Occurrence and Distribution of DNA methylation in Insects

Veiko Krauss

Lehrstuhl für Genetik

Department of Genetics

UNIVERSITÄT LEIPZIG
Fakultät für Biowissenschaften, Pharmazie und Physiologie
Institut für Biologie II

DNA methylation

- is exclusively found as methylcytosine in eukaryotes
- concerns between 0 and 50 % of all cytosines
- deamination of methylcytosine to thymine is about 10 times more abundant than the analogous substitution of cytosine
- so, DNA methylation has a mutational cost
- general molecular function seems to be suppression of transcription, either of initiation (animals, plants) or of elongation (fungi)
- this function is suggested to be important for
- a) suppression of transposon activity
- b) regulated silencing of genes
- c) suppression of initiation of transcription inside of genes

Abundance and distribution of DNA methylation

- Thus, DNA methylation should become more important with

- a) the rise of somatic cell divisions and differentiations
- b) larger genes which increase the risk of internal transcriptional initiation

- At the same time, DNA methylation should be suppressed due to larger population sizes which tend to

- a) stronger select against the methylcytosine hypermutation effect
- b) shrink gene sizes
- c) stronger select directly against transposon activity

Conclusion: Strong differences in abundance and distribution of DNA methylation are expected in different eukaryotic species.

Why look for DNA methylation in insects ?

- Homo sapiens: 5%
- Drosophila melanogaster: <1% (only found in embryos)

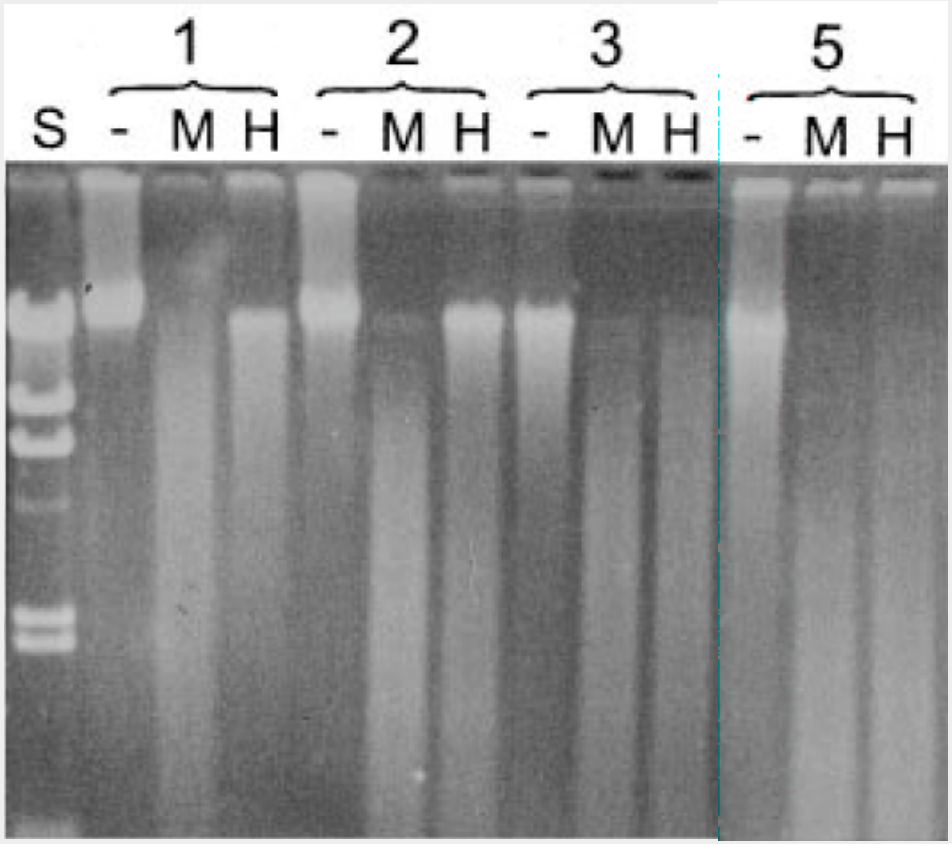
- according to the literature insects (mainly represented by Drosophila) might

a) have nearly lost DNA methylation or
b) have developed novel patterns (exclusively inside genes, not in heterochromatin) and functions of DNA methylation (genome-wide imprinting, genome stability)

- however, data on insect DNA methylation are rare and difficult to compare to other species

- Therefore, we searched for insect species with a relatively low population size and with high somatic cell turnover which may have patterns of DNA methylation more similar to vertebrates

A simple screen for genome-scale symmetric (CpG) cytosine methylation
=
A digestion using two differently sensitive iso-endonucleases

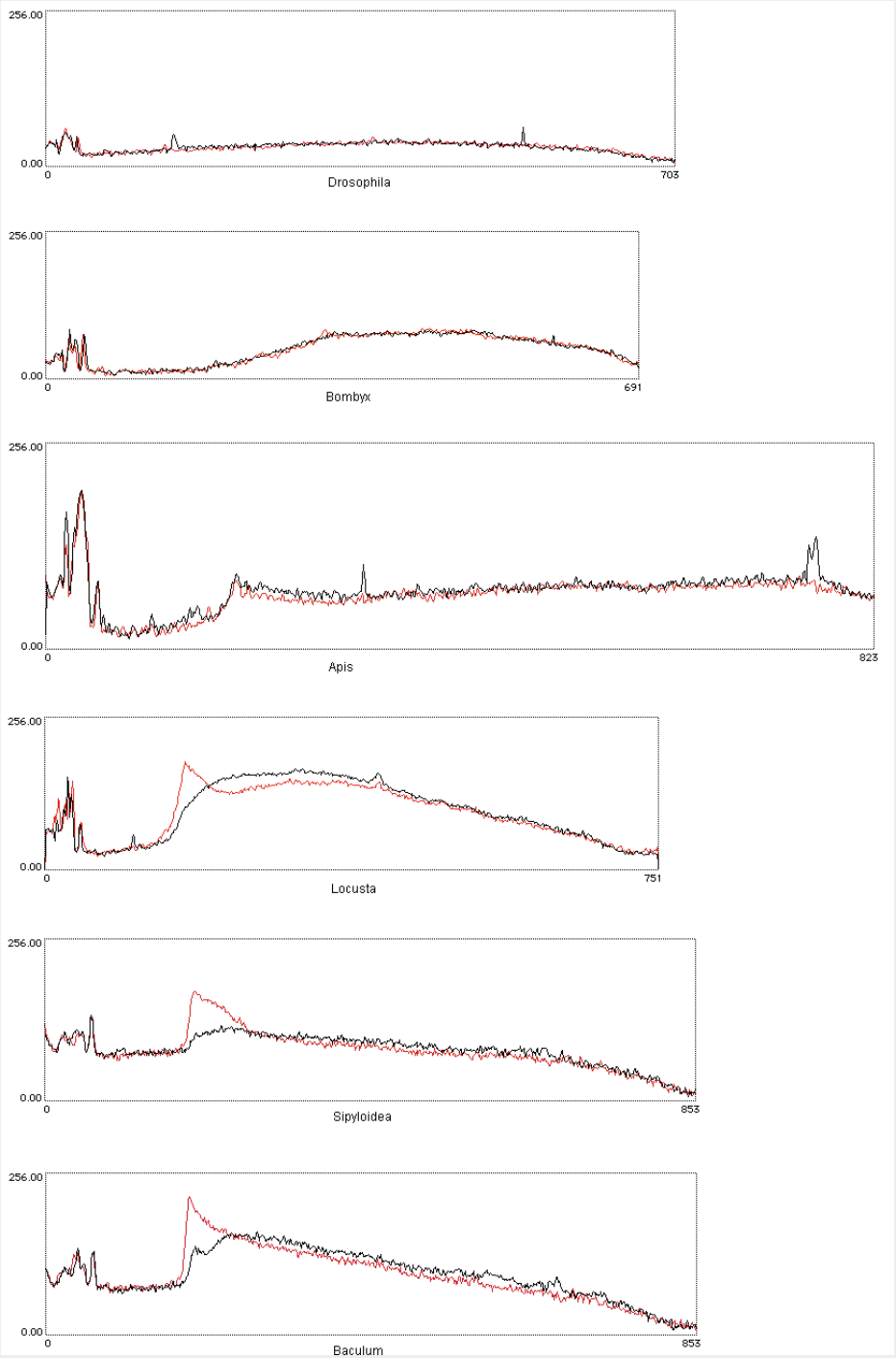


Genomic DNA of humans (1), of a sea urchin (2), a aphid (3) and of Drosophila melanogaster (5) was used undigested (-) or digested with MspI (M, unsensitive for methylation of CpG positions) and HpaII (H, unsensitive for methylation of CpG positions). The recognition site of both enzymes is CCGG. S = DNA standart.

from Field 2000

Differential digest of selected insect species
(Tina Unger)

- the ethidium bromide signal was plotted
- MspI (black), HpaII (red)



Walking sticks (Phasmatodea)

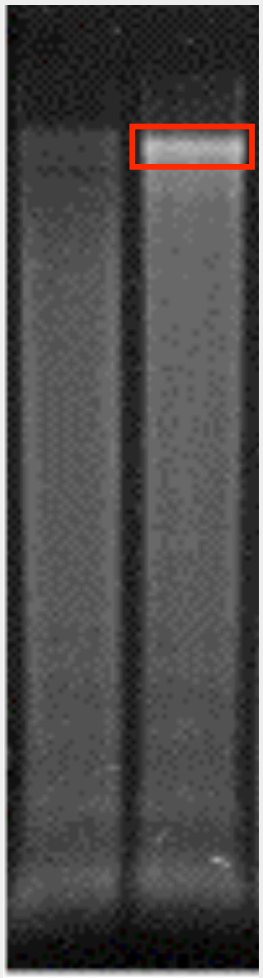
Medauroidea extradentata

features:

- large genoms (1C = 2-8 Gb), second to orthopterans (locusts, crickets and grasshoppers)
- facultative parthenogenetic reproduction
- long generation time (0.5 years)
- monophyletic line since 300 million years

Isolation of methylated DNA from Medauroidea extradentata (Tina Unger)

MspI HpaII



- this gel region was eluted and cloned
- results in 23 different DNA fragments with methylated CCGG recognition sites
- none of these 23 sequences contained a cognizable part of a functional gene

DNA methylation was confirmed for 10 fragments which are tested by Southern Blotting

(Carina Eisenhardt)

- at least 14 of 23 sequences are repetitive inside the Medauroidea genome

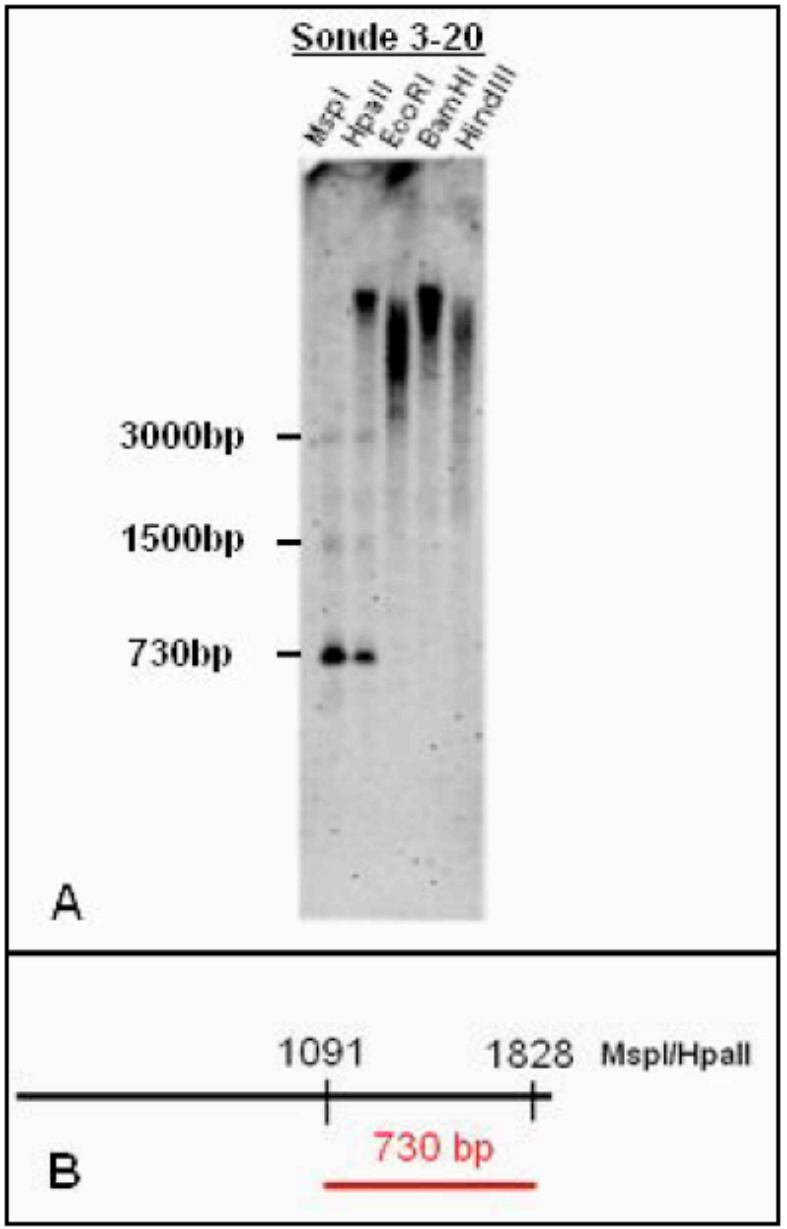


Abbildung 3.2.1:

A: Southern-Blot Sonde 3-20

B: Restriktionskarte Sonde 3-20

Bisulfit Sequencing in Medauroidea

(Carina Eisenhardt and Julia Johnke)

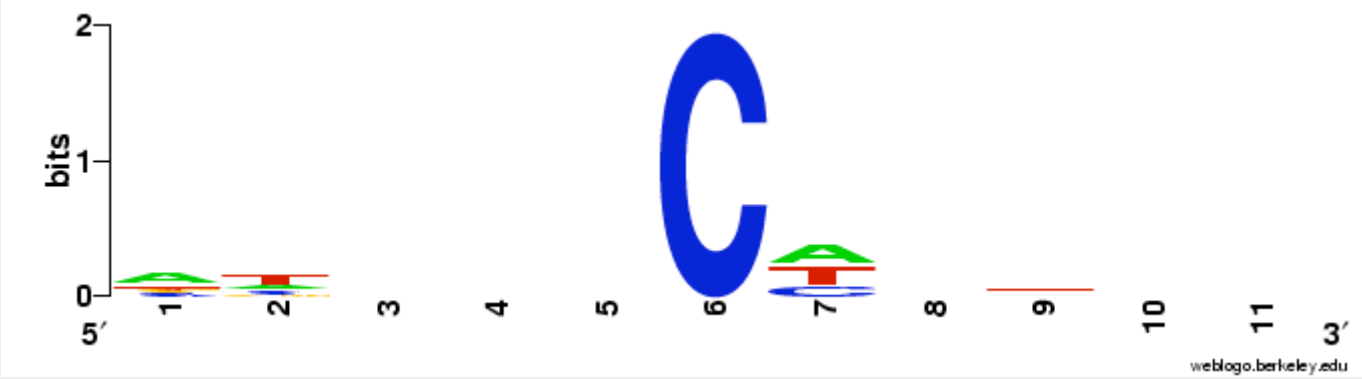
- Method for sequence-specific detection of DNA methylation
- based on the exclusive change of unmethylated cytosine to thymine (sodium bisulfit)

7-29 (retrotransposon fragment, methylation confirmed by Southern blot)	Phosphatase 2a gene fragment (includes an intron)
39 of 196 C methylated (19,9%)	**238 of 1513 C methylated (15,7%)**
38 of 40 CpG methylated (95,0%)	**184 of 197 CpG methylated (93,4%)**

Medauroidea shows mainly CpG-specific methylation

Consensus of surrounding sequences (all 272 methylated cytosines)

Consensus of surrounding sequences (53 non-CpG-^{5m}cytosines)



Germline CpG methylation

- methylcytosine is especially sensible against deamination to thymine (CpG hypermutation)
- in all sequences which show CpG methylation in the germline, CG are depleted and resulting TG and CA sequences are enriched
- in two sequence classes of Medauroidea, we found footprints of this CpG germline methylation

Sequence class	Frag-ments	bp	G+C%	CpG*	(TpG+CpA)/ 2*	(TpG+CpA)/ 2CpG*
Protein-coding genes	5	2525	47.4	0.59**	1.295**	2.19**
Repetitive DNA	15	14788	38.4	0.76**	1.17	1.54
Unclassified DNA	15	5885	40.8	0.97	1.075	1.11
28S and 18S ribosomal RNA genes	2	4058	56.5	1.10	0.86	0.78
∑	37	27256	42.4	0.89	1.095	1.23

*observed/expected

**significant different from 1 according to Karlin and Cardon (1994)

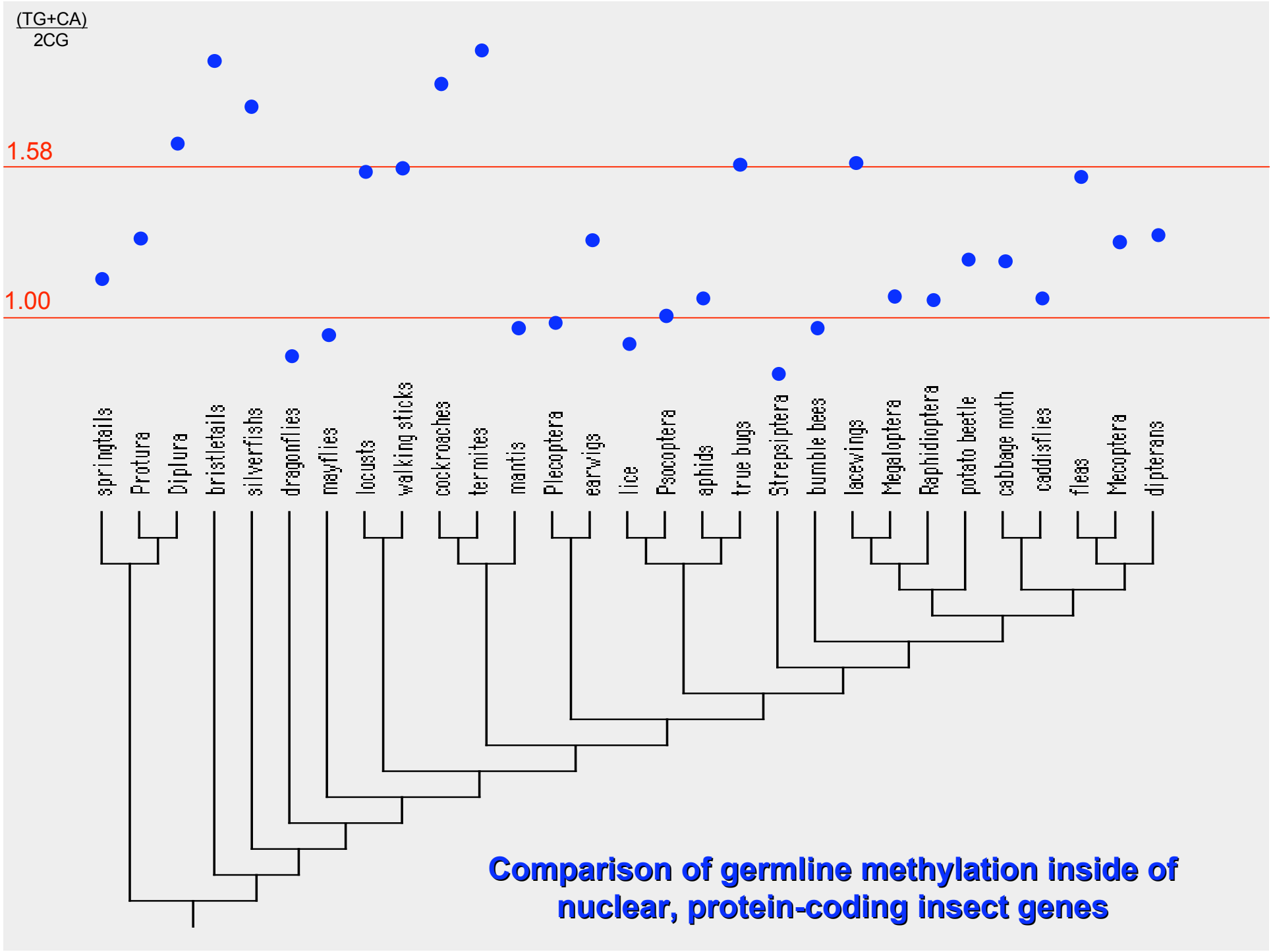
Comparison of germline methylation inside of conserved genes

Organism	CpG	(TpG+CpA)/2	(TpG+CpA)/2CpG
Danio	0.53*	1.365*	2.58*
Nematostella	0.56*	1.290*	2.30*
Medauroidea	0.59*	1.295*	2.19*
Mus	0.62*	1.275*	2.06*
Homo	0.60*	1.190	1.98*
Strongylocentrotus	0.63*	1.190	1.89*
Ciona	0.66*	1.215	1.84*
Acyrthosiphon	0.75*	1.260*	1.68*
Apis	0.72*	1.195	1.66*
Pediculus	0.79	1.145	1.45
Nasonia	0.82	1.140	1.39
Drosophila	0.92	1.245*	1.35
Mamestra	0.92	1.100	1.20
Daphnia	0.94	1.085	1.15
Aedes	1.03	1.165	1.13
Bombyx	1.05	1.025	0.98
Anopheles	1.15	1.105	0.96
Caenorhabditis	1.12	0.990	0.88
Tribolium	1.19	0.995	0.84

*significant different from 1 according to Karlin and Cardon (1994)

above the line, CpG germline methylation is supported

used are exact homologous fragments of 5 COGs


(TG+CA)
2CG
1.58
1.00
springtails
Protura
Diplura
bristletails
silverfishs
dragonflies
mayflies
locusts
walking sticks
cockroaches
termites
mantis
Plecoptera
earwigs
lice
Psocoptera
aphids
true bugs
Strepsiptera
bumble bees
lacewings
Megaloptera
Raphidioptera
potato beetle
cabbage moth
caddisflies
fleas
Mecoptera
dipterans
Comparison of germline methylation inside of
nuclear, protein-coding insect genes

Conclusion

- Medauroidea shows DNA methylation in

- a) genes
- b) transposons

- DNA methylation appear to be significant stronger inside genes, which is confirmed by literature and CpG hypermutation for many insect groups

Function	In insects
Suppression of internal transcriptional initiation	Strongly supported by distribution
Suppression of transposon activity	Supported in Medauroidea and Drosophila
Regulated silencing of genes	No data
Novel functions	No conclusive data

Outlook

1. Analysis of CpG hypermutation

- a) EST / mRNA data
- b) transposon sequences
- c) whole, annotated genomes

2. Comparison of cellular turnover and population sizes between the analyzed species

3. Identification of an animal model for DNA methylation which represents a typical methylation pattern and which allows the complete erasure of methylation without immediate vital effects

Thanks to

Tina Unger
Carina Eisenhardt
Julia Johnke
Caroline Schmidt

For some experiments,
which were often not successful,
but always salutary

and You

for Your attention

Lehrstuhl für Genetik
Department of Genetics

UNIVERSITÄT LEIPZIG
Fakultät für Biowissenschaften, Pharmazie und Physiologie
Institut für Biologie II