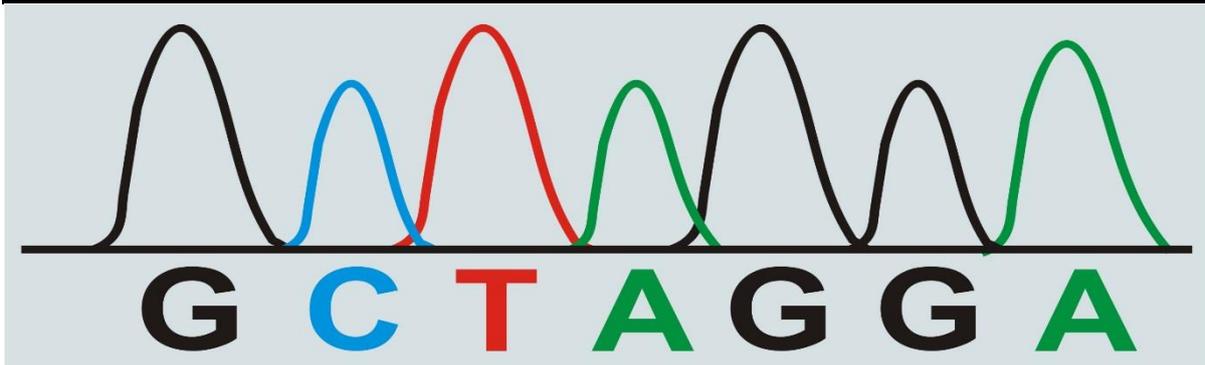


# UK-FRANCE JOINT MEETING ON APHIDS

April 3<sup>rd</sup> – 5<sup>th</sup> 2019, Rothamsted Research, Harpenden, UK

## PROGRAMME AND ABSTRACTS



# PROGRAMME

(For author details see abstracts)

**Wednesday 3<sup>rd</sup> April**

**Chair: Richard Harrington**

**13.00 Lunch**

**14.00 Welcome from Richard Harrington and Jean-Christophe Simon**

**14.10 Welcome from Professor Achim Dobermann, Director of Rothamsted Research**

**14.30 Invited keynote: James Bell:** Automatic identification in the field and lab: what's possible with aphids?

**15.10 Steve Foster:** New challenges with aphid control after the EU ban of neonicotinoid seed treatments on all outdoor crops

**15.25 John Pickett:** New opportunities to manage ecosystem services for conservation biological control of pest aphids

**15.40 Estelle Postic:** Unravelling trophic webs between aphids and parasitoids in strawberry greenhouses to improve a biological control program

**15.55 Refreshments**

**16.25 Amandine Cornille:** Invasion history of a major apple aphid pest in Europe and beyond

**16.40 Gaël Le Trionnaire and Marilyne Uzest:** Development of a robust CRISPR-Cas9 mutagenesis protocol in the pea aphid to knock out *stylin-01* gene and study the role of Stylin-01 cuticular protein in *Cauliflower mosaic virus* transmission

**17.10 Maurice Hullé:** Encyclop'Aphid: a website for aphid enthusiasts

**17.25 Please fix posters if you have not already done so, and load tomorrow's orals**

**18.00 Coach to Aubrey Park Hotel**

**Thursday 4<sup>th</sup> April**

**Chair: Jean-Christophe Simon**

**08.45 Coach from Aubrey Park Hotel**

**09.30 Invited keynote: Julie Jaquiéry:** Sex loss in aphids: mechanisms and evolutionary consequences

**10.10 Ramiro Morales-Hojas:** Genetic structure of cereal aphids in the UK

**10.25 Mélanie Ribeiro-Lopez:** IAP (Inhibitor-of-Apoptosis Protein) gene family expansion and functional diversification in aphids

**10.40 Hana Platkova:** Vertical stratification in temperate floodplain forest – where to look for aphids

**10.55 Hugh Loxdale:** Generalism in nature...the great misnomer: aphids as examples

**11.10 Refreshments**

**11.40 Gia Aradottir:** Aphid resistant wheat: is it probable or even possible?

**11.55 Amma Simon:** Elucidating mechanisms of resistance to *Rhopalosiphum padi* (L.) (bird cherry–oat aphid) in ancestral wheat

**12.10 Felix Feistel:** The role of toxic quinolizidine alkaloids (QA) from *Genista* (Fabaceae) in aphid–plant interactions

**12.25 Maria Paulmann:** Forisomes – possible key players in legume defence against aphids and *Acyrtosiphon pisum* host race development

**12.40 Yvan Rahbé:** From stylet to cuticle: transcriptomics and genomics of aphid cuticular proteins

**13.00 Lunch and posters**

**Chair: Simon Leather**

**14.00 Invited keynote: Tsutomu Tsuchida:** Molecular basis and ecological relevance of symbiont-mediated body colour change in aphids

**14.40 Chen Luo:** *Acyrtosiphon pisum* humoral immunity and life traits are modulated selectively by facultative symbionts

**14.55 Ailsa McLean:** Aphid symbiont confers multiple phenotypes without trade-offs

**15.10 Gaurav Pandharikar:** Cross-talk between aphid facultative symbionts and nitrogen fixation symbiosis in the aphid and legume interaction

**15.25 Inès Pons:** Evolution of symbiosis in insects: mechanisms involved in associations between aphids and the gut-associated *Serratia symbiotica* bacterium

**15.40 Refreshments**

**16.10 Corentin Sochard:** Testing for co-adaptation potential through experimental evolution of aphid–symbiont interactions

**16.25 Christoph Vorburger:** Parasitoid pre-adaptation improves biological control of symbiont-protected aphids

**16.40 Jean-Christophe Simon:** Towards the mechanisms underlying host plant adaptation in the pea aphid complex

**16.55 Helmut van Emden:** Artificial diet for aphids – any good, any use?

**17.10 Mariusz Kanturski:** Ole E. Heie – entomologist, teacher, friend

**17.25 Posters**

**18.30 Coach to Aubrey Park Hotel (currently booked for 18.00)**

**19.30 Conference dinner**

**Friday 5<sup>th</sup> April**

**Chair: Richard Harrington**

**08.45 Coach from Aubrey Park Hotel**

**09.30 Special invitation: Roger Blackman:** How (or why) do they do that?

**10.10 Chris Bass:** Resistance to natural and synthetic xenobiotics in the aphid *Myzus persicae*: a tale of three P450s

**10.25 Maëlle Deshoux:** The acrostyle, an organ at the tip of aphid stylets, interacts with an effector from aphid saliva

**10.40 Michael Giolai:** Spatially resolved transcriptomics reveals plant host responses to the aphid pest *Myzus persicae*

**10.55 Matteo Gravino:** Plant immunity triggered by oligogalacturonides in defence against *Myzus persicae* requires calcium-dependent protein kinases and is inhibited by the aphid effector Mp10

**11.10 Refreshments**

**11.40 Urs Wyss (*in absentia*) FILM:** The dangerous life of the linden aphid *Eucallipterus tiliae* in the lime tree microcosm

**12.30 Conclusions**

**13.00 Lunch**

**14.00 Meeting ends**

# POSTERS

(For author details see abstracts)

## **Mariska Beekman**

Symbionts to the rescue? The presence and effect of protective endosymbionts in biologically controlled greenhouse aphids

## **Federica Calevro**

Bacteriocyte reprogramming to cope with nutritional stress in a phloem sap feeding hemipteran, the pea aphid *Acyrtosiphon pisum*

## **Jurij Danilov**

First records of amphigonic morphs of *Cinara (Cinara) piniphila* (Ratzeburg, 1844)

## **Helena Donner**

Aphids out of control – how defensive symbiosis affects parasitoid biocontrol success

## **Nadine Douglas**

Distinguishing the watery and gelling saliva of the pea aphid (*Acyrtosiphon pisum*) by quantitative mass spectrometry

## **Helmut van Emden**

Changing the host selection responses of aphids through a short experience of a novel secondary plant compound

## **Varvara Fazalova**

How rapid is the pea aphid radiation?

## **Dion Garrett**

Predicting the future: migration patterns, first flight and evolution of resistant biotypes of the trap-shy currant–lettuce aphid, *Nasonovia ribisnigri*

## **Sylvie Hudaverdian**

An integrated protocol for efficient CRISPR-Cas9 mutagenesis in the pea aphid

## **Joshua Joyce**

Investigating early aphid-induced Ca<sup>2+</sup> signals and their role in determining aphid–host compatibility

## **Mariusz Kanturski**

Antennal sensilla of Lachninae (Hemiptera Aphidomorpha): a new approach

## **Mariusz Kanturski**

The comparative study of the reproductive system of sexual morphs of the model organism – the pea aphid *Acyrtosiphon pisum* (Hemiptera, Aphididae)

## **Torsten Knauer**

Stylectomy for analyzing legume phloem sap substances after pea aphid infestation – evaluation of a method

**Beth Moore**

Do patterns of relatedness determine the spatial structure of insecticide resistance in *Sitobion avenae*?

**Stéphanie Morlière**

Optimization of protocols for secondary symbiont introduction and elimination in the pea aphid

**Hana Platková**

The effect of wheat variety on demography of *Metopolophium dirhodum*

**Tom Pope**

Host selection by the English grain aphid (*Sitobion avenae*): is Maris Huntsman still the number one choice for aphids?

**François Renoz**

Revealing the microbiota of the subterranean aphid *Anoecia corni* through metagenomic sequencing

**Mélanie Ribeiro Lopes**

A novel cell death process eliminates both bacteriocytes and their symbionts in the pea aphid/*Buchnera* symbiotic system

**Joe Roberts**

Biological crop protection: a new ‘slow down–speed up’ strategy for aphid management

# **ABSTRACTS**

**(Alphabetical by presenter)**

**In all cases, the presenting author has been placed as the first author to make the abstract easy to find during the meeting.**

**Only the address of the presenting author is given.**

**All abstracts refer to oral presentations unless headed “POSTER”.**

**Aphid resistant wheat: is it probable or even possible?**

**Gudbjorg I. Aradottir, Amma Simon, Beant Singh, Lesley Smart, Janet Martin and Jackie Freeman**

**Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK**

Cereal aphids have long been problematic in wheat production. They are hard to spot in the crop and although they don't often cause direct damage in the UK, they transmit the barley yellow dwarf viruses that cause yield losses, often up to 30%. Resistant wheat varieties are not commercially available to the two aphid species of economic importance in the UK, the bird cherry–oat aphid (*Rhopalosiphum padi*) and the English grain aphid (*Sitobion avenae*).

We have screened over 1000 wheat lines, ranging from wild relatives to commercial wheat varieties for resistance to cereal aphids and in this talk I will discuss the progress we have made in identifying aphid resistant germplasm, some of the possible mechanisms for wheat resistance or resilience against these two aphid species, and the potential for breeding.

## **Resistance to natural and synthetic xenobiotics in the aphid *Myzus persicae*: a tale of three P450s**

**Chris Bass**

**University of Exeter, College of Life and Environmental Sciences, Penryn, UK**

The process by which genetic novelty is created and drives the evolution of key innovations required for ecological adaptation is still relatively poorly understood. In this talk I will outline recent work investigating the role of cytochrome P450s in driving host-range expansion and xenobiotic resistance in the peach–potato aphid, *Myzus persicae*, the most economically important aphid pest worldwide. *M. persicae* is globally distributed and highly polyphagous with a host range of over 400 species including many important crop plants. Relatively recently this species host-shifted to tobacco, and we have previously demonstrated that the constitutive overexpression of a cytochrome P450, *CYP6CY3*, allows tobacco-adapted races of *M. persicae* to efficiently detoxify nicotine and has preadapted them to resist neonicotinoid insecticides. Our recent work has characterised the remarkable mutational events leading to the overexpression of *CYP6CY3* and two other P450s of the *CYP6CY* subfamily in resistant clones of *M. persicae*. The role that each of the three P450s plays in conferring resistance to natural and synthetic xenobiotics will be discussed.

## **POSTER**

### **Symbionts to the rescue? The presence and effect of protective endosymbionts in biologically controlled greenhouse aphids**

**Mariska M. Beekman, Bart A. Pannebakker, Eveline C. Verhulst, Bas J. Zwaan and Marcel Dicke**

**Wageningen University, Droevendaalsesteeg 1 (Building 107), 6708 PB, Wageningen, The Netherlands**

Aphids are major pests in greenhouses, largely because they transmit many agriculturally important plant viruses. Since chemical control of insect pests is no longer sustainable, aphids are increasingly controlled using biological control, which mainly relies on the use of natural enemies of aphids, often parasitoid wasps. Unfortunately, biological control of greenhouse aphids is not always successful, for unknown reasons. A possible explanation could be the presence of facultative endosymbionts that can protect their hosts against natural enemies.

Currently, we know of at least nine facultative aphid endosymbionts that can fulfil many different ecological functions for their aphid host. This project studies the presence and distributions of endosymbionts in greenhouse aphid populations. The dynamics of greenhouse aphid–endosymbiont populations under parasitoid pressure are studied, to test if populations of biologically controlled greenhouse aphids are enriched with protective endosymbionts. By curing and creating artificial infections in the lab, we will study how different endosymbionts affect the fitness and resistance of different aphid genotypes. When aphids infected with protective endosymbionts are found to be present in greenhouses, the mechanisms underlying this protection will be studied at a genomic level.

Ultimately, we will develop tailor-made strategies for growers to deal with outbreaks of endosymbiont-protected aphids. This study will help us gain insight into the ecological and genetic mechanisms driving endosymbiont–aphid–parasitoid interactions. By increasing our understanding of these interactions, we can improve biological control practices and contribute to a more sustainable, insecticide-free, agricultural sector.

## KEYNOTE

### Automatic identification in the field and lab: what's possible with aphids?

James R. Bell, Alex Dye, Kirsty L. Hassall and Ilyas Potamitis

Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK

In the last five years, there has been an exponential interest in real-time detection of insect threats. Researchers have used electronics to explore the possibilities of detection and have focussed efforts on camera traps with image analysis, radar and lidar, all with varying degrees of success. Bioacoustic analysis is playing an increasingly important role in entomology, especially for large enigmatic insects such as moths, flies and cryptic insect pests found within timber that literally knock on wood. The sound and therefore the vibration generated by an insect betrays its presence; most humans can hear the buzz of bees, wasps and mosquitoes approaching and even the tapping of deathwatch beetles within timber, given they all fall well within the audible range. Other insects have weaker bioacoustics and evade detection, but these stealthy insects include some of the most serious pests to agriculture, notably aphids.

The problem of detection is compounded by the fact that to a computer image algorithm, aphids are no more than small blobby insects that lack any identifiable markings to pin them down further than just a bug. Instead, to discriminate between aphid species, wingbeat signatures using information generated during wing flap is perhaps the only remaining method that promises discrimination. We know that species identification is an uphill challenge because aphids generate a wingbeat signal that is an order of magnitude weaker than mosquitoes, making this group hard to detect, even using very sophisticated methods. However, this has not dampened a mild enthusiasm by some entomologists to attempt to discriminate: Byrne (1987) showed that within the Hemiptera, whiteflies and aphids could be distinguished by their wingbeat frequency alone as early as the 1980s. Moore & Miller (2002) demonstrated twenty years later that five aphid species had different mean wingbeat frequencies and different harmonics, intimating the possibility of automatic species identification. There, research on aphid wingbeat frequencies came to an abrupt stop with seemingly little activity on automatic detection until very recently.

Here, we discuss the technical challenges of identifying aphid pests to species level using opto-acoustic methods. Using fast Fourier transform, a high-pass filter and a detrending step to 'clean' the signal, we plot the frequency spectra generated by a small number of aphid species to indicate the high variability of wingbeat recordings within and between species. Yet, from these we can estimate to reasonable confidence the fundamental frequency, the dominant frequency, the bioacoustic index and the temporal entropy, all of which show some role in aphid species classification. We will argue that a one index approach is flawed, and instead show support for combining indices for species discrimination.

Byrne, D.N. (1987) An examination of whitefly flight. *Vegetable Report* **1987**, 47-50.  
<https://repository.arizona.edu/handle/10150/221405>

Moore, A. and Miller, R.H. (2002) Automated identification of optically sensed aphid (Homoptera: Aphidae) wingbeat waveforms, *Annals of the Entomological Society of America* **95**, 1–8, [https://doi.org/10.1603/0013-8746\(2002\)095\[0001:AIOOSA\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2002)095[0001:AIOOSA]2.0.CO;2)

## **SPECIAL INVITATION**

**How (or why) do they do that?**

**Roger Blackman**

**Department of Life Sciences, The Natural History Museum, London SW7 5BD, UK**

Despite substantial progress in recent years, some elements of the developmental and reproductive processes of aphids are still shrouded in mystery. How do viviparous aphids feed their embryos? Why do chromosome numbers differ so much in some genera but remain stable in others? What is the function of the “B-chromosomes” of birch aphids - do they enable certain genes to be inherited only through the male line? What is the significance of the remarkable behaviour of aphid chromosomes during spermatogenesis? How and why has the seemingly ritualistic “tug-of-war” for the X chromosome between the spermatocytes survived for at least 150 million years? Yet there is another aspect of chromosome behaviour during the process of sperm production that differs between species, in a way which may have important consequences. Plenty of questions but, unfortunately, I shall not be providing any answers!

## POSTER

### **Bacteriocyte reprogramming to cope with nutritional stress in a phloem sap feeding hemipteran, the pea aphid *Acyrtosiphon pisum***

**Federica Calevro, Nicolas Parisot, Mélanie Ribeiro Lopes, Karen Gaget, Gabrielle Duport, Patrice Baa-Puyoulet, Hubert Charles and Patrick Callaerts**

**Univ Lyon, INSA-Lyon, INRA, BF2I, UMR0203, F-69621, Villeurbanne, France**

Nutritional symbioses play a central role in the ability of insects to thrive on unbalanced diets and in ensuring their evolutionary success. A genomic model for nutritional symbiosis comprises the hemipteran *Acyrtosiphon pisum*, and the gamma-3-proteobacterium, *Buchnera aphidicola*, with genomes encoding highly integrated metabolic pathways. *A. pisum* feeds exclusively on plant phloem sap, a nutritionally unbalanced diet highly variable in composition, thus raising the question of how this symbiotic system responds to nutritional stress.

We addressed this by combining transcriptomic, phenotypic and life history trait analyses to determine the organismal impact of deprivation of tyrosine and phenylalanine. These two aromatic amino acids are essential for aphid development, are synthesized in a metabolic pathway for which the aphid host and the endosymbiont are interdependent, and their concentration can be highly variable in plant phloem sap. We found that this nutritional challenge does not have major phenotypic effects on the pea aphid, except for a limited weight reduction and a 2-day delay in onset of nymph laying. Transcriptomic analyses through aphid development showed a prominent response in bacteriocytes (the core symbiotic tissue which houses the symbionts), but not in gut, thus highlighting the role of bacteriocytes as major modulators of this homeostasis. This response does not involve a direct regulation of tyrosine and phenylalanine biosynthetic pathway and transporter genes. Instead, we observed an extensive transcriptional reprogramming of the bacteriocyte with a rapid down-regulation of genes encoding sugar transporters and genes required for sugar metabolism. Consistently, we observed continued overexpression of the *A. pisum* homolog of RRAD, a small GTPase implicated in repressing aerobic glycolysis. In addition, we found increased transcription of genes involved in proliferation, cell size control and signaling. We experimentally confirmed the significance of these gene expression changes detecting an increase in bacteriocyte number and cell size *in vivo* under tyrosine and phenylalanine depletion.

Our results support a central role of bacteriocytes in the aphid response to amino acid deprivation: their transcriptional and cellular responses fine-tune host physiology providing the host insect with an effective way to cope with the challenges posed by the variability in composition of phloem sap.

## **Invasion history of a major apple aphid pest in Europe and beyond**

**Cornille, A., Grandcolas O., Remoué C., Venon A., Rousselet A., Alhmedi A., Beliën T., Momont L., Alins G., Franck P., Haddioui A., Jacobsen S.K., Andreev R., Simon S., Sigsgaard L., Guibert E., Ricard J.M., Tournant L., Mody K., Khachtib Y., Roman A., Ursu T., Zakharov I.A., Belcram H., Oram S., Agnello A., Beers E.H., Engelman J., Mottet C., Barrès B., Degrave A., Simon J.C., Harry, M., Giraud T., Falque M., Jardillier L., Deschamps P., Dapena E., Miñarro M. and Jousselin E.**

**GQE – Le Moulon, INRA, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91190, Gif-sur-Yvette, France**

Understanding how evolutionary processes have shaped the distribution and genetic structure of emerging pests is critical to tackling the processes driving species invasion, diversification, distribution and persistence. With frequent host shifts involving the colonization of new hosts across large geographic ranges, crop pests are relevant models to study the mechanisms of rapid diversification and colonization. Here we investigated the genetic diversity and population structure of the rosy apple aphid, *Dysaphis plantaginea*, the major aphid pest of the cultivated apple (*Malus domestica*), and we reconstructed its invasion history in Europe using approximate Bayesian computation. We used 30 newly developed highly evolving genetic markers (microsatellites) amplified on a comprehensive collection of 760 *D. plantaginea* individuals from Europe, Maghreb and North America. We showed a weak spatial population structure with four main clusters: a Moroccan, an American, a Spanish and a European excluding Spain. Genetic diversity in the rosy apple aphid was moderate suggesting that the dominance of asexuality over the year does not impair the maintenance of diversity in the population. We are currently running coalescent-based simulations (ABC) to provide further insights into the demographic history of this major aphid apple pest. Altogether these results show that the rosy apple aphid is an outstanding model to study the genetic consequences of rapid range expansion.

## POSTER

### First records of amphigonic morphs of *Cinara (Cinara) piniphila* (Ratzeburg, 1844)

Jurij Danilov

Institute of Biosciences, Vilnius University, Saulėtekio al. 7, LT-10257 Vilnius, Lithuania

*Cinara (Cinara) piniphila* (Ratzeburg, 1844) feed on shoots and twigs of *Pinus sylvestris* in Europe and Turkey, also recorded on *P. tabuliformis* in China. Life cycle and sexuales have not been recorded yet ([www.aphidsonworldsplants.info](http://www.aphidsonworldsplants.info)).

Amphigonic morphs of *C. piniphila* inhabiting shoots and 1-2 year-old twigs of young *P. sylvestris* 2-4 meters high were observed in 2 localities of Lithuania. Colonies of this aphid species have been explored there since summer 2017. They were recorded for the first time on 15 October 2017 in Alksnynė (Neringa district, Baltic coast). In 2018, the same host plants were monitored on a monthly basis from May until October. The first oviparae and males were collected on 11 August. By this time pine shoots had become entirely wooden. Amphigonic morphs dominated against parthenogenetic ones from 14 September, when the oldest needles had turned yellow. Oviparous females were available until 6 October, whilst males occurred until 24 September. Oviparae laid eggs in line along needles of shoots and twigs. Fresh eggs were yellow, later they turned into shining black. The first eggs were found on 14 September. They were both yellow and black. The last fresh eggs were found on 24 September. The second location was Puvočiai (Varėna district, South Eastern Plains of Lithuania). Oviparae and males of *C. piniphila* were collected there once on 27 September 2018.

Samples of viviparous females, oviparous females and males were collected from both localities for morphological preparations and DNA analysis (partial COI sequences). Oviparae of *C. piniphila* are egg-shaped, 2.8-3.7 mm long. They are dark brown to blackish, very similar to apterous viviparous females. Hind tibiae are slightly swollen, with 165-245 pseudosensoria. Males are winged, greyish-brown, smaller (2.2-2.9 mm long) and elongate. Their dorsal abdomen is pale, with small sclerites (up to 79  $\mu\text{m}$ ). HT I 0.51-0.59 x HT II. Length of hairs exceeds typical values for viviparous females. Hairs on ANT III are up to 84  $\mu\text{m}$  long, on ABD TERG V 112  $\mu\text{m}$  long and on the hind tibia 155  $\mu\text{m}$  long.

This research was funded in part by grant No P-MIP-17-365 from the Research Council of Lithuania. I gratefully acknowledge methodological help provided by professor Rimantas Rakauskas (Vilnius University). His colleague Jekaterina Havelka kindly carried out DNA analysis of samples of *C. piniphila*.

## **The acrostyle, an organ at the tip of aphid stylets, interacts with an effector from aphid saliva**

**M. Deshoux, B. Monsion, E. Pichon, C.G. Webster, V. Masson, K. Arafah, S. Voisi, P. Bulet, J. Jimenez, A. Moreno, A. Fereres, S. Blanc and M. Uzeit**

**BGPI, Univ Montpellier, INRA, CIRAD, Montpellier SupAgro, Montpellier, France**

Sustained feeding requires intimate associations between the insect and its host plant. In compatible plant–aphid interactions, effectors secreted in aphid saliva can modulate plant defenses triggered by the mechanical injury caused by the penetration of the stylets into plant tissues, and by the release of elicitor molecules also contained in the saliva.

The acrostyle is an organ located at the tip of aphid maxillary stylets shown to promote plant virus transport from host to host through specific protein-protein interactions (Webster *et al.*, 2018). This organ is located at the confluence of the salivary and food canals. It is therefore in contact with plant sap, cell content and aphid saliva, and we suspect that it could have a specific role during the aphid feeding process through the transient retention of effectors and their delivery within the plant.

To decipher the physiological function of the acrostyle, we identified proteins with peptides exposed at the surface of the organ – named stylins – susceptible to interact with molecules from aphid saliva. We will here present the ongoing characterization of Stylin-01 and Stylin-03. We first showed that the knockdown of *stylin-01* and *stylin-03* gene expressions had no impact on the fecundity and survival rate of *Myzus persicae*. However, we demonstrated by electropenetography (EPG) that silenced aphids were affected in their feeding behavior, particularly in phases linked to aphid salivation. In addition, Stylin-03 was shown to interact with the effector Mp10 (Bos *et al.*, 2010) in the yeast two-hybrid system and finally, we observed that Mp10 binds the acrostyle of *M. persicae* dissected stylets. All these results suggest that the acrostyle may be involved in aphid–plant compatible interactions.

Bos, J.I.B., Prince, D., Pitino, M., Maffei, M.E., Win, J. and Hogenhout, S.A. (2010) A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (Green Peach Aphid). *PLoS Genetics*, 6:e1001216

Webster, C.G., Pichon, E., van Munster, M., Monsion, B., Deshoux, M., Gargani, D., Calevro, F., Jimenez, J., Moreno, A., Krenz, B. *et al.* (2018) Identification of plant virus receptor candidates in the stylets of their aphid vectors. *Journal of Virology*, 92.

## **POSTER**

### **Aphids out of control – how defensive symbiosis affects parasitoid biocontrol success**

**Helena Donner, Bart Pannebakker, Eveline Verhulst, Bas Zwaan and Marcel Dicke**

**Laboratory of Genetics, Wageningen University, Droevendaalsesteeg 1, 6708PB  
Wageningen, The Netherlands**

Biological control of crop pests is becoming more important with the regulations on pesticide use becoming stricter. While for the biocontrol of aphid pests, parasitoid wasps are considered especially effective, they have been employed with varying degrees of success, and optimization is still necessary. Since facultative symbionts have been found in almost all pest aphid species studied so far, this could pose an underestimated threat to biocontrol efficiency. Laboratory studies on endosymbiont-conferred resistance in aphids have shown that parasitoid biocontrol can be seriously affected by defensive symbiosis. Studies on endosymbiont-conferred resistance in aphids have been limited to laboratory and field situations. Information on the effects of symbiont-conferred resistance in greenhouses is currently lacking. A greenhouse study is necessary considering the possible consequences of endosymbiont-conferred resistance on parasitoid augmentative biocontrol strategies. We are currently undertaking the first large-scale greenhouse study on the effects of endosymbiont-conferred resistance on parasitoid biocontrol success.

## **POSTER**

### **Distinguishing the watery and gelling saliva of the pea aphid (*Acyrtosiphon pisum*) by quantitative mass spectrometry**

**Nadine E. Douglas, Tom L. Wilkinson and James C. Carolan**

**UCD School of Biology and Environmental Science, University College Dublin, Dublin, Ireland**

**Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland**

The pea aphid (*Acyrtosiphon pisum*) is an agriculturally significant insect pest, which feeds on the phloem sap of the legume family, causing damage directly through feeding and indirectly as a vector of many plant-infecting viruses. Aphid feeding involves the secretion of two types of saliva: i) watery saliva injected into plant cells and the sieve elements, and ii) gelling saliva which forms a protective sheath around the stylets. The aphid's ability to circumvent the plant defence system is determined by the salivary proteome. To gain insight into the significance of the salivary proteome in the aphid–plant interaction we have used mass spectrometry to characterise and compare equivalent collections of watery and gelling saliva from the pea aphid biotype ND1, which is virulent on resistant varieties of the legume model plant *Medicago truncatula*. Numerous proteins were common to both watery and gelling saliva including several structural proteins, M1 and M2 metalloproteases, GMC oxidoreductases and peroxidases. However, quantitative analyses resolved groups of proteins that were statistically enriched in, or unique to, either fraction and a clear functional distinction was evident between the watery and gelling saliva components. The watery saliva profile comprises numerous enzymes and candidate effectors, whereas the gelling saliva predominantly comprises large structural proteins and some proteins with potential enzymatic activity which may be involved in sheath solidification and evading/suppressing plant defence responses at the interface between sheath and plant tissue. This research has identified numerous candidate proteins for future functional analysis and RNAi-based pest control strategies.

## Artificial diet for aphids – any good, any use?

Helmut van Emden

School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AJ, UK

On 9th February 1976, we established a clone of the aphid *Myzus persicae* (Sulzer) on a modification of Mittler & Dadd's (1962) fully-defined diet, and this recipe has proved capable of sustaining the insect for over 30 years.

In spite of its ability to sustain aphid growth and reproduction, the diet is still clearly an inferior substrate to plant (Brussels sprout) leaves. Development to adult is extended from about 9 to 15 days, and on diet there is then an additional 4-day pre-reproductive phase almost missing in plant-reared aphids. Aphids on diet also have a much-reduced fecundity (the mean per aphid is only 16.5 compared with 47.1 on plants).

One important difference between the diet and plants is that the diet gives constant phago-stimulation, whereas chemicals in the phloem fluctuate in concentration, particularly diurnally. A short interval when *Aphis fabae* Scopoli were given ½ strength diet before being returned to diet was followed by a 2-3-fold increase in honeydew production for 5-6 hours. Alternating 17 h on full diet with 7 h of ½ strength diet for three days gave slightly heavier *A. fabae* than those reared only on full diet. A second obvious difference between diet and plants is the high pressure of the phloem sap which effectively 'force-feeds' the aphids. However, work, again with *A. fabae*, putting a pressure of 2 kg/cm<sup>2</sup> behind the diet, also made some (but very little) difference.

A major breakthrough occurred while studying the failure of the cornicle secretion of diet-reared *M. persicae* to stimulate an alarm response in neighbouring aphids. We added a little acetate (in the form of 32 mg sodium acetate to 100 ml diet) as an early stage in the metabolic pathway in the synthesis of the alarm pheromone. To our surprise both development time and adult weight were close to that on plants.

Although originally intended for studies on aphid nutrition, the diet has not proved useful for this. Any change in any direction is deleterious. It is, however, useful for looking at compounds affecting aphid behaviour and for introducing chemicals into aphids, such as antibiotics for work on aphid symbionts. The parasitoid *Aphidius colemani* Viereck can be reared in aphids on the diet, but survival is limited.

Our diet-reared culture of *M. persicae* died out suddenly after 33 years. Almost simultaneously, a culture on diet started at Imperial College with our *M. persicae* also died out. Do the populations of what are believed to be permanently parthenogenetic aphid genotypes have a limited life span?

Mittler, T.E. and Dadd, R.H. (1962) Artificial feeding and rearing of the aphid *Myzus persicae* (Sulzer) on a completely defined synthetic diet. *Nature* **195**, 404.

## POSTER

### Changing the host selection responses of aphids through a short experience of a novel secondary plant compound

Helmut van Emden, John Kilbane and the late Jan Pettersson

School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AJ, UK

We have tested how far it is possible to change the host selection responses of *Myzus persicae* (Sulzer) as a polyphagous species, *Brevicoryne brassicae* (L.) as oligophagous and *Macrosiphum albifrons* Essig (lupin aphid) as monophagous, by rearing them on Brussels sprouts or lupin (for *M. albifrons*) and then testing their host plant selection after they had spent 24 h on a fully defined artificial diet with or without the addition of 0.05% tomatine, a secondary compound characteristic of tomato.

*Myzus persicae* from Brussels sprouts did not discriminate between leaves of normal host plants (sprouts and potato) and tomato in a simple arena after 24 h on normal diet, but selection clearly shifted towards tomato after time on diet containing tomatine. A technique involving systemic insecticide to detect feeding from the phloem region (van Emden *et al.*, 1991) confirmed this shift in host selection.

The same two experiments were carried out with *B. brassicae*, but here sprouts or Chinese cabbage represented normal hosts. In the arena experiment, experience of diet with tomatine again caused a clear shift in host selection towards tomato. In the pair-wise systemic insecticide experiment, a shift in selection towards tomato did not occur in the comparison with sprouts, but was observed in the comparison with Chinese cabbage. The oligophagous *B. brassicae* was therefore less positively influenced by experience of tomatine than the polyphagous *M. persicae*.

With the monophagous *M. albifrons* offered lupin or tomato in the arena, 24 h experience of tomatine caused only a slight and statistically insignificant shift towards tomato, but such experience did not change the far higher proportion of aphids feeding from lupin than from tomato plants in the systemic insecticide experiment.

We conclude that the degree by which a 24 h experience of a novel secondary compound influences subsequent host selection behaviour is greatest for the polyphagous *M. persicae* and virtually zero for the monophagous *M. albifrons*. The most interesting result is that *B. brassicae*, a specialist on plants containing glucosinolates, does show a more positive response to a quite unrelated plant (tomato) after a brief experience of the relevant secondary compound (the glycoalkaloid tomatine).

van Emden, H.F., Vidyasagar P. and Kazemi, M.H. (1991) Use of systemic insecticide to measure antixenosis to aphids in plant choice experiments. *Entomologia Experimentalis et Applicata* **58**, 69-74

## POSTER

### How rapid is the pea aphid radiation?

Varvara Fazalova and Bruno Nevado

Department of Zoology, University of Oxford, 11a Mansfield Rd, Oxford OX1 3SZ, UK

The pea aphid (*Acyrtosiphon pisum*) complex is a well-studied adaptive radiation consisting of multiple host-plant races. Previous research suggests that the pea aphid complex diversified extremely rapidly, with estimated divergence time of 8,000-16,000 years ago [1]. This time calibration was based on an unusually high mutation rate on pea aphids' maternally transmitted bacterial endosymbiont *Buchnera aphidicola* [2].

We recently showed that distantly related host races from the pea aphid complex are at the late stage of speciation, as they exhibit strong genomic divergence and intrinsic post-mating pre-zygotic isolation [3]. This suggests that completion of speciation in pea aphids proceeds extremely rapidly.

In this study, we investigated if time of divergence of the most distantly related host races of pea aphids – estimated with genome-wide population data – supports the view of extremely rapid speciation. For this, we performed mutation accumulation experiments for 12 parthenogenetic lines (24 genomes) of different pea aphid host races during 27 generations, and estimated genome-wide divergence between host races (2 populations, 12 individuals each, 15X whole-genome resequencing).

Our preliminary results suggest that the spontaneous mutation rate of pea aphids falls within range of other insect taxa. Together with the genome-wide estimate of divergence between host races, our results suggest much older divergence times in the pea aphid radiation than previously assumed. These results may point to novel scenarios of diversification for this important model of ecological speciation.

[1] Peccoud, J. *et al.* (2009) Post-Pleistocene radiation of the pea aphid complex revealed by rapidly evolving endosymbionts *PNAS* **106**, 7495–7500

[2] Moran, N. A. *et al.* (2008) The dynamics and timescale of ongoing genomic erosion in symbiotic bacteria. *Science* **323**, 379–382.

[3] Fazalova, V. *et al.* (2018) Intrinsic pre-zygotic reproductive isolation of distantly related pea aphid host races. *Biology Letters* **14**, 20180332

## **The role of toxic quinolizidine alkaloids (QA) from *Genista* (Fabaceae) in aphid–plant interactions**

**Felix Feistel, Torsten Knauer, Christian Paetz, Filip Kaftan, Aleš Svatoš, Jean-Christophe Simon, Jonathan Gershenzon and Grit Kunert**

**Max-Planck-Institute for Chemical Ecology, Hans-Knöll-Strasse 8, Germany**

The pea aphid (*Acyrtosiphon pisum*), native to legume plants, comprises at least 15 genetically different host races (biotypes) with each of them being native to just one or a very few different host plants and which can just perform there. One of these host races is specialized on plants of the genus *Genista*. These plants are known for their direct chemical defence based on quinolizidine alkaloids (QAs). These compounds stimulate and paralyze the central nervous system of insects, ultimately leading to their death. Thus, it seems likely that QAs are involved in the different performance of native *Genista* pea aphid host race and pea aphid host races non-native to *Genista* plants. However, information about *Genista* QAs is sparse. Furthermore, nothing is known about how *Genista* host race aphids have adapted to their host plants.

In this study, we isolated and identified the most abundant QAs within the extracts of *Genista tinctoria* and *G. sagittalis* by means of NMR spectroscopy and high resolution MS. This revealed the presence of eight QAs, with one new for *Genista* species and one never described before. To evaluate the distribution of these QAs within the pea aphid system, they were quantified by LC-MS/MS in *G. tinctoria* phloem sap, leaf and stem extracts, as well as in *Genista* host race aphid tissue and honeydew. Additionally, cross-sections of leaves from *G. tinctoria* were investigated by MALDI-MS. Our findings indicate that only a few QAs of the identified ones are present within the *G. tinctoria* phloem sap and further in the aphids and their honeydew.

## **New challenges with aphid control after the EU ban of neonicotinoid seed treatments on all outdoor crops**

**Steve Foster, Linda Oliphant and Martin Williamson**

**Rothamsted Research, Harpenden, Herts., AL5 2JQ, UK**

Neonicotinoid seed treatments were first used in Europe on sugar beet in the early 1990s to provide targeted protection against a range of different pests including virus-carrying aphids. Over time, they also became an important tool for growers of other crops, such as cereals and oilseed rape, to protect against yield losses. However, in April 2018 a decision was taken by the EU to extend neonicotinoid restrictions, first introduced on flowering crops in 2013, to cover all outdoor uses of these seed treatments. The presentation will discuss the consequences and implications of this EU ban in relation to the remaining insecticide treatments available and the presence of insecticide resistance in some virus-transmitting aphid pests.

## POSTER

### **Predicting the future: migration patterns, first-flight and evolution of resistant biotypes of a trap-shy aphid pest**

**Dion Garrett**

**Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK**

The currant–lettuce aphid, *Nasonovia ribisnigri*, is the most damaging species of aphid on outdoor lettuce and is responsible for large economic losses. The use of insecticides and host-resistant lettuce cultivars were the best method to reduce aphid outbreaks. However, insecticide-resistant biotypes and others that break host-plant resistance (resistant-breaking (*Rb*)) have recently been found in the UK. In the absence of lettuce cultivars with a new resistance mechanism, *N. ribisnigri* infestations will escalate.

Early warning information from local and national surveillance schemes could improve targeted control of *N. ribisnigri* before aphids enter lettuce crop. Paradoxically, whilst winged aphids are quick to colonise lettuce in summer, they are unusually scarce in existing trapping methods. Incorporating a variety of trapping methods to identify a more effective technique to capture *N. ribisnigri* in the field last year was found to yield comparable results. Therefore, it is important to understand the movement of this pest to infer its dispersal capacity and design efficient methods of control to minimise impact to agriculture.

It is envisaged that utilising molecular techniques could provide insight into *N. ribisnigri* population structure throughout the UK. Using the Rothamsted Insect Survey archive, all *N. ribisnigri* captured between 2003 and the present from all the UK suction-traps have been collected. By identifying potential candidate regions of resistance within the genome, it will be possible to understand the spread and evolution of *Rb* biotypes over time.

Additionally, by incorporating over 50 years of phenological data from the Rothamsted suction-trap network, combined with overwintering monitoring experiments, a forecasting model will be constructed to help predict *N. ribisnigri* movement into the lettuce crop.

## **Spatially resolved transcriptomics reveals plant host responses to the aphid pest *Myzus persicae***

**Michael Giolai, Walter Verweij, Ashleigh Lister, Iain Macaulay, Sam Mugford, Matthew D. Clark and Saskia Hogenhout**

**John Innes Centre / Earlham Institute, Norwich Research Park, NR4 7UH, UK**

Phloem sap feeding insect pests cause devastating agricultural losses with poorly understood mechanisms of plant defence responses to the insect and very few efficient means to mitigate agricultural damages. A striking feature of these pests is the characteristic pattern of how plants are colonised; *i.e.* by continuous manipulation of the host's immune system until the point of successful phloem sap feeding. Plants disseminate these events with a complex network of defence processes locally, *i.e.* in an entire leaf and systemically throughout the plant. How can we use forefront genomics approaches to gain insight in this complex spatial and temporal process and, doing so, gain understanding about how plants respond to phloem sap feeding insects? With my work, I will show that by linking electrophysiological insect assays to recent progress in spatial transcriptomics, we can unravel some of the universal features in *Arabidopsis thaliana* to *Myzus persicae* responses and, more generally, plant–insect interactions.

**Plant immunity triggered by oligogalacturonides in defence against *Myzus persicae* requires calcium-dependent protein kinases and is inhibited by the aphid effector Mp10**

**Matteo Gravino, Sam T Mugford, Felice Cervone, Giulia De Lorenzo and Saskia A Hogenhout**

**Department of Crop Genetics, John Innes Centre, Norwich, UK**

Aphids are among the most destructive pests in agriculture, causing direct damage to crops by feeding on phloem, as well as indirect losses by transmitting more than half of all described plant viruses. Moreover, during feeding aphids secrete effector proteins into their hosts to manipulate host cell processes and promote infestation.

Aphids are largely controlled by pesticides that are environmentally damaging and are being withdrawn from the market. Therefore, there is an urgent need to develop new strategies for controlling aphid infestations, such as the use of insect-resistant crops. The development of such crops can be greatly enhanced by knowledge on plant resistance mechanisms against these pests.

The cell wall is a major line of plant defence against pathogens and pests. Plant cells harbour sophisticated mechanisms for sensing the alteration of cell wall integrity during a biotic stress. For instance, they perceive endogenous molecules released as a consequence of the cellular damage caused by pathogens, the so-called damage-associated molecular patterns (DAMPs). A well-known class of DAMPs is represented by oligogalacturonides (OGs), oligomers of  $\alpha$ -1,4-linked galacturonic acid released upon partial hydrolysis of the cell wall pectic component. Cell walls and OGs play a role in defence to fungal and bacterial pathogens, but their role in defence against aphids is not known.

We show here that OGs enhance *Arabidopsis thaliana* resistance to the peach–potato aphid *Myzus persicae*. This resistance response is mediated by calcium-dependent protein kinases that are known to be involved in immune signaling. Intriguingly, the OG-induced immunity is inhibited by the aphid effector Mp10. *A. thaliana* transgenic plants with increased level of pectin methyl esterification were more susceptible to *M. persicae* colonization. Our work shows that plant cell walls and OGs are components of the plant immune response to aphids. This is important, because aphids are likely to release these DAMPs when they penetrate plant cell walls with their stylets.

## POSTER

### **An integrated protocol for targeted mutagenesis with CRISPR-Cas9 system in the pea aphid**

**S. Hudaverdian, G. Le Trionnaire, M. Uzest, S. Tanguy, M. Deshoux, B. Cayrol, B. Monsion, E. Pichon, T. Kosuth, F. Gleonnec, G. Richard, C.G. Webster, A. Herpin and D. Tagu**

**UMR 1349 IGEPP INRA, Agrocampus Ouest Rennes, Universit. Rennes 1, France**

CRISPR-Cas9 technology is a very efficient functional analysis tool and has been developed in insects to edit their genome through injection of eggs with guide RNAs targeting coding sequences of the gene of interest. Developing and using this technology in aphids is challenged by their mode of reproduction, being most of the time asexual (parthenogenetic). The switch to a sexual reproductive mode allowing the production of eggs can be triggered by carefully controlled photoperiod conditions.

Here we present the key steps of the protocol of egg injection with CRISPR-Cas9 reagents we developed in the pea aphid to target *stylin-01*, a cuticular protein gene. The first step lasts for 2 months and consists of the photoperiod-triggered induction and synchronization of sexual morphs. Males and females are then placed together for mating to allow egg production. Eggs are then collected and injected with Cas9 protein and sgRNAs previously validated for their ability to cleave *stylin-01 in vitro*. Once melanized, eggs must experience a 3-month obligate diapause period before hatching. Newly hatched colonies are then maintained as clones. The overall procedure is spanned over a 7-month period.

As major results, mutation rates within eggs before diapause were estimated at 70 to 80%. The hatching rate of diapausing injected eggs ranged from 1 to 11% depending on the cross. Finally, six lineages showing stable mutations of one or both *stylin-01* alleles were generated and maintained clonally. The germline transmission rate could be thus estimated at 35%, which fully validated our CRISPR-Cas9 mutagenesis protocol in the pea aphid.

## **Encyclop'Aphid: a website for aphid enthusiasts**

**Maurice Hullé, Evelyne Turpeau, Bernard Chaubet and Jean-Christophe Simon**

**INRA, UMR 1349 IGEPP, F-35653 Le Rheu, France**

Encyclop'Aphid ([https://www6.inra.fr/encyclopedie-pucerons\\_eng/](https://www6.inra.fr/encyclopedie-pucerons_eng/)) (Hullé *et al.*, 2018) is a numeric encyclopedia in both English and French devoted to aphids and their natural enemies, created for aphid enthusiasts and developed by INRA's Insect Ecology and Genetics Research team of IGEPP (Institute of Genetics and Environment applied to Plant Protection).

The site includes five main sections describing aphid features (taxonomy, morphology, biology), their interactions with other organisms (antagonists, viruses, ants and Diptera), their agronomic incidence and some current research themes such as genomics. It also includes presentation and identification files for 124 aphid species and 65 natural enemies (predatory insects, parasitoids and hyperparasitoids, pathogenic fungi).

Encyclop'Aphid contains several identification keys for aphids of the main crop plants and cultivated trees. It offers a large iconography and a video library, both of which are freely accessible.

The site is made to evolve and to be constantly enriched by new contributions from different international specialists, in both English and French. We are looking forward to receiving your contribution on any topic related to aphids and their associates or antagonists.

Hullé M., Turpeau E. and Chaubet B. (2018) Encyclop'aphid, INRA,  
<http://doi.org/10.15454/1.5367482425330007E12>

## **KEYNOTE**

### **Sex loss in aphids: mechanisms and evolutionary consequences**

**Julie Jaquiéry**

**INRA UMR 1349 IGEPP, Domaine de la Motte, Le Rheu, France**

Asexual lineages occur in most groups of organisms and arise from loss of sex in sexual species. Yet, the genomic basis of these transitions in reproductive modes remains largely unknown. We addressed this issue in the pea aphid where both sexual and obligate asexual lineages coexist in natural populations. These lineages may occasionally interbreed because some asexual lineages maintain a residual production of males potentially able to mate with the females produced by sexual lineages. We combined quantitative genetic and population genomic approaches to unravel the genetic control of shifts towards permanent asexuality in the pea aphid. We found that permanent asexuality was determined as a recessive character by a single locus and identified a 2.5 Mb genomic region responsible for permanent asexuality located on the X chromosome. Also, population genetic data indicated substantial gene exchange between these reproductively distinct lineages, potentially leading to the conversion of some sexual lineages into asexual ones in a contagious manner. In order to identify the candidate gene(s) controlling reproductive mode variation in the pea aphid, we are currently narrowing down the size of the control region and investigating patterns of coding and non-coding polymorphisms within and outside the causal locus in sexual and asexual lineages. Using genetic markers linked to reproductive modes and applied to pea aphids caught in suction-traps over the last two decades, we are also analysing retrospectively the influence of winter climate on the frequencies of sexual and asexual lineages. Because asexual lineages are susceptible to frost, this would allow identification of the critical temperature below which their survival is at risk.

## **POSTER**

### **Investigating early aphid-induced Ca<sup>2+</sup> signals and their role in determining aphid–host compatibility**

**Joshua Joyce, James Canham, Michael Giolai, Tony Miller, Dale Sanders and Saskia Hogenhout**

**Department of Crop Genetics, John Innes Centre, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, UK**

Aphids pose a great threat to plant health as insect pests and vectors of many significant plant viruses. Despite this, little is still known about the mechanisms by which plants can detect, and defend themselves against, aphid attack. Recently, elevations in cytosolic calcium have been identified as key signals for the plant defence response against aphid feeding. How are these calcium signals produced and how do they function to coordinate specific plant defences? By live *in vivo* imaging of early calcium signalling responses to aphid species of different host compatibilities, we are beginning to unravel some of the key features of these signals and their roles in aphid–plant interactions. Such insights will be fundamental in understanding the colonization ability of different aphid species and the mechanisms underlying aphid–host compatibility.

**Ole E. Heie – entomologist, teacher, friend**

**Mariusz Kanturski**

**Department of Zoology, Faculty of Biology and Environmental Protection, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland**

Ole Engel Heie is undoubtedly one of the greatest Hemipterologists of the past 100 years, not only from the scope of his work but the number of papers published, and the number of species described. He has published dozens of papers and books for several years, describing families, genera and species of extinct and extant aphids. He is also perhaps one of the last specialists to contribute substantially to descriptive studies in both fossil and contemporary taxa. His well-known “green books” – The Aphidoidea of Fennoscandia and Denmark, are still fundamental and one of the most important elements for every aphidologist not only in Europe but also in other parts of the world.

In the year of his passing I have the honour and pleasure to present a review of his achievements and discuss his legacy.

## **POSTER**

### **Antennal sensilla of Lachninae aphids (Hemiptera: Aphidomorpha): a new approach**

**Mariusz Kanturski, Łukasz Depa, Łukasz Chajec and Sebastian Student**

**Department of Zoology, Faculty of Biology and Environmental Protection, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland**

Aphids are one of the economically most important groups of insects due to the great evolutionary success they have achieved through their ecological plasticity, life cycles and polymorphism. Undoubtedly, one of the equally important aspects of this success is their specific sensory organ system on the antennae.

Lachninae representatives differ from members of other subfamilies by often much larger body size, abundant body chaetotaxy, morphology of the antennae, siphunculi and cauda. Important diagnostic features for Lachninae are also located on tarsi, the second segments of which often tend to lengthen and the empodial setae are more or less developed. Also, in the anatomy of the digestive and reproductive systems this subfamily is characterized by specific features that clearly distinguish all the representatives from other groups of Aphididae.

Since lachnids were distinguished as a higher systematic level; their classification and division underwent constant changes depending on different authors and adopted classification systems of aphids. Despite recent molecular research made with representatives of this subfamily, the morphology, and specific features of each groups of taxa are unknown or known only poorly.

In this study, we used light and scanning electron microscopy to present the preliminary results of detailed morphological and ultrastructural studies of antennal sensilla of representatives of all genera belonging to Lachninae. The antennae in Lachninae are five- or six-segmented and bear two kinds of trichoid sensilla, big multiporous placoid sensilla, small multiporous placoid sensilla, and coeloconic sensilla which are known as primary, secondary rhinaria and rhinariola. The greatest variety is shown by placoid and coeloconic sensilla of the last antennal segment. We present their morphology and different arrangement in selected species belonging to all genera from all tribes. Characters of sensilla and their arrangement seem to be stable on generic and tribal levels and may have important significance in systematics and phylogeny not only in Lachninae but Aphidomorpha as a whole.

This research was partly funded by the Ministry of Science and Higher Education grant for young scientists, 2016.

## **POSTER**

### **The comparative study of the reproductive system of sexual morphs of the model organism – the pea aphid *Acyrtosiphon pisum* (Hemiptera, Aphididae)**

**Mariusz Kanturski, Karina Wieczorek and Piotr Świątek**

**Department of Zoology, Faculty of Biology and Environmental Protection, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland**

The pea aphid *Acyrtosiphon pisum* (Harris) is a worldwide serious pest of cultivated plants from the family Fabacea. As a model organism, it is intensively studied, and an enormous literature on this species has been published. However, the structure of the reproductive system of the sexual generation – male and oviparous female, remains unstudied.

Here we describe the morphology, histology and ultrastructure of the reproductive system with particular emphasis on genital tracts, using histology, light and fluorescent microscopy as well as transmission and scanning electron microscopy. Comparing sexuales of the previously studied species of aphids and the present results, we conclude that histologically, the components of the reproductive system are broadly similar and their ultrastructure is simple. Moreover, the epithelial cells of the walls of vasa deferentia, male and oviparous female accessory glands have secretory functions, correlated with age. Such a simple and conservative structure of the reproductive system results from the aphid's biology.

As the pea aphid is listed among the 14 species of the greatest economic importance, the examination and comparison of the structures of the reproductive system is potentially important in aphid management, plant protection and the global need of food security.

## **POSTER**

### **Stylectomy for analyzing legume phloem sap substances after pea aphid infestation - evaluation of a method**

**Torsten Knauer, Michael Reichelt, Jonathan Gershenzon and Grit Kunert**

**Max Planck Institute of Chemical Ecology, Hans-Knöll-Straße 8, 07745 Jena, Germany**

The pea aphid (*Acyrtosiphon pisum*), specialized on the legume family, encompasses ecologically and genetically distinct host races. All of them perform well on their universal host plant *Vicia faba* and their native host plant. However, if they are confronted with a non-host plant, this results in a decreased performance or death. Previous work has revealed that responsible factors may be localized in the sieve elements of the plant phloem. Amongst others, phloem-located chemical compounds might be important for the different aphid performance.

There are two main techniques for sampling phloem sap: phloem exudation and stylectomy, but stylectomy is the only existing technique which allows collection of pure phloem sap. However, phloem sap sampled from one aphid stylet represents the phloem sap of just one sieve element. Thus, the question is whether this is representative for a whole plant. To answer this question, we sampled phloem sap from several single sieve elements from individual *Trifolium pratense* plants infested with different pea aphid host races and analysed the amino acid composition. Afterwards we investigated changes in amino acid and phytohormone composition in the phloem sap of *Vicia faba* plants infested with different host races of the pea aphid.

## **Development of a robust CRISPR-Cas9 mutagenesis protocol in the pea aphid to knock out *stylin-01* gene and study the role of Stylin-01 cuticular protein in *Cauliflower mosaic virus* transmission**

**G. Le Trionnaire<sup>1</sup>, M. Uzest<sup>2</sup>, M. Deshoux, B. Cayrol, B. Monsion, E. Pichon, T. Kosuth, S. Tanguy, S. Hudaverdian, F. Gleonnec, G. Richard, C.G. Webster, A. Herpin and D. Tagu**

**<sup>1</sup> UMR 1349 IGEPP INRA, Agrocampus Ouest Rennes, Universit. Rennes 1, France**

**<sup>2</sup> BGPI, Univ Montpellier, Inra, Cirad, Montpellier SupAgro, Montpellier, France**

CRISPR-Cas9 technology is a very efficient functional analysis tool that has been developed in many insects to edit their genome through the injection of eggs with guide RNAs targeting coding sequences of the gene of interest. However, its implementation in aphids is more challenging since aphids alternate between clonality and sexual reproduction during their annual life cycle. The production of eggs after mating of sexual individuals is a unique yearly event and is necessarily triggered by a photoperiod decrease. Fertilized eggs must then experience an obligate 3-month diapause period before hatching as new clonal colonies. Taking into consideration these particularities, we developed in the pea aphid a step-by-step protocol of targeted mutagenesis based on the microinjection within fertilized eggs of CRISPR-Cas9 components designed to target the *stylin-01* gene. This gene encodes a cuticular protein of the acrostyle in aphid maxillary stylets that is currently the best candidate receptor for noncirculative plant viruses.

In a first part, we will detail the critical steps of the 7 months procedure necessary to create stable mutant lineages for *stylin-01* gene in the pea aphid. This includes: i) the photoperiod-triggered induction of sexual morphs (2 months), ii) the mating and egg collection step followed by egg microinjection of CRISPR-Cas9 components and their melanisation (1 month), iii) the obligate 3-months diapause that eggs must experience and iv) the hatching of new lineages from injected eggs and the maintenance of stable lineages (1 month). Mutation rates within eggs before diapause were estimated at 70 to 80%. The hatching rate of diapausing injected eggs ranged from 1 to 11% depending on the cross. The maximum germline transmission rate could then be estimated at 35%. Finally, six lineages showing stable mutations of one or both *stylin-01* alleles were generated and maintained clonally.

In a second part, we will present a phenotypic analysis of *stylin-01* knocked out lineages. These lineages indeed represent so far the first mutant aphids ever generated. The role of Stylin-01 in the transmission of *Cauliflower mosaic virus* (CaMV) is currently under evaluation. Our data indicate that Stylin-01 KO mutants are strongly affected in their CaMV transmission capacity. These encouraging data open exciting opportunities to estimate the role of Stylins as receptors of plant viruses, and should help better defining of the intrinsic function of this cuticular protein.

## **Generalism in nature...the great misnomer: aphids as examples**

**Hugh D. Loxdale and Adalbert Balog**

**School of Biosciences, Cardiff University, Museum Avenue, Cardiff, Wales, CF10 3AX, UK**

This year marks the centenary of the death of the great German biologist and evolutionist, Ernst Haeckel (1834-1919), who, having read Darwin's 1859 *Origin of Species*, formulated the concept of 'ecology' in his 1866 writings (Richards, 2008). Later, following on from the realisation by the English ecologist Charles Elton (1900-91) of the existence of the ecological 'niche' – the interactions of an organism in its habitat with its surroundings, abiotic and biotic, including other organisms directly or indirectly competing with it – the ecologist George Hutchinson (1903-91) defined the niche further as: "a highly abstract multi-dimensional hyperspace in which the organism's needs and properties were defined as dimensions". In light of these definitions, when a species population A evolves to become species population B, for whatever reason, including chromosomal, then that is clearly the act of specialisation and it is a unique event. No other organism fills that niche, except the organism in question and it will remain in that niche until it becomes extinct, is displaced for whatever reason/s, or evolves to fill another niche. Hence, the organism/species population, as it evolves to fill a new niche in time and space, effectively creates a new niche, perhaps for the first time ever, or re-fills a vacant, previously filled niche, or displaces an extant and resident organism in an invasive manner. Furthermore, because of the inherent uniqueness of the ecological niche, an organism can never be 'generalist'. It may perhaps be polyphagous to some greater or lesser extent (but see below), yet it is surely never generalist, rather it always remains a specialist. If a species were truly generalist, then its evolution would stop, as the act of evolving to fill a new niche, perhaps due to intra- or interspecific, or in the case of asexual aphids, intra- or interclonal competition, is the main driving force of evolution itself. Such interspecific pressure may also relate indirectly to predator/parasitoid pressure, and the requirement to evolve into a new 'enemy free' space, or more exactly, niche.

In this talk, we discuss the reasons why we do not believe aphids are ever generalist. Indeed, we dispute the long-held view that many of them are even polyphagous *sensu stricto*, mainly because of our ignorance in such matters, *i.e.* lack of empirical DNA evidence. In fact, very few species appear to be highly polyphagous (< 1% of ~ 5,000 recorded species), more especially pests such as the peach–potato aphid, *Myzus persicae*; in the case of this particular species, this ability seems to be a rather unique specialisation, as most aphid species do not show such ecological-genetic-biochemical flexibility. We also posit that the apparent extensive polyphagy seen by pest aphids on crops is the direct result of relaxed selection arising from a reduction in secondary plant antifeedant titre in artificially designed and reared crops within the manicured agroecosystem, cossetted plants that would normally be unsuitable as aphid hosts in their wild form. In a truly wild scenario, both chemical defences and predator pressure would exclude such flexible behaviour and would limit the insects to their original, long co-evolved natal host/s, either a single host or taxonomically closely related hosts within the same plant family.

Richards, R.J. (2008) *The Tragic Sense of Life: Ernst Haeckel and the Struggle Over Evolutionary Thought*. *Chicago University Press*, Chicago, Illinois (see p. 144)

## ***Acyrtosiphon pisum* humoral immunity and life traits are modulated selectively by facultative symbionts**

**Chen Luo, Nicolas Desneux, Jean-Luc Gatti and Marylène Poirié**

**Université Côte d'Azur, INRA, CNRS, Institut Sophia Agrobiotech, Sophia Antipolis, 06903, France**

The pea aphid *Acyrtosiphon pisum*, a major pest in agronomy, is a highly symbiotic organism. It hosts an obligate symbiote, *Buchnera aphidicola*, and can harbour several facultative symbionts (FS), most of which (e.g. *Hamiltonella defensa*, *Regiella insecticola*, *Serratia symbiotica*) provide extended phenotypes to the host, ranging from plant-specific adaptation to resistance to abiotic (heat) or biotic (fungi, parasitoid wasps) stress. The impact of some FS on the immune parameters of the pea aphid has also been described (Schmitz *et al.*, 2012), but it is surprisingly less well documented for the humoral aspect. This is certainly due to the fact that none of the genes encoding typical insect antimicrobial peptides was found in the *A. pisum* genome. On the other hand, this genome encodes two phenoloxidases (PO2 and POA3) involved in the still little studied aphid phenoloxidase cascade. We questioned therefore the effect of the presence of facultative symbionts mainly on this last component as well as its correlation with the fitness of the host. We have shown that the presence of some, but not all, of the symbionts selectively decreases the expression of the PO (PO2 and POA3) genes, the amount of the encoded proteins, and the PO activity in the haemolymph. Besides, lower amounts of PO correlate with a reduction in host fertility and a shorter life. When evaluating the amounts of PO at various stages of host development, we observed a reduction after the L3 nymph stage for the same impacting symbiont. Finally, we quantified life traits and PO expression in aphid lines harbouring different FS symbionts and subjected to different biotic stresses (latex beads, *E.coli* and Yeast). The presence of the symbionts was either neutral or prejudicial, but never beneficial to the host defence. It is interesting that aphid lines containing the most PO had higher survival under stress. Altogether, FS affect the humoral immunity of *A. pisum* both at the gene expression and protein levels which may in turn affect response to stress and thus fitness.

Schmitz, A., Anselme, C., Ravallec, M., Rebuf, C., Simon, J.C., Gatti, J.L. and Poirié, M., (2012) The cellular immune response of the pea aphid to foreign intrusion and symbiotic challenge. *PLoS One* **7**, e42114

## **Aphid symbiont confers multiple phenotypes without trade-offs**

**Ailsa McLean, Jan Hřček, Ben Parker, Chantal Paine, Hugo Mathe-Hubert, Heidi Kaech and Charles Godfray**

**Department of Zoology, University of Oxford, UK**

Many aphids are known to harbour symbiotic bacteria that are not required for successful development or reproduction. These facultative symbionts impact aphid biology in a variety of ways, and some aphid symbiont species are known to confer multiple diverse effects. However, little is known about how different symbiont-mediated effects might relate to one another.

In pea aphids (*Acyrtosiphon pisum*), the bacterial symbiont *Spiroplasma*, has previously been reported to cause reproductive manipulation via male-killing and to confer protection against fungal pathogens. We tested whether *Spiroplasma* affects aphid resistance to parasitoids, a phenotype which has been reported for other aphid symbionts and for *Spiroplasma* in other insects. Our results show that *Spiroplasma* can provide strong and specific protection against parasitoids in pea aphids.

We then investigated whether there was any correlation between the three different phenotypes of the symbiont *Spiroplasma* in pea aphids (male-killing, fungal pathogen resistance and parasitoid resistance) in order to distinguish between three different hypotheses for maintaining multiple symbiont phenotypes: (a) beneficial phenotypes compensate for reproductive manipulation phenotypes (a trade-off for the host); (b) reproductive manipulation and different beneficial effects represent alternative strategies by different symbiont strains (trade-offs for the symbiont); or (c) all symbiont-mediated phenotypes are evolving independently of one another, potentially in response to interactions with hosts and natural enemies. There were no correlations between any of the phenotypes and we conclude that the multiple phenotypes conferred by aphid *Spiroplasma* are evolving independently.

## **POSTER**

### **Assessing the role of climate and land use in driving patterns of genotypic diversity and insecticide resistance**

**Beth Moore, Gaynor Malloch, Fiona Hight and Lesley Lancaster**

**Institute of Biological and Environmental Sciences, University of Aberdeen, UK.**

Changes in land use and climate can impact the spatial distribution of genetic diversity across a species' range. For pest insects, these shifts in genetic diversity may be linked to changes in resistance and abundance, which influence their impact on crops. Thus, it is particularly important to understand how environmental change affects the spatial distribution of pest species genetic diversity and resistance across a landscape.

For my PhD thesis work, I am interested in understanding how climate and land use impact the distribution of diversity and resistance in UK aphids. Recent data from Scottish suction-traps have indicated that changes in climate may have impacted the recent spread of insecticide-resistant *Sitobion avenae* clones into Scotland. These resistant clones have historically been restricted to warmer parts of the UK, however recently they have been found more frequently in Scotland and in conjunction with an overall decline in genetic diversity. Climate change or changing land use patterns may drive this process, but the generality of this phenomenon remains to be tested more rigorously. We plan to collate time series data on genotype, abundance and sex ratio for as many aphid species as possible, relying on existing data from the suction-trap network. We will combine these data with GIS land use and climate data (time series) to assess whether and how these environmental factors are responsible for spatial and temporal changes in genetic diversity and insecticide resistance. We have varying amounts of data for three aphid species (*Sitobion avenae*, *Myzus persicae* and *Macrosiphum euphorbiae*) across Scotland, and are seeking to expand this to more species, across more years, and a greater geographical area to complete our study. Results will provide large-scale insight into the drivers of aphid population dynamics, with relevance for predicting response to future climate change and land use change.

## **Genetic structure of cereal aphids in the UK**

**Ramiro Morales-Hojas, Fernando Alvira Iraizoz, Todd Jenkins, Alex Greenslade, Lynda Alderson, Tracey Kruger, Mike Hall and James Bell**

**Insect Survey, Biointeractions and Crop Protection, Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK**

Cereal aphids are among the most economically relevant pest species in the UK. They can reduce crop yield by direct feeding and are vectors of *Barley yellow dwarf viruses* (BYDV). Their abundance has been monitored in the UK for over 50 years by the Rothamsted Insect Survey. Analyses of this dataset show long-term stability of aphid population size despite climate change. However, little is known about their population dynamics from the genetic perspective. With the recent ban on neonicotinoids, understanding the dispersal dynamics of these aphids is becoming critical to improving their management and control. Studying the population genetics of these pests is fundamental to determining the geographic distribution of genetic diversity, patterns of gene flow and dispersal, and hence to identifying potential barriers to their movement. Here, we present results of population genetic analyses of cereal aphids in England using genome-wide molecular markers. We compare the genetic structure of two species, *Rhopalosiphum padi* and *Sitobion avenae*, and investigate gene flow patterns across England to understand their dispersal and potential natural barriers to their long-distance migration. We have also investigated the evolution of *R. padi* by looking at the genetic differentiation of populations in the past using the Rothamsted Insect Survey's archive of samples. We have improved knowledge of the genome of *R. padi* and generated the first genome draft for *S. avenae*.

## POSTER

### **Optimization of protocols for secondary symbiont introduction and elimination in the pea aphid**

**Stéphanie Morlière<sup>1</sup>, Corentin Sochard<sup>1</sup>, Grégory Toussaint, Yannick Outreman<sup>2</sup>, Jean-Christophe Simon<sup>2</sup> and Akiko Sugio<sup>2</sup> (<sup>1</sup>*first authors equal contribution*, <sup>2</sup>*senior authors equal contribution*)**

**INRA, UMR 1349, Institute of Genetics, Environment and Plant Protection, Domaine de la Motte, BP 35327, Le Rheu, France**

The effects of facultative symbionts have been closely studied in the pea aphid (*Acyrtosiphon pisum*), a complex of multiple plant-specialized biotypes that feed on specific host legume plants and differ in their symbiont composition and prevalence. However, the influence of facultative symbionts on *A. pisum* phenotypic variation and fitness is not always clear. To study the effects of different secondary symbionts on *A. pisum* phenotype and performance, we aimed to create aphid lines with specific symbiont compositions. In this process, we optimized techniques of facultative symbiont introduction and elimination by injection of hemolymph harbouring the facultative symbionts or antibiotics. The age of injected aphids and quantity of hemolymph or antibiotics to be injected have strong effect on the success rate of symbiont introduction or elimination. The success rates of symbiont manipulation were variable depending on the combination of symbiont and aphid genotypes. Nonetheless, with the optimized protocol, we achieved up to 84 and 80 % of success rate for introduction and elimination of symbionts, respectively.

## **Cross-talk between aphid facultative symbionts and nitrogen fixation symbiosis in the aphid and legume interaction**

**Gaurav Pandharikar, Jean Luc Gatti, Pierre Frendo and Marylène Poirié**

**Université Côte d'Azur, INRA, CNRS, France  
Institut Sophia Agrobiotech, Sophia Antipolis, France**

Plants, animals, and humans live in a microbial world and a wide range of interactions with microbes has evolved in host-specific symbioses, which are usually beneficial for both the host and the microbe(s). These symbiotic interactions include aphids that live in associations, obligatory and facultative, with certain bacteria. Aphids are major agricultural pests through their plant feeding damage and virus transmission and because of their asexual reproductive capacity. Aphid growth and impact on plant fitness are strongly influenced by the interaction with other partners such as the facultative endosymbionts (SS) they harbour. Leguminous plants are important agricultural plants attacked by aphids, notably the pea aphid (*Acyrtosiphon pisum*). These plants are able to reduce atmospheric nitrogen in ammonia to satisfy the plant nitrogen need via a symbiosis with the soil *Rhizobium* bacteria. This symbiosis takes place in a plant organ, the root nodule. In this context, it is important to know whether and how the presence of different facultative symbionts in the pea aphid and in the host-plant modulate the legume–aphid interaction and vice-versa.

In order to address this question, we have evaluated the effect of the Nitrogen-Fixing Symbiosis (NFS, inoculated plant) compared to watering with nitrates (non-inoculated plant) in *Medicago truncatula*, a leguminous plant model, on the development and growth of five lines of *A. pisum*. These lines are either deprived of SS or host only one SS (*Hamiltonella defensa*, *Regiella insecticola* natural or artificial, *Serratia symbiotica*) in an YR2 clone background. We also tested for an effect of infestation by the aphid lines on inoculated vs non-inoculated plants, and then on the symbiotic nitrogen fixation. We have also analysed the *M. truncatula* defence response to the different aphid lines by expression analysis of plant defence gene markers. We showed that the NFS reduces aphid fitness independent of the aphid lines. The infection by the majority, but not all, of the aphid facultative symbionts decreases significantly plant nitrogen fixation efficiency. Finally, a specific defence response was observed in the nitrogen-fixing plants compared to nitrate-fed plants independently of the aphid lines. Overall, our results demonstrate that plant–aphid interactions are influenced both by the plant and the aphid symbiotic partners.

**Forisomes – possible key players in legume defence against aphids and *Acyrtosiphon pisum* host race development**

**Maria K. Paulmann, Jonathan Gershenzon, Ralf Oelmüller, Alexandra C. U. Furch and Grit Kunert**

**Department of Biochemistry, Max-Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, 07745 Jena, Germany**

*Acyrtosiphon pisum* is an aphid species specialized on Fabaceae and comprises a complex of at least 15 different host races. These host races are in the process of speciation, which raises the question about factors involved in maintaining them. Previous work has shown that plant specific factors may be localized in the sieve elements of the plant phloem.

Plants have evolved mechanisms to occlude their sieve elements in response to the possible loss of phloem sap, for example through phloem feeding insects or damage to the vascular system. This occlusion is mediated via callose deposition and phloem (p)-proteins. In the *Papilionoids* of the Fabaceae family a special form of p-protein – the forisome - has developed. It can reversibly change its conformation in a  $\text{Ca}^{2+}$ - instead of an ATP-dependent manner. The biological role of forisomes has been subject to frequent discussions and has previously been indicated to lie in legume defence against aphids.

Most recently we investigated the influence of pea aphid feeding on forisome reactivity in the host plants *Vicia faba* and *Trifolium pratense*. Comparing the effect of different *A. pisum* host races showed that the suppression of forisome reactivity does depend on the plant – host race interaction and may play a role in *A. pisum* host race maintenance.

## **New opportunities to manage ecosystem services for conservation biological control of pest aphids**

**John A. Pickett, Wynand van der Goes van Naters and Irene Castellan**

**Schools of Chemistry, and Biosciences, University of Cardiff, CF10 3AT, Wales, UK**

With the widely accepted need for sustainable intensification of agricultural food production, there will be increased pressure on ecosystem services to deliver more, probably by land sparing strategies. In addition to reducing the impact of pesticides on pollinators such as honey bees by methods conceived long ago, we must start to consider enhancing populations of other beneficial Hymenoptera such as the parasitoids used against pests, including aphids, by conservation biological control methods. Although slow to be developed in regions practicing industrialised agriculture, conservation biological control of lepidopterous pests by managing parasitoids using semiochemicals delivered by companion cropping, for example in the push-pull system for controlling stem borers and more recently the fall armyworm in small holder cereal cropping agriculture in sub-Saharan Africa, clearly demonstrates the value of such approaches. However, for high input broad acre agriculture as is the main route to food production in industrialised regions we must consider ways to maximise the ecosystem service of reliably high parasitoid populations. Simply growing marginal strips of plants to encourage beneficial insects, as with so-called beetle banks, does not work in practice and leaves the beneficials happily living in their refugia. For aphid parasitoids, we can draw them into the crop, using plant stress related semiochemicals, as with the push-pull. Here we describe an opportunity by which to create a push from the ecosystem service so that the learning by parasitoids of chemical cues, associated with the value of the ecosystem service directly to parasitoid development, is overcome thereby promoting foraging where we need them.

## **Vertical stratification in temperate floodplain forest – where to look for aphids?**

**Hana Platková, Petr Pyszko, Armelle Coeur d'Acier, Emmanuelle Jousselin and Pavel Drozd**

**Department of Biology and Ecology, Faculty of Science, University of Ostrava,  
Chittussiho 10, 710 00 Ostrava, Czech Republic;  
Crop Research Institute, Drnovska 507, 161 06 Praha 6 – Ruzyne, Czech Republic**

Knowledge on aphid ecology has a crucial economic importance due to their impact on most temperate crops. Less attention is paid to general patterns of host specificity (excluding economically important species), other ecological requirements and patterns of their diversity and density. Contrary to herbs, tree canopies provide large scale microhabitats and such heterogeneity of conditions could influence dispersal strategies. Unfortunately, there is a lack of complex spatial studies describing patterns of aphid dispersal along a canopy gradient.

We aimed to study if the density and diversity of aphids change along vertical gradients and whether the relative position (percentile of canopy) or absolute position within the canopy (meters of canopy) play more a substantial role. We sampled within a 0.2 ha plot of temperate floodplain forest in south Moravia, Czech Republic, during two seasons on all 58 trees within the plot (*Acer campestre*, *Carpinus betulus*, *Fraxinus spp.*, *Quercus cerris*, *Quercus robur*, *Tilia cordata* and *Ulmus laevis*). In total, we found 2676 free-living aphids and 2205 galls, belonging to 31 aphid species. Due to the low frequency, but high density, of galls, they were excluded from analysis of density, but not diversity.

There were large differences in density and species diversity among tree species and during the season. Both, density and diversity change in a vertical gradient differently for particular tree species. Density is better explainable with a model based on relative position, whereas diversity is better explainable by absolute position within the canopy. We also analysed these patterns separately for each of the six most abundant species: *Euceraaphis betulae*, *Hoplocallis rupertii*, *Myzocallis boernerii*, *Myzocallis carpini*, *Periphyllus testudinaceus* and *Tuberculatus annulatus*.

We aim to broaden models by considering the stochasticity of aphid dispersion from the original host to substitutional “hosts” (tree species not providing optimal conditions). Our goal is to compare the occurrence of aphids considering their known affinities and dispersion capability (apterous vs alate) along the vertical gradient. We hypothesize that lower parts of the canopy will host a larger proportion of xenocenosus species.

This work was supported by Grant Agency of the Czech Republic (14-04258S) and SGS14/PfF/2018 project.

## POSTER

### **The effect of wheat variety on demography of *Metopolophium dirhodum***

**Platková H., Skuhrovec J., Kosová K., Vítámvás P., Tylová E. and Saska P.**

**Crop Research Institute, Drnovska 507, 161 06 Praha 6 –Ruzyně, Czech Republic**

On six varieties of spring wheat (*Triticum aestivum* L.) we tested how particular parameters of the life table vary in relation to the declared tolerance of varieties to drought. We used *Metopolophium dirhodum* (Walker, 1849), which is one of the main pests on cereals in the temperate zone, as a model species. Two varieties tolerant to drought (Septima, Jarissa), three conventional (Quintus, Kabot, Kadrill) and one intermediate (Seance) were used. At the beginning of the experiment, 10 adult wingless aphids were placed to each pot that contained 10 plants (8 reps per variety) and they were allowed to produce nymphs overnight. Adult aphids were then removed and 10 of the new-born aphids entered the life table study. Pots were observed every day and the number of aphids and their instar were recorded. After reaching adulthood, the production of nymphs was recorded until death. Data were processed using the TWOSEX-MSChart program. Bootstrap testing showed that varieties differ from each other in most of the population parameters – finite rate of increase varied between 1.23 and 1.27 d<sup>-1</sup>, intrinsic rate of increase between 0.21 and 0.24 d<sup>-1</sup>, mean generation time between 14.3 and 16.3 d and fertility from 32 to 42 nymphs per female. Fertility and mean number of offspring per female were highest for the intermediate variety. Overall, we showed that varieties tolerant to drought were less resistant to aphids.

This work was supported by Grant Agency of the Czech Republic (18-13174J).

**Evolution of symbiosis in insects: mechanisms involved in associations between aphids and the gut-associated *Serratia symbiotica* bacterium**

**Inès Pons, François Renoz, Christine Noël and Thierry Hance**

**Biodiversity Research Centre, Earth and Life Institute, Université catholique de Louvain, Croix du sud 4-5, 1348 Louvain-la-Neuve, Belgium**

Symbiotic microorganisms are widespread in nature and can play a major role in evolution. A key question in evolutionary ecology is how mutualistic associations are established and evolve. The aphid-*Serratia symbiotica* bacterium interaction provides a valuable model to study mechanisms behind this question. *S. symbiotica* is one of the most common symbionts among aphid populations and includes a wide variety of strains whose degree of interdependence on the host may vary considerably. *S. symbiotica* strains with free-living capacity have recently been isolated from aphids. By using these strains, we established artificial associations by simulating new bacterial acquisitions involved in aphid gut infections to decipher their infection processes, associated biological effects on their new hosts as well as the way they infect aphids and their transmission behaviour. Our study showed that the cultivable *S. symbiotica* colonized the aphid digestive tract following infection, after which the bacterium multiplied exponentially during aphid development. Our results further revealed that gut colonization by the bacteria induce a fitness cost. However, it appeared that they also offer an immediate protection against parasitoids that should probably in some conditions compensate the costs. Moreover, cultivable *S. symbiotica* seem to be extracellularly transmitted, partly through the honeydew and the plant sap. Indeed, we showed that the circulation of the cultivable *S. symbiotica* in aphids can be mediated by plants. We discovered that the cultivable *S. symbiotica* can be absorbed by plant roots and induce new bacterial infections in aphids feeding on these new infected plants. Furthermore, our results demonstrated that cultivable *S. symbiotica* can be horizontally transferred from infected to uninfected aphids sharing the same plant, providing first direct evidence that plants can mediate horizontal transmission of certain strains of this symbiont species. These findings provide new insights into the nature of symbiosis in aphids and highlight the importance of considering symbiotic associations in complex systems where microorganisms can circulate between different compartments.

## POSTER

### Host selection by the English grain aphid (*Sitobion avenae*): is Maris Huntsman still the number one choice for aphids?

Tom Pope, Dan Hawes, Joe Roberts and Ed Dickin

Harper Adams University, Shropshire, TF10 8NB, UK

The wheat variety Maris Huntsman was added to the NIAB Recommended List in 1972, and for many years was the most widely grown winter wheat in the UK. Maris Huntsman was superseded in the late 1970s by Hobbit, the first semi-dwarf variety in the UK, which shared the parent Professor Marchal with Maris Huntsman; then Hobbit-derived varieties such as Brock, Galahad and Norman dominated wheat production through the 1980s. The period during which these varieties were widely grown was one characterised by repeated outbreaks of cereal aphids such as the English grain aphid, *Sitobion avenae*, and the rose-grain aphid, *Metopolophium dirhodum*. As Maris Huntsman and its relatives were replaced with other more modern semi-dwarf varieties in the 1990s these severe outbreaks of aphid pests have become less common, although the reasons for the link between aphid outbreaks and the growing of Maris Huntsman remain unclear.

In the work presented here the landing behaviour of alate *S. avenae* was recorded under semi-field conditions to a range of wheat varieties. Three wheat seedlings (14 varieties from the current AHDB winter wheat Recommended List, 3 ancient wheats as well as Maris Huntsman and Flanders, a variety grown widely at the same time as Maris Huntsman) were grown in John Innes No2 compost under controlled conditions, each in 9 cm diameter pots to growth stage 12 - 13 before being moved to the glasshouse for testing. Each group of seedlings had approximately the same leaf area. The pots of wheat seedlings were arranged, with equal spacing between pots, in random order around the circumference of a circle approx. 100 cm in diameter within a large mesh cage. In the centre of the circle 60 alate aphids, previously reared on barley, were released from a 50 ml specimen tube. The numbers of alates and nymphs on each plant was then recorded 24, 96 and 168 hours later.

From these results, significantly more adult alate *S. avenae* and nymphs produced by these alates were recorded on Maris Huntsman than on almost all of the current AHDB Recommended List wheat varieties tested. There was, however, no difference between numbers of alates or nymphs recorded on Maris Huntsman and Flanders. While an important first step in breeding for resistance to crop pests is phenotyping of varieties or wild ancestors to these varieties to search for beneficial plant traits such as resistance to insect pests, here we present results that indicate a preference for Maris Huntsman over modern varieties. Understanding what makes *S. avenae* more likely to land on Maris Huntsman may, however, also prove useful in breeding programmes and may lead to development of useful tools in the management of this pest.

## **Unravelling trophic webs between aphids and parasitoids in strawberry greenhouses to improve a biological control program**

**Estelle Postic, Christelle Buchard, Yannick Outreman and Anne Le Ralec**

**Agrocampus Ouest, 65 rue de Saint-Brieuc, CS 84215, 35042 Rennes cedex, France**

In French strawberry greenhouses, aphids are major pests, leading to yield and quality losses. Biological control of these pests relies on the inundative release of predators (e.g. *Chrysoperla carnea*) and parasitoids (mainly belonging to the subfamily Aphidiinae). However, these releases are often inefficient, so chemical applications are often needed. The lack of reliability of biological control of aphids prevents the implementation of a global Integrated Pest Management strategy in strawberry greenhouses. In order to improve biological control of aphids, our study focuses on structure and spatio-temporal variability of trophic webs between strawberry aphid species, their insect parasitoids and hyperparasitoids.

During two successive years, we sampled aphid and parasitoid individuals in French strawberry greenhouses at the national scale. First, we investigated aphid communities in spring and summer in various production contexts. Second, identification of parasitized aphids and parasitoid individuals enabled us to build quantitative food webs. In spring, four aphid species dominated the communities and their relative abundance was related to the production context. Two generalist parasitoid species, *Aphidius ervi* and *Praon volucre*, parasitized the dominant aphid pest species. Also, we observed some specific relationships within trophic webs: one aphid species, *Chaetosiphon fragaefolii* was parasitized by a single specialist parasitoid species, *Aphidius eglanteriae*. Temporal variation in food web structure was found as a shift in aphid community composition between spring and summer was observed, leading to both change and diversification of the parasitoid community composition. This study also highlights the negative role of hyperparasitoid species in the success of biological control as a lot of hyperparasitoid individuals emerged from parasitized aphids.

This study is a first step in the unravelling of aphid–parasitoids interaction networks in strawberry greenhouses. It will help identify parasitoid species or populations well adapted to the aphids found in this system.

## **From stylet to cuticle: Transcriptomics and genomics of aphid cuticular proteins**

**Yvan Rahbé, Natalia Guschinskaya, Manuela van Munster and Marilyne Uzest**

**UMR5240 CNRS INSA-Lyon MAP, FR BioEnviS (BioEnvironnement Santé),  
Microbiologie, Adaptation, Pathogénie, Bat. A.-Lwoff, 11 rue Dubois,  
F 69622 Villeurbanne Cedex, France**

Aphid stylets are an example of the specialized mouthparts of Hemiptera, the design of which started more than 200 MY ago. They have specific features, some shared with hemipteran groups and some not.

In the course of a vector biology project, we identified the complete set of cuticular proteins from the maxillary and mandibular stylets of the model aphid *Acyrtosiphon pisum*. An extension of the definition of cuticular proteins is proposed, unravelling almost 80% new cuticular protein class(es), based on compositional biases and not on sequence homology. We will review our findings in this context, and the opening of a project on comparative genomics of cuticular proteins within the Hemiptera, and eventually beyond.

## POSTER

### Revealing the microbiota of the subterranean aphid *Anoecia corni* through metagenomic sequencing

François Renoz, Samir Fakhour, Jérôme Ambroise, Jean-Luc Gala and Thierry Hance

Earth and Life Institute, Université catholique de Louvain, Croix du sud 4-5, 1348 Louvain-la-Neuve, Belgium

Many insects harbour symbiotic bacteria in their cells and tissues that can significantly influence their evolutionary ecology. Insects can also harbour a fairly rich microbiota composed of transient microorganisms, pathogens, intestinal bacteria and environmental contaminants. Microbial diversity associated with aphids has the reputation of being poor compared to that associated with other insect groups. This could be explained by the quasi-sterility of the phloem these insects feed on. Microbial diversity associated with aphids is generally restricted to the presence of the obligate nutritional symbiont *Buchnera*, and the more sporadic presence of facultative symbionts.

In this study, we used deep 16S rRNA sequencing to evaluate the bacterial diversity associated with dogwood–grass aphids (*Anoecia corni*) collected in Morocco. By living on the roots of grasses, this aphid species spends much of its life cycle in a subterranean environment where the surrounding microbial flora is vast. We assessed the bacterial diversity associated with *A. corni* to test the hypothesis that the lifestyle of this species would promote the acquisition of a richer bacterial diversity compared to aphid species that exclusively feed on the aerial parts of their host plant. Indeed, living surrounded by bacteria in the rhizosphere, it is expected that root aphids will easily pick up environmental bacteria.

Our study has identified 23 bacterial operational taxonomic units (OTUs) corresponding to 11 bacterial genera. In addition to *Buchnera*, only two facultative symbionts were detected: *Arsenophonus* and *Serratia symbiotica*. These two species of symbionts were generally found in single infections and detected with a prevalence of up to about 25% in the sampled colonies. The majority of the OTUs corresponds to 8 bacterial genera in addition to previously identified symbionts. Among them, the genera *Delftia*, *Dactylopiibacterium* and *Anaerobacillus* were found with a high prevalence (81%, 56% and 31% respectively). The other identified genera (*Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Enhydrobacter* and *Undibacterium*) were detected more sporadically in the different aphid colonies. These bacterial taxa correspond to environmental bacteria that are probably present in the rhizosphere. While some of these taxa have already been identified in previous studies, others have been identified for the first time here.

How can insects acquire new bacterial partners to benefit from new ecological traits is an important issue in evolutionary ecology. Our results shed light on the microbiota harboured by “unconventional” aphids. Further analyses applied to larger samples are needed to determine whether the soil, and more particularly the rhizosphere, can be a reservoir for the acquisition of new microbial partners by insects. There is no doubt that subterranean aphids are excellent candidates to tackle this issue.

## **IAP (Inhibitor-of-Apoptosis Protein) gene family expansion and functional diversification in aphids**

**Mélanie Ribeiro Lopes, Nicolas Parisot, Karen Gaget, Gabrielle Duport, Korneel Hens, Patrick Callaerts and Federica Calevro**

**Univ Lyon, INSA-Lyon, INRA, BF2I, UMR0203, F-69621, Villeurbanne, France**

Apoptosis is a widespread form of regulated cell death, controlled by a genetically-encoded machinery conserved in almost all metazoan clades. In the highly diverse group of insects, apoptotic pathways have been characterized in only a few dipteran and lepidopteran species, where they have been shown to be essential for development, metamorphosis and immunity-related processes. The lack of studies in other insect orders clearly limits our understanding of the role of apoptosis in insects' lives.

In this study, we combined phylogenetic analyses and conserved domain identification to annotate the apoptosis pathway in a hemipteran model: the pea aphid *Acyrtosiphon pisum*. We found that the executive part of the pathway is incomplete, with homologs for only 4 out of the 8 proteins present in *Drosophila melanogaster*, while the inhibitory part underwent a large expansion, with over 30 Inhibitor-of-Apoptosis Proteins (IAPs) in contrast to the 4 to 7 found in other insect species. Tissue-specific qRT-PCR and RNAseq analyses have shown that, in senescent aphids, only two apoptotic effectors are induced in bacteriocytes, the specialized cells housing symbiotic bacteria, while several apoptosis inhibitors are concomitantly over-expressed. This provides a possible mechanism whereby apoptosis is prevented in these cells, enabling the emergence of a novel cell death process. We have confirmed the anti-apoptotic role of these bacteriocyte cell death-associated IAPs in heterologous expression experiments using a tractable in vivo model, the drosophila developing eye.

These results suggest that a restricted set of aphid IAPs have a key role in bacteriocyte homeostasis. Other aphid IAPs are differentially expressed in different life stages and tissues, suggesting an as yet not understood functional diversification. Interestingly, this IAP functional diversification is expected to also occur in other aphid species as, using comparative phylogenomics analyses, we have demonstrated that IAP expansion is widespread in aphid lineages.

## POSTER

### **A novel cell death process eliminates both bacteriocytes and their symbionts in the pea aphid/*Buchnera* symbiotic system**

**Mélanie Ribeiro Lopes, Pierre Simonet, Karen Gaget, Séverine Balmand, Nicolas Parisot, Kurt Buhler, Gabrielle Duport, Veerle Vulsteke, Abdelaziz Heddi, Hubert Charles, Patrick Callaerts and Federica Calevro**

**Univ Lyon, INSA-Lyon, INRA, BF2I, UMR0203, F-69621, Villeurbanne, France**

Symbiosis is a key source of ecological and evolutionary diversification of eukaryotic organisms throughout the animal and plant kingdoms. In insects that are obligatorily dependent on intracellular bacterial symbionts, novel host cells, the bacteriocytes, have evolved for harboring beneficial microbial partners. These cells constitute a fascinating riddle in developmental cell biology, as their embryonic origin and the molecular mechanisms governing their development and organogenesis, as well as their elimination in response to host physiology, remain largely unsolved.

Here we report the discovery of a hitherto unknown cell-death process involved in the degeneration of bacteriocytes in the pea aphid *Acyrtosiphon pisum*/*Buchnera aphidicola* symbiotic system, which emerged in recent years as a powerful model for studying symbiotic relationships. This new form of cell death is activated progressively throughout aphid adulthood and exhibits morphological features distinct from evolutionary-conserved pathways, including apoptosis- or autophagy-dependent cell deaths. By combining electron microscopy, immunohistochemistry, and molecular analyses, we demonstrated that the initial event of bacteriocyte cell death is the cytoplasmic accumulation of non-autophagic vacuoles, followed by a sequence of cellular stress responses including the formation of autophagosomes in intervacuolar spaces, swelling of bacteriocyte mitochondria, activation of reactive oxygen species, and *Buchnera aphidicola* endosymbiont degradation by the lysosomal system. This multistep cell-death process originates from the endoplasmic reticulum, an organelle exhibiting a unique reticular network organization in these cells, probably imposed by the presence of millions of *Buchnera* endosymbionts in each bacteriocyte.

Our findings reveal novel mechanisms by which both bacteriocyte cell and symbiotic bacterial numbers are controlled to maintain organismal homeostasis. They also shed light on previously unknown consequences of persistent obligatory symbiotic bacteria on the cellular organization and functioning of bacteriocytes in their eukaryotic hosts.

## POSTER

### **Biological crop protection: a new ‘slow down–speed up’ strategy for aphid management**

**Joe Roberts, Simon Leather, Tom Pope, Jamin Ali, William Kirk, Toby Bruce, Elaine Fitches, Ari Sadanandom, Gill Prince, Graham Teakle and Dave Chandler**

**Centre for Integrated Pest Management, Department of Crop and Environment Sciences, Harper Adams University, Newport, Shropshire, TF10 8NB, UK; and  
Centre for Applied Entomology and Parasitology, School of Life Sciences, Keele University, Keele, Staffordshire, ST5 5BG, UK**

Aphids are important insect pests of a wide range of crop plants, causing over \$1billion of crop losses annually. For field crops, the ‘standard’ method of managing aphids has been to apply synthetic chemical insecticides. While this approach has been an effective method of control for many years, overreliance on insecticides has led to the evolution of insecticide resistance in a number of key aphid pests. Furthermore, there are increasing concerns regarding the environmental impact of insecticide use, leading to many products being withdrawn from sale. This has left farmers with few effective options for controlling the aphid pests that have now become a major threat to global crop production. A sustainable aphid management strategy that can help to protect harvests from pests is urgently needed to address this threat.

To achieve more sustainable crop protection methods that are less prone to resistance, farmers should not over-rely on any one intervention. Diversification of crop protection can be achieved through integrated pest management (IPM), which promotes the use of an appropriate combination of environmentally sustainable methods of pest control. These can include pest resistant crop varieties, the use of natural enemies, and 'biopesticide' products based on beneficial microbes or natural products. Unfortunately, as farmers have been so reliant on synthetic chemical insecticides as their principal form of pest control, there is currently no effective IPM system in place for the majority of aphid pests in field crops. The development of IPM for aphid pests has largely been restricted by a lack of new pest control methods being developed and an understanding of how control methods can be used together.

This project aims to develop the tools and knowledge needed for an effective IPM system to manage aphid pests of field crops using a model system comprising of *Brassica* plants, the peach–potato aphid (*Myzus persicae*) and parasitoid wasps such as *Aphidius ervi*. We will use this model system to test the hypothesis that plants with partial resistance ‘slow down’ aphid development and increase their susceptibility to biological control agents while pest control can be ‘speed up’, using *cis*-jasmonate to induce plant defence priming to attract and retain parasitoid wasps within a *Brassica* crop.

## **Elucidating mechanisms of resistance to *Rhopalosiphum padi* (L.) (bird cherry–oat aphid) in ancestral wheat**

**Amma Simon, John Caulfield, Lesley Smart, Kim Hammond-Kosack, Lin Field and Gudbjorg Inga Aradottir**

**Department of Biointeraction and Crop Protection, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK**

Wheat is an economically important crop as it is a staple in many diets around the world. Pest species such as aphids are one of the main biotic threats to wheat. Bird cherry–oat aphid (*Rhopalosiphum padi* (L.)) infestations can cause up to 30% yield loss (Voss *et al.*, 1977). With the emergence of insecticide resistance and restrictions on insecticidal compounds, there has been increased interest in understanding resistance traits, as part of Integrated Pest Management (IPM).

No modern commercial wheat with resistance to *R. padi* is available in the UK. *Triticum monococcum* (L.) lines MDR045 and MDR049 have been identified to possess some post-alighting resistance (antibiosis) to *R. padi* at seedling stage (Greenslade *et al.*, 2016). However, it is unknown whether this antibiosis is present in older wheat, whether these lines confer pre-alighting resistance (antixenosis) to *R. padi* and whether resistance is present in field conditions.

To address whether antibiosis is growth stage dependant, *R. padi* development and fecundity studies were carried out on susceptible *T. monococcum* line MDR037 and *Triticum aestivum* variety Solstice, and the resistant *T. monococcum* MDR045 and MDR049 at four growth stages. It was found that antibiosis was dependant on the interaction between plant age and genotype with MDR045 and MDR049 adult plants having varying levels of resistance.

Volatile organic compounds emitted from susceptible and resistant wheat were collected by air entrainment and behavioural bioassays were used to determine whether MDR045 and MDR049 conferred antixenosis resistance to *R. padi* and whether this was stable with the onset of aphid herbivory. Antixenosis was identified in one of the antibiosis resistant lines, the compounds underpinning this resistance were identified through electroantennography (EAG) and gas chromatography-mass spectrometry (GC-MS).

Two years of field trials with susceptible, resistant and intermediate wheat lines have been carried out to determine if the levels of resistance observed under controlled conditions are present in the field. Populations of three aphid species and three beneficial insects' presence were recorded. Whilst resistance was present in the field, an interaction between plant genotype and drought affecting aphid populations was observed.

Voss, T. S., Kieckhefer, R. W., Fuller, B. W., Mcleod, M. J. and Beck, D. A. (1977) Yield losses in maturing spring wheat caused by cereal aphids (Homoptera: Aphididae) under laboratory conditions. *Journal of Economic Entomology* **90**, 1346–1350

Greenslade, A. *et al.* (2016) *Triticum monococcum* lines with distinct metabolic phenotypes and phloem based resistance to the bird cherry oat aphid *Rhopalosiphum padi*. *Annals of Applied Biology* **168**, 435–449

## **Towards the mechanisms underlying host plant adaptation in the pea aphid complex**

**Jean-Christophe Simon, Pierre Nouhaud, H el ene Boulain, Fabrice Legeai, Mathieu Gautier, Renaud Vitalis, Julie Jaqui ery, Jean Peccoud and Akiko Sugio**

**INRA, UMR IGEPP, 35650 Le Rheu, France**

The pea aphid, *Acyrtosiphon pisum*, shows a complex of plant-specialized biotypes, ranging from sympatric host races to incipient species, and resulting from a recent adaptive radiation about 10,000 years ago. However, we know very little on the mechanisms underlying biotype specialization on their legume hosts. Host plant selection, acceptance and exploitation by aphids rely on a series of behavioural and physiological traits involving chemosensory, salivary, detoxifying and digestive systems. Because we have limited knowledge on which functions and genes determine biotype specialization, we used population genomics and transcriptomics approaches to identify candidate genes controlling plant adaptation in different biotypes. We found about 400 hotspots of genomic differentiation between biotypes that may contain targets of selection on plant usage. Interestingly, salivary genes, which are considered as main effectors of plant–aphid interactions, were enriched in these hotspots. Further comparative transcriptomic analyses on salivary glands of different biotypes allowed identification of differentially expressed genes that may represent candidate effectors determining host plant specificity. Altogether, these findings open future research directions towards functional studies for validating the role of these genes in biotype specialization.

## **Testing for co-adaptation potential through experimental evolution of aphid–symbiont interactions**

**Corentin Sochard\***, **Grégory Toussaint**, **Fabrice Vavre**, **Yannick Outreman\***, **Jean-Christophe Simon\*** (\*=share senior authorship)

**INRA, UMR 1349, Institute of Genetics, Environment and Plant Protection, Domaine de la Motte, BP 35327, Le Rheu, France**

In recent years, evidence has accumulated that all functions of macro-organisms are influenced by the microbial communities they host, that shape their ecology and evolution. A host and its associated microbes form a holobiont but whether this entity constitutes a unit of selection is matter of debate. If holobionts respond to natural selection as a whole, co-adaptation between hosts and their microbiota should be favoured (*i.e.* hosts and symbionts would align their respective interests). However, this co-adaptation hypothesis has been rarely tested. In this study, we tested this co-adaptation hypothesis by modifying the symbiotic cortege of a host species, the pea aphid (*Acyrtosiphon pisum*) and by monitoring the evolution of these new symbiotic interactions over 20 generations in laboratory conditions.

To assess the co-adaptation between pea aphid lines and their microbiota, we first eliminated the secondary symbionts of several aphid lines by an antibiotic treatment. We then reintroduced the eliminated symbionts in control lines and created new lines by swapping secondary symbionts. We compared life history and morphological traits between control lines and new symbiotic interactions at three time points: soon after symbiont injection, 10 and 20 generations after symbiont acquisition.

Because the experiments are still under progress, this joint meeting will be the perfect occasion to present and discuss our freshly acquired results.

## KEYNOTE

### **Molecular basis and ecological relevance of symbiont-mediated body colour change in aphids**

**Tsutomu Tsuchida**

**Graduate School of Science and Engineering, University of Toyama, 3190  
Gofuku, Toyama, Toyama 930-0887, JAPAN**

In the pea aphid *Acyrtosiphon pisum*, red and green colour morphs are found in the same populations. Early ecological studies showed that the colour variations are important traits, often involved in the prey–predation relationships. The red morph of the aphid is more attractive to a predator ladybird beetle, while the green morph is more cryptic. In the previous study, we found an aphid endosymbiont of the genus *Rickettsiella* that modifies the aphid body colour from red to green. Therefore, the symbiont-induced body colour change seems to have an effect not only on the infected aphid but also on prey–predation relationships and food webs. We examined phylogenetic relationships, microbiological natures, and phenotypic traits of the *Rickettsiella* symbiont, and revealed that the symbiont is distinctly different from previously reported *Rickettsiella* species. Therefore, we proposed the designation “*Candidatus Rickettsiella viridis*” for the symbiont clade.

We chemically characterized the main green pigments that are increased by *Rickettsiella* infection. Quantification using LC-MS confirmed that both *Rickettsiella*-infected and uninfected aphids contain the same green pigments, although the amounts of the pigments were remarkably different between them. From those results, *Rickettsiella* probably does not synthesize the green pigments by itself but somehow interferes with aphid metabolism to activate green pigment production.

In this meeting, I will also present our recent outcomes regarding molecular mechanisms underlying *Rickettsiella*-induced body colour change.

## **Parasitoid pre-adaptation improves biological control of symbiont-protected aphids**

**Christoph Vorburger and Silvan Rossbacher**

**Eawag & ETH Zürich, Überlandstrasse 133, 8600 Dübendorf, Switzerland**

Aphids are commonly infected by heritable bacterial endosymbionts that increase their resistance to parasitoid wasps. Such defensive symbionts may have the potential to reduce the effectiveness of controlling pest aphids with parasitoids. Using laboratory cage experiments with black bean aphids (*Aphis fabae*) and their main parasitoid *Lysiphlebus fabarum*, we have shown that the release of parasitoids rapidly selects for aphids protected by the defensive symbiont *Hamiltonella defensa*, thus compromising biological control. However, other experiments have shown rapid counter-adaptation of parasitoids to the presence of *H. defensa* in their hosts. Taken together, these findings suggest that parasitoid pre-adaptation could be a viable strategy to improve biological control of symbiont-protected aphids. We tested this prediction in laboratory cage populations of *A. fabae*, comprising a mixture of *H. defensa*-free aphids, aphids infected with a moderately protective strain of *H. defensa*, and aphids with a strongly protective strain. These populations were exposed to five different treatments: (i) no parasitoids/control, (ii) parasitoids experimentally evolved on *H. defensa*-free aphids, (iii) parasitoids evolved on aphids protected by the less protective strain of *H. defensa*, (iv) parasitoids evolved on aphids protected by the more protective strain, and (v) a mixture of iii and iv. As expected, all treatments with parasitoids resulted in strong selection for *H. defensa*-protected aphids, but parasitoids adapted to *H. defensa*-free aphids and parasitoids adapted to the less protective strain of *H. defensa* failed to reduce aphid infestations relative to the control. Parasitoids adapted to the more protective strain and the mixed parasitoid treatment, on the other hand, strongly reduced aphid densities, resulting in significantly improved host plant condition. This study thus showed, for the first time, that parasitoid pre-adaptation can improve the control of partly-resistant aphid populations harbouring a protective endosymbiont.