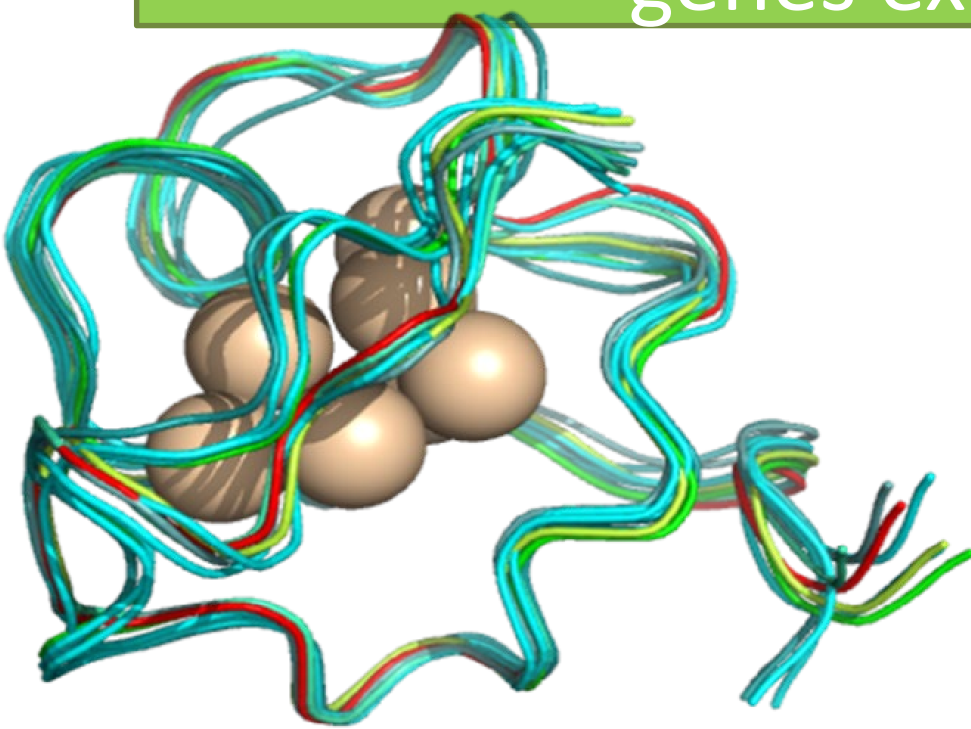
Response to heavy metal stress in *Drosophila* species – the metallothionen genes expression



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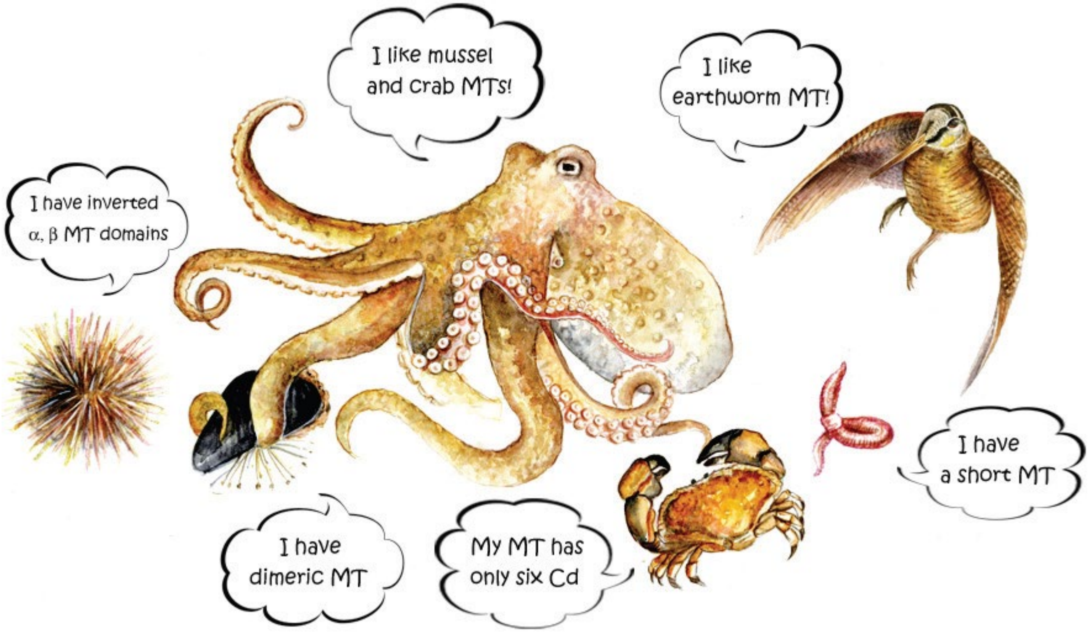
Metallothioneins

- Discovered in 1957
- Key to **metal metabolism**
- In eukaryotes and some prokaryotes
- Isoforms are species dependent

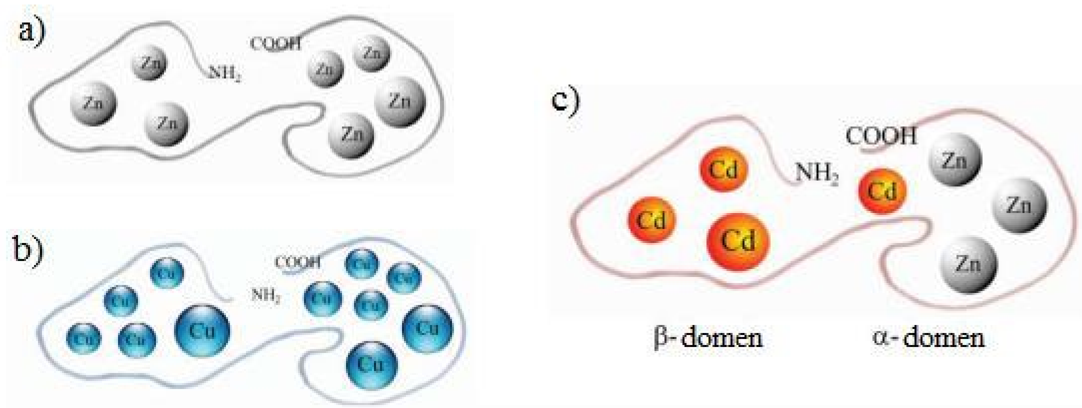
- Multifunctional

1. homeostasis of Zn and Cu
2. Cd and Hg detoxification
3. "Scavenging" of free radicals



Metallothioneins

- Small proteins, **24-85**aa long – cystein reach (**15-33%**)
- Low molecular weights
- They can chelate different metal ions simultaneously



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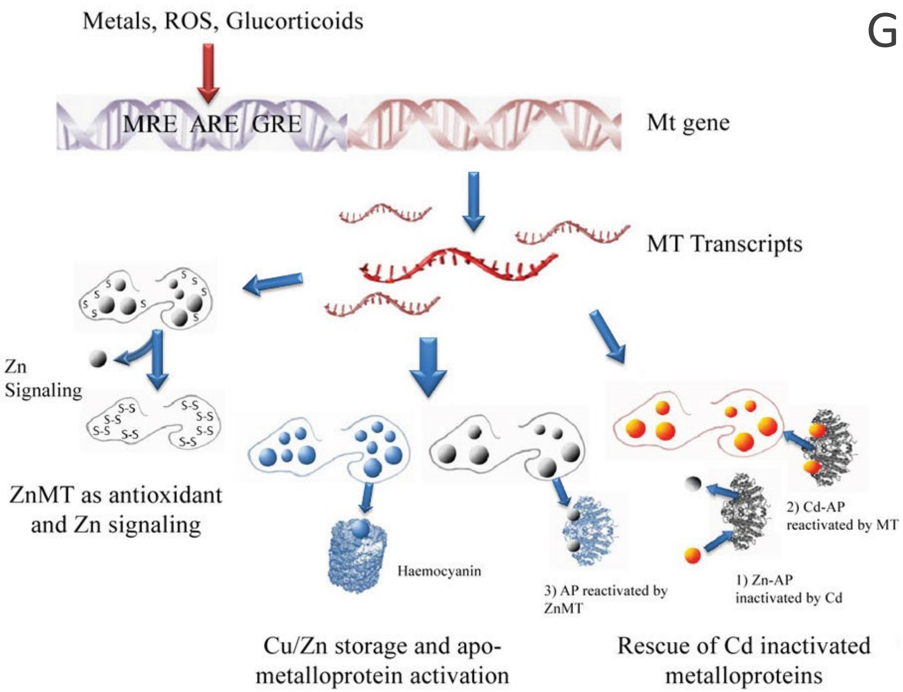
Gene structure

- usually 3 exons, 2 introns (insects: 2 exons, 1 intron)
- In humans at least 10 genes on the same chromosome (only 4 genes functional?)
- Transcription as response to different stimuli: promoter sequence has a few response elements: MRE (*metal-response element)*, ARE (*antioxidant response elements*), GRE (*glucocorticoid response elements*)



the number and position of MREs analyzed in 12 *Drosophila* species indicates greater diversity in these sequences than in coding ones

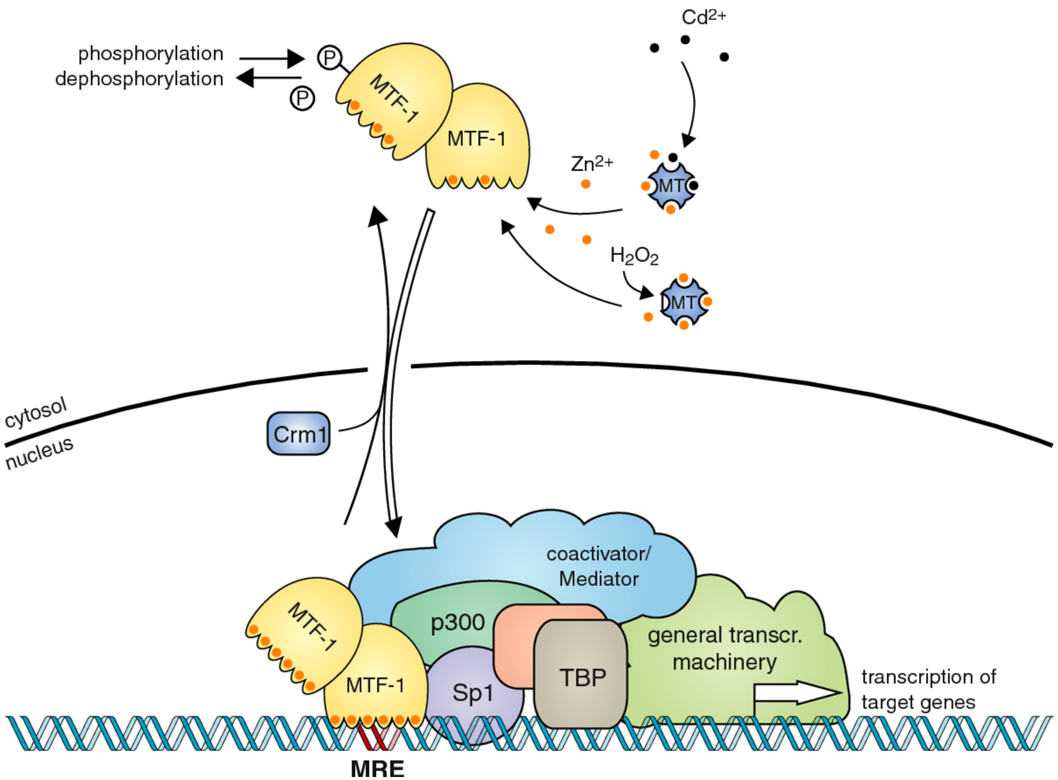
Regulation of expression

- MTF-1 metal-regulatory/ responsive transcription factor 1

  (MRE-binding transcription factor 1)

- Feedback mechanism:

  in the event of an increase in metal concentration, the released zinc binds to MTF-1, which then binds to DNA (to the MRE), initiating transcription



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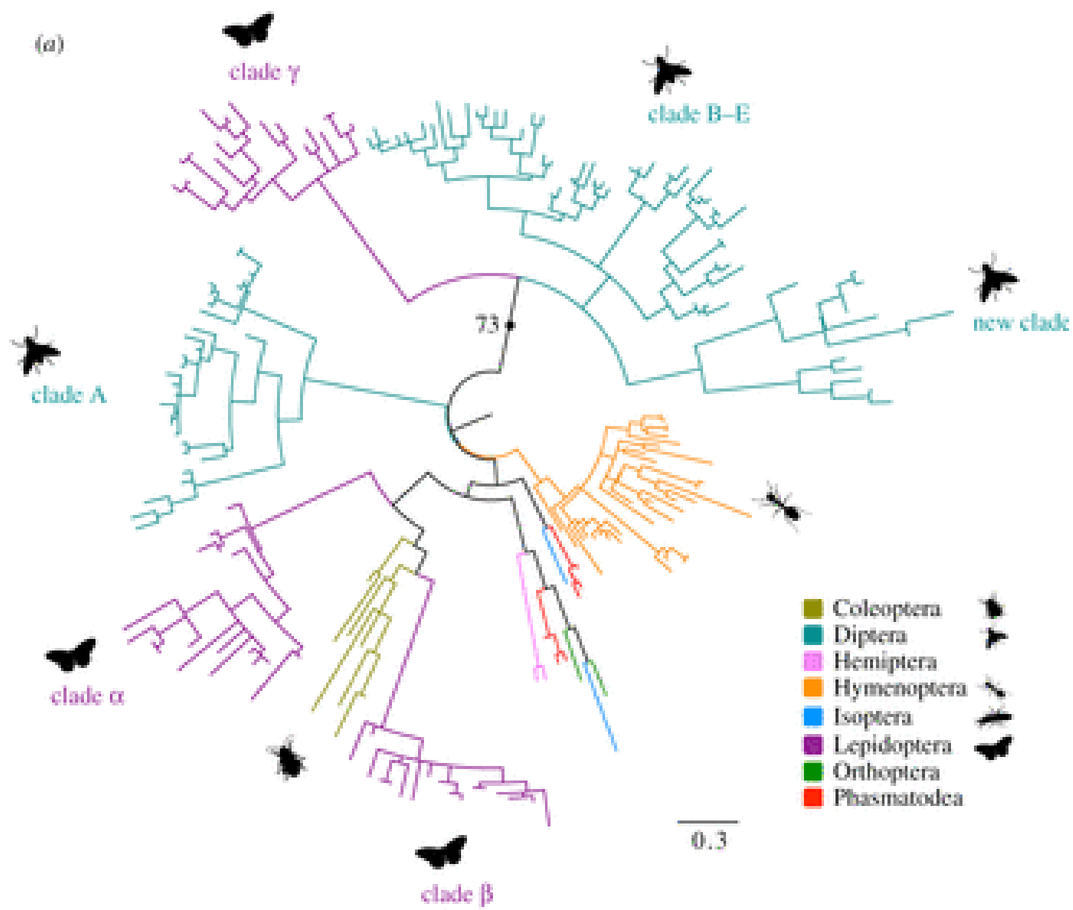
Insects

- Until two years ago:
  bees 1MT, grasshoppers 2MT, mosquito – 2MT, frut fly 5MT …
  (in total 19MT)
- sequenced genomes and transcriptomes of more than 100 insect species
- More than **300MT** was found
- All descended from a single ancestral gene, evolved prior the diversification of insects
- Phylogenetics analysis and synteny analysis
- Disadvantage: transcriptome analysis - low-expressed genes and pseudogenes cannot be detected

Phylogenetic analysis

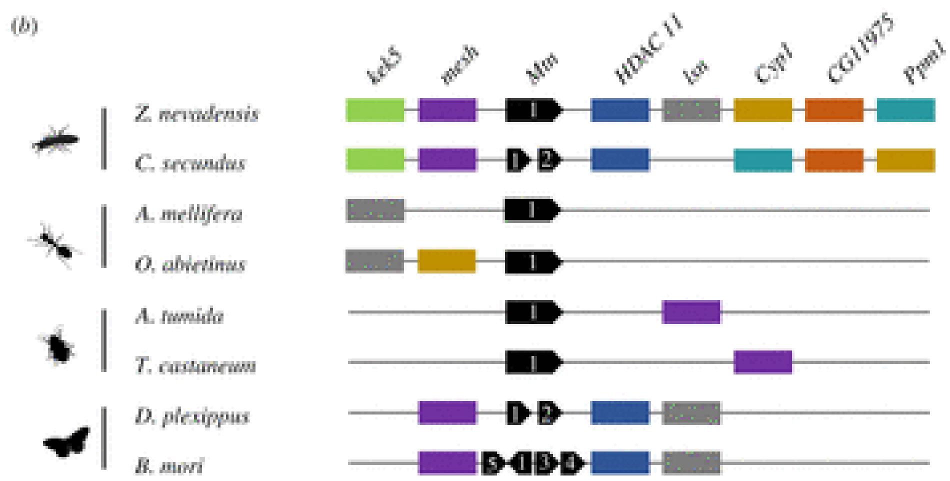
- Coleoptera – metallothioneins from 1 MT from their last common ancestor
- Hymenoptera - from 1 MT from their last common ancestor
- Lepidoptera - at least 3 MT in the last common ancestor
- Diptera - several MT in the last common ancestor
- MT duplications before separating Lepidoptera and Diptera



Sinteny analysis

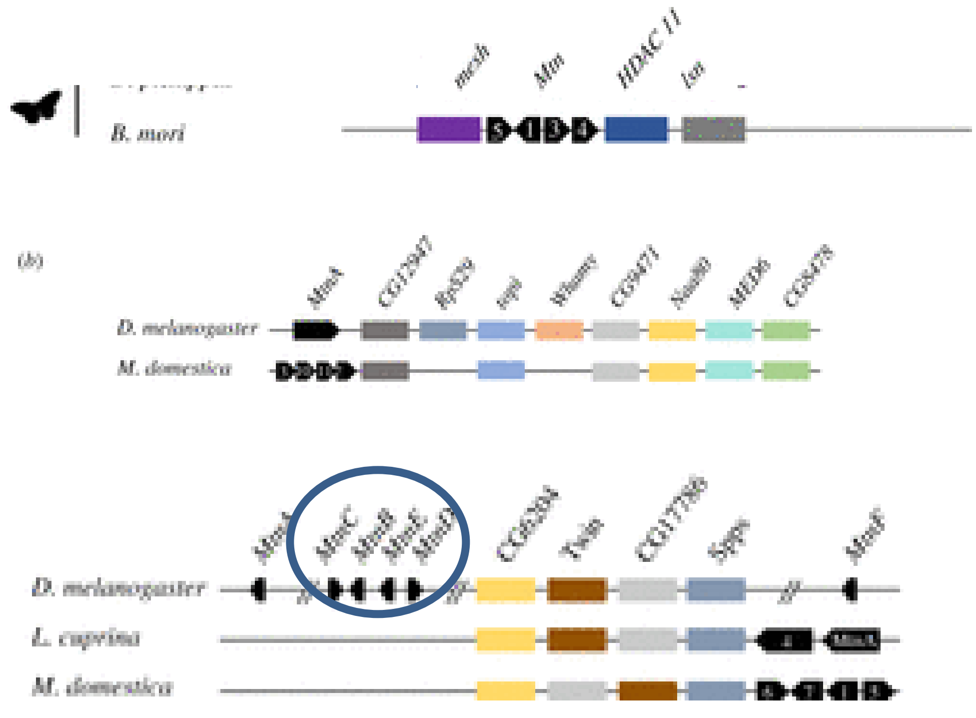
- In most species Mtn gene(s) flanked by the same orthologous genes (except in Hemiptera)

- In Diptera flanked with some of these genes (*mesh, HDAC 11, Isn, CG11975*) but in other genomic region (3R chromosome in Drosophila)
- Consequence of chromosomal inversions!



Tandem duplications

- *B. mori* - 4MTs
- *Musca domestica* – 4 clustered copies of *MtnA*
- *Drosophila* – tandem repeats for a clade B-E
- The most duplications in Lepidoptera – average 2,7 tandem copies for the species



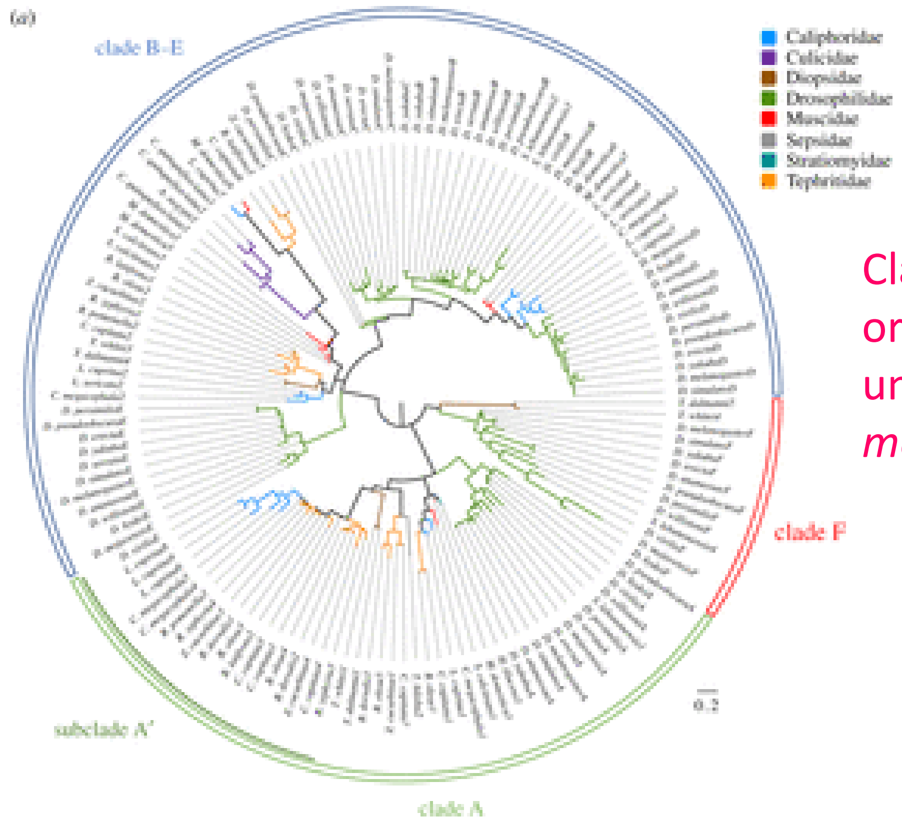
The number of Mtn genes is a consequence of lineage specific tandem duplications

Consequence of different environmental conditions, nutrition, heavy metal exposure?

Evolution in Diptera – MT sequences (141 proteins)

- 3 major branches – at least 3MT genes in common ancestor

Clade B-E – homologous MtnB-like cluster *D. melanogaster*

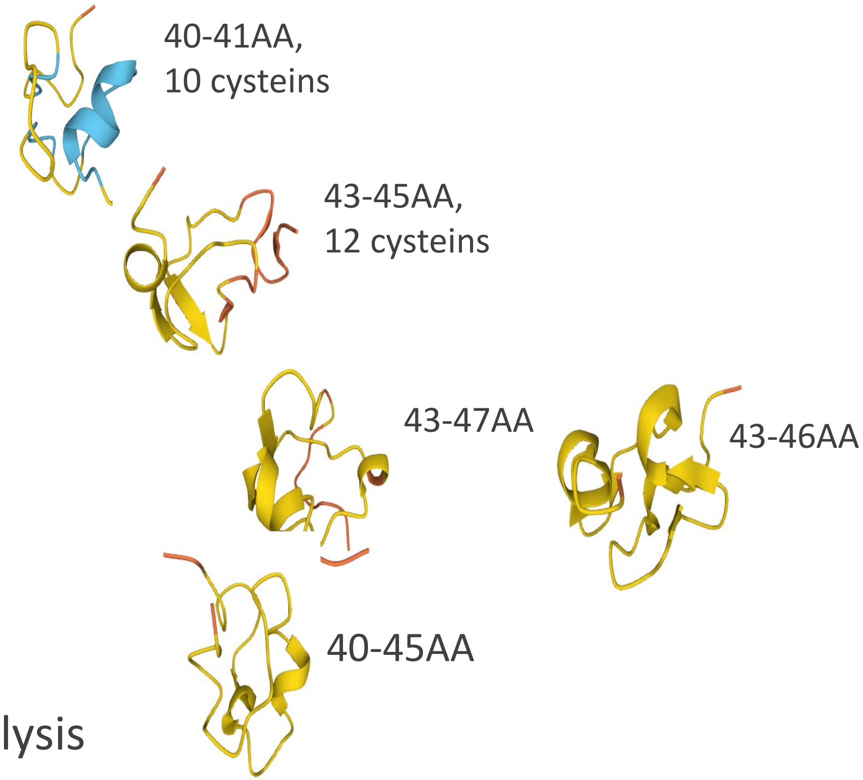


Clade F – orthologous uncharacterized *D. melanogaster* ORF

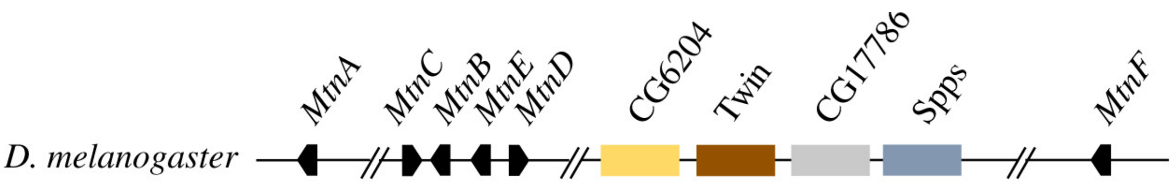
uncharacterized ORF CG43222

Clade A – orthologous of *MtnA* gene *D. melanogaster*

Drosophila melanogaster



- ***MtnA*** – 1985 year; role in Cu detoxification
- ***MtnB*** – 1987 year; role in Cd detoxification
- ***MtnC*** and ***MtnD*** – 2000 and 2002 years; role?
  not crucial for detoxification of Cu and Cd
- ***MtnE*** – 2011. revealed by bioinformatic analysis
- ***MtnF*** – 2020. revealed by bioinformatic analysis



•MtnA and MtnB and MtnE share a telomeric–centromeric orientation (according to their 5′/3′ expression sense), while MtnC and MtnD, flank MtnB in the opposite strand (centromeric–telomeric orientation)

MtnF

(*a*)

- N-terminal end conserved, very similar to other Mtn
- 3-D structure pediction: it should be functional (upstream 2 putative sites for binding MTF-1)
- Compared to the other two clades– more changes:

  around 62aa – longer at C terminus – origin???
- Reacher in cystein
- Zn binding???

D. subobcura larvae treated with different heavy metals

expression analysis of all 6 metallothionein genes (*MtnA-F*)

Lower concentration

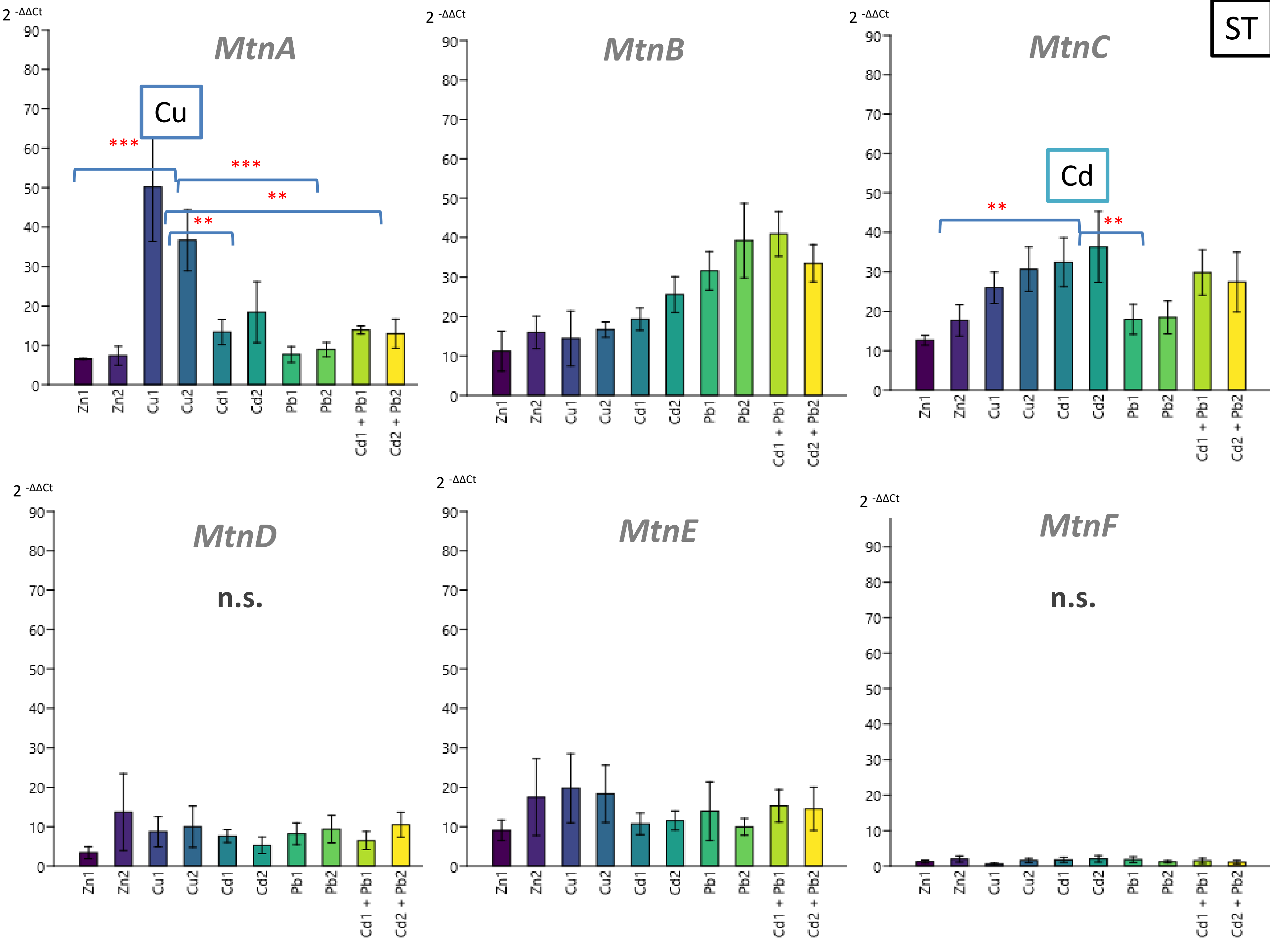
Zinc (Zn)	Copper (Cu)	Cadmium (Cd)	Lead (Pb)	Cadmium and Lead (Cd+Pb)
2.5mM	0.5mM	0.05mM	0.25mM	0.05mM + 0.25mM
5mM	1mM	0.1mM	2.5mM	0.1mM + 2.5mM

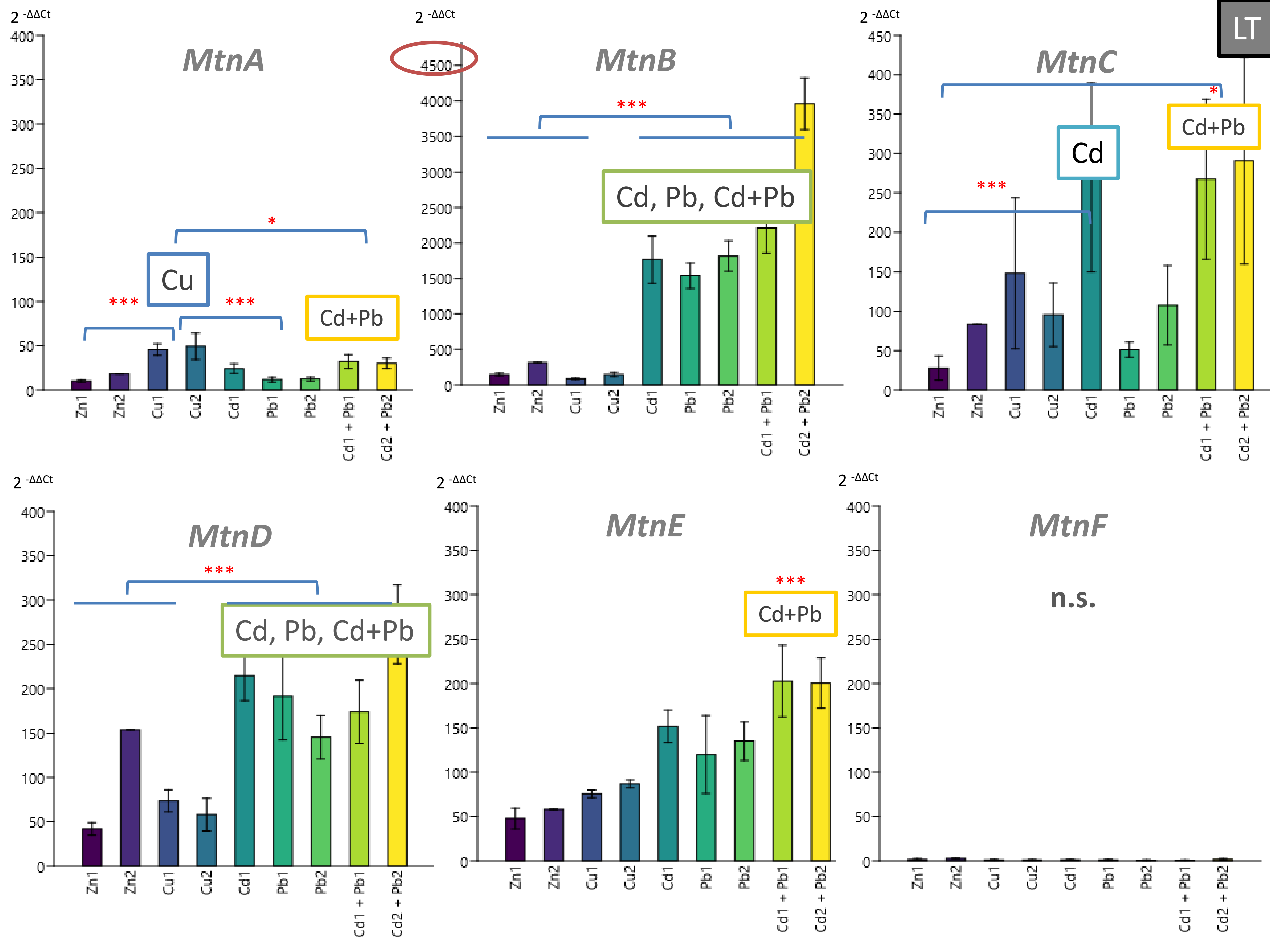
Higher concentration

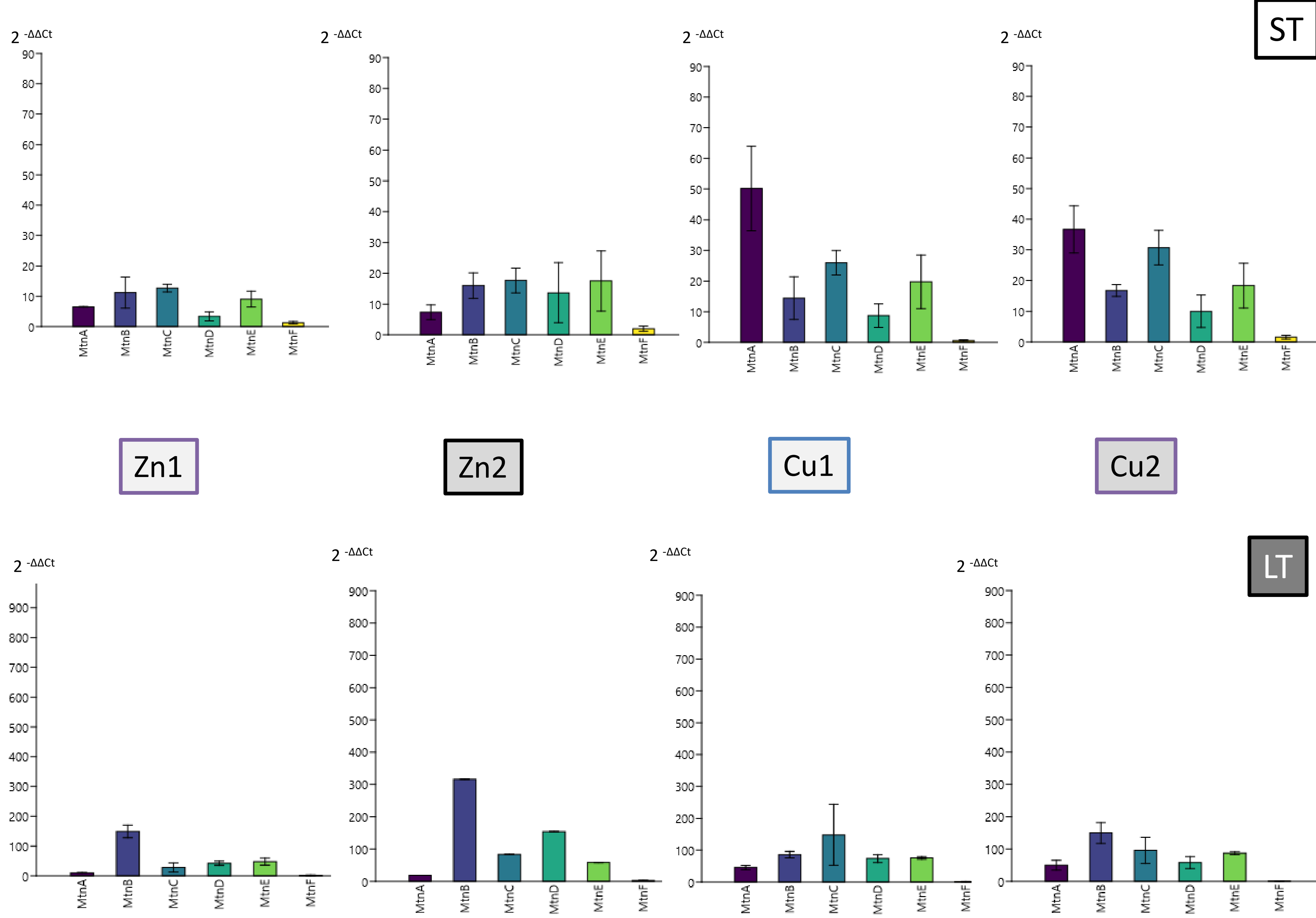
Short term 24h (ST)
Long term ~ 10 days (DT)
Control group (St)

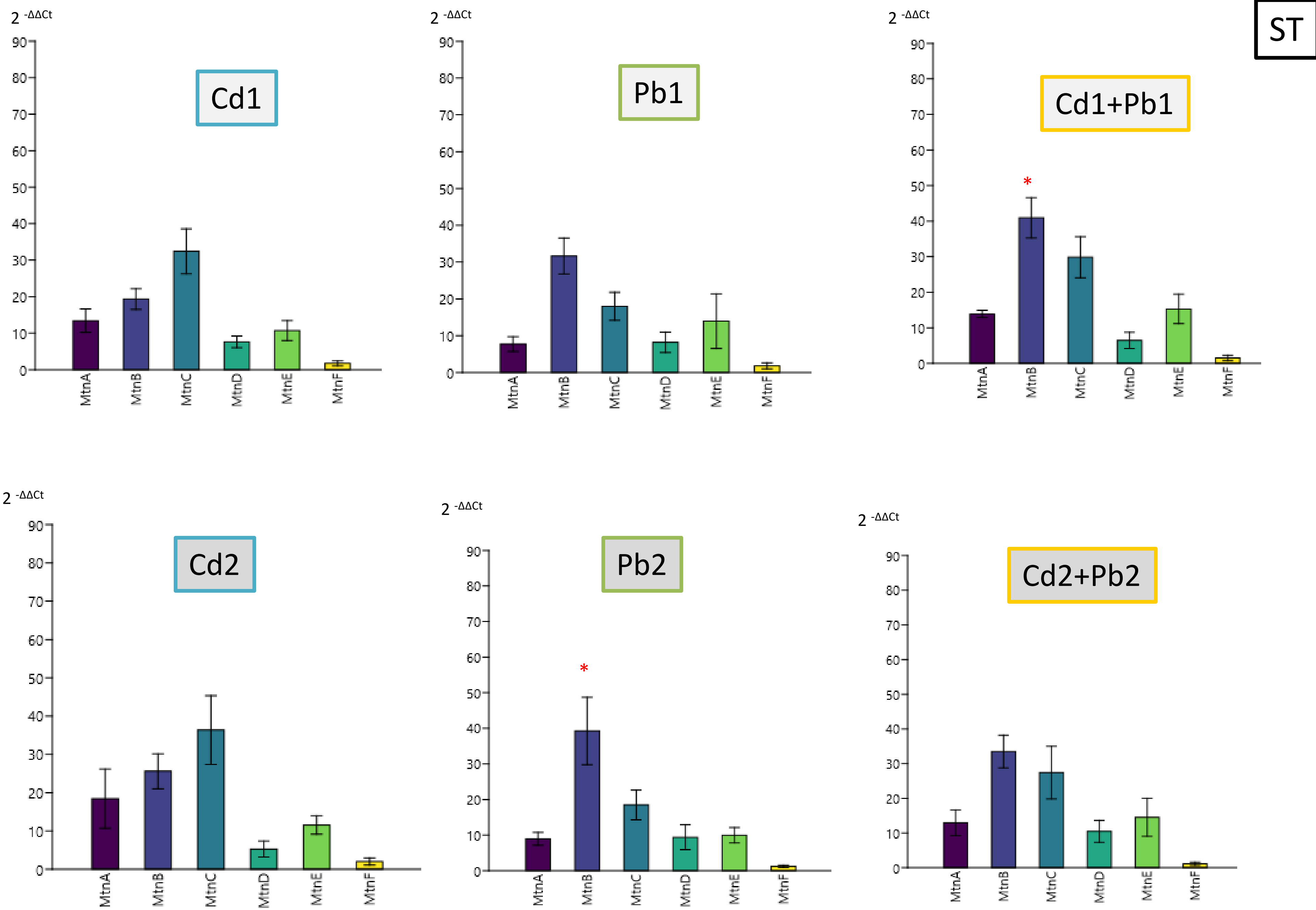
Short term 24h (ST)
Long term ~ 10 days (DT)
Control group (St)

- Relative gene expression
- Reference genes: *Actin* and *RpL32*
- SYBR Green

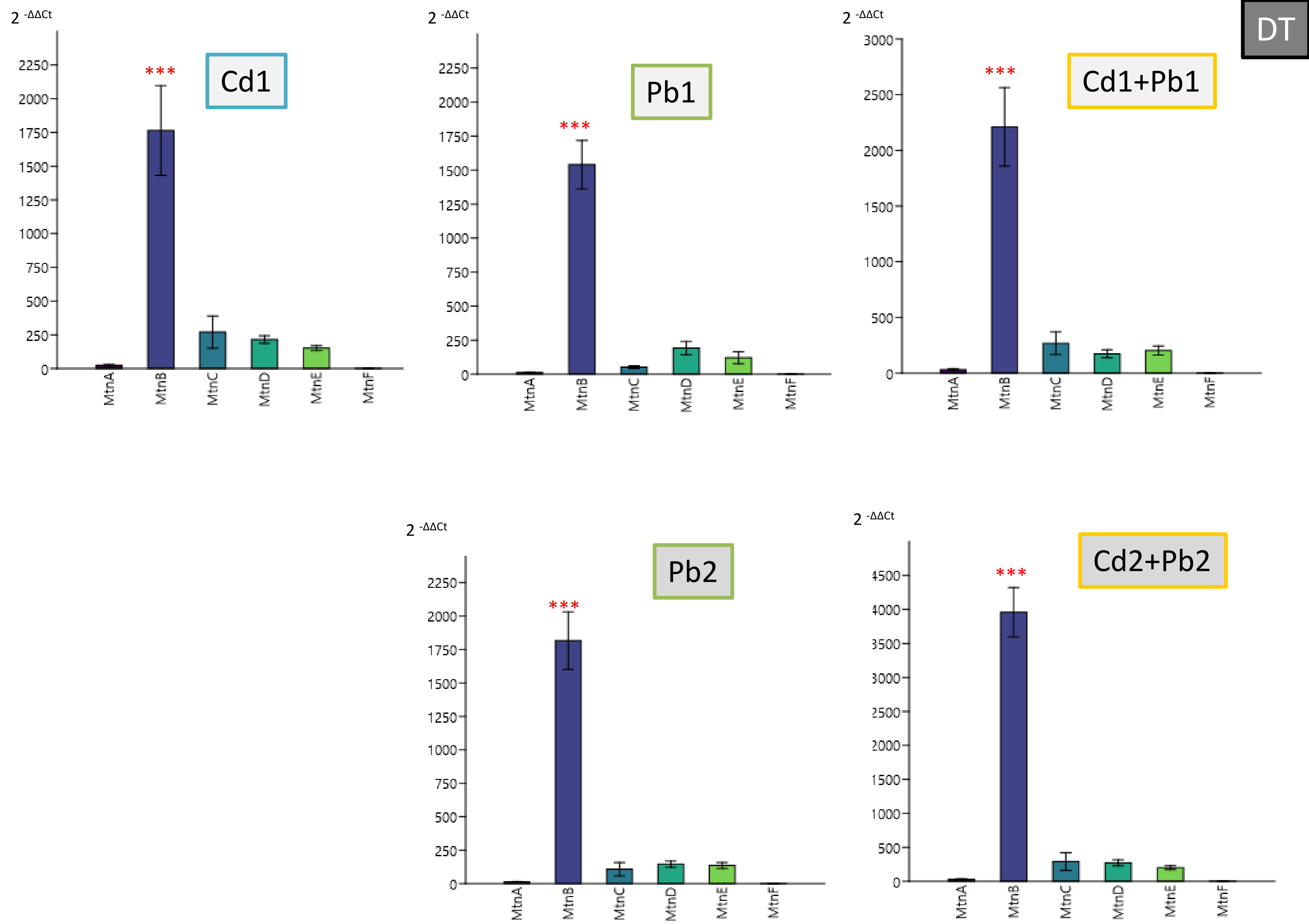

ST
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF
Cu
Cd
n.s.
n.s.
2 -ΔΔCt
***
***
**
**
**
**
Zn1
Zn2
Cu1
Cu2
Cd1
Cd2
Pb1
Pb2
Cd1 + Pb1
Cd2 + Pb2


LT
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF
2 -ΔΔCt
Cu
Cd+Pb
Cd, Pb, Cd+Pb
Cd
n.s.
***
*
Zn1
Zn2
Cu1
Cu2
Cd1
Pb1
Pb2
Cd1 + Pb1
Cd2 + Pb2


ST
LT
Zn1
Zn2
Cu1
Cu2
2 -ΔΔCt
0
10
20
30
40
50
60
70
80
90
100
200
300
400
500
600
700
800
900
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF



ST
Cd1
Pb1
Cd1+Pb1
Cd2
Pb2
Cd2+Pb2
2 -ΔΔCt
0
10
20
30
40
50
60
70
80
90
*
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF


DT
Cd1
2 -ΔΔCt
0
250
500
750
1000
1250
1500
1750
2000
2250
***
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF
Pb1
2 -ΔΔCt
0
250
500
750
1000
1250
1500
1750
2000
2250
***
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF
Cd1+Pb1
2 -ΔΔCt
0
500
1000
1500
2000
2500
3000
***
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF
Pb2
2 -ΔΔCt
0
250
500
750
1000
1250
1500
1750
2000
2250
***
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF
Cd2+Pb2
2 -ΔΔCt
0
500
1000
1500
2000
2500
3000
3500
4000
4500
***
MtnA
MtnB
MtnC
MtnD
MtnE
MtnF

Conclusions

- It seems that newly discovered ***MtnF*** does not have assumed role as others metallothioneins – **an unclear role** given the established expression level!

- The expression levels of **other 5 Mtn genes are always increased** in all heavy metal treatments, except for *MtnD* that is increased only after long term exposure.

- **The duration of the exposure** influences only the expression levels of *MtnB* **cluster** (the expression of *MtnB* is especially increased – the more important role relative to *MtnC* and *MtnD*), while the *MtnA* expression level does not change significantly in long term treatments.

- **The effects** of **two non essential (Cd+Pb)** heavy metal stress**:**

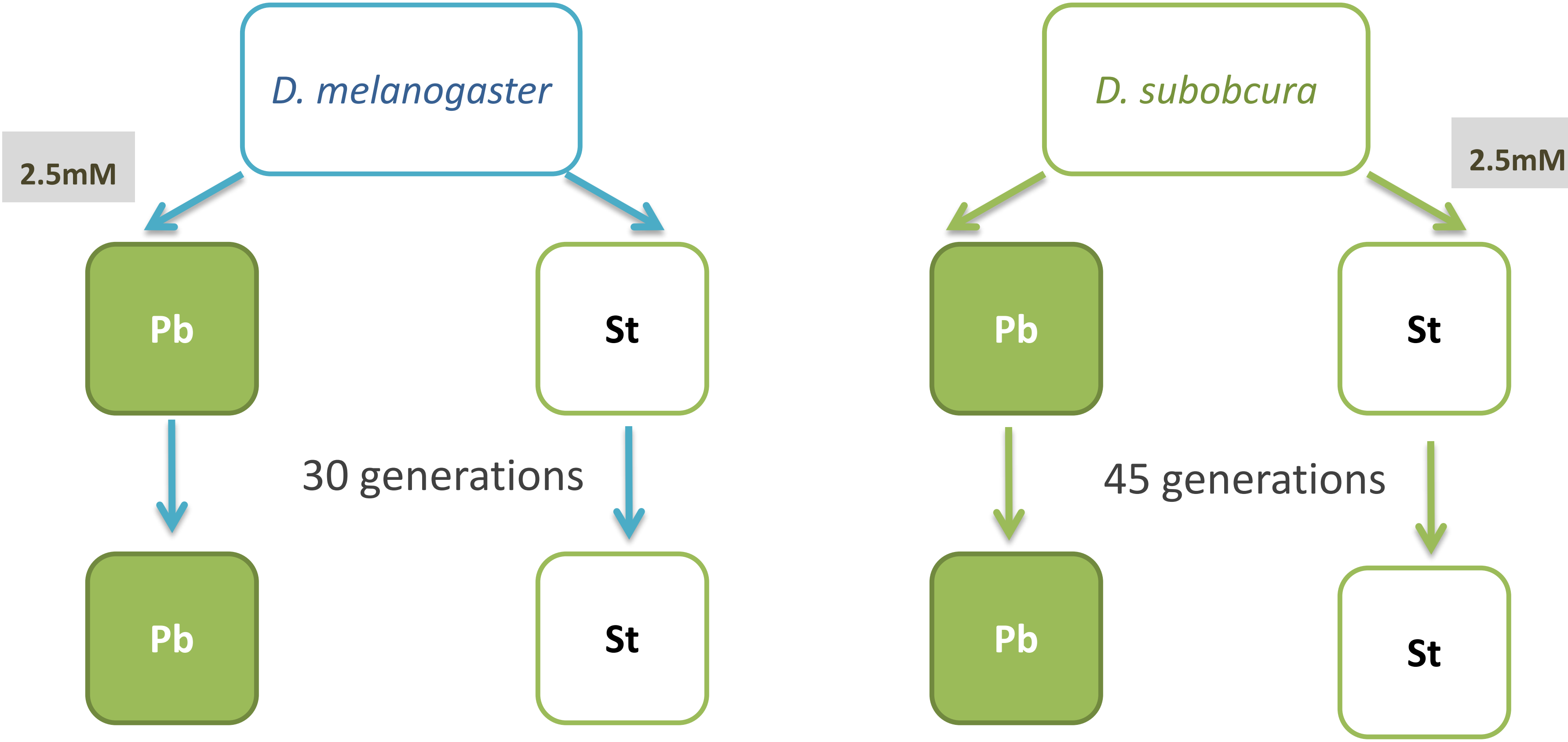
- Metal concentrations do not influence the expression levels of *Mtn* genes (except for Cd and Pb combination long term treatment)

- Influences significant increase of *MtnA* expression

-Although the expression of the *MtnE* significantly increases in comparison to control, it is significant only in long term combined stress

D. melanogaster and *D. subobcura*

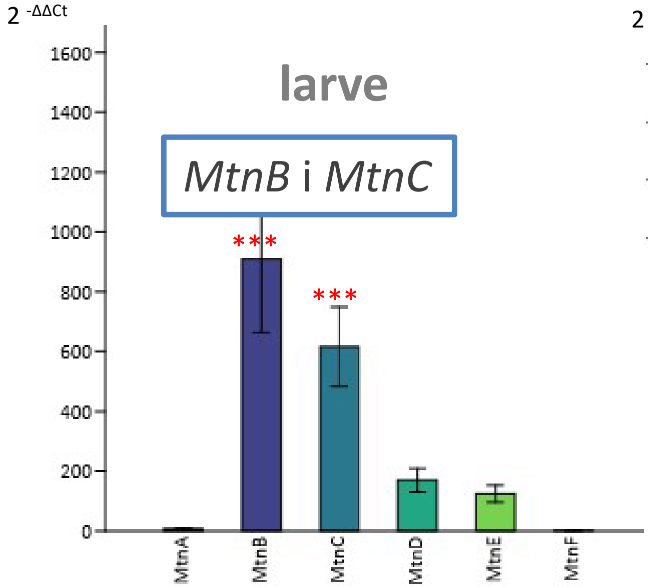
expression analysis of all 6 metallothionein genes (*MtnA-F*)

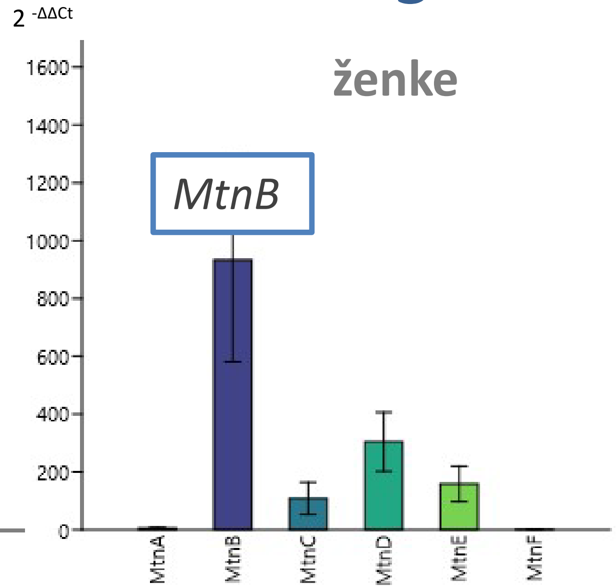


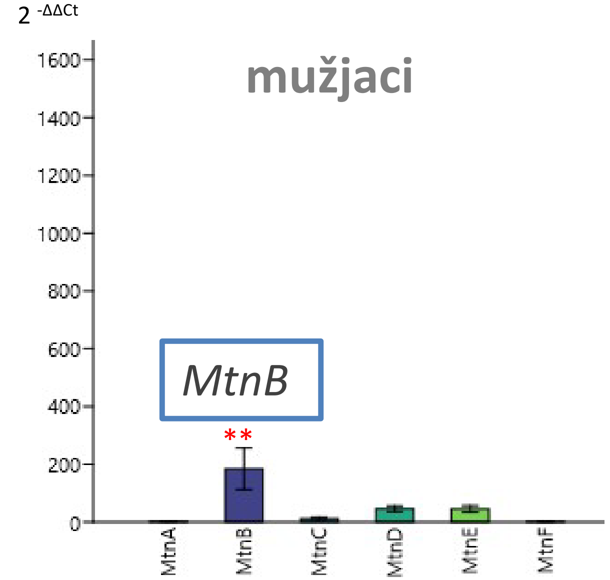
- Relative gene expression
- Reference genes: *Actin* and *RpL32*
- SYBR Green

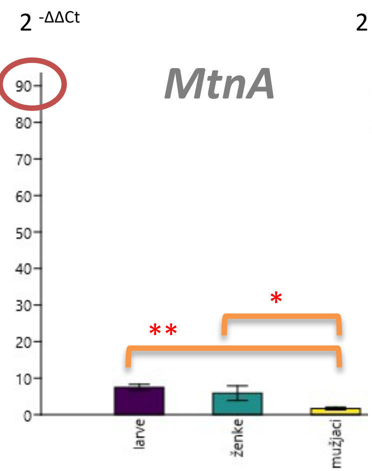
- LARVAE
- ADULTS – females
  males

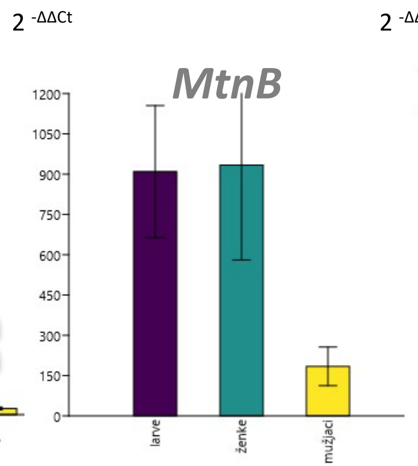
D. melanogaster

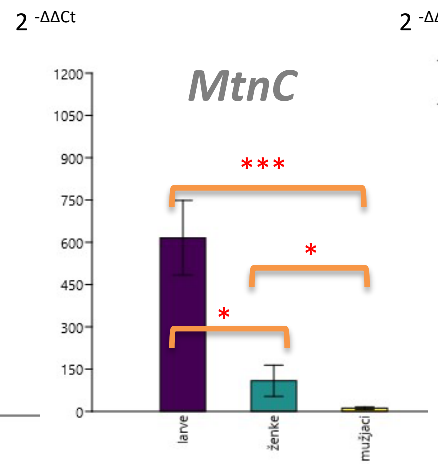


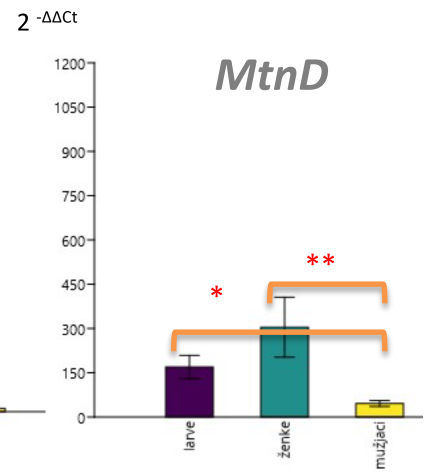


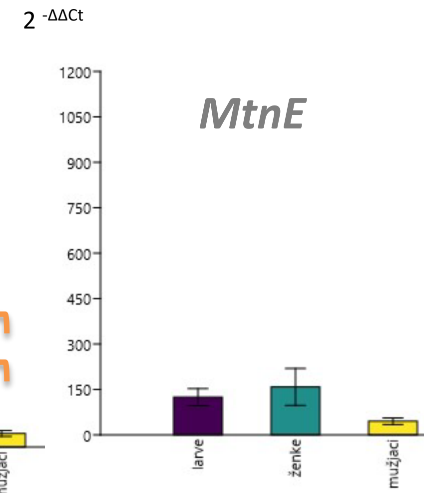




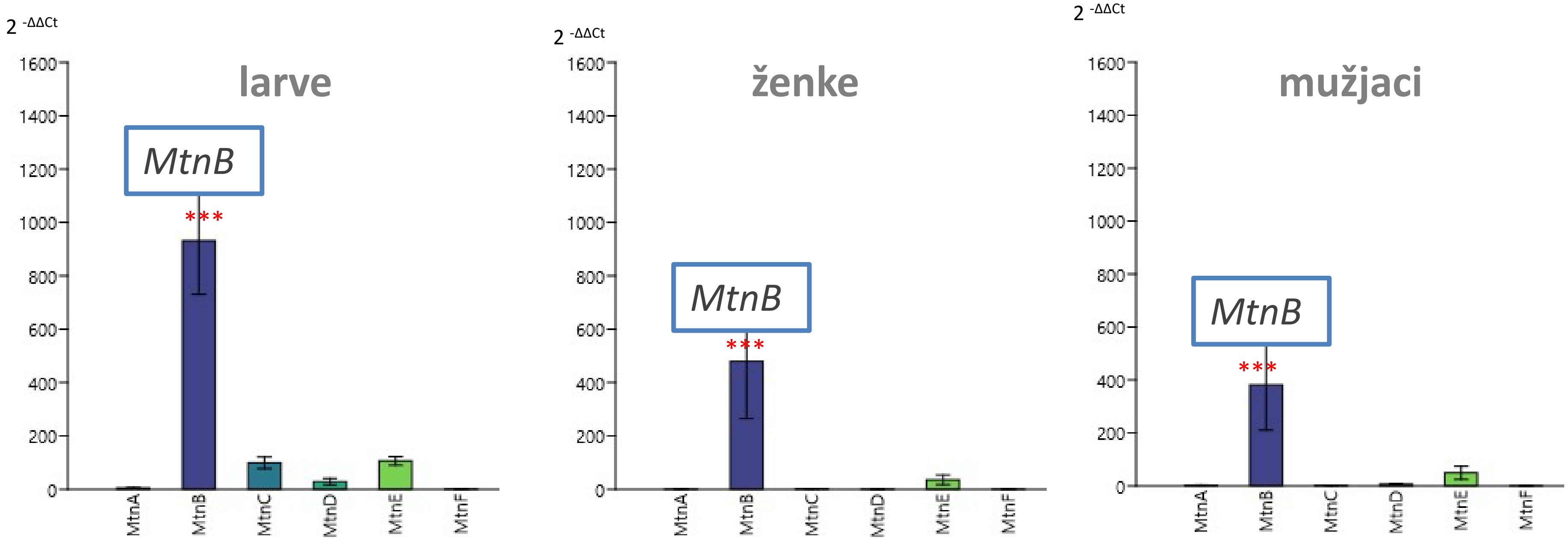


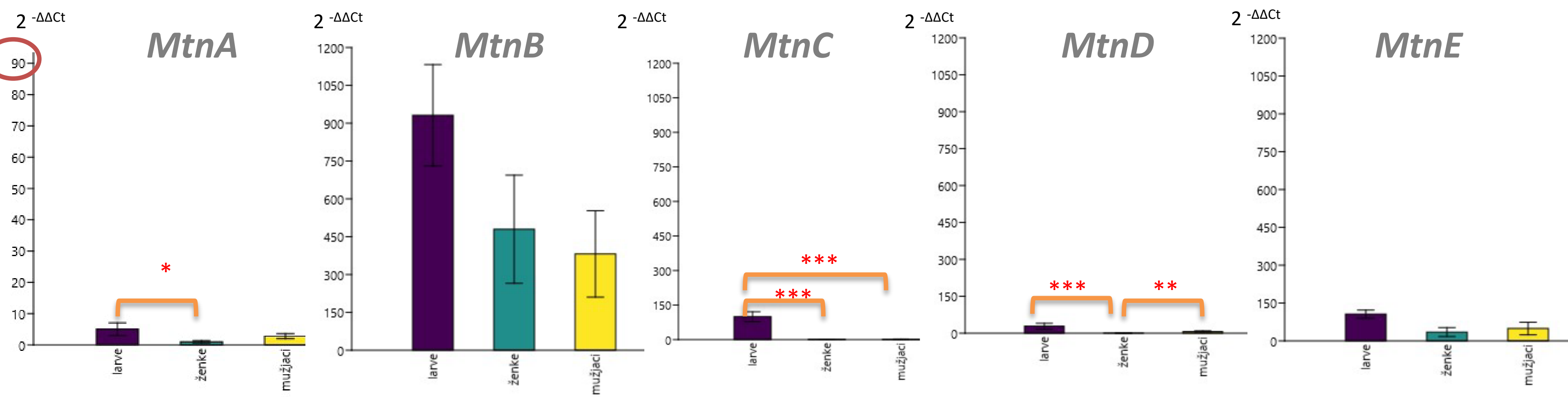






D. subobscura



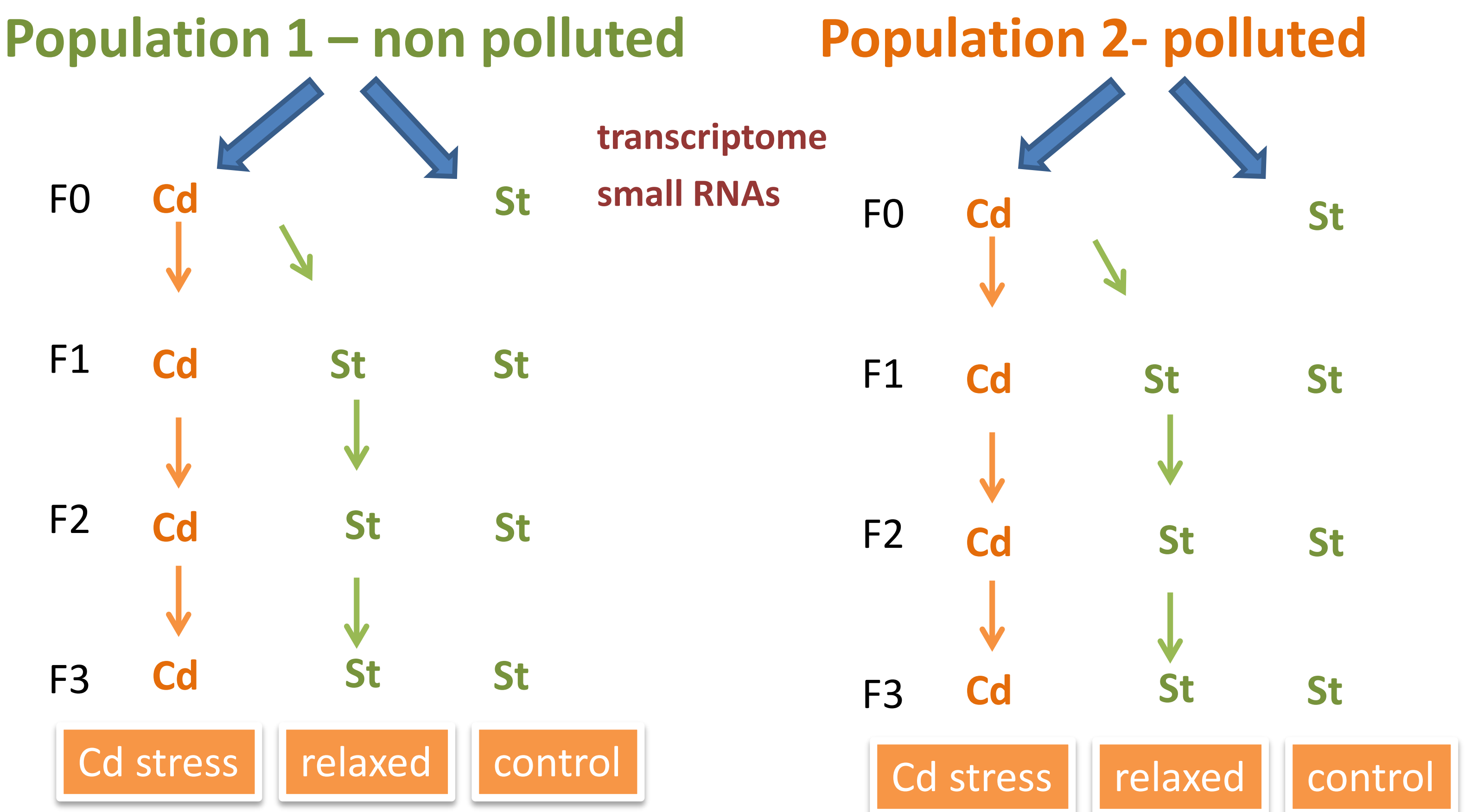


Conclusions

- The same results for ***MtnF*** gene in both species - still **unclear role**
- Gene expression differences between two species - in ***D. melanogaster*** significantly increase expression **of all five Mtn** genes with chronic exposure to lead, but in *D. subobscura* species only of *MtnB* and *MtnE*
- It has been confirmed that lead exposure notably increases *MtnB* gene expression in both species, of both sexes and larvae (there was no differences in expression between them).
- Difference between sexes only for *MtnD?*
- In larvae somewhat higher expression of Mtn genes regard to adults – for Mtns that are not crucial in response to heavy metal stress (***MtnC*** i *MtnD*)

Current experiment

Drosophila melanogaster – mass populations



fitness: egg to adult development, survival, longevity

gene expression: candidate genes

Repeat after 15 generations