



Des stylet(s) à leur cuticule: transcriptomique et génomique des protéines cuticulaires de puceron



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abdomen

10µm

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DES SCIENCES
APPLIQUÉES
LYON



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26-28 Juin 2019

Plan

1

- ❖ **Introduction:** the biological issue, or why do we need stylet « analytics » ?
- ❖ What is really « stylet transcriptomics » ? : Retort organ revisited.
- ❖ Two (x2) stylets for one bundle = maxillary vs mandibular.

2

- ❖ What is a cuticular protein ? Domain conservation is chitin-binding

3

- ❖ Cuticular protein profiles and CuP (=CP) compositional complexity: Shannon again.
- ❖ Compositional bias in cuticular proteins: Structure, composition, function.
- ❖ Parsing genome for compositional bias: A brand new world ? (in retort organs)
- ❖ **Conclusions:** Homology and orthology in cuticular proteins

The biological issue

- ❖ Hemipteran stylet and mouth cuticle territories do bind microbes:
 - viruses (*non-circulative* , *CaMV*... -aphids-)
 - bacteria (*Xylella* -sharpshooters-)

- ❖ What is mouthpart cuticle made of ? Structure ? Components ?
 - cuticular proteins ?
 - lipids ?

- ❖ How to achieve such characterization ?

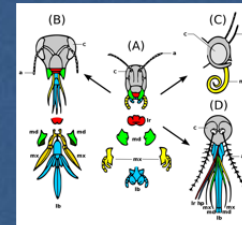
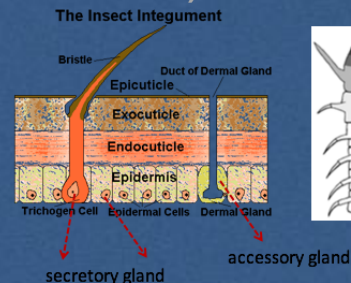
Analysis of mouthpart cuticular proteins

Very scarce data at this time (a single analysis on mosquitoes rostrum)

standard (model) cuticle

appendage cuticle

mouth-part cuticle



- ❖ Combined analysis with proteomics, transcriptomics & genomics (structural, annotational, comparative).
- ❖ The advantage of transcriptomics (in identifying the main -gene- players)
 - Sensitivity
 - Insensitivity to post-translational modifications
 - Expression (levels), quantitative: therefore a partial link to function

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Stylet transcriptomics

Retort organ revisited

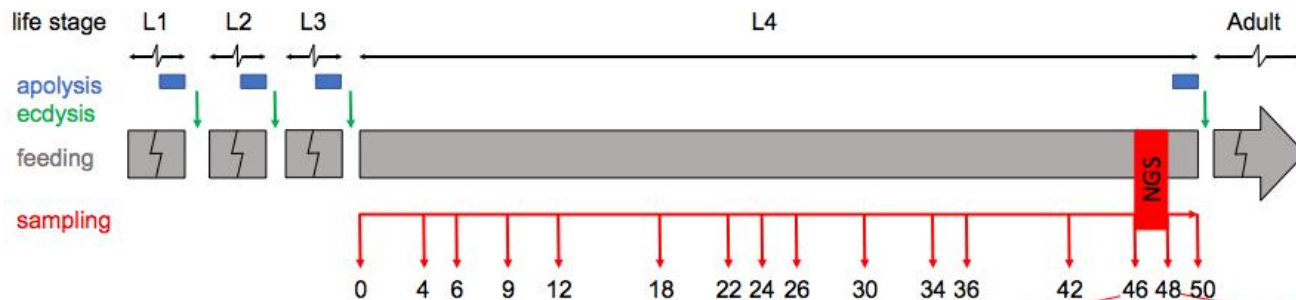


200µm

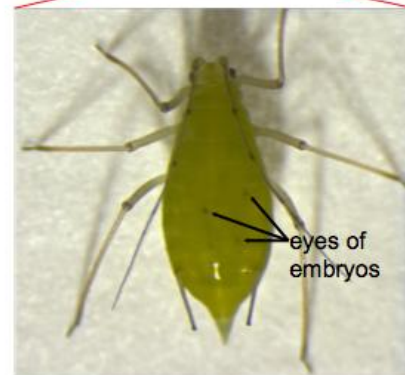
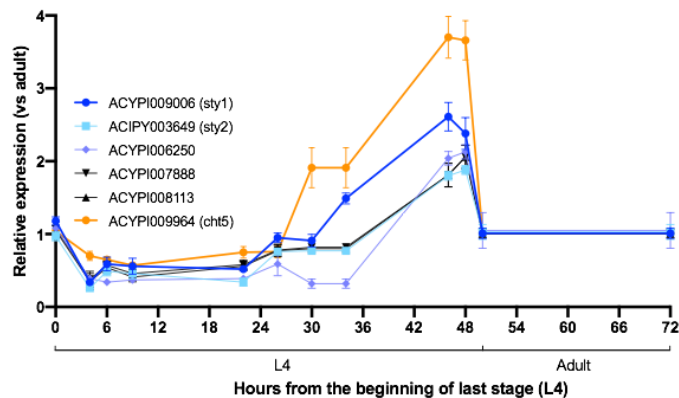
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The retort organ

in time



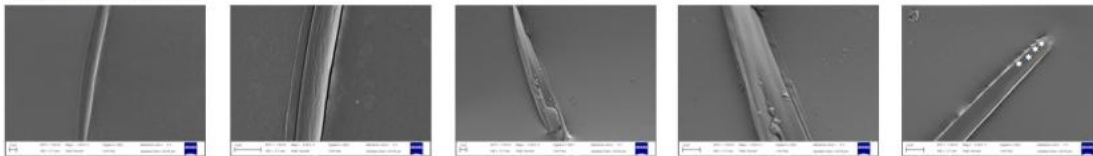
A) Expression level in aphid's heads



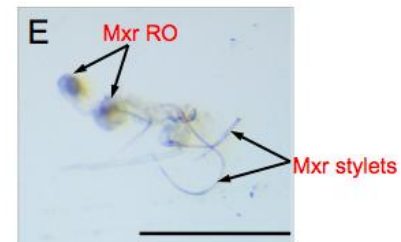
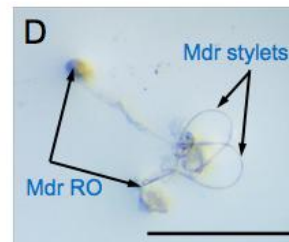
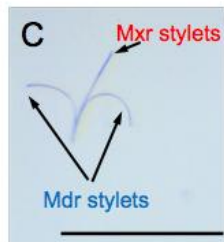
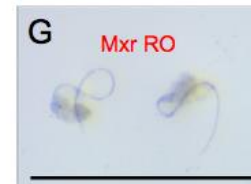
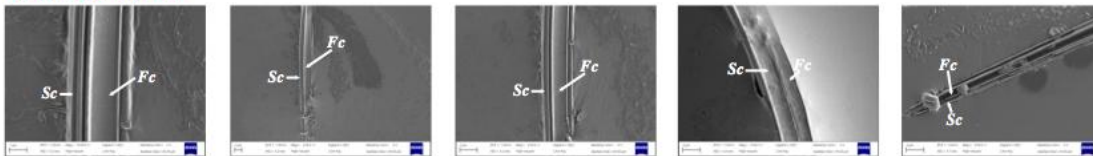
The retort organ(s)

in vitro: dissection of maxillary and mandibular stylet glands

Mandibular stylets:



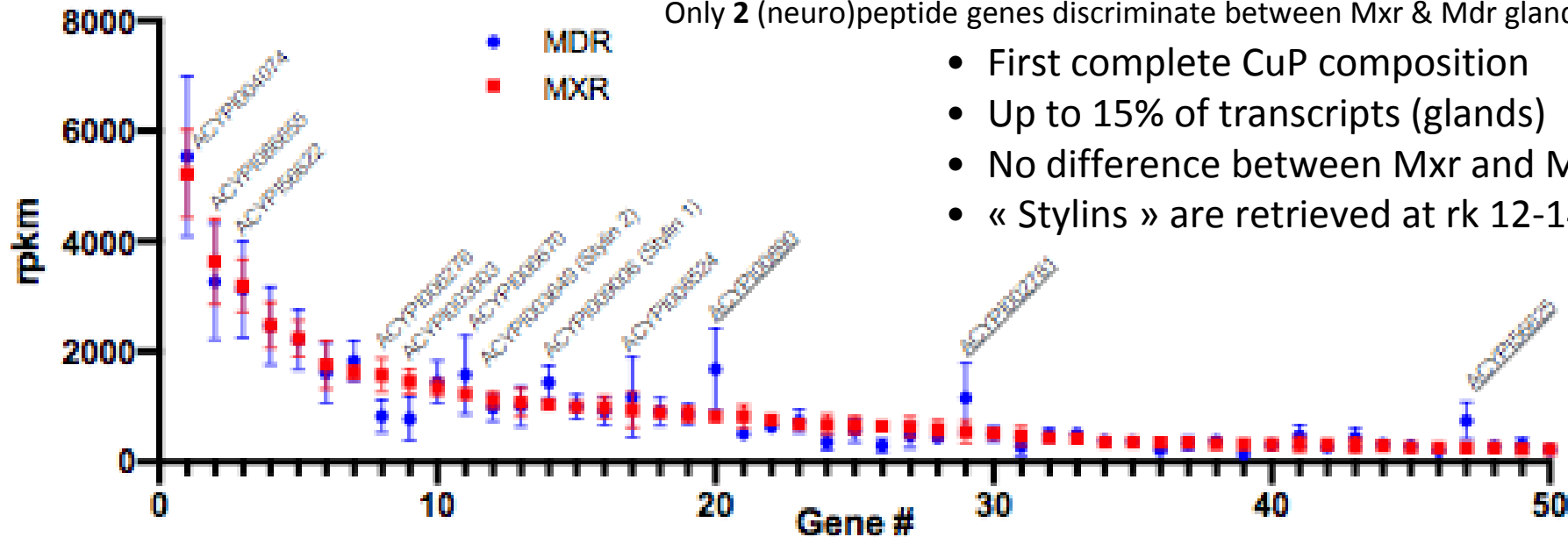
Maxillary stylets:



The retort organ

in (cuticular) proteins

C



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Cuticular proteins

and Cuticle Biopolymer



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200µm

What are cuticular proteins ?

in aphids

Basically: Proteins with a (set of specific) **chitin-binding** domain(s)

Table 5
Proteins^a identified from 14 arthropod proteomes using CutProtFam-Pred (see Section 2.6).

Family	<i>Drosophila melanogaster</i>	<i>Glossina morsitans</i>	<i>Culex quinquefasciatus</i>	<i>Aedes aegypti</i>	<i>Anopheles gambiae</i>	<i>Bombyx mori</i>	<i>Danaus plexippus</i>	<i>Tribolium castaneum</i>	<i>Apis mellifera</i>	<i>Nasonia vitripennis</i>	<i>Acyrtosiphon pisum</i>	<i>Pediculus humanus corporis</i>	<i>Daphnia pulex</i>	<i>Tetranychus urticae</i>
CPR_RR-1 ^b	61	33 ^c	49	66	43	47	47	34	13	19	9	9	101 ^c	0
CPR_RR-2 ^b	42	27 ^c	97	150	103	78	57	55	15	32	84	15	36	7
CPR_Uncl. ^b	34 ^a	17	30	28 ^c	21 ^c	19	18 ^c	21 ^c	10	18 ^c	20 ^c	17	152 ^c	31 ^c
CPAP1	29	11	10	14	13	13	16	13	15	16	10	12	20	14
CPAP3	10	6	8	9	10	6	10	7	7	6	8	6	12	5
CPCFC	1	1	1	1	1	1	1	2	0	0	1	1	0	0
CPF	5	1	5	3	4	1	1	5	4	5	2	1	0	0
CPLCA	13	9	3	3	3	2	1	1	0	0	0	0	0	0
CPLCG	4	4	41	18	25	0	1	1	0	0	0	0	0	0
CPLCW	0	0	0	7	10	0	0	0	0	0	0	0	0	0
Tweedle	29	9	9	6	12	4	5	3	2	2	3	2	0	0
Total	228	118	253	305	245	171	157	142	66	98	137	63	321	57

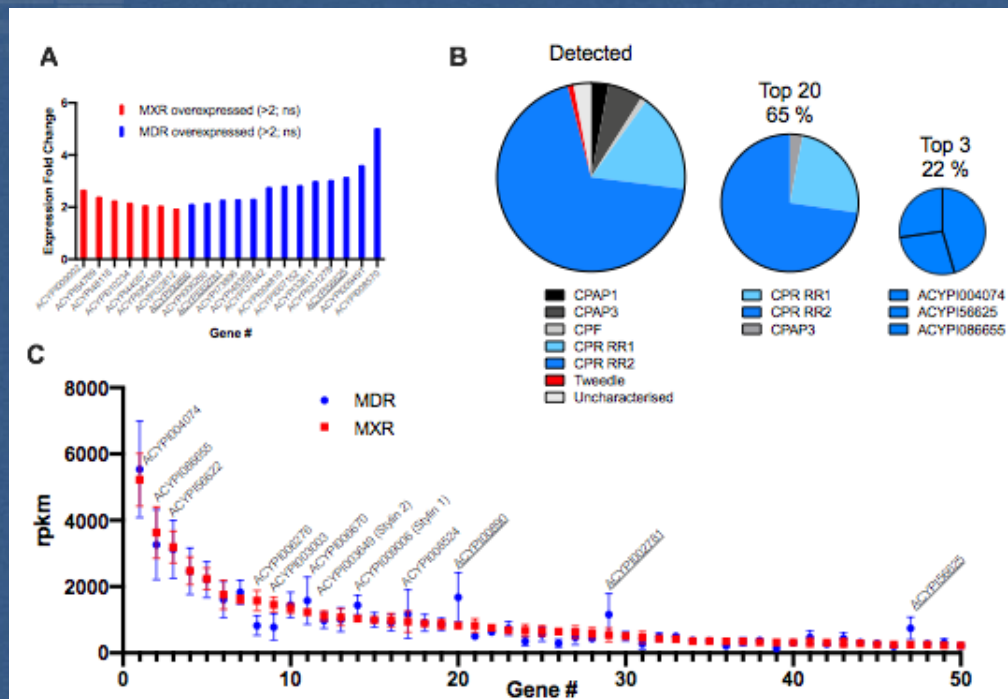
^a Numbers include all splice variants. See [Supplementary File 2](#) and [Supplementary File 3](#) for values based on gene numbers.

^b Sequences that score equal or above the assigned score cutoffs for the CPR_RR-1 and CPR_RR-2 models are classified in the corresponding family, while sequences that score below the assigned score cutoffs and above 0 for

Cuticular protein compositional complexity in aphid stylets

Shannon's diversity index

$$H' = - \sum_{i=1}^R p_i \ln p_i$$

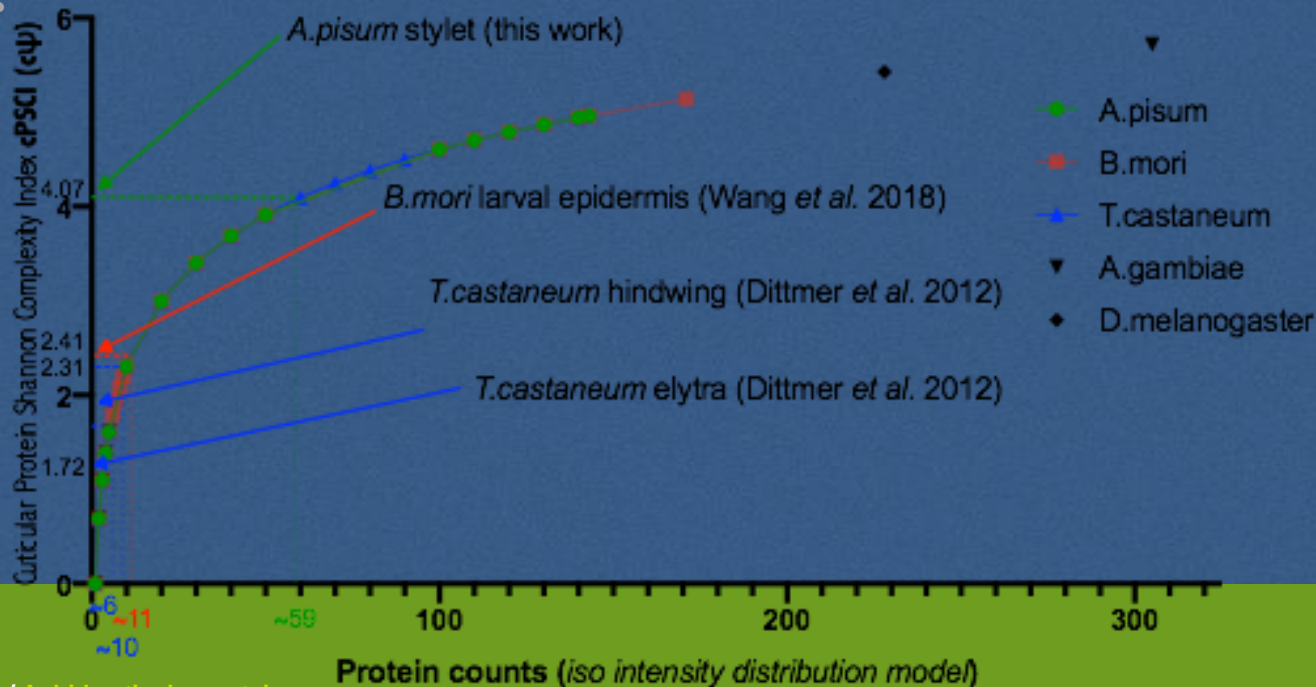


Cuticular protein compositional complexity

in insect cuticles

Figure 11: c ψ Plots

Compared Complexity
of insect cuticles
analyzed for
whole genome transcripts



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Compositional bias

vs homology domains

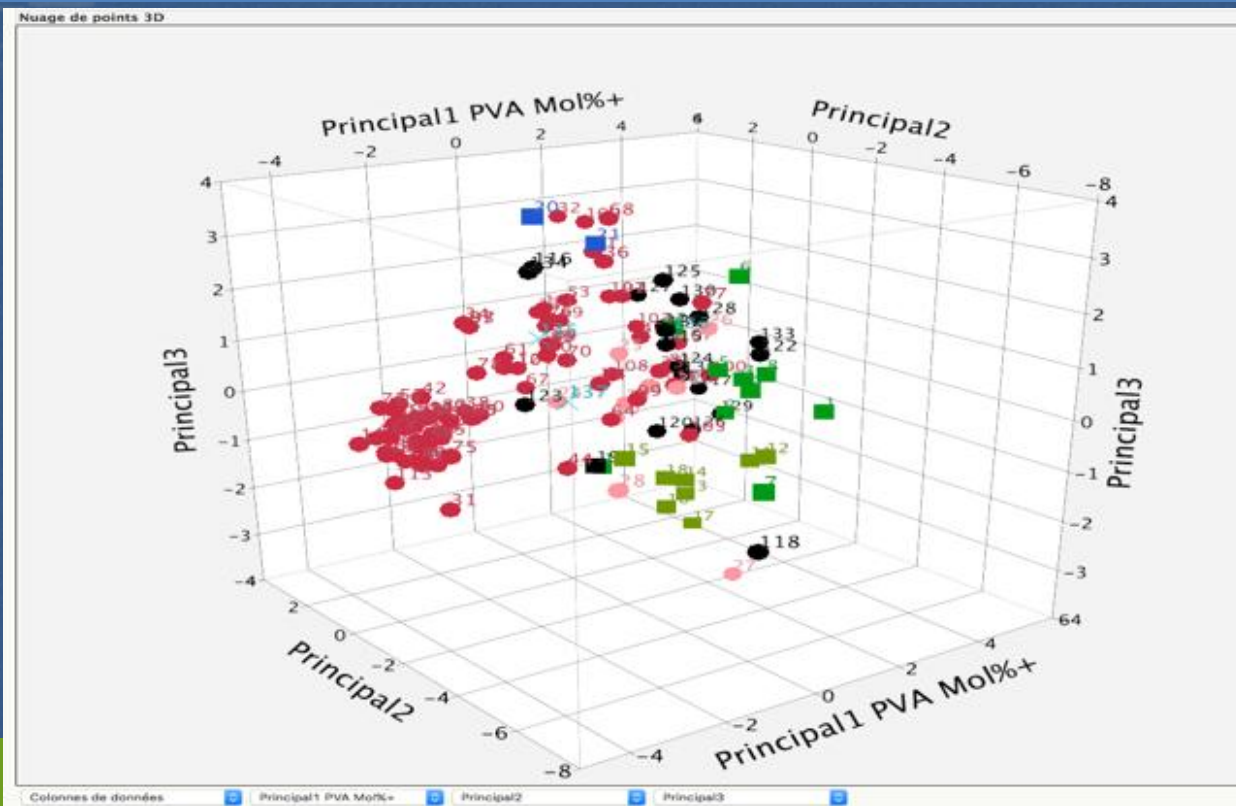


200µm

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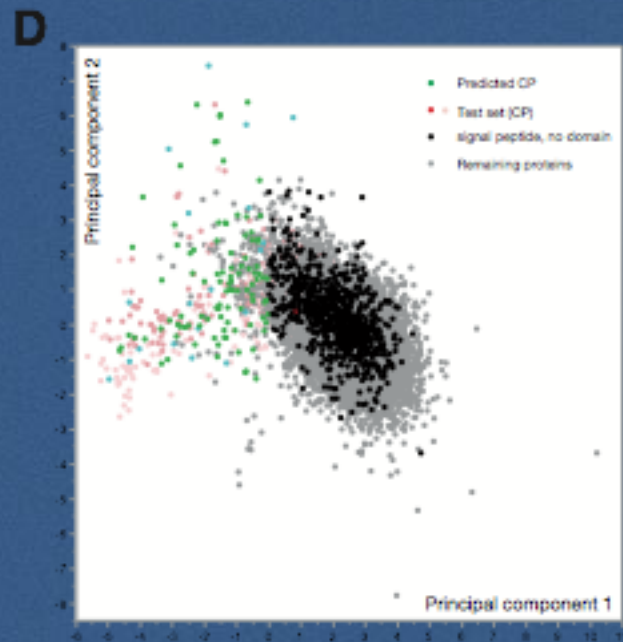
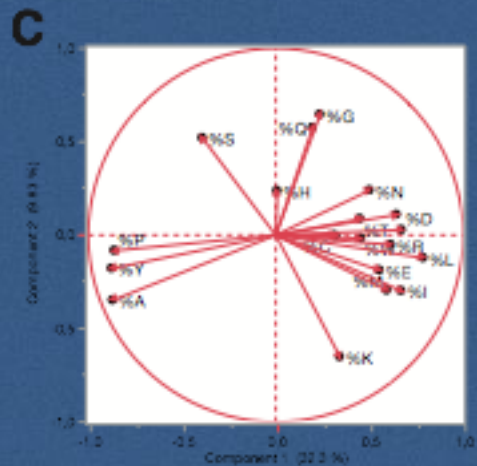
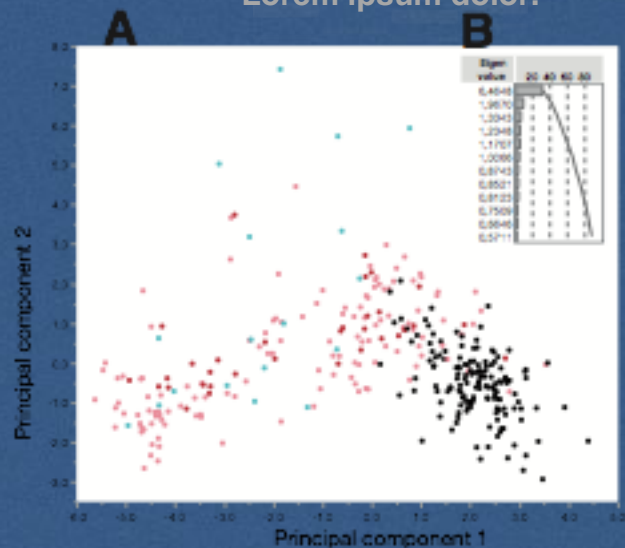
Compositional bias in cuticular proteins

in « standard » (recognized) cuticular proteins



Compositional bias in (cuticular?) proteins in aphid retort organ and aphid whole genome

Lorem ipsum dolor.



sample sequences

>ACYPI087730 grydary

EEAKKETKVVVEKRSDKSDLTPAATGRSFLGGYNSYPTGYSSGYNSGYNSGYNSYPSTYSGGYSSYPSYSSYPSSGY

>ACYPI082258 «paysa»ine

QEAPKAEEQVGAEAAARVKKHAYIAAPALAYSSPAYPYAYSAYPSAYPYSYSYPAAYPTAYASYPSYAYAHDDGKY

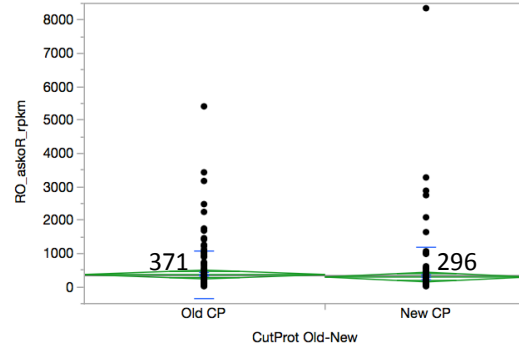
>ACYPI001038 hyperstylin

APSHGFISPVILPKVHLASPDVTVVRQPYVVQQTEPVFRHVYYAGDSVPYVSHHVQAYHQHPAVVAAYQTAPA

>J9JUP8_ACYPI

SVHSINFSVYSISTVHNTLTKMAAKLIIFTVCVASAFAQYSAPAYKPAYSSPAYAAPAY/
APKAYAPEPAYAPAPY**NFEYSVNDPHTYDVKSQSEYSDGNGYVKGSYSLL**LEADGST
YTADDYNGFNNAVVKNEGGYKAPAYAAPAYAAPAYSAPAYKPAYSAPAYSAPAYKPA
AYSAPAYSAPAYKPAYKPAY

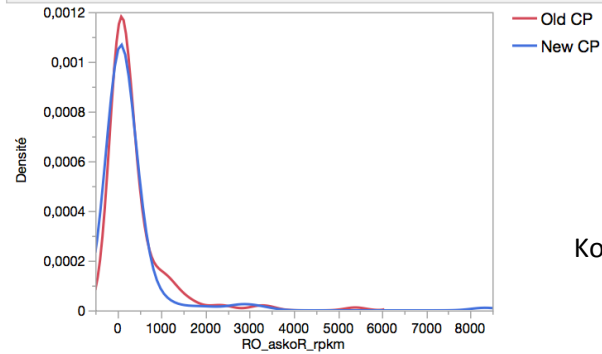
ANOVA à un facteur de RO_askoR_rpkm en fonction de CutProt Old-New



Anova test
 $p=0.44$

ANOVA à un facteur de RO_askoR_rpkm en fonction de CutProt Old-New

Comparer les densités



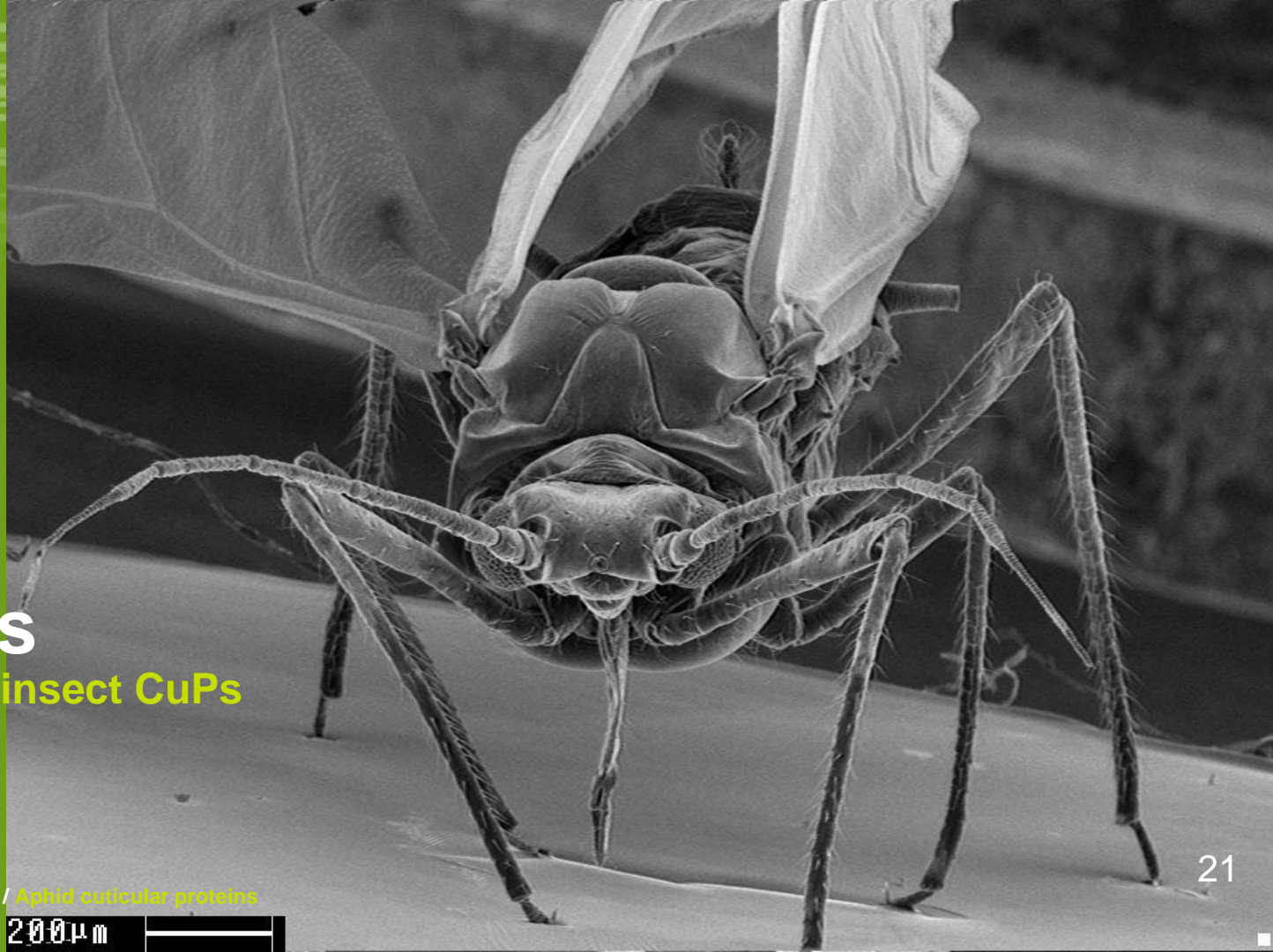
Kolm/Smir test

$p=0.01$

_the end

Conclusions

homology in insect CuPs



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Natalia Guschinskaya, Denis Ressnikoff, Karim Arafah, Sébastien Voisin, Philippe Bulet, Marilyne Uzest, Yvan Rahbé. Insect mouthpart transcriptome unveils extension of cuticular protein repertoire and complex organization, *iScience*, in revision.

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