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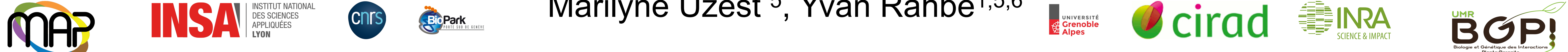
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Analysis of the retort organs, producing mouthpart cuticle, in (the pea) aphid

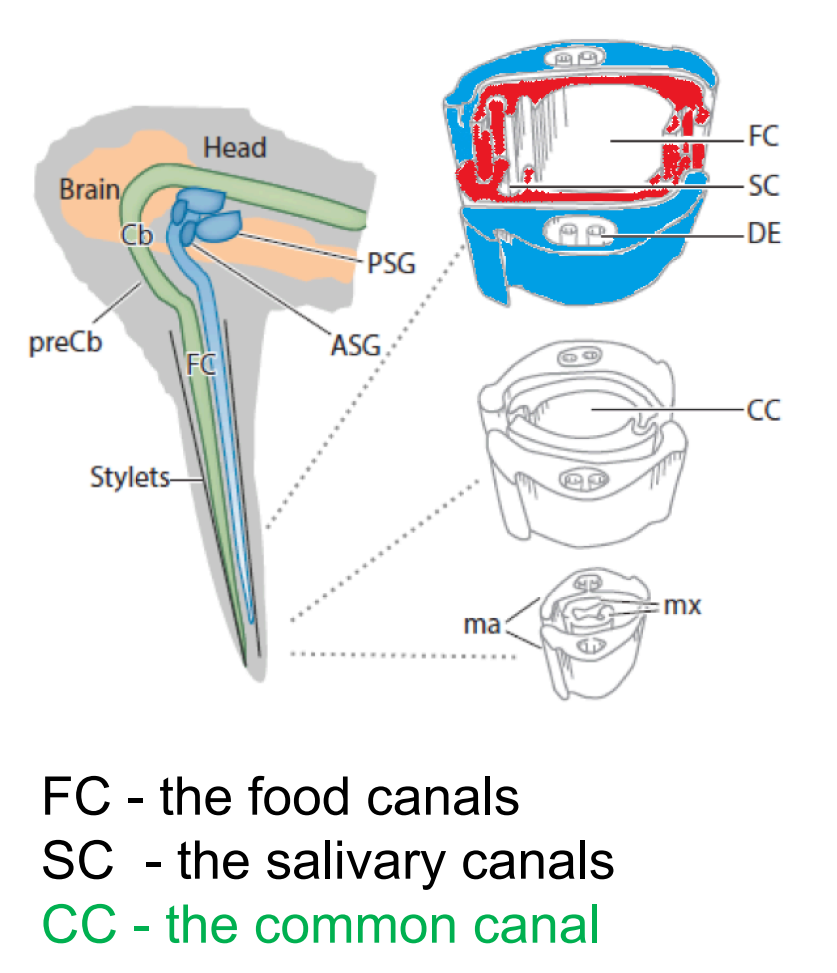
Natalia Guschinskaya^{1,6}, Denis Ressnikoff^{2,6}, Karim Arafah^{3,4}, Sébastien Voisin^{3,4}, Philippe Bulet^{3,4},
Marilyne Uzest⁵, Yvan Rahbé^{1,5,6}



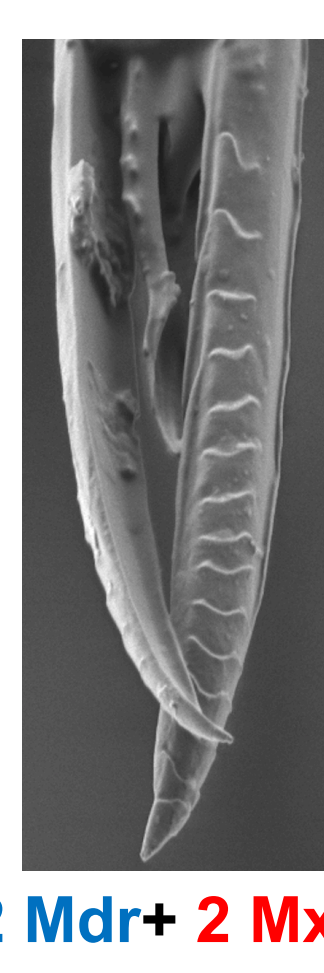
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Introduction

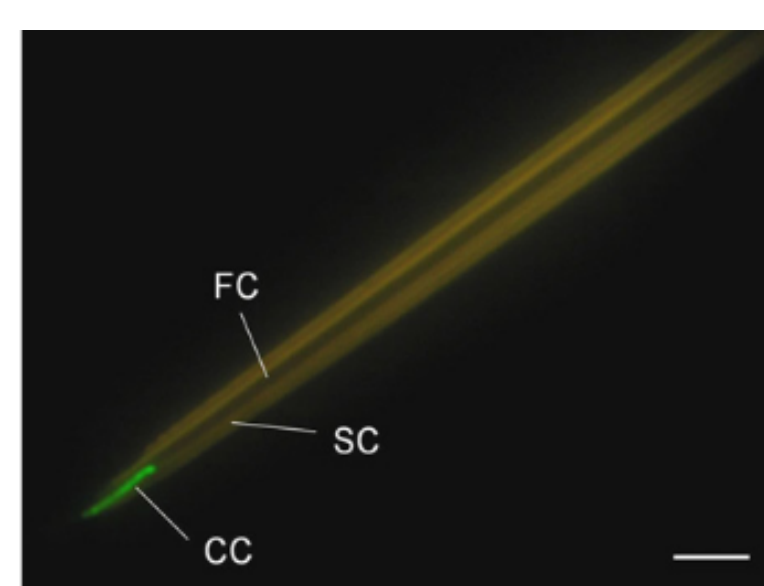
Insects display specialized cuticle territories with very specific molecular and architectural structures and functions, among which micro-structures governing their interactions with transmitted microbes (viruses, bacteria). Very often, these territories are displayed by mouthparts, a very specialized subset of insect cuticular structures, and yet poorly studied. In aphids, the main transmitters of noncirculative phytoviruses, a piercing-sucking mouthpart composed of two external mandibular stylets that surround two inner maxillary stylets bearing the virus receptor. We provide the first analysis of any insect's mouthpart cuticle, through aphid "retortiforms organs" (ROs) specialized in producing these (stylet, mandibular and maxillary) mouthparts.



FC - the food canals
SC - the salivary canals
CC - the common canal



2 Mdr + 2 Mxr

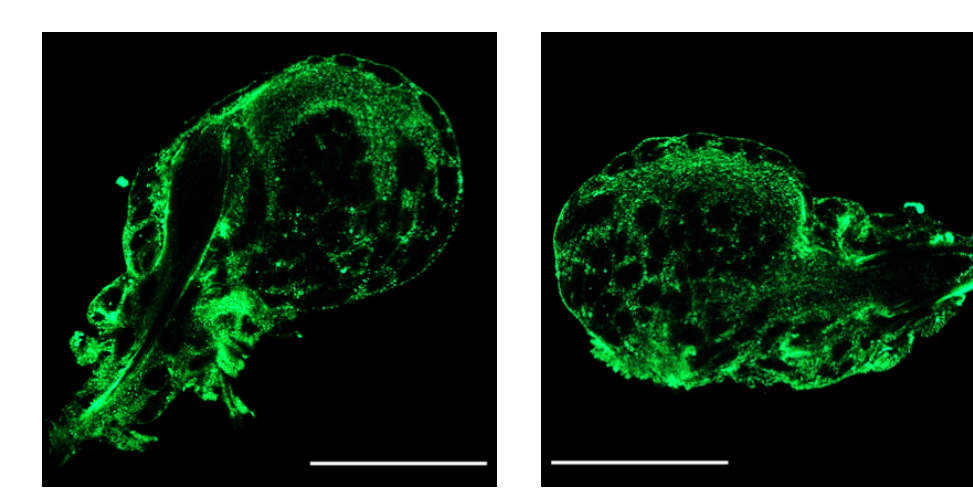


In CC, the CaMV P2 protein fused to GFP

Mxr stylets bearing the virus receptor (Uzest et al., 2010)

Imaging of Retort Organs

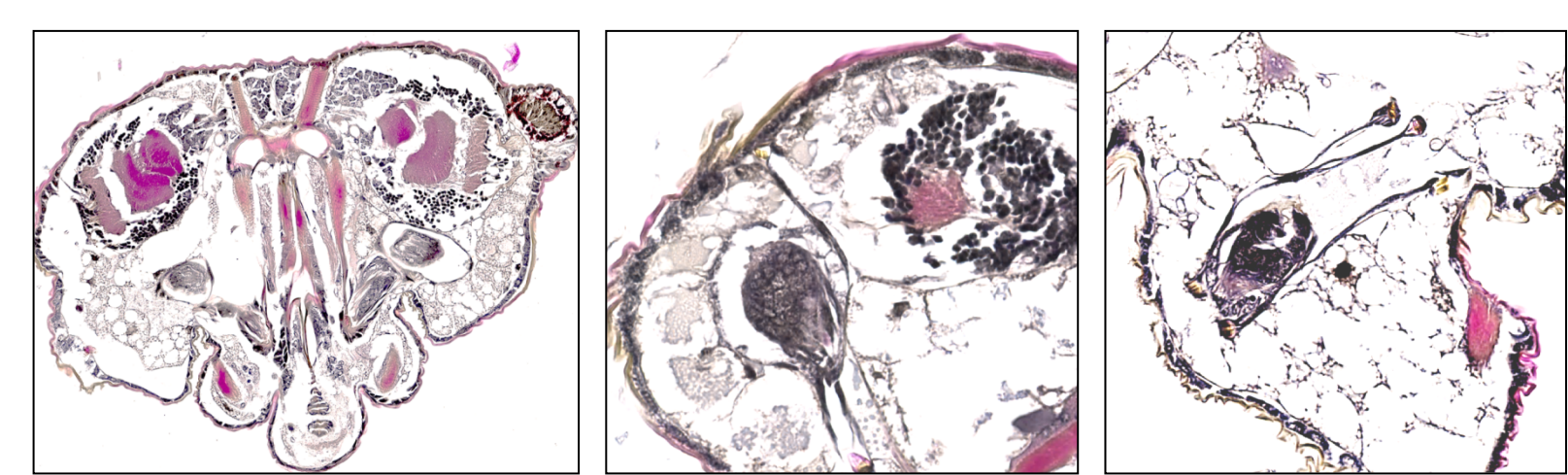
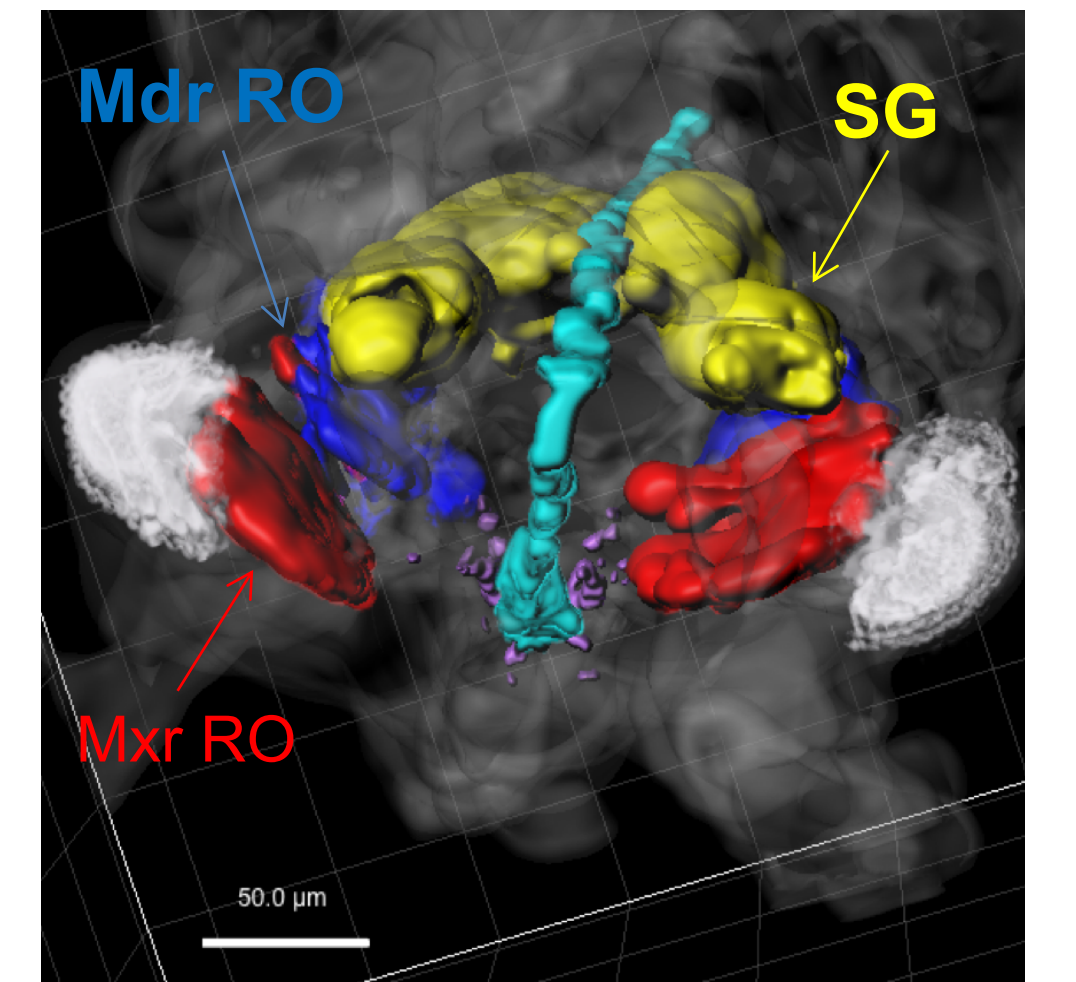
❖ *in situ* laser-scanning Microscopy
[Confocal autofluo; Imaris3D]



Mxr RO

Mdr RO

❖ Cutting sections of paraffin-embedded tissues and 3D-reconstruction [optical, \$\$\$ staining; Imaris3D]

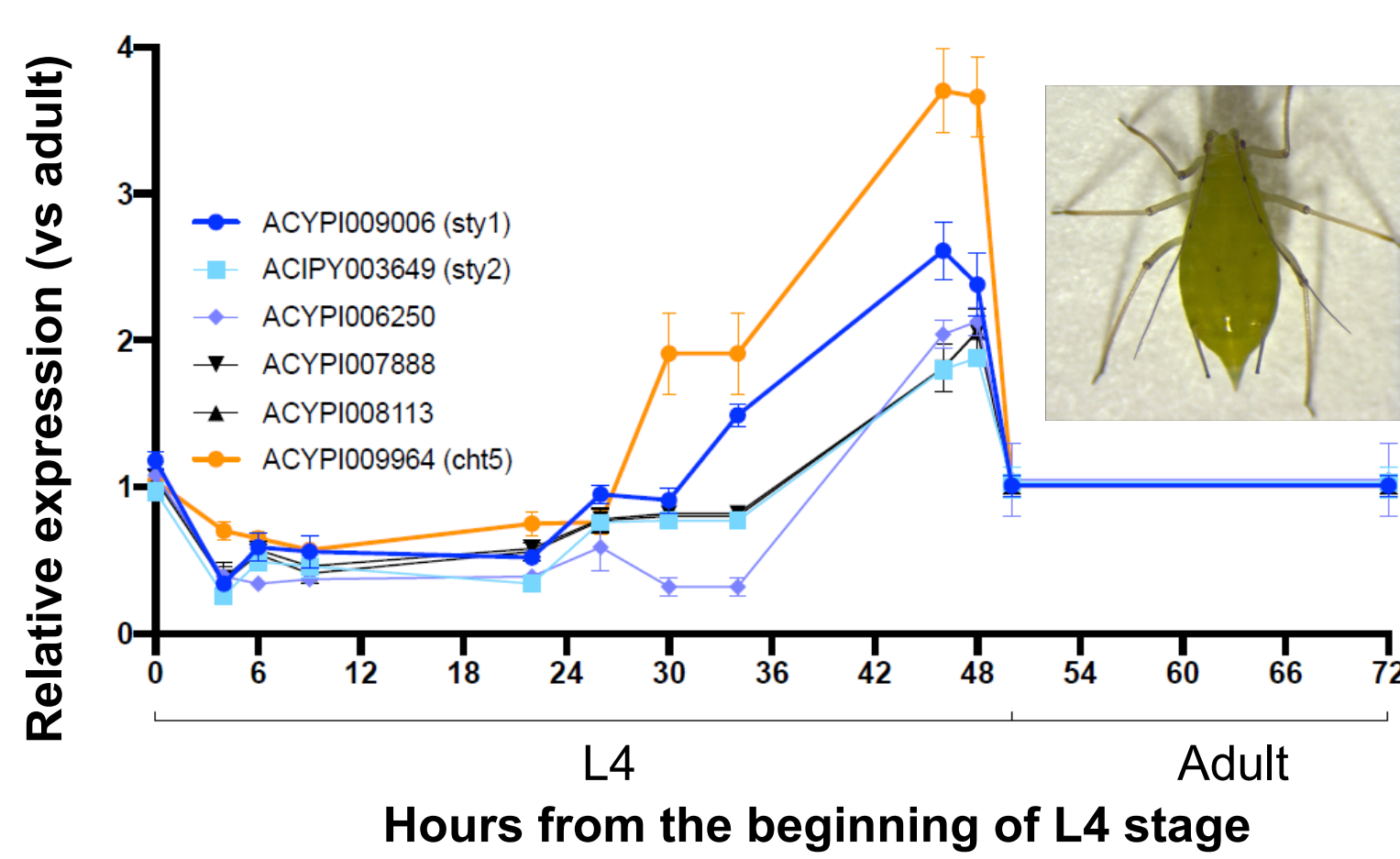


Mxr RO

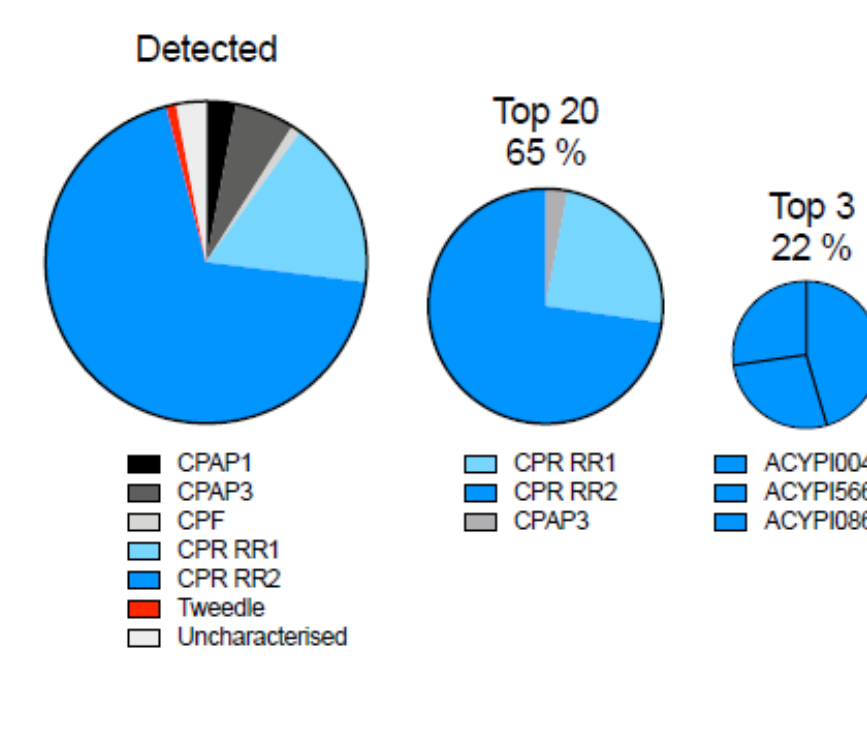
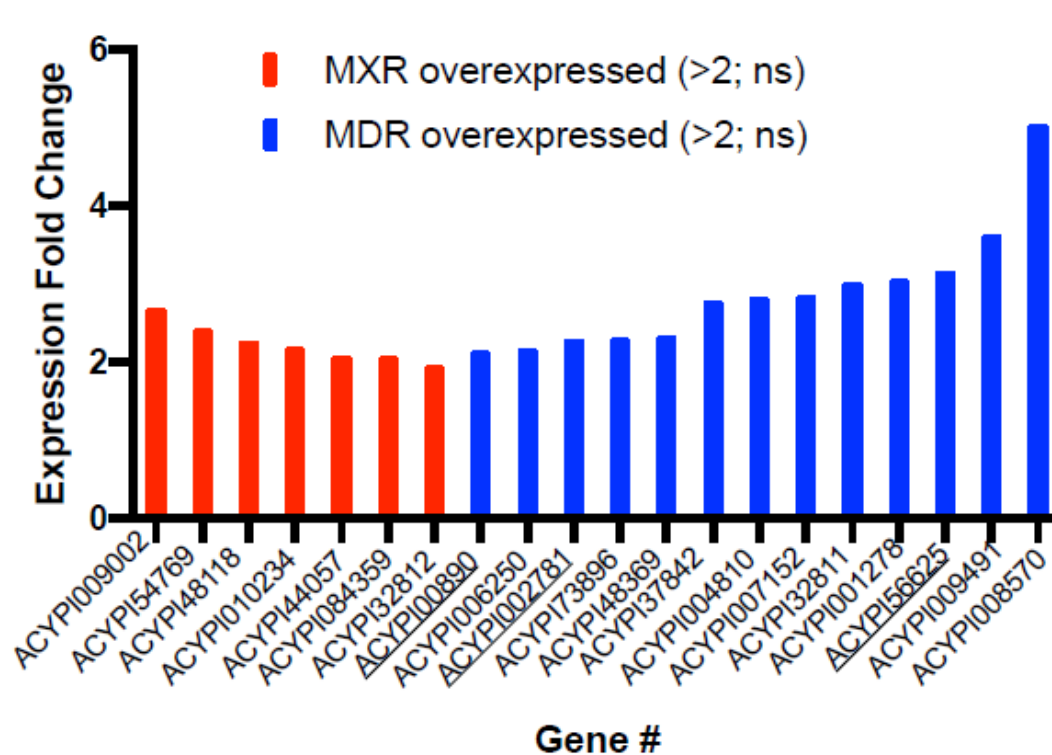
Mdr RO

Transcriptomics of Retort Organs

Aphid genes coding for cuticular protein have expression peaks at L4-48h

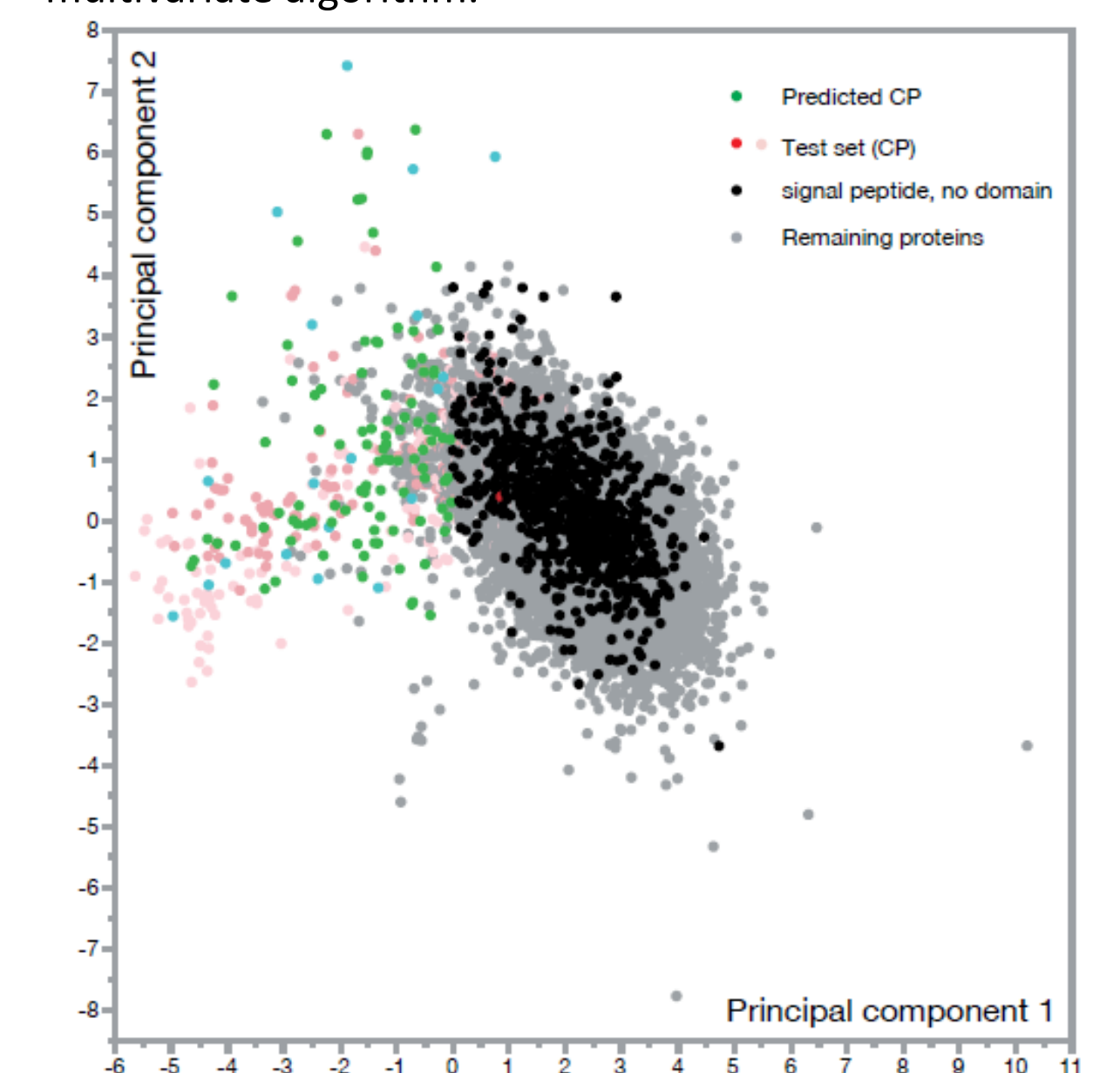


Mxr and Mdr stylets share a same global cuticular protein composition

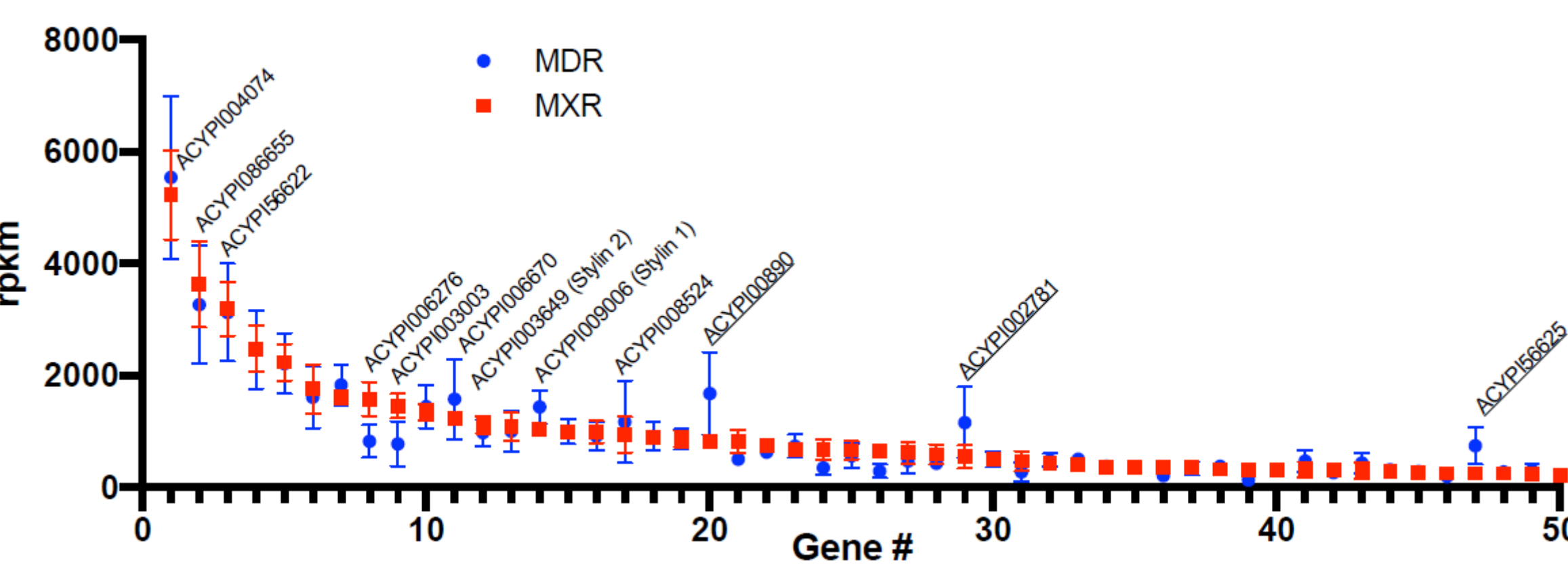
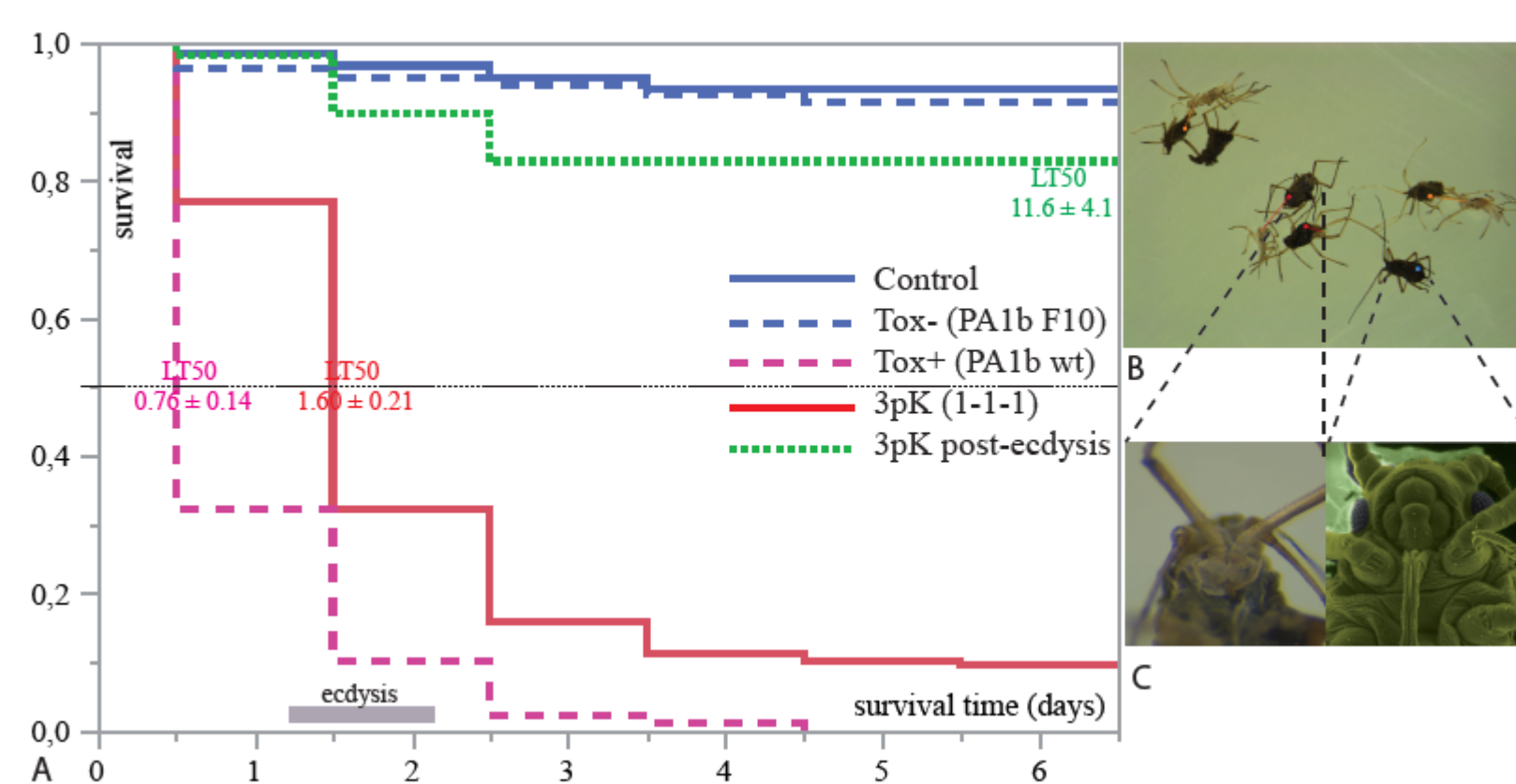


RO expression analysis defines a potential new class of cuticular proteins

We identified, within the 200 RO-most expressed genes, a series of unannotated secreted proteins that show a striking imbalance in amino-acid composition, similar to that of cognate CUPs. From this, we identified 121 new cuticular proteins (in the whole genome) with such an multivariate algorithm.



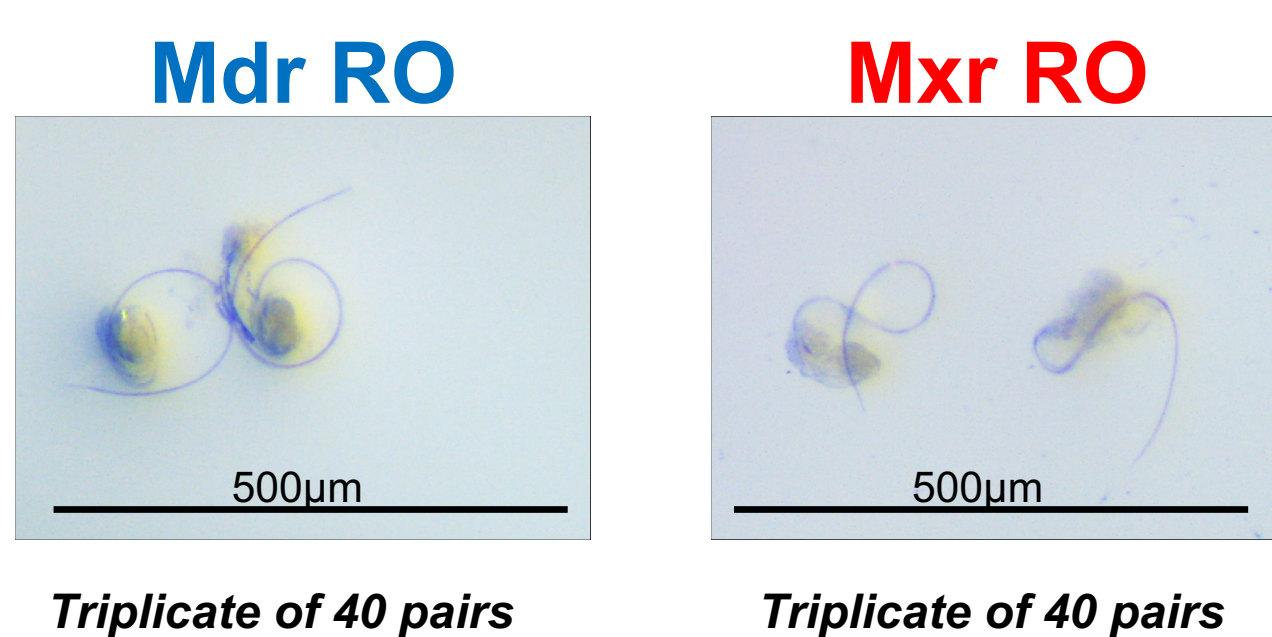
Pyrokinin signs mandibular gland expression and alters imaginal moult



Proteomics of Retort Organs

Methods:

❖ Sample preparation by direct dissection



Triplicate of 40 pairs

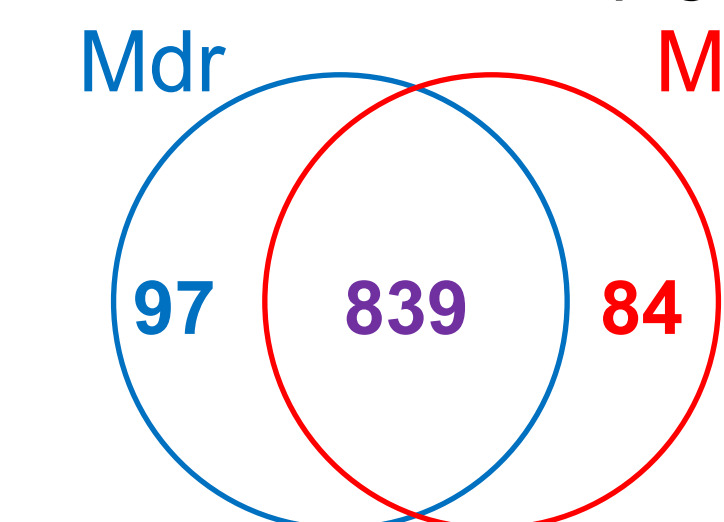
Triplicate of 40 pairs

❖ Extraction with 0,1% Rapigest and HFIP
❖ nano-LC/MS-MS
❖ Identification with Proteome Discoverer 2.0
❖ Label Free Quantification

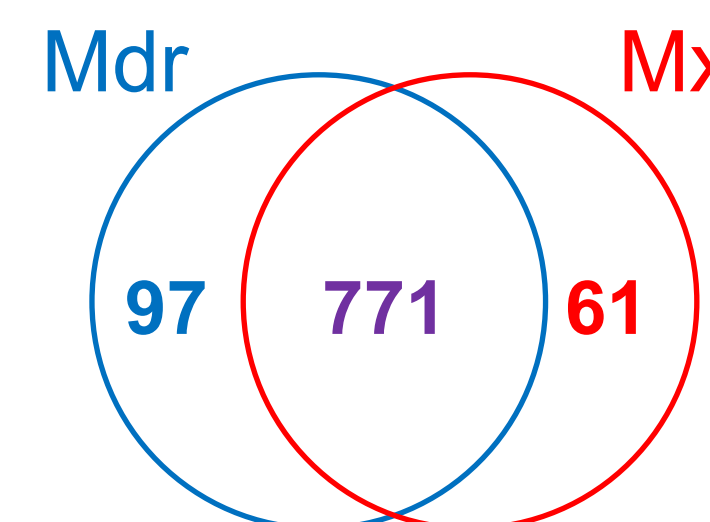
Results:

a) First proteome of retort glands as cuticle producing organs

Extracted with Rapigest



Extracted with HFIP



b) Detection of cuticular proteins:

Known CP families:
53 of 143 (37%)

New CP family:
31 of 121 (31%)

Mdr Mxr

3 50 3

ACYPI003003 RR-2
ACYPI000983 RR-2
ACYPI005387 RR-2

ACYPI001932 CDA
ACYPI002005 RR-2
ACYPI045536 CPAP1

Core "cuticulome" of RO:

1 CPAP1 34 CPR_RR-2
6 CPAP3 1 Tweedle
7 CPR_RR-1 1 CP Unchar



Proteomics of Stylets

❖ First package of WP1 (Stylet analytics from ANR StyliHook)
❖ Separate work by Deshoux, Masson et al. (in prep, see refs)
❖ First proteomic analysis of stylet (mouthpart) cuticle of any insect

Publications from ANR StyliHook WP1

Guschinskaya, N., Ressnikoff, D., Arafah, K., Voisin, S., Bulet, P., Uzest, M., and Rahbé, Y. Insect mouthpart transcriptome unveils extension of cuticular protein repertoire and complex organization. *iScience* (2019) in revision
Deshoux, M., Masson, V., Guschinskaya, N., van Munster, M., Cayrol, B., Webster, C. G., Arafah, K., Voisin, S., Blanc, S., Rahbé, Y., Uzest, M., and Bulet, P. Cuticular structure proteomics in the pea aphid *Acyrtosiphon pisum* reveals new plant virus receptor candidates at the surface of the acrostyle. *Mol. Cell. Proteomics* (2019) (2019 in prep)
Rahbé, Y. Pathogen transmission by insects: impact of vector evolutionary histories, & comparative vector biology between plant and animal pathogens (2019). in *Microbes 2019*, invited (SFM ed., SFM, Cité des Sciences et de l'Industrie, Paris (Fra))
Guschinskaya, N., and Rahbé, Y. Comparison of whole genome expression profiles of a liquid- vs solid-secreting gland in insects: the stylet and salivary glands of the pea aphid *Acyrtosiphon pisum*. *PLoS One* (in prep 2019)
Guschinskaya, N., Arafah, K., Voisin, S., Uzest, M., Rahbé, Y., and Bulet, P. Differential proteomic analysis of a couple of cuticular insect glands, the aphid stylet (retort) organ. *Mol. Cell. Proteomics* (in prep 2019)
Guschinskaya, N., Yvon, M., Bulet, P., Rahbé, Y., and Uzest, M. Apoptotic (pea) aphids display altered salivary behaviour and proteome, but are not impeded in their transmission behaviour for non-circulative viruses. (in prep 2019)
Guschinskaya, N., Deshoux, M., Ressnikoff, D., Uzest, M., and Rahbé, Y. Morphology and immunological / transcriptomic characterization of the Retort Glands of pea aphid *Acyrtosiphon pisum*. *Arthropod Structure and Development* (prev 2020)
Guschinskaya, N., Ribot, C., and Rahbé, Y. Insect interactions with cellular pathogens at the cuticular interface. (prev 2020)
Rahbé, Y., Guschinskaya, N., and Uzest, M. Comparative genomics of cuticular proteins in the pea aphid and other insect models. (prev 2020)