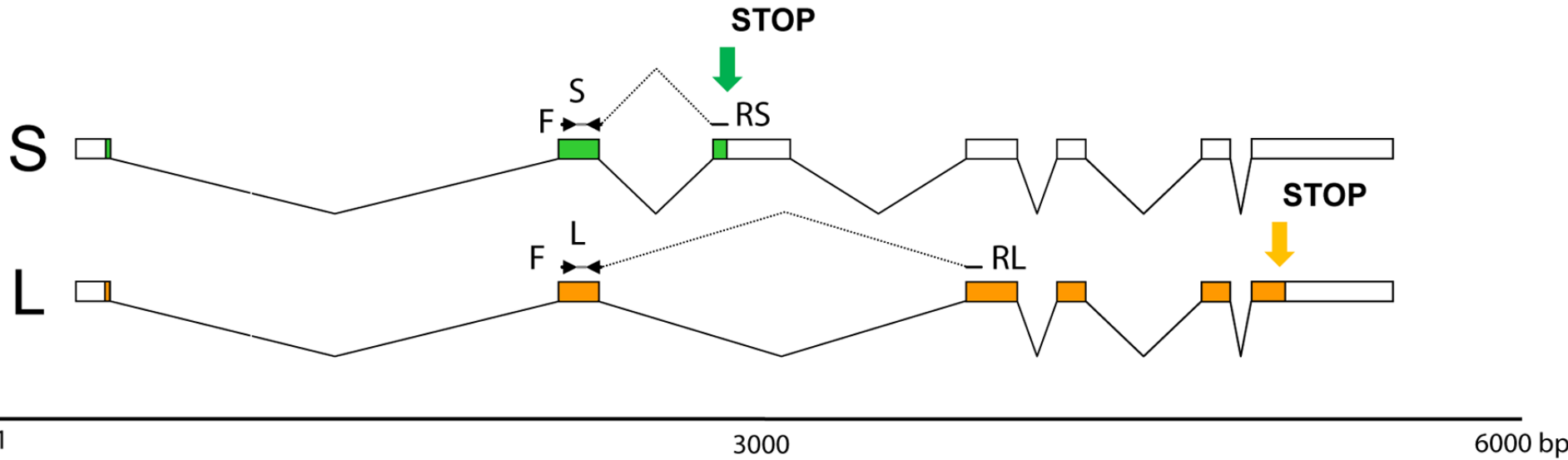
# Identification of Differentially Methylated Genes

Gene ID	No. of CpGs	Antenna	Brain	Relative Expression		Ovary	Thorax	Gene Annotation
				HPG	Larva			
GB18602	30	1	1	1	1	1	1	Transmembrane protein YhhN
GB18303	13	1	1	1	1	1	1	Activator protein of Rab-like small GTPases
GB13368	9	2	1	2	10	1	3	3-hydroxyacyl-CoA dehydrogenase, NAD-binding
GB13215	34	1	1	1	1	1	1	Glycine cleavage system P-protein,
GB15588	9	1	1	1	1	1	1	Low-density lipoprotein receptor domain class A
GB15132	24	1	1	1	1	1	1	TAP42 (regulates the TOR signaling pathway)
GB12560	12	1	1	1	1	9	1	RNA-binding protein
GB11648	13	1	1	1	1	2	1	Catalase
GB19645	12	1	1	1	1	1	1	Phosphodiesterase 6
GB12929	39	1	1	1	1	1	1	Paralytic - Na channel
GB11421	31	1	1	1	1	1	1	Tight junction associated protein
GB19503	33	1	1	1	1	1	1	Heat shock protein 8
GB13740	24	1	1	1	1	1	1	Dysfusion, TF with PAS domain
GB10394	8	1	1	1	1	1	1	TNF-receptor-associated factor 1
GB16628	9	10	6	8	10	10	10	Ribosomal protein L6

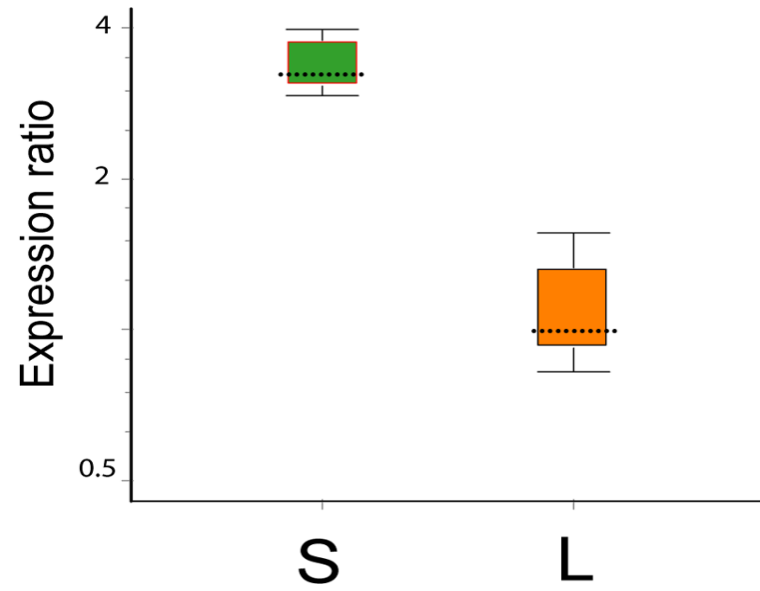
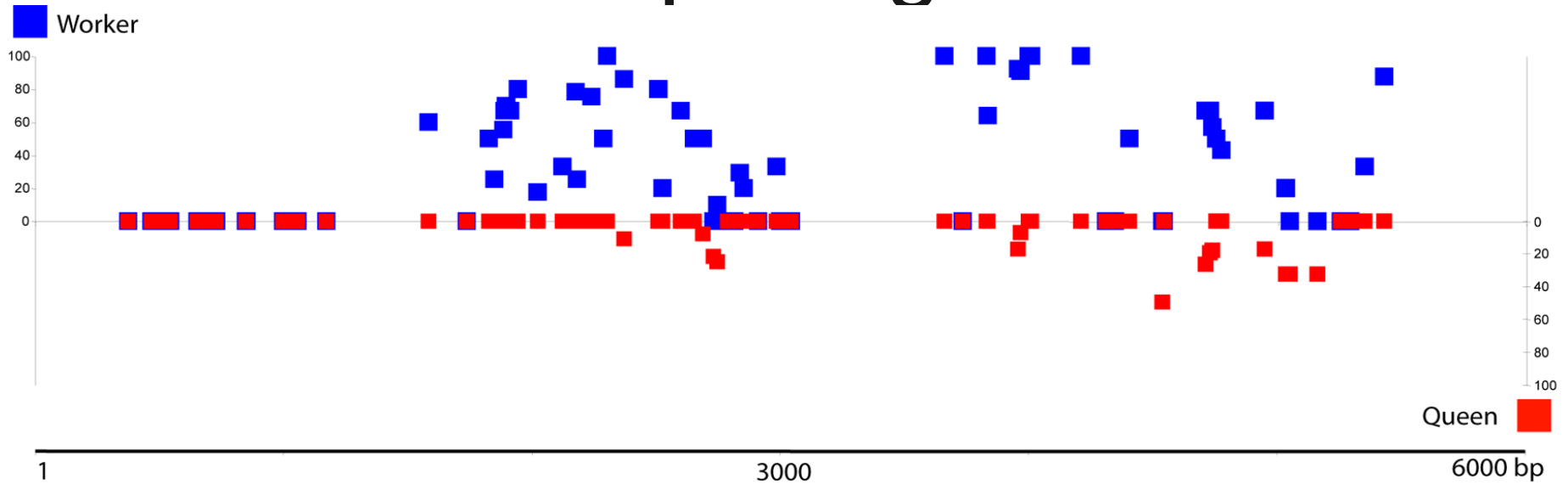
# Different methylation and splicing



# Different methylation and splicing



# Different methylation and splicing





# Conclusions

- At least 560 different genes are differently methylated in the brains of Queens and Workers
- Different methylation can lead to different protein expression profiles which can partially explain the differences in the behaviour and phenotype of these animals

Thank you  
for the  
attention!!!



# Link 454 sequencing

[http://www.roche-applied-science.com/publications/multimedia/genome\\_sequencer/amplicon\\_07/wbt.htm](http://www.roche-applied-science.com/publications/multimedia/genome_sequencer/amplicon_07/wbt.htm)