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ΚΡΗΤΙΚΟ ΕΡΓΑΣΤΗΡΙ
ΟΙΚΟΓΕΝΕΙΑ ΛΑΜΠΑΚΗ

General Agenda

Sunday- June 11	Monday- June 12	Tuesday- June 13	Wednesday- June 14	Thursday- June 15	Friday- June 16
	7:30–9:00 Breakfast	7:30–9:00 Breakfast	7:30–9:00 Breakfast	7:30–9:00 Breakfast	7:30–9:00 Breakfast
		Pest and Disease Control (C) I- Rafael Maciel de Freitas	Reproductive Manipulations (A) I- Sarah Bordenstein	Molecular and Cellular Biology (B) I- Wolfgang Miller	11:30 Check out and departure
	9:00-9:10 Welcome words	9:00-9:20 C1-Taylor, M	9:00-9:20 A1-Bordenstein, Se.	9:00-9:20 B1-Lindsey, A.	
	9:10-9:50 Bandi, C. History of *Wolbachia* research	9:20-9:40 C2-Maciel de Freitas, R.	9:20-9:40 A2-Dormus, M.	9:20-9:40 B2-Suliman, Y.	
	Wolbachia in Nematodes (E) - Mark Taylor	9:40-10:00 C3-Rasgon, J.	9:40-9:55 A3-Riegler, M.	9:40-10:00 B3-Strunov, A.	
	9:50-10:10 E1-Brown, A.	10:00-10:15 C4-Stark, N.	9:55-10:10 A4-Namias, A.	10:00-10:20 B4-Russel, S.	
	10:10-10:30 E2-Perlman, S.	10:15-10:30 C5-Dhokiya, N.	10:10-10:25 A5-Charlat, S.	10:20-10:40 B5-Jones, M.	
	10:30-10:50 Coffee break	10:30-10:50 Coffee break	10:30-10:50 Coffee break	10:40-11:00 Coffee break	
	Wolbachia in Nematodes (E) cont.	Pest and Disease Control (C) II- Eric Caragata	Reproductive Manipulations (A) II- Daisuke Kageyam	Molecular and Celullar Biolgy (B) II- Julie Reveillaud	
	10:50-11:10 E3-Cantin, L.	10:50-11:05 C6-Caragata, E.	10:50-11:05 A6-Fricke, L.	11:00-11:20 B6-Schrieke, H.	
	11:10-11:30 E4-Behrmann, L.	11:05-11:20 C7-Watson, K	11:05-11:20 A7-Arai, H.	11:20-11:40 B7-Reveillaud, J.	
	11:30-11:50 E5-Voronin, D.	11:20-11:35 C8-Flores, H.	11:20-11:35 A8-Muro, T.	11:40-12:00 B9-Asgari, S.	
	Ecology and Evolution (F) I- Steve Perlman	11:35-11:50 C9-Asselin , A.	11:35-11:50 A9-Harumoto, T.	12:00-12:20 B10-Makepeace, B.	
	11:50-12:05 F1-Bordenstein, Sa.	11:50-12:05 C10-Wolfe, T.	11:50-12:05 A10-Gerth, M.		
	12:05-12:20 F2-Maia, T.	12:05-12:20 C11-Yizraeli, Y.			
	12:20-12:30 Discussion and posters promotion (E; B; H)	12:20-12:30 Discussion and posters promotion (C)	12:05-12:30 Discussion and posters promotion (A)		
	12:30 Lunch	12:30 Short Lunch	12:30 Lunch	12:30 Lunch	
	Ecology and Evolution (F) I cont.	13:30-23.00 Excursion and dinner at Chania	Reproductive Manipulations (A) III- Laura Fricke	Experimental evolutionary approaches to symbiosis (H) I- Takema Fukatsu	
	14:00-14:15 F3-Serga, S.		14:00-14:15 A11-Wybouw, N.	14:00-14:20 H1-Fukatsu, T.	
	14:15-14:30 F4-Papachristos, K.		14:15-14:30 A12-Imchen, M.	14:20-14:45 H2-Koga, R.	
	14:30-14:45 F6-Hornett, E.		14:30-14:50 A13-Loppin, B.	14:45-15:10 H3-Furusawa, C.	
	14:45-15:00 F7-Vancaester, E.		14:50-15:10 A14-Bordenstein, Se.	15:10-15:25 H4-Drew, G.	
	15:00-15:15 F8-White, J.		15:10-15:30 A15-Kageyama, D.		
15:00- Arrival, check-in, registrations	15:15-15:30 Discussion and posters promotion (G; F)				
	15:30-15:50 Coffee break		15:30-15:50 Coffee break	15:25-15:45 Coffee break	
	Ecology and Evolution (F) II		Other symbionts (G)- Einat Zchori-Fein	Experimental evolutionary approaches to symbiosis (H) II	
	15:50-16:05 F9-Rashid, A.		15:50-16:05 G1-Miller, W.	15:45-16:05 H5-Wakamoto, Y.	
	16:05-16:20 F10-Trouche, B.		16:05-16:20 G2-Hunter, M.	16:05- 16:30 H6-Fukuda, S.	
	16:20-16:35 F11-Nowak, K.		16:20-16:35 G3-Siozos, S.	16:30-16:50 H7-Kakizawa, S.	
	16:35-16:55 Sponsor presentatoins: ERATO; Frontiers MB		16:35-16:50 G4-Leclerc, L.	16:50-17:05 H8-Pons, I.	
			16:50-17:05 G5-Ross, P.	17:05-17:20 H9-Martinez, J.	
	17:30-19:00 Poster session: B, E, F, G, H		17:30-19:00 Poster session: A, C	17:20- 18:30 Final discussion	
19:00-21:00 Welcome reception	19:00 Dinner		19:00 Cretan night	19:00 Dinner	

Poster session I		Poster session II	
#	Author	#	Author
B1	Phelps, M.	A1	Inoue, M.
E1	Chappell, L.	A2	Koskinioti, P.
F1	Bordenstein, S.	A3	Mackevicius, V.
F2	Higashi, C.	A4	Makepeace, B.
F3	Khoo, J.	A5	Numajiri, Y.
F4	Palanichamy, P.	A6	Ohata, Y.
F5	Strunov, A.	A7	Papaopoulos, N.
G1	Koivisto, E.	A8	Papaopoulos, N.
G2	Singh, P.	A9	Porter, J.
G3	Tsiamis, G.	A10	Takamatsu, T.
H1	Bao, Q.	C1	Carron, E.
H2	Kanai, Y.	C2	Elnagdy, S.
H3	Moriyama, M.	C3	Garcia, G.
H4	Noda, T	C4	Fowler, S.
H5	Nozaki, T.	C5	Kotsadam, E.
H6	Wang, Y.	C6	Layton, E.
		C7	Marinho, P.
		C8	Masters, E.
		C9	Miskinyte, M.
		C10	Rooney, S.
		C11	Ross, P.
		C12	Wodowski, G.

Sunday June 11

15:30- Arrival, check-in, registrations

19:00-21:00 Welcome reception

Monday, June 12

7:30–9:00 Breakfast

9:00-9:10 Welcome note

9:10-9:50 Claudio Bandi "The Big Sweep. A personal account on the history of *Wolbachia* (1992-2002)"

9:50-10:30 *Wolbachia* in Nematodes (E)

Chair: Mark Taylor

09:50-10:10 E1- *A. Kaur, T. Kaur, E. Sharma, A. M. V. Brown* "Widescale sampling and analysis of plant-parasitic nematode *Wolbachia* uncovers new strains and unsolved puzzles"

10:10-10:30 E2- *S. J. Perlman* "Evolution of obligate *Wolbachia* and *Symbiopectobacterium* endosymbionts in insect-parasitic tylenchid nematodes"

10:30-10:50 Coffee break

10:50-11:50 *Wolbachia* in Nematodes, Cont.

10:50-11:10 E3- *L. Cantin, J. Dunning Hotopp, J. Foster* "Enrichment of *Wolbachia* genomic DNA from nematodes using ATAC-seq"

11:10-11:30 E4- *L. V. Behrmann, K. Bosse-Plois, A. J. Hennenfent, M. Franz-Wachtel, T. A. Harbig, H. Neufeld, B.Maček, K. Nieselt, A.Hörauf, K. M. Pfarr* "Mode of action of cell wall biosynthesis inhibitors in *Wolbachia* spp."

11:30-11:50 E5- *D. Voronin, E. Ghedin* "Identification of *Brugia malayi* miRNAs involved in *Wolbachia*-Host symbiosis"

11:50-12:30 Ecology and Evolution (F.I.)

Chair: Steve Perlman

11:50-12:05 F1- *S. R. Bordenstein & S. R. Bordenstein* "Phage WO: Comparative genomics and taxonomic classification"

12:05-12:20 F2- *T. G. Maia, C. R. Carmo, N. E. Martins, M. Silva, M. Sporniak, L. Teixeira* "Genetic bases of the variation of *Wolbachia* titres and antiviral protection in *Drosophila*"

12:20- 12:30 *Poster promotion slides*: *Wolbachia* in Nematodes (#E1); Molecular and Cellular Biology (#B1); Experimental evolutionary approaches to symbiosis (#H1-6).

12:30 Lunch

14:00-15:30 Ecology and Evolution (F.I, Cont.)

14:00-14:15 F3- *S. Serga, A. Lavrinienko, V. Tyukmaeva, J. Kesäniemi, J. Máca, J. Oboňa, O. Krstic, I. Tosevski, C. L. Phooi, M. Lapegue, A. Estoup, A. Loiseau, C. Deschamps, M. Gautier, E. Knott, I. Kozeretska, N. O. Rode* "Factors driving *Wolbachia* prevalence in native and invasive populations of *Drosophila suzukii*"

14:15-14:30 F4- *K. Papachristos, M. Montoliu-Nerin, W. J. Miller, L. Klasson* "*Wolbachia* in the neotropical fruit flies: A genome-wide study to reveal factors associated with long-term symbiont-host co-evolution and horizontal transmission events"

14:30-14:45 F6- *E. A. Hornett, L. A. Reynolds, D. Kageyama, G. D. D. Hurst* "How many ways can you rescue a male?"

14:45-15:00 F7- *E. Vancaester, M. Blaxter* "Phylogenomic analysis of *Wolbachia* genomes from the Darwin Tree of Life biodiversity genomics project"

15:00-15:15 F8- *J. White, M. Doremus, J. Proctor, L. Rosenwald* "Within and among host interactions among symbionts, as mediated by temperature"

15:15- 15:30 *Poster promotion slides*: Other Symbionts (#G1-3); Ecology and Evolution (#F1-F5).

15:30-15:50 Coffee Break

15:50-17:05 Ecology and Evolution (F.II.)

15:50-16:05 F9- *A. H. Rashid, K. Kus, M. Blagrove, E. Chrostek* "Effects of *Wolbachia* on host oviposition preferences"

16:05-16:20 F10- *B. Trouche, F. Trigodet, J. Reveillaud* "Reconstruction of a near-complete *Wolbachia* genome from long-read sequencing of *Culex quinquefasciatus* ovaries"

16:20-16:35 F11- *K. Nowak, C. Valdivia, M. Kolasa & P. Łukasik* "Two birds with one stone–Validating COI barcoding as a low cost, high throughput, and high resolution method of *Wolbachia* screening across insects"

16:35-16:55 Sponsor presentations: ERATO; Frontiers in Microbiology

17:30-19:00 Poster Session I

Wolbachia in Nematodes; Ecology and Evolution; Molecular and Cellular Biology; Other Symbionts; Experimental evolutionary approaches to symbiosis

19:00 Dinner

Tuesday, June 13

7:30–9:00 Breakfast

9:00-10:30 Pests and disease control (C.I.)

Chair: Rafael Maciel de Freitas

9:00-9:20 C1- *A. Yousef, E. Masters, Y. Wu, H. Kirk, R. Clare, A. Steven, W. D. Hong, J. D. Turner, P. M. O'Neill, S. A. Ward, M. J. Taylor* "Autophagy is essential for anti-*Wolbachia* drug efficacy"

9:20-9:40 C2- *J. Corrêa-Antônio, F. J. Gnonhoue, D. Couto-Lima, M. R. David, M. Galvão Pavan, R. Maciel-de-Freitas* "Integrated Vector Management is the key to fasten *Wolbachia* introgression and enhance its effectiveness on mitigating arbovirus transmission"

9:40-10:00 C3- *J. L. Rasgon* "It doesn't always block: *Wolbachia*-induced pathogen suppression is a surprisingly complex and variable phenotype"

10:00-10:15 C4- *N. Stark, I. L. G. Newton, R. W. Hardy* "The interactive effects of *Wolbachia* and *Nop60B* on Sindbis virus replication"

10:15-10:30 C5- *V. Dhokiya, M. Madhav, J. Bandibabone, M. M. Sandeu, C. Antonio Nkondjio, T. Walker, G. Hughes* "Towards the use of novel high density *Anopheles*-specific *Wolbachia* strains for *Anopheles* vector control."

10:30-10:50 Coffee Break

10:50-12:30 Pests and disease control (C.II.)

Chair: Eric Caragata

10:50-11:05 C6- *E. P. Caragata, L. Husdell, E. Rancès, L. E. Hugo, J. Popovici, S. L. O'Neill, H. J. Schirra, E. A. McGraw* "*Wolbachia*, larval nutrition, and metabolomics in *Aedes aegypti* mosquitoes."

11:05-11:20 C7- *K. Watson, A. Lindsey, T. Bhattacharya, M. Gasser, R. Hardy, I. Newton, J. C. Dunning Hotopp* "The role of epitranscriptomics in *Wolbachia*-mediated pathogen blocking through the lens of direct RNA sequencing"

11:20-11:35 C8- *H. A. Flores, K. R. Dainty, J. Hawkey, L. M. Judd, K. E. Holt, C. P. Simmons* "High genomic stability of wMel *Wolbachia* after introgression into three geographically distinct *Ae. Aegypti* populations"

11:35-11:50 C9- *A. K. Asselin, J. Engelstaedter, K. N. Johnson* "*Wolbachia*-mediated changes in viral dynamics"

11:50-12:05 C10- *T. M. Wolfe, D. J. Bruzzese, L. Klasson, E. Corretto, S. Lečić, C. Stauffer, J. L. Feder, H. Schuler* "The influence of horizontally acquired *Wolbachia* on invasive cherry fruitfly populations: A multi-perspective study"

12:05-12:20 C11- *Y .Izraeli, G. Wodowski, D. Lepetit, N. Mozes-Daube, J. Varaldi, E. Chiel, E. Zchori-Fein* "An RNA virus in the parasitoid wasp *Anagyrus vladimiri* inhibits encapsulation by the mealybug host"

12:20-12:30 Poster promotion slides: Pests and disease control (#C1-C12)

12:30- Short Lunch

13:30-23:00 Excursion and dinner at Chania

Wednesday, June 14

7:30–9:00 Breakfast

9:00-10:30 Reproductive manipulation (A.I.)

Chair: Sarah Bordenstein

9:00-9:20 A1- *R. Kaur, C. J. Meier, S. O'Neill, J. F. Hillyer, S. Bordenstein* "The cell biological mechanism of cytoplasmic incompatibility"

9:20-9:40 A2- *M. R. Doremus, O. L. Mathieson, D. L. Shultz, S. E. Kelly, S. Schmitz-Esser, M. Kleiner, M. S. Hunter* "Of sperm and symbionts: Identification of candidate *Cardinium* CI factors by integrating symbiont cellular localization, CI modification timing, and protein expression"

9:40-9:55 A3- *A. Kalav, J. M. Cook & M. Riegler* "*Cardinium* and *Wolbachia* affect host fitness and sex allocation via egg size provisioning in different ways, yet are stably maintained as coinfection"

9:55-10:10 A4- *A. Namias, I. Boussou, K. Terretaz, F. Justy, P. Makoundou, M. Perriat-Sanguinet, F. Landmann, M. Sicard, M. Weill* "Digging deeper into the genetic bases of CI patterns in *Culex pipiens:* does the toxin-antidote model still hold?"

10:10-10:25 A5- *D. Kuperberg, A. Namias, M.Sicard, M.Weill, S. Charlat* "Modeling the mechanism of cytoplasmic incompatibility: new mathematical tools for more data"

10:30-10:50 Coffee Break

10:50-12:30 Reproductive manipulation (A.II.)

Chair: Daisuke Kageyama

10:50-11:05 A6- *L. Fricke, ARI. Lindsey* "Identification of parthenogenesis inducing *Wolbachia* proteins"

11:05-11:20 A7- *H. Arai, S. Katsuma, N. Matsuda-Imai, T. Sasaki, H. Anbutsu, A. Wijinarko, S. R.Lin, M. N.Inoue, D. Kageyama* "*Wolbachia*-induced male-killing and phage WO: genes, diversity of mechanisms, and evolution"

11:20-11:35 A8- *T. Muro, S. Katsuma* "Characterization of the evolutionary process of the symbiotic relationship establishment between *Ostrinia* moths and *Wolbachia* endosymbiont"

11:35-11:50 A9-*T. Harumoto* "Self-stabilization mechanism encoded by a bacterial toxin facilitates reproductive manipulation"

11:50-12:05 A10- *M. Tischer, A. Beliavskaia, A. C. Darby, B. L. Makepeace, J. J. Khoo, C. Bleidorn, & M. Gerth* "New branches on the *Wolbachia* tree of life"

12:05-12:30 Poster promotion slides: Reproductive manipulation (#A1-A10).

12:30 Lunch

14:00-15:30: Reproductive manipulation (A.III.)

Chair: Laura Fricke

14:00-14:15 A11- *N. Wybouw, F. Mortier, E. Van Reempts, J. Zarka, F. Zélé, D. Bonte* "Complex host modifier systems control two distinct *Wolbachia*-induced reproductive phenotypes in mites"

14:15-14:30 A12- *M. Imchen, S. R. Bordenstein* "Impacts of the host extracellular microbiome on an animal-microbe endosymbiosis"

14:30-14:50 A13- *B. Horard, M. Caron, B. Loppin* "Cytological analysis of *Wolbachia* Cif proteins"

14:50-15:10 A14- *R. Kaur, J. D. Shropshire, B. A. Leigh, S. R. Bordenstein* "CifA and CifB are both *in vitro* nucleases that promote spermatid DNA damage"

15:10-15:30 A15- *D. Kageyama* "Male killing induced by maternally inherited viruses in arthropods"

15:30-15:50 Coffee Break

15:50-17:10 Other Symbionts (G)

Chair: Einat Zchori-Fein

15:50-16:05 G1- *E. Koivisto, A. Strunov, M. Mühlbauer, M. Gerth, W. J. Miller* "To Kill or not to Kill: Evolutionary long-term relationships with facultative Male-Killing *Spiroplasma* in Neotropical flies"

16:05-16:20 G2- *E. F. Umanzor, S. E. Kelly, Y. Matsuura, M. S. Hunter* "The role of the intracellular symbiont *Lariskella* in a leaf-footed bug"

16:20-16:35 G3- *S. Siozios, P. N. Jimenez, T. Azagi, H. Sprong, C. L. Frost, A. Rice, R. Connell, S. R. Parratt, L. Brettell, K. C. Liew, J. Hinton, K. C. King, M. A Brockhurst, V. Hypša, E. Novakova, A. Darby, G. D. D. Hurst* "Genome dynamics across the evolutionary transition to endosymbiosis"

16:35-16:50 G4- *L. Leclerc, B. P. Burns, N. Lo* "How can *Wolbachia* transcriptomic methods be used to determine the role of *Midichloria Mitochondrii* in *Ixodes* ticks?"

16:50-17:05 G5- *X. Gu, P. A. Ross, A. Gill, Q. Yang, E. Ansermin, S. Sharma, S. Soleimannejad, K. Sharma, A. Callahan, P. A. Umina, T. N. Kristensen, A. A. Hoffmann* "Deleterious endosymbionts for aphid pest control"

17:30-19:00 Poster Session II

Pests and disease control; Reproductive manipulation

19:00 Cretan Night

Thursday, June 15

7:30–9:00 Breakfast

9:00-10:30 Molecular and Cellular Biology (B.I.)

Chair: Wolfgang Miller

9:00-9:20 B1- *A. R. I. Lindsey* "*Wolbachia* supports development and buffers against nutritional stress"

9:20-9:40 B2- *Y. Suliman, Z. Li, A. Sinha, P. D. Dyer, C. S. Hartley, L. Ettwiller, A. C. Darby, C. K. Carlow, B. L. Makepeace* "*Wolbachia* and the transcriptional landscape of stress: A comparison between strains wMelPop-CLA and wAlbB at single-nucleotide resolution"

9:40-10:00 B3- *A. Strunov, K. Schmidt, M. Kapun, W. J. Miller* "The taming of a mighty bug: how neotropical *Drosophila* flies restrict *Wolbachia* infection"

10:00-10:20 B4- *S. L. Russell, Jennie Ruelas Castillo, William T. Sullivan* "*Wolbachia* endosymbionts manipulate GSC self-renewal and differentiation to enhance host fertility"

10:20-10:40 B5- *M. W. Jones, A. R. I. Lindsey* "Co-transmitted *Wolbachia* exhibit distinct patterns of distribution in host ovarian tissue"

10:40-11:00 Coffee Break

11:00-12:30 Molecular and Cellular Biology (B.II.)

Chair: Julie Reveillaud

11:00-11:20 B6- *H. Schrieke, O. Duron, A. Murat Eren, J. Reveillaud* "Multiple *Wolbachia* sub populations co-occur in single *Culex pipiens* mosquitoes"

11:20-11:40 B7- *A. Ghousein, J. Tutagata, M. Etienne, V. Chaumeau, S. Boyer, N. Pages, D. Roiz, A. Murat Eren, G. Cambray, J. Reveillaud* "pWCP is a widely distributed and highly conserved *Wolbachia* plasmid in *Culex pipiens* and *Culex quinquefasciatus* mosquitoes worldwide"

11:40-12:00 B8- *S. Asgari, M. Hussain, G. Zhang, M. Leitner, L.M. Hedges* "Antisense nucleic acids as tools to explore *Wolbachia*-host interactions"

12:00-12:20 B9- *L. Bell-Sakyi, J.J. Khoo, C. Hartley, E. Chrostek, W. Miller, B.L. Makepeace* "Isolation and propagation of novel *Wolbachia* strains in tick and insect cell lines from the Tick Cell Biobank"

12:30 Lunch

14:00-15:25: Experimental evolutionary approaches to symbiosis; ERATO sponsored session (H.I.)

Chair: Takema Fukatsu

14:00-14:20 H1- *T. Fukatsu* "Experimental evolutionary approaches to symbiosis"

14:20-14:45 H2- *R. Koga* "The things have small beginnings: various single mutations could make *Escherichia coli* an insect mutualist"

14:45-15:10 H3- *C. Furusawa* "Exploring the universality of symbiosis: constructive and quantitative approaches to symbiotic evolution"

15:10-15:25 H4- *G. C. Drew, K.C. King* "Experimental coevolution in a defensive symbiosis community"

15:25-15:45 Coffee Break

15:45-17:10 Experimental evolutionary approaches to symbiosis II

Chair: Takema Fukatsu

15:45-16:05 H5- *Y. Wakamoto* "Bacterial growth and competition in geometrically structured habitats"

16:05-16:30 H6- *S. Fukuda* "Genetic mutation in *Escherichia coli* genome during adaptation to the murine intestine is optimized for the host diet"

16:30-16:50 H7- *S. Kakizawa* "Whole genome cloning of genome-reduced insect symbionts"

16:50-17:05 H8- *I. Pons, C. Henzler, M. Á. González-Porras, M. García-Lozano, H. Salem* "Specificity of an obligate leaf beetle-bacterial symbiosis"

17:05-17:20 H9- *J. Martinez, A. W. Wairimu, S. P. Sinkins* "Parallel evolution in *Wolbachia*-infected mosquito cell lines"

17:20-18:30 Final discussion and next meeting selection

19:00 Dinner

Friday, June 16

7:30–9:00 Breakfast

11:30 Check out and Departure

ABSTRACTS

Oral presentations:

Wolbachia in Nematodes (E)

E1: Widescale sampling and analysis of plant-parasitic nematode *Wolbachia* uncovers new strains and unsolved puzzles

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Wolbachia strains in plant-parasitic nematodes (PPNs) are important as the earliest branch in this genus, and as infections in some of the most globally significant agricultural pests, yet their distribution, diversity, function, and host-manipulative mechanisms remain elusive. Despite efforts to understand their similarities to strains of *Wolbachia* in filarial nematodes and arthropods, they remain mysterious due to the scarcity of fully sequenced representatives and the difficulties of culturing and manipulating their tiny nematode hosts. This study sought to address the paucity of data on PPN *Wolbachia* through broad sampling and fine-tuned bioinformatic analyses. We sampled 410 nematode communities collected from across grasslands, forests, and many agricultural settings across 23 U.S. states using a new, highly sensitive PCR assay. We also bioinformatically screened diverse rhizosphere, soil, plant, and nematode sequences in NCBI's sequence read archive (SRA), including 3200 amplicon sequencing experiments, and >2000 whole genome shotgun sequencing and RNAseq experiments. In U.S. field-collected nematodes, we found ~46% of communities hosted *Wolbachia*. Our rapid screen of SRA data revealed ~0.7% of sampled runs had novel PPN *Wolbachia*. These *Wolbachia* strains included diverged sequences in *Heterodera* and *Globodera* spp. (cyst nematodes), Criconematinae (ring nematodes), and *Helicotylenchus* (spiral nematodes). Given the previous discovery of dual *Wolbachia* and *Cardinium* infection in *Pratylenchus penetrans*, we investigated co-occurrence of *Cardinium* in these samples and found numerous novel *Cardinium* strains. Although present at a higher prevalence than *Wolbachia*, *Cardinium* appeared to have a negative co-occurrence, suggesting exclusion. Analysis of horizontally transferred genes, genome repertoires, and relative selection (dN/dS) on genes and pathways amongst PPN *Wolbachia* suggested an important role of lysine synthesis, highly conserved heme biosynthesis and iron regulation functions, and relaxed purifying selection on protein folding and transport, chaperone binding, and aromatic amino acid synthesis. Together, these results highlight weak mutualism in PPN *Wolbachia*, but leave *Wolbachia*'s long residency and scarcity in PPNs an unsolved puzzle.

E2: Evolution of obligate *Wolbachia* and *Symbiopectobacterium* endosymbionts in insect-parasitic tylenchid nematodes

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Compared to their insect counterparts, symbioses between nematodes and intracellular bacteria are much less studied. In this talk, I will discuss our recent work on obligate endosymbionts in insect-parasitic tylenchid nematodes. Within a clade of nematodes that

parasitize flies, two lineages have independently acquired obligate symbionts. A small group of *Drosophila*-parasitic nematodes, including *Howardula aoronymphium*, harbours a strain of *Symbiopectobacterium*, a recently characterized lineage of bacteria closely related to plant-pathogenic *Pectobacterium*, and that forms numerous and diverse symbioses with insects. The nematode-associated *Symbiopectobacterium* exhibits the hallmarks of a recent transition to obligate symbiosis, with a large genome containing over a thousand pseudogenes. In contrast, an undescribed *Howardula* species that infects sphaerocerid flies, harbours a highly divergent strain of *Wolbachia* that likely represents a distinct supergroup. This strain has the smallest genome of all sequenced *Wolbachia* thus far, at 550 kb, and has lost numerous genes and pathways, including ones involved in cell division and cell wall synthesis. Both symbiont-bearing nematode lineages have evolved a viviparous lifestyle with very large motherworms, and we speculate that each symbiont provides their host with essential nutrients to fuel nematode growth and reproduction. Studying diverse obligate symbioses will provide new insights in the biology and convergent evolution of animal parasitism.

E3: Enrichment of *Wolbachia* genomic DNA from nematodes using ATAC-seq

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While *Wolbachia* is normally described as a reproductive parasite in arthropods, the bacteria is an obligate endosymbiont in filarial worms. These parasitic nematodes cause filariasis, a leading cause of morbidity in humans worldwide. Current anthelmintic treatment programs are only effective at preventing transmission, but do not cure active infections with adult worms. However, antibiotics, such as doxycycline, can be used to kill the *Wolbachia,* leading to the sterility and eventual death of the adult worms. Comparative genome analyses can be used to better understand the complex evolutionary relationship between the nematodes and their endosymbiont. *Wolbachia*, like many bacterial symbionts, cannot be cultured away from host cells in the lab, making it difficult to obtain adequate bacterial DNA and to limit host DNA contamination for genome assembly. Here, we use ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) with *Brugia malayi* worms as a proof of principal to increase the number of *Wolbachia* sequences 50-fold over standard gDNA library preps. ATAC-seq, normally used as an epigenetic technique, uses the Tn5 transposase to cut and attach Illumina sequencing adapters to open chromatin, unbound by histones, in eukaryotic cells. Bacterial and mitochondrial DNA in these preps will be cut preferentially, as they do not have histones, leading to the enrichment of these sequences. The benefits of this method over other enrichment strategies include minimal tissue input (<1 mg of tissue), quick protocol (<4 hours), low sequencing coverage, less biased and no prior sequence knowledge required. We were able to assemble the wBm genome with as little as 1 million Illumina short paired-end reads with greater than 97% coverage of the published genome, compared to only 12% with the standard gDNA libraries. Using BlobTools, we were able to partition the bacterial sequences from the worm sequences in the assembly using either the read coverage or BLAST taxonomic matches of each contig. This method can extend to additional bacterial genome sequencing projects. We were able to identify bacterial sequence enrichment and assemble bacterial genomes from previously published ATAC-seq data sets in human cells infected with *Mycobacterium tuberculosis* and *C. elegans* contaminated with their food source, the OP50

strain of *E. coli*. These results demonstrate the feasibility and benefits of using ATAC-seq to easily obtain bacterial genomes to aid in symbiosis, infectious disease, and microbiome research.

E4: Mode of action of cell wall biosynthesis inhibitors in *Wolbachia* spp.

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Filarial nematodes infect ~100 million humans and can cause lymphatic filariasis (Elephantiasis) or onchocerciasis (River Blindness). *Wolbachia* spp., obligate endobacteria of filarial worms, are essential for embryogenesis, larval development and adult survival, making anti-wolbachial therapy a potent treatment of filarial infections. Fosfomycin inhibits the first dedicated cytoplasmic enzyme for lipid II synthesis (MurA). Although *Wolbachia* have a reduced genome and lack a canonical cell wall, key enzymes needed to synthesize lipid II, including MurA, are conserved. Fosfomycin treatment leads to enlarged *Wolbachia* cells, demonstrating that lipid II is necessary for cell division. Consistent with this, we found that D-cycloserine, an inhibitor of the D-alanine:D-alanine ligase (Ddl) that synthesizes the terminal dipeptide of the lipid II pentapeptide chain induces the same phenotype.
To elucidate the cellular effects of fosfomycin treatment, *Wolbachia*-infected C6/36 insect cells were treated for 9 days. Total RNA and proteins were extracted from the same assay and used for transcriptomics and proteomics to characterize the expression levels of fosfomycin-treated *Wolbachia*. Pathways of interest, besides cell wall biosynthesis and cell division, are branched fatty acid synthesis, starvation and oxidative stress phenotypes shown to be upregulated in other fosfomycin-treated bacteria, and stress-response elements.
Different assay setups and RNA preservation methods led to the identification of conditions yielding high-quality RNA for RNA-Seq. Depletion of rRNA with riboPOOLs worked best and was combined with insect mRNA depletion using Dynabeads, increasing coverage of the wolbachial transcripts from 0.1% to ~1%; RNA-Seq of the fosfomycin assay is running. For proteomics, coverage was increased from 3% to ~60% using an optimized extraction method; we are currently analyzing the data. First recovery assays suggested that *Wolbachia* are able to regrow after antibiotic removal. Using fluorescence microscopy, we will elucidate whether enlarged cells are still present after removal of the antibiotic or whether the phenotype is reversible or may indicate a fosfomycin-induced persistence-like state.
These analyses will clarify if depletion of *Wolbachia* by fosfomycin is the result of active damage or the failure of cells to divide. Understanding the *Wolbachia* spp. cellular response to fosfomycin will provide insight into its mechanism of action as well as clues about the mechanism of action of other cell wall biosynthesis-inhibiting antibiotics such as D-cycloserine in this reduced-genome endosymbiont.

E5: Identification of *Brugia malayi* miRNAs involved in *Wolbachia*-Host symbiosis

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Wolbachia is an obligatory symbiont of filarial nematodes, parasites that are causative agents of human lymphatic filariasis and onchocerciasis. These bacteria are essential for the successful development, reproduction, and survival of filarial worms. The mechanism for such dependency is being studied to understand bacteria-eukaryotic cell endosymbiosis and to explore the unique potential of using *Wolbachia* as a chemotherapeutic target against human filarial infections. *Wolbachia* can affect different aspects of the worm's biology and impact survival within the host. We propose that *Wolbachia* infection in filarial worms modifies the pathways used by the worms to maintain the mutualistic relationship and that this may be in part controlled by parasite microRNAs (miRNAs). To test this, we compared the expression of miRNAs (bma-miRNAs) in *Brugia malayi* treated with doxycycline (anti-*Wolbachia* agent) as compared to untreated control worms. We identified 13 upregulated and 20 downregulated bma-miRNAs in worms treated with doxycycline, as compared to controls**,** four of which are unique to *Brugia* with no homologues in other filaria. Using a miRNA target prediction algorithm (miRanda), we identified potential target genes and selected those *Brugia* genes that belong to pathways essential for *Wolbachia*-worm endosymbiosis. We validated predicted pairs of miRNAs and their targets by using a dual-luciferase assay. We then applied miRNA-mediated interference to silence specific miRNAs highly expressed in adult female worms to study miRNA-mediated processes in *Wolbachia* and *B. malayi*. The treatment with miRNA inhibitors significantly reduced *Wolbachia* numbers and induced apoptosis in treated worms, as compared to control worms treated with a scrambled miRNA inhibitor. The experiments showed that bma-miRNAs, which are highly expressed in adult females, downregulate lysosomal activity in worm cells. This allows *Wolbachia* to escape from autophagy and survive in host cells. Decreasing expression of selected miRNAs in doxycycline-treated adult females activates autophagy and lysosomal activity, which eliminate *Wolbachia*. Moreover, suppression of the bma-miRNAs in worms by anti-*Wolbachia* treatment significantly increases the expression of CEDs (apoptotic proteins) and apoptosis in developing embryos. These results helped better understand the *Wolbachia*-filaria mutualistic relationship and allowed us to dissect fundamental aspects of intracellular bacteria-eukaryotic host symbiosis.

Ecology and Evolution (F)

F1: Phage WO: Comparative genomics and taxonomic classification

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Phage WO genes are associated with nearly all arthropod-infecting *Wolbachia,* facilitating reproductive parasitism, horizontal gene transfer, and chromosomal rearrangement within the bacterial host. Its modular genome encodes core structural genes as well as a unique Eukaryotic Association Module (EAM) that is enriched with ankyrin repeats and eukaryotic-like domains. First observed in 1978, the virus remained unclassified in the absence of purified phage lysates from cell-free *Wolbachia* cultures. Here we propose a new taxonomic family, Symbioviridae, based on genomes from phage WO and closely related viruses of other symbionts. We describe general characteristics of the Symbioviridae and survey prophage regions from over 150 *Wolbachia* assemblies to reveal patterns of distribution, putative functions of gene clusters, and specific WO-associated rearrangements of the bacterial chromosome. Finally, we assess its relationship with Octomom – a genetic region linked to

Wolbachia over-proliferation – to present a phage origin model for this pathogenic island. Taken together, genomic evidence supports the perspective that phage WO may be the ultimate puppeteer behind many aspects of *Wolbachia*'s success.

F2: Genetic bases of the variation of *Wolbachia* titres and antiviral protection in *Drosophila*

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Wolbachia are maternally transmitted intracellular bacteria, commonly found in insects and filarial nematodes. These bacteria can establish from mutualistic to parasitic relationships with their hosts and are responsible for the induction of several phenotypes, such as reproductive manipulation and antiviral protection. Deployment of *Wolbachia*-carrying mosquitos in the wild is an effective strategy to block the transmission of arboviruses, such as dengue. *Wolbachia* titre regulation is important in the maintenance of the symbiotic relationship. High proliferation of *Wolbachia* has a cost for the host, but also positively correlates with the strength of induced phenotypes, including antiviral protection. Understanding how *Wolbachia* titers are regulated is, therefore, important to understand its biology and modulation of antiviral activity. To assess the contribution of natural genetic variation of *Wolbachia* in these phenotypes, we used a collection of 180 *Wolbachia* variants all introduced into the same *D. melanogaster* isogenic background and determined *Wolbachia* titres at two time points and measured the antiviral strength of these variants upon infection with *Drosophila* C virus. Afterwards, we performed a Genome Wide Association Study to associate *Wolbachia* levels and antiviral strength with both published and newly obtained genomic data, with the goal of identifying polymorphisms associated with these phenotypes. We observed extensive variation within and between clades, for both *Wolbachia* titres and antiviral protection, and confirmed a positively correlation between these traits. The analysis allowed us to associate specific genetic differences with the phenotypic differences between some clades. Furthermore, we found two *Wolbachia* variants that are highly proliferative and protective. Due to the low genetic variation between these variants and closely related ones, we identified 2 and 6 genes, respectively, as potentially involved in the regulation of *Wolbachia* titers in *Drosophila*. These results will contribute for a better understanding of the interactions between *Wolbachia* and host which can be applied in the improvement of arbovirus fighting strategies.

F3: Factors driving *Wolbachia* prevalence in native and invasive populations of *Drosophila suzukii*

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Understanding the factors that drive the prevalence of endosymbionts in natural populations is a central goal of evolutionary ecology. The success of maternally transmitted symbionts can be explained by three parameters: reproductive manipulations of the host, vertical transmission rates and effects on host fitness. Those parameters can be modulated by both environmental and genetic factors. The *Wolbachia* strain *w*Suz, which infects the invasive pest species *Drosophila suzukii*, is a canonical example of maternally transmitted symbiont with low to intermediate prevalence in host populations and weak reproductive manipulation. For *Wolbachia* strains with such properties, the mechanisms inherent to the infection dynamics remain poorly understood. We investigated infection frequencies and *w*Suz intra-strain polymorphism in 24 natural populations of *D. suzukii* from both native (China and Japan) and invasive areas (Europe and America). Infection frequencies in populations from China were on average significantly higher than those in populations from invasive areas. Based on the study of an insertion sequence site and a genomic rearrangement polymorphism in *w*Suz genome, we could identify three *w*Suz variants (i.e. European, American and Asian) corresponding to the initial sample location. More in-depth investigations indicated that the European variant was also present in some Asian populations and that one population from Europe included the European and American variants. Additional analyses based on whole-genome sequencing of 70 *D. suzukii* population samples showed lower *Wolbachia* genomic diversity in invasive populations (America and Europe) than in native (Japan, Korea and China) populations, consistent with a bottleneck in invasive populations. Finally, we analyzed two types of factors that could affect *Wolbachia* infection frequencies in natural populations: climatic variation and *Wolbachia*-induced cytoplasmic incompatibility. We found that variations in the strength of cytoplasmic incompatibilities or in monthly temperatures were not sufficient to fully explain observed prevalence pattern. Overall, our results show that, despite reduced intra-strain genomic diversity, both population prevalence and phenotypic effects on host reproduction can vary greatly among *Wolbachia* variants, suggesting complex interactions with host genetic background and environmental factors.

F4: *Wolbachia* in the neotropical fruit flies: A genome-wide study to reveal factors associated with long-term symbiont-host co-evolution and horizontal transmission events

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Wolbachia, an α-proteobacterium, is the most common intracellular symbiont of insects. The main mode of inheritance for *Wolbachia* is vertical, but phylogenies of *Wolbachia* and their hosts also show that *Wolbachia* is capable of jumping between hosts. Almost all neotropical *Drosophila* species of the saltans and willistoni groups are infected with very similar *Wolbachia* strains, hinting at a long-term symbiosis that was established before the divergence of these species. To test this, we assembled and annotated *Wolbachia* strains from neotropical *Drosophila* species. By comparing phylogenies, we see that the *w*Pau *Wolbachia* strains have a

position on the tree that contradicts the host tree, but is in agreement with the host mtDNA tree. This could suggest a horizontal transmission event in *D. paulistorum* from some other insect, resulting in replacement of an older *Wolbachia* strain and a *Wolbachia*-driven replacement of mitochondria of the host. Overall, the comparison of their phylogenies could suggest concordance between *Wolbachia* and fly phylogenies. We find evidence of horizontal transmission from the Saltans and Willistoni groups to other insects and conclude that long-term co-speciation of *Wolbachia* with the Neotropical flies is a possibility.

F5: Comparative genomics of three feminizing *Wolbachia* strains from a single isopod host.

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Three feminising *Wolbachia* strains, called *w*VulC, *w*VulM and *w*VulP, have been identified in the pillbug *Armadillidium vulgare* (Bouchon *et al.* 1998, Cordaux *et al.* 2004, Verne *et al*. 2007). These three strains, belonging to the B supergroup of *Wolbachia*, are different according to MLST genotyping. In natural populations, no individual with multiple infections was detected. These facultative symbionts are vertically transmitted and the corresponding host lineages have been stably maintained in the laboratory for many years. The *w*VulC strain has a higher transmission rate than the *w*VulM strain and is the most geographically widespread in host populations, while the *w*VulP strain identified in a few populations appears to be more virulent than the other two.
We used an enrichment procedure (Geniez *et al.* 2012) to extract *Wolbachia* DNA from ovaries of females of the three host laboratory lineages. The three genomes (*w*VulC, *w*VulM and *w*VulP) were then sequenced using both Illumina and Oxford Nanopore technologies. After assembly, we were able to obtain the complete genomes of the three *Wolbachia* strains as well as the mitochondrial genomes of the hosts. In the three *Wolbachia* genomes, we paid particular attention to the prophages and their eukaryotic association modules (EAMs) and to the biotin metabolic pathway. The comparison of *Wolbachia* and mitogenomes allowed us to test the congruence between hosts and symbionts.

F6: How many ways can you rescue a male?

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Diverse inherited bacteria have evolved to kill the male progeny of their hosts, and these male-killing symbionts are found in host species with distinct sex determination mechanisms. Male-killing produces intense selection on their host to restore male function, and the spread of suppressor mutations has been observed in two field systems. In the butterfly *Hypolimnas bolina*, the spread of host suppression of MK *Wolbachia* was observed in Samoa between 2001 and 2005, while in the lacewing *Mallada desjardinsi,* the spread of suppression of MK *Spiroplasma* was observed in Japan between 2011 and 2016. In this study, we used a population genomic approach to examine the host loci under selection in both cases. We hypothesized that the diverse nature of male-killers and their hosts would be reflected in the mechanism of suppression: with the genetics underpinning suppression being context dependent. In *H. bolina*, we demonstrate that the target of suppression maps to the core sex determining gene,

doublesex. Contrastingly, our data indicate that *doublesex* is unlikely to be involved in host suppression in *M. desjardinsi*. These data indicate that the host response to male-killers is driven through diverse genetic mechanisms.

F7: Phylogenomic analysis of Wolbachia genomes from the Darwin Tree of Life biodiversity genomics

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The Darwin Tree of Life project aims to sequence all eukaryotic life occurring on the British Isles. However, in addition to the target genome, sequenced samples often contain genetic material from organelles, endosymbionts and contaminants. One such a frequently co-occurring species is *Wolbachia,* an alphaproteobacterial endosymbiont of terrestrial arthropods and some nematodes that can infect more than 40% of all arthropod species. Here, I screened 368 insect genomes generated within the Darwin Tree of Life project and was able to assemble 110 complete *Wolbachia* genomes derived from 93 hosts. Despite being maternally inherited and its constant association with the germline of their hosts, we couldn't detect a bias towards female *Wolbachia*-positive samples. *Wolbachia* are divided into supergroups, with groups A and B common among insects. Most detected genomes in Lepidoptera belonged to supergroup B, while other insect orders mainly harboured A-type *Wolbachia* genomes. Nonetheless, we can show that many host switching events have occurred within the evolutionary history of *Wolbachia*, sometimes spanning multiple insect orders. Furthermore, *Wolbachia* within arthropods can be infected by a temperate double-stranded DNA bacteriophage, WO. We show that genome size in *Wolbachia* can be inflated due to phage integration and its remaining relics.

F8: Within and among host interactions among symbionts, as mediated by temperature

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Maternally-transmitted microbes are pervasive in arthropods, yet are often studied only as singular infections. The Linyphiid spider, *Mermessus fradeorum*, represents an opportunity to investigate how interactions among a community of coexisting endosymbionts within a shared host shape the host's phenotype and population biology, and vice versa. This spider can be co-infected by up to five maternally-inherited strains of bacteria: *Rickettsiella*, *Tisiphia*, and three strains of *Wolbachia*. In field populations in Kentucky, USA, all spiders are infected with *Rickettsiella*, and a substantial minority of the population (~20%) is co-infected with all 5 symbionts. The *Rickettsiella*-only spiders exhibit cytoplasmic incompatibility (CI), whereas the 5-fold infected spiders are feminized. In these feminized spider lineages, the presence of one strain of *Wolbachia* (W1) appears crucial for the phenotype, but the third strain of *Wolbachia* (W3) also contributes. In the absence of W3, average spider sex ratio is reduced to 85% female, rather than the nearly 100% female exhibited by spiders with a 5-fold infection. The second strain of *Wolbachia* (W2) has no apparent effect on sex ratio. The expression of both CI and feminization phenotypes is modulated by temperature: when reared at moderately high temperatures, infected male spiders exhibit reduced ability to induce CI, and feminized spiders produce offspring that have reverted to an even sex ratio. Male offspring produced by these females are fertile, generally retain the 5-fold infection, and also retain the ability to induce CI. Intriguingly, for feminized spiders that exhibit less-than-perfect feminization, mating with a

Rickettsiella-infected male appears to improve the feminization rate, through an unknown mechanism. We therefore hypothesize that coexistence of two reproductive manipulations at relatively stable rates in Kentucky field populations may result from mutual reinforcement by the surprisingly collaborative phenotypes, despite periodic destabilization of both CI and feminization phenotypes by high temperatures.

F9: Effects of *Wolbachia* on host oviposition preferences

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Wolbachia is a bacterium commonly found in insects. They do not naturally occur in *Aedes aegypti*. However, *Ae. aegypti* has been artificially infected with *Wolbachia* since this bacterium can confer protection against RNA viruses. Moreover, *Wolbachia* is also known for their manipulative reproductive phenotypes in mosquitoes, which enable its spread through populations. Due to these abilities *Wolbachia* is used for vector and arbovirus control. More recently studies have shown that *Wolbachia* can also change the behavior of its hosts, such as thermal preference in *Drosophila melanogaster*. Here, we have studied thermal preference of *Wolbachia*-infected female *Ae. aegypti* and compared them to the uninfected controls using a thermal gradient setup. Knowing that developmental temperature is crucial for the *Wolbachia*-conferred antiviral protection, we have compared temperatures where infected and uninfected mosquitoes choose to lay eggs. We observed that *Wolbachia* alters the mosquito oviposition preference. We have also observed interaction between the presence of *Wolbachia*, mosquito developmental time, and the temperature where they choose to oviposit. Our results will help us to understand the relationship between *Wolbachia*, *Ae aegypti,* and temperature. Such knowledge is useful to evaluate the influence of environment on releasing *Wolbachia*-infected mosquitoes and may be important for vector control programs.

F10: Reconstruction of a near-complete *Wolbachia* genome from long-read sequencing of *Culex quinquefasciatus* ovaries

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Wolbachia bacteria are of particular interest due to their selfish manipulation of their host's reproduction and their interactions with viral pathogens, leading to important applications to vector control strategies. In this context, next-generation sequencing technologies are key to characterize the naturally occurring diversity of obligate intracellular *Wolbachia* that remain recalcitrant to cultivation. Nevertheless, the presence of repeated prophage regions in *Wolbachia* genome leads to fragmented assemblies when relying on short reads generated by Illumina sequencing technologies. Here we present a near-complete genome of *Wolbachia* from *Culex quinquefasciatus* from Martinique (French Caribbean Island), reconstructed from

long-read sequencing (ONT) data of mosquito ovaries. Lambda phage DNA was added to the sequencing library to estimate a final read accuracy of 99.9%. The final assembly contains 4 linear contigs and 1 circular contig corresponding to the plasmid previously described by Reveillaud and Bordenstein *et al*. (1). The full sequence is 1,467,850 bp long (longest contig 980,141 bp) and estimated to be 94.8% complete and 1.57% redundant. It harbors 1525 protein-coding sequences, 1 rRNA operon and an overall GC content of 34.27%. Its average nucleotide identity with reference genomes *w*PipPel (2), *w*PipJHB (3) and *w*PipMol (4) is 98.2%, 98.5% and 85.1%, respectively. A pangenomic analysis with these three reference genomes revealed that 88.2% of gene clusters were shared by all genomes, while our genome presented 21 new genes, most of which had no assigned function. The acquisition of this fairly unfragmented genome is a stepping stone for further investigation of *Wolbachia* genomic diversity and rearrangement in *Culex* mosquitoes collected from distinct geographic locations and settings around the globe.

F11: Two birds with one stone – Validating COI barcoding as a low cost, high throughput, and high resolution method of *Wolbachia* screening across insects

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DNA barcoding is a powerful tool for high-throughput identification of species, increasingly applied to large insect collections from around the world. However, cytochrome c oxidase I (COI) gene, the standard mitochondrial marker targeted during insect barcoding, is also present in the *Wolbachia* genome. Due to high similarity of sequences within priming sites, *Wolbachia* sequence co-amplifies with the insect host sequence. These *Wolbachia* reads, often interfering with the reconstruction of insect barcodes, are generally regarded as a nuisance and filtered out or ignored.
The goal of this study was to evaluate the reliability of *Wolbachia* detection and assess the resolution of *Wolbachia* genotyping in insect barcoding datasets. To achieve it, we compared COI and 16Sv4 rRNA amplicon data for ca. 9000 diverse, wild-caught insects. We demonstrate ~95% agreement between the *Wolbachia* presence assessed using 16Sv4 amplicon data, and the assessment based on COI amplicons. Furthermore, *Wolbachia* COI data provided much higher phylogenetic resolution than the 16Sv4 data routinely used for microbiome surveys. Genotyping based on COI data provided also biologically realistic patterns, such as individuals from the host species being often infected with the same *Wolbachia* genotype.
Our study shows that the COI amplicon data can be used for reliable detection and characterisation of *Wolbachia* infections across diverse insects. The careful extraction and annotation of symbiont data, a by-product in massive insect barcoding datasets, can be an extremely cost-effective means of surveying *Wolbachia* across unprecedented numbers of insects. Combining this information with host-barcode data provides a unique opportunity for tracking *Wolbachia* strain distribution and transmission within and across species, multi-species communities, and entire ecosystems, offering an exciting novel window into its ecological and evolutionary roles.

Pests and disease control (C)

C1: Autophagy is essential for anti-*Wolbachia* drug efficacy

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Onchocerciasis and lymphatic filariasis (LF) cause significant global public health burdens with more than 900 million individuals at risk and over 60 million people currently living with symptomatic manifestations caused by filarial diseases. Due to the importance of *Wolbachia* for the survival of adult filarial nematodes, anti-*Wolbachia* therapy has been validated as a safe macrofilaricidal treatment for LF and onchocerciasis. The A-WOL consortium was established with the goals of defining registered anti-*Wolbachia* antibiotics, as well as developing new drugs effective in a regimen of 7 days or less. We have previously shown autophagy has a core role in the regulation of *Wolbachia* populations across a diverse range of associations. In this study, we investigated the role of autophagy in the mode-of-action and efficacy of the portfolio of anti-*Wolbachia* drugs. This work has demonstrated a consistent increase in autophagy flux with four broad-spectrum anti-*Wolbachia* antibiotics from different classes (doxycycline, rifampicin, moxifloxacin and sparfloxacin) exposed to three different insect cell lines (C6/36, SF9 and S2 cells) and in *B. malayi*. This autophagy activation was also observed for selected-candidates from the A-WOL consortium (flubentylosin [TylAMac], AWZ1066S and fusidic acid). Drugs ineffective against *Wolbachia* failed to induce autophagic flux. Antibiotic-induced autophagy was observed in the absence of *Wolbachia*, indicating its effect occurs independent of the bacteria. The activation of autophagy by anti-*Wolbachia* drugs is not observed in mammalian cells, is restricted to insect cells and nematodes and is independent of ROS activation. Through concentration-dependency testing of anti-*Wolbachia* antibiotics, we show that only concentrations that induced autophagy resulted in effective *Wolbachia* depletion (of >90%), the empirical threshold of delivering the desired macrofilaricidal activity. The contribution of autophagy in the efficacy of anti-*Wolbachia* drugs and their ability to reduce bacterial viability was demonstrated in *B. malayi* mf and adult worms when autophagy was inhibited during anti-*Wolbachia* drug exposure. Moreover, a role for autophagy was observed in the continued decline in *Wolbachia* post-drug exposure. *Wolbachia* purified from C6/36 cells and exposure to anti-*Wolbachia* drugs showed no impact on their viability re-enforcing the essential requirement for autophagy flux activation in the efficacy of anti-*Wolbachia* drugs.

C2: Integrated Vector Management is the key to fasten *Wolbachia* introgression and enhance its effectiveness on mitigating arbovirus transmission

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The effectiveness of *w*Mel releases on the incidence of dengue and chikungunya in the highly endemic city of Rio de Janeiro has been recently published. After releasing 67 million *w*Mel-infected *Aedes aegypti* between 2017-2019, an average 32% introgression into the wild population resulted in a 38% and 10% reduction of dengue and Chikungunya, respectively. A Randomized Control Trial in Yogyakarta, Indonesia, showed a better outcome, with intervention clusters reaching >70% *w*Mel invasion after 4-7 months of release, and a 77% reduction in dengue cases[2]. The results of Rio de Janeiro are welcomed, but the obstacles that hinder the invasion of *Wolbachia* and limit its epidemiological effect need further scrutiny. Herein, we provide an independent dataset involving field entomology and vector competence assays to explore these issues further. A total of 75 BG-Sentinel traps have been distributed across 12 neighborhoods in the southern limit of the *w*Mel release area. We report data from 28,613 adult mosquitoes collected between August 2021 and September 2022, i.e., 13 months of fortnightly trapping. *Culex quinquefasciatus* and *Ae. aegypti* were the most abundant species, with 15,825 and 12,783 specimens collected, respectively. For *Ae. aegypti*, 5,349 (41.84%) mosquitoes were individually screened for *w*Mel presence. The frequency of wMel dropped dramatically in the study area, reaching an average of 12% invasion, with a 0% of wMel-infected individuals being trapped in some neighborhoods. Experimental infections with field-gathered *Ae. aegypti* from five sites and two DENV-1 doses (3 x 10^4 FFU/mL (low titer) and 6 x 10^8FFU/mL (high titer)) revealed that under high DENV-1 dose challenge, the presence of *w*Mel did not avoid viral infection in mosquitoes' bodies and saliva, but DENV-1-infected *w*Mel mosquitoes produced lower viral loads than in *w*Mel-free mosquitoes. *w*Mel mosquitoes submitted to low DENV-1 dose were less susceptible than *w*Mel-free mosquitoes, even though once infected *w*Mel and *w*Mel-free mosquitoes exhibited similar viral loads in the body and the saliva. Noteworthy, however, lower viral loads were observed in the saliva of Tubiacanga samples, where *w*Mel releases first started in Brazil. As previously observed[3], body infection and saliva infectivity were not strongly associated with *Wolbachia* density in mosquito bodies. The high density of native *Ae. aegypti* populations are negatively affecting *w*Mel frequency in Rio, by hindering its introgression into release areas and beyond. Furthermore, the potential DENV-1 leakage in the saliva of *Wolbachia*-infected individuals could reduce the epidemiological effectiveness of this strategy.

C3: It doesn't always block: Wolbachia-induced pathogen suppression is a surprisingly complex and variable phenotype

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Multiple Wolbachia strains can block pathogen infection, replication, and/or transmission in mosquitoes under both laboratory and field conditions. However, Wolbachia effects on pathogens can be highly variable across systems and the factors governing this variability are not well understood. Multiple studies have demonstrated that Wolbachia pathogen blocking phenotypes are dependent not just on the Wolbachia strain used for control, but also on the pathogen, the arthropod species, the genetics of the host, the host microbiome, and the environment, and these factors may interact in unpredictable ways to modulate pathogen suppression phenotypes. In this talk I will attempt to synthesize what is known about Wolbachia pathogen blocking, the parameters that influence this phenotype, and underlying mechanisms, with important considerations for measuring pathogen blocking in laboratory experiments as well as potential issues for the application of Wolbachia for disease control in the field

C4: The interactive effects of *Wolbachia* and *Nop60B* on Sindbis virus replication

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Arboviruses pose a threat to more than half of the world's population. The intracellular bacterium, *Wolbachia,* is found in 40-60% of insect species and can share a mosquito host with a number of human pathogens. Indeed, *Wolbachia* colonization protects invertebrate hosts against infection from single-stranded, positive-sense RNA viruses, a phenomenon known as pathogen blocking. Despite a lack of understanding of the mechanism of pathogen blocking, *Wolbachia*-infected mosquitoes are being released in the field to reduce transmission of mosquito-borne viruses. We performed a screen to identify host factors involved in *Wolbachia*-mediated pathogen blocking of Sindbis virus (SINV) replication in *Drosophila melanogaster*. We identified a host pseudouridine synthase gene, *Nop60B* that displays significant differential isoform usage in flies colonized with *Wolbachia* compared to *Wolbachia*-free counterparts. We previously showed that RNA modifications play a role in *Wolbachia*'s antiviral activity. Thus, we hypothesized that *Wolbachia*-induced changes to *Nop60B* may affect SINV replication. Interestingly, we found that *Wolbachia* and *Nop60B* have interactive effects on SINV replication. SINV RNA levels decrease when *Nop60B* is knocked down in *Wolbachia*-colonized flies. In contrast, an insertion at the 3' end of *Nop60B* leads to an increase in SINV replication in *Wolbachia*-free flies. To narrow down on a potential mechanism, we sought to investigate *Nop60B* in a *D. melanogaster* cell line cleared of *Wolbachia*. We found that *Nop60B* knockdown increased SINV RNA levels and protein production in JW18-tet cells. In conclusion, we hypothesize that specific isoforms of *Nop60B* may have different effects on Sindbis replication. Future experiments will focus on investigating individual *Nop60B* isoform effects on Sindbis virus replication in whole flies and cell culture. The results of these experiments will contribute to our understanding of *Wolbachia*-virus-host interactions and antiviral mechanisms in general.

C5: Towards the Use of Novel High Density *Anopheles*-Specific *Wolbachia* Strains for *Anopheles* Vector Control.

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The use of *Wolbachia* as a novel vector control strategy has been highly successful, demonstrating a significant impact on disease prevalence in field trials targeting *Aedes* mosquitoes. Long thought to be absent from natural populations of *Anopheles* mosquitoes, the highly effective malaria vectors, *Wolbachia* based interventions have proven challenging. Our recent discovery of natural high density *Wolbachia* strains in populations of *Anopheles moucheti* and *Anopheles demeilloni* has reinvigorated efforts to create transinfections in medically

relevant *Anopheles* mosquitoes. Here we present our work demonstrating high density maternally transmitted strains of *Wolbachia* in *An. moucheti* and *An. demeilloni* from sub-Saharan Africa, providing concrete evidence for resident *Wolbachia* strains in this genera. In addition, we report on our recent endeavours transferring these novel strains of *Wolbachia* to cell lines, thereby providing a tractable source of *Wolbachia* for further experiments. We discuss our findings in the context of developing novel *Wolbachia*-based control approaches in *Anopheles* to reduce the burden of malaria.

C6: *Wolbachia*, larval nutrition, and metabolomics in *Aedes aegypti* mosquitoes.

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Stable, maternally inherited *Wolbachia* infections have been generated in the dengue mosquito, *Aedes aegypti*, through transinfection. *Wolbachia* induces many physiological changes to the host, including those that allow it to rapidly spread into wild mosquito populations and confer resistance to arboviral infection. These mosquitoes are currently being utilized in large-scale interventions that seek to reduce the incidence of dengue transmission. The metabolic impact of *Wolbachia* on the host has not been well characterized, and may underlie many key changes to host physiology, and the ability to resist arboviral infection. *Wolbachia* lack vital metabolic genes and biosynthetic pathways, and likely scavenge key resources from their hosts. To investigate the metabolic impact of *Wolbachia* in *Ae. aegypti*, we employed 900MHz ^{1}H NMR spectroscopy- and UPLC-based metabolic profiling of two *Wolbachia*-infected mosquito lines: the *w*Mel line, currently being used in field releases, and the more virulent, life-shortening *w*MelPop line. We observed distinct metabolic effects associated with *Wolbachia* infection, *Wolbachia* strain, and larval nutrition status, with the most profound impacts associated with *w*MelPop infection in the high nutrition treatment. We observed increased levels of lipids and amino acids associated with *Wolbachia* infection, as well as an impact of nutrition on pyruvate, and on sugars such as trehalose, outlining a metabolic tug-of-war between host and symbiont. Critically, we also identify metabolic interactions linking *Wolbachia* infection, glutathione, tyrosine, and oxidative metabolism, which could explain key aspects of *Wolbachia* transinfection in mosquitoes.

C7: The Role of Epitranscriptomics in *Wolbachia*-mediated Pathogen Blocking Through the Lens of Direct RNA Sequencing

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Pathogen blocking occurs when the presence of a bacterial *Wolbachia* endosymbiont reduces viral replication and transmission in its insect host by altering insect-virus interactions. Messenger RNA modifications are proposed as a mechanism of *Wolbachia*-mediated pathogen blocking between *Drosophila melanogaster* and Sindbis virus. In *Drosophila melanogaster, Wolbachia* modulates the transcription of Dnmt2, which methylates cytosine in RNA. RNA modification detection methods are continuously improving with advances in sequencing technologies, but there are numerous barriers including the relatively low accuracy and precision of these methods. We use a combining strategy to detect RNA modifications in Oxford Nanopore Technologies (ONT) direct RNA sequencing data, relying on both basecalling data and statistical differences in signal. We validated this combining strategy with published yeast direct RNA sequencing data supplemented with our own data from a 5-methylcytosine methyltransferase knockout. Subsequently we compared completely unmodified *in vitro* transcribed Sindbis viral RNA to *in vivo* viral RNA isolated from the *Drosophila melanogaster* host. We identified a handful of positions with putative modifications to be interrogated further, including positions with putative 5-methylcytosine, *N*6-methyladenosine, and pseudouridine. We expect that the *Wolbachia*-mediated alterations in transcription of Dnmt2 not only modulates the viral transcript modification, but also the insect transcript modification. Detection of differential modification of *Drosophila* mRNA is limited by variation in mRNA transcript abundance that only allows us to interrogate 645 genes. Yet differential RNA modification can still be observed between *Drosophila* mRNA from *Wolbachia*-colonized and uncolonized flies. Taken together, this provides us with candidates to further characterize the effects *Wolbachia* endosymbionts exert on their host.

C8: High genomic stability of *w*Mel *Wolbachia* after introgression into three geographically distinct *Ae. Aegypti* populations

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The introgression of *Wolbachia* into *Aedes aegypti* populations is being used for the biocontrol of arboviruses such as dengue, Zika, and chikungunya. *w*Mel was transinfected from *Drosophila melanogaster* into *Ae. aegypti* more than a decade ago and is currently the most common *Wolbachia* strain used for biocontrol. A *w*Mel infection in *Ae. aegypti* both reduces the

transmission of arboviruses such as dengue by the mosquito and causes a reproductive manipulation, cytoplasmic incompatibility, that aids *w*Mel introgression into wildtype mosquito populations. The long-term success of this method relies, in part, on the evolution of *w*Mel not impacting these critical features that make it successful as a biocontrol tool. *w*Mel introgression and prevalence have been both spatially and temporally variable across some release sites, and we hypothesized this could be due to the evolution of the *w*Mel genome in response to its novel host and/or environments. To test this, we obtained mosquitoes from Colombian, Indonesian, and Vietnamese *Ae. aegypti* populations and sequenced the *w*Mel genome shortly after, then again, several years after *w*Mel introgression into these three sites. We show very limited genomic changes to the *w*Mel genomes from each population and time point suggesting that evolution of *w*Mel does not explain the heterogeneity of *w*Mel in these populations. More broadly, our results suggest *w*Mel is not undergoing rapid sequence evolution despite its introduction into novel hosts and environments. These results are consistent with previous results from Australia and supports the application of *w*Mel for biocontrol of *Ae. aegypti* transmitted arboviruses.

C9: *Wolbachia*-mediated changes in viral dynamics

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Arboviruses are a major cause of human mortality and morbidity. *Wolbachia* interferes with viral replication and accumulation in arthropod hosts, thus reducing transmission and ultimately burden of disease on humans. The mechanisms of *Wolbachia*-mediated viral interference are not fully understood. To dissect the complex tripartite interactions between host-virus-*Wolbachia*, we employed a distinctive approach, modelling the impact of *Wolbachia* on the dynamics of viral replication across the course of an infection within a single host. Here we used *Drosophila melanogaster* with or without *Wolbachia* strain *w*MelPop to measure the impact of *Wolbachia* on viral dynamics. We measured accumulation of viral genomic RNA and infectious virus, following systemic infection with three viral doses of 50, 500, or 5000 infectious units of Drosophila C virus. Accumulation was assayed at 10-to-14-hour time intervals until flies died from infection; approximately 17- and 6-days post infection, for flies with and without *Wolbachia* respectively. This empirical data represents the parameter space used to investigate and understand the impact of *Wolbachia* and viral dose on viral dynamics. Following infection, viral RNA and infectious virus increases in both *Wolbachia* and *Wolbachia*-free flies, however the rate of accumulation is lower in *Wolbachia* infected flies. Both infectious virus and virus RNA increase to a maximum stationary phase after which no further increase is observed, suggesting a limit on maximum viral load. Notably, the maximum viral load is lower for *Wolbachia*-infected flies. Despite being different between flies with and without *Wolbachia,* the maximum viral load is consistent across flies with different initial viral doses. Interestingly there is a larger amount of variation in measured viral RNA and virus in flies with *Wolbachia* which is more pronounced at lower doses. Taken together, the results demonstrate that viral dynamics are impacted by *Wolbachia* and may be dependent on viral dose. Modelling of this data will guide research into the mechanisms likely to be involved in *Wolbachia*-mediated viral interference.

C10: The influence of horizontally acquired *Wolbachia* on invasive cherry fruit fly populations: A multi-perspective study

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Despite diverse and often intense reproductive parasitism, *Wolbachia* has successfully spread throughout arthropod species. Currently, mounting evidence indicates that *Wolbachia* does not only cause reproductive parasitism but can also benefit its host, particularly in infected females where *Wolbachia* is inherited. Such positive fitness effects are now believed to help *Wolbachia* spread through new host populations after horizontal transfers and include nutrient enhancement, defense against certain viruses, and influences on various life history traits. However, ongoing spreads into new species after horizontal transfer in natural systems are typically transient and go undetected. Here we study the sweep of a newly acquired *Wolbachia* strain in the invasive American cherry fruit fly pest *Rhagoletis cingulata* using a combination of spatial and temporal population screening, genomics, and lab crosses. The genomics confirmed that the new strain originated from *Rhagoletis cerasi*, a cherry fruit fly congener from Europe, and that all the necessary components for cytoplasmic incompatibility are present in this strain. We found from our extensive screening of European populations that infection frequency variation of the new strain forms a north-south cline and that fixation has yet to occur in distant populations from the nearly fixed populations. Surprisingly, lab crosses showed conditional positive effects on the host egg hatch rate by the new strain but no cytoplasmic incompatibility, despite carrying the loci that cause it. Our results demonstrate a novel example of ongoing horizontally acquired *Wolbachia* strain spread through positive effects on its host. We anticipate that similar results could be found in other invasive insect species and might have direct applications in the fight against crop pests in the future.

C11: An RNA virus in the parasitoid wasp *Anagyrus vladimiri* inhibits encapsulation by the mealybug host

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While DNA viruses are known to engage tight symbiotic relationships with parasitoid wasps, the roll of RNA viruses in the biology of those insects remains largely unknown. Recently we described the virome of the parasitoid *Anagyrus vladimiri* (Hymenoptera: Encyrtidae), an important natural enemy of mealybug pests, by sequencing extracted viral nucleic acids. Three new viruses were found, one of which is a double-stranded RNA virus from the order *Reoviridae*, named AnvRV. The genome of this virus is composed of ten genomic segments, varying in

length from 1.5-4.2 Kbp. One open reading frame was detected on each segment, but only two of the predicted proteins have a known function. Phylogenetic analysis placed AnvRV together with other insect-associated viruses in the genus *Idnoreovirus*. While AnvRV was present in all 40 mass-reared specimens tested, its' prevalence in field-collected *A. vladimiri* was only ~12%. AnvRV is transmitted maternally but not paternally, and electron microscopy validated the presence of this virus in the ovaries of female wasps. Enabling AnvRV-infected and non-infected female wasps to share mealybugs for oviposition, revealed that the AnvRV can be horizontally transmitted between wasp larvae developing together inside the same mealybug host. This transmission mode enabled the establishment of a new wasp line by introducing the AnvRV into the virus-free line. Comparing fitness parameters between the newly infected wasp line with the virus-free line, revealed no substantial differences in longevity of females, developmental time, sex ratio and total fecundity. Instead, encapsulation rate of eggs was significantly lower when laid by virus-carrying wasps, and more of them hatched. In the talk we will discuss the overall phenotypic effect of the AnvRV on its' parasitoid host. Our findings offer application solutions to improve the biological control of mealybugs.

Reproductive manipulation (A)

A1: The Cell Biological Mechanism of Cytoplasmic Incompatibility

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Wolbachia's widespread distribution in arthropods and success in global vector control efforts are due to their ability to manipulate host reproduction. Cytoplasmic incompatibility (CI) is the most prevalent reproductive anomaly that manifests as a paternal-effect, embryonic lethality upon mating between *Wolbachia*-infected males and uninfected females. Infected females, however, rescue this lethality for increased transmission of the symbiont. Successful spread and maintenance of *Wolbachia* in vector populations heavily relies on CI, yet the details of how CI modifies host reproduction has only recently come into view. Here, we investigated the cellular and molecular mechanisms of CI in *w*Mel-transinfected *Aedes aegypti* mosquitoes released to the field by the World Mosquito Program (WMP). We performed cellular and chromatin integrity analyses and show that (i) the *w*Mel CI proteins CifA and CifB in mosquito testes invade nuclei of developing sperm and modify paternal genome integrity by inducing abnormal histone retention and protamine deficiency; (ii) the Cif proteins do not paternally transfer to the fertilized mosquito embryos, instead the sperm genome modified during pre-fertilization, spermatogenic events transfers to bestow CI; (iii) the rescue protein CifA also invades ovarian and embryonic nuclei of infected female mosquitoes. Overall, we demonstrate that the CI drive system in the released WMP mosquitoes follows the Host Modification model of CI. These results also constitute the first characterization of the evolutionary-conserved histone-to-protamine transition during spermiogenesis in a mosquito vector species.

A2: Of sperm and symbionts: Identification of candidate *Cardinium* CI factors by integrating symbiont cellular localization, CI modification timing, and protein expression

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The most common form of symbiont associated reproductive sabotage is cytoplasmic incompatibility (CI), in which the symbiont lethally modifies males to kill the offspring of matings with uninfected females. The symbiont then reverses this modification when sperm arrives in the cytoplasm of infected oocytes, rescuing the embryo from CI. CI is caused by an ever-growing list of diverse symbionts, including the symbiont *Cardinium hertigii*, which infects ~10% of arthropod species. *Cardinium* causes CI in a range of hosts including minute parasitic wasps of whiteflies in the genus *Encarsia*. Little has been known concerning the mechanism underlying *Cardinium*-induced CI, including when this symbiont induces CI in males, how it localizes in relevant tissues during CI induction, and the identity of the genetic factors responsible for CI. Using a combination of antibiotic assays and fluorescent microscopy, we characterized the timing of CI modification and *Cardinium* localization in male reproductive tissues in two *Encarsia* species. We found that while both *Cardinium* strains modify developing hosts during pupation, they exhibit strikingly different localization patterns and may ultimately use different methods to modify their hosts. We next compared differentially expressed *Cardinium* proteins across host life stage, sex, and tissue type using LC-MS/MS to identify candidate factors involved in *Cardinium* CI. We detected more than 130 symbiont proteins and identified 10 candidate CI factors expressed in a pattern indicative of CI. These candidates will undergo further testing using heterologous gene expression assays in yeast to confirm their role in CI.

A3: *Cardinium* and *Wolbachia* affect host fitness and sex allocation via egg size provisioning in different ways, yet are stably maintained as coinfection

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Cardinium and *Wolbachia* have evolved diverse strategies to manipulate host reproduction. A common reproductive manipulation is cytoplasmic incompatibility (CI) between differently infected individuals. In diplodiploid hosts, CI leads to mortality of embryos irrespective of their sex. In haplodiploid hosts, however, CI can result in either mortality or male development of fertilized eggs, yet, unfertilized eggs still develop. Therefore, females experiencing CI produce male offspring which poses a challenge for endosymbiont invasion. Recently, an additional endosymbiont effect in sexually reproducing haplodiploids has been recognized in the modification of sex allocation. This was expected due to the maternal inheritance of endosymbionts and natural selection for them to increase infected female production, yet the underlying mechanism remained unknown. We tested whether and how *Cardinium* and *Wolbachia* causing different CI types interact to increase female production in naturally coinfected haplodiploid Kelly's citrus thrips (*Pezothrips kellyanus*), an Australian-native pest species that is invasive in other parts of the world. This species has an egg size-dependent fertilization mechanism, and sex allocation depends on egg size provisioning and maternal condition. We found that *Cardinium* augmented female production by increasing maternal

fitness and egg size, thereby boosting fertilization rate and offspring fitness. *Wolbachia*, in contrast, reduced the beneficial effects of *Cardinium*. Our results demonstrate different invasion strategies and antagonistic effects of endosymbiotic bacteria on host fitness and evolution of sex allocation. Furthermore, we found that *Cardinium* occurs at a high prevalence in all field populations; *Wolbachia*, on the other hand, is restricted to populations in eastern Australia, where it occurs at high prevalence, but it is absent from other populations in Australia and elsewhere. However, the *Wolbachia-Cardinium* coinfection is linked to particular mitochondrial haplotypes and an overall lower haplotype diversity, suggesting that *Wolbachia* more recently infected this host species already infected by *Cardinium*, and currently invades populations despite *Wolbachia*'s deleterious fitness effects.

A4: Digging deeper into the genetic bases of CI patterns in *Culex pipiens*: does the toxin-antidote model still hold?

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Wolbachia induce cytoplasmic incompatibility (CI), a reproductive manipulation leading to reduced embryo viability in many arthropods. CI is formalized as a *modification* (*mod*) – *rescue* (*resc*) system, whereby *Wolbachia* introduces a *mod* factor in the sperm, leading to embryonic defaults unless the appropriate *resc* factor is present in the egg. In *Culex* mosquitoes, the *Wolbachia* *w*Pip cause extraordinarily diverse CI patterns, suggesting the involvement of multiple genetic factors. This prediction has first been confirmed by showing that *cidA* and *cidB*, the CI causing genes, are amplified and diversified in all examined *w*Pip genomes; the set of all different copies present within a genome being called a repertoire. Diversification was previously found to be restricted to two specific regions for both *cidA* and *cidB*, later shown to be part of the CidA-CidB interaction interface regions. We previously established that CidB variations in these interaction regions strongly correlate with variations in *mod* patterns.
To get better insights into the genetic bases of *w*Pip-induced CI patterns, we used a combination of full-genome long reads sequencing and Nanopore sequencing of *cidA* and *cidB* PCR products. Acquiring *cidA* and *cidB* variants from more than 80 *w*Pip strains worldwide, we showed clear correlations between *cidA* variations in interaction regions and *resc* patterns. On top of diversification in CidA-CidB interaction regions, we discovered two new layers of genetic polymorphism, shedding light to some to date unexplained CI patterns: 1) we observed that most *cidB* variants were truncated, missing their DUB domain, and 2) we discovered a new polymorphic region, outside of previously identified interaction regions. Can these various layers of genetic polymorphism, in and out the interaction zones, and their intertwined influence on CI patterns be reconciled with a toxin-antidote model of CI?

A5: Modelling the mechanism of cytoplasmic incompatibility: new mathematical tools for more data

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Despite major recent breakthrough on the genetics of *Wolbachia*-induced cytoplasmic incompatibly (CI), a complete and consensual understanding of the underlying mechanism has not been reached. While empirical studies are obviously on the front line in this matter, they can be relevantly complemented by theoretical treatments aimed at integrating diverse facts into a coherent framework. The *Culex pipiens* case, where population genomics have been combined with phenotypic, cellular and molecular data, constitutes a system of high interest in this regard. Here, complex patterns of incompatibility are known to be associated with a monophyletic but diversified Wolbachia lineage named *w*Pip, and recent comparative analyses of the cif genes have converged with theory to indicate that single Wolbachia genomes contain multiple active copies of these genes. Yet, inferring a minimal genetic architecture, under various plausible mechanistic models and from a growing body of phenotypic data, remains a computationally challenging task, one that belongs more specifically to the well-defined class of NP-complete problems. We tackle this problem using computational tools specifically designed to address such problems, namely SAT-solvers, that make it possible to analyze even very large data-sets and compare predictions from diverse mechanistic models, from a classic one-to-one toxin-antidote interaction, to more elaborate, and perhaps more realistic hypotheses regarding the respective contributions of the Cif proteins to CI induction and rescue.

A6: Identification of Parthenogenesis Inducing *Wolbachia* Proteins

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In some arthropods, *Wolbachia* is the causative agent of asexual reproduction: i.e., parthenogenesis induction (PI). Despite the discovery of this phenotype more than thirty years ago, the bacterial genes mediating PI have remained unknown. We used comparative genomics to uncover two candidate genes, hereinafter “parthenogenesis inducing factors” (Pifs). Pif proteins contain predicted eukaryotic domains, indicative of interaction with host biology. The genes are expressed by two PI-*Wolbachia, w*Tpre and *w*Lcla, and their expression varies across host development. As both PI-*Wolbachia* and their hosts lack tools for genetic manipulation, we used surrogate approaches including expression in *Drosophila melanogaster* to characterize the function of the Pif proteins. When transgenically expressed in *Drosophila*, the Pif proteins impact the development of sexually dimorphic characteristics and fly sex ratios. Full characterization of the proteins and host targets will provide further insight into the parthenogenetic mechanisms employed by some *Wolbachia* strains, as well as host-symbiont interactions more broadly.

A7: *Wolbachia*-induced male-killing and phage WO: genes, diversity of mechanisms, and evolution

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Male killing (MK) is a notable feature of the maternally transmitted bacterium *Wolbachia*, whereby the sons of infected females are killed during embryogenesis; however, the origin and diversity of *Wolbachia*-induced MK mechanisms remain unclear. Here, we identified a prophage region WO-76 specific to an MK *Wolbachia* *w*Hm-t by comparing the genomes of MK *w*Hm-t and non-MK *Wolbachia* strains (*w*Hm-a, *w*Hm-b and *w*Hm-c) isolated from the moth *Homona magnanima* (Tortricidae). The prophage encoded a homolog of the *oscar* gene, which induces MK in *Ostrinia* moths, and two tandemly arrayed homologs of the *wmk* gene, which induces various toxicities in *Drosophila* flies. Upon overexpressing the *wmk* and *oscar* in *Drosophila melanogaster*, some of the *wmk* homologs killed male individuals, whereas the *oscar* homolog (*Hm-oscar*) had no effect on male flies. Notably, we found the evidence that *Hm-oscar* suppressed the functions of a crucial male sex determining gene masculinizer (*masc*) of *H. magnanima*. These findings highlight that *Wolbachia* utilise different mechanisms to achieve MK in dipteran insects (Drosophila) and lepidopteran insects (*Ostrinia* and *Homona*). Further genome analyses of *Wolbachia* in lepidopteran insects revealed that the MK strain *w*CauA from *Ephestia cautella* (Tsuchiura, Japan), the MK strain *w*Bol1Y from *Hypolimnas bolina* (Yogyakarta, Indonesia), and the feminising strain *w*Fem from *Eurema mandarina* (Tanegashima, Japan) harboured Oscar homologs, whereas non-MK *Wolbachia* strains of *w*CauB from *E. cautella* (Tsuchiura, Japan), *w*CI from *E. mandarina* (Tanegashima, Japan), *w*KueA from *Ephestia kuehniella* (Tsuchiura, Japan), *w*Ni from *Trichoplusia ni* (Matsudo, Japan), and *w*Ano from *Hypolimnas anomala* (Ishigaki, Japan) did not. Intriguingly, the *w*Bol1Y, which is not closely related to the *w*Hm-t, harboured the *Hm-Oscar* and the prophage region homologous to the WO-76. These results suggest that Oscar is a major causative factor for MK in lepidopteran insects and that horizontal phage transfer may have contributed to the acquisition of MK in *Wolbachia*.

A8: Characterization of the evolutionary process of the symbiotic relationship establishment between *Ostrinia* moths and *Wolbachia* endosymbiont

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Infection with male-killing *Wolbachia* is found in Japanese populations of *Ostrinia* moths (Lepidoptera: Crambidae) including the economically important maize pest *O. furnacalis* and the dicot crop pest *O. scapulalis*. *Wolbachia* elimination by antibiotics in infected lineages results in the death of female progeny in these insects, which is not the case with other lepidopteran insects infected with male-killing *Wolbachia* (e.g., *Hypolimnas bolina* butterfly and *Homona magnanima* moth). The goal of this study is to characterize the evolutionary process of the establishment of the unique symbiotic relationship between *Ostrinia* moths and *Wolbachia*. To understand evolutionary dynamics of *Wolbachia* infection in the moth clade including *Ostrinia*

genus, 27 species belonging to Pyraustinae or Spilomelinae in Japan were screened for *Wolbachia* infection. The infection was detected in eight species other than *Ostrinia* spp., and genome analysis of newly identified *Wolbachia* is currently underway. Additionally, to characterize the evolutionary property of male killing, we investigated the distribution pattern of a recently identified male-killing factor, *Oscar*, across *Wolbachia* genomes available in the public databases. *Oscar* homologs were identified from a small subset of strains associated with lepidopterans. The evolutionary dynamics of *Oscar* and its homologs will be discussed.

A9: Self-stabilization mechanism encoded by a bacterial toxin facilitates reproductive manipulation

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Insect symbioses, in which microorganisms are living inside insect hosts, are extreme examples of host-microbe interaction. Several symbiotic bacteria, like *Wolbachia* and *Spiroplasma* manipulate host reproduction in selfish manners to spread their infection into populations. *Spiroplasma* is a helical and motile, Gram-positive bacterium, which resides in diverse *Drosophila* species. The most remarkable feature of this bacterial symbiont is male killing, whereby male offspring from infected females is selectively killed during development. Recent successes in identifying the responsible factors in the bacterial genomes have highlighted the common appearance of deubiquitinase domains, although their functional roles in distinct manipulative phenotypes remain unknown. For example, *Spiroplasma* in *Drosophila* selectively kill male progeny with a male-killing toxin Spaid that encodes an OTU deubiquitinase domain. Here I show that without the function of OTU, the male-killing activity of Spaid is attenuated, though not eliminated, since it is polyubiquitinated and degraded through the host ubiquitin-proteasome degradation pathway. Furthermore, I show that Spaid utilizes its OTU domain to deubiquitinate itself in an intermolecular manner. Thus, the deubiquitinase activity of Spaid serves as a self-stabilization mechanism to facilitate male killing in flies, enabling the efficient manipulation of the host at low-cost.

If time permits, I will also present our ongoing project regarding a novel male-killing gene, discovered in a symbiotic virus in wild-caught *Drosophila*.

A10: New branches on the *Wolbachia* tree of life

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Wolbachia is the most widespread inherited symbiont on earth, occurring in around half of all terrestrial arthropod species. *Wolbachia* taxonomy is largely unresolved, with only a single species described and strains being assigned to “supergroups”, for which no formal definition exists. In recent years, the number of *Wolbachia* complete and metagenome-assembled genomes have drastically increased, however a unified phylogenetic framework of the genus *Wolbachia* is lacking.

We have compiled the most phylogenetically comprehensive dataset of *Wolbachia* genomes to date, including 46 strains from all supergroups for which genomic information is available and adding novel data for 4 strains from 3 supergroups, 2 of which are novel. Our analyses indicate that the core genome of *Wolbachia* evolves tree-like and that there is very little conflict in the phylogenetic signal between loci and phylogenetic approaches. Most *Wolbachia* strains cluster within two highly supported, reciprocally monophyletic clades: one clade includes supergroups A and B, and thus the majority of described *Wolbachia* strains. The other clade is shows a higher genetic diversity and comprises *Wolbachia* from filarial nematodes and various arthropods (supergroups C, D, F, and others). Several host shifts from nematodes to arthropods can be inferred for the evolutionary history of this clade. The strains outside of the two major clades are mostly represented by MAGs only and were isolated predominantly from soil dwelling animals. Further, we find that the *Wolbachia* tree, contrary to previous reports, can be confidently rooted. The position of the root is robust to outgroup choice and the inclusion of long-branched taxa.
This new phylogeny of the *Wolbachia* tree of life will be an important resource for research on *Wolbachia* evolution and prompts a re-evaluation of *Wolbachia* taxonomy.

A11: Complex host modifier systems control two distinct *Wolbachia*-induced reproductive phenotypes in mites

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Wolbachia symbionts induce sex allocation distortion and cytoplasmic incompatibility (CI) in haplodiploid arthropods such as spider mites. Both reproductive phenotypes are typified by great variation in strength across host-symbiont systems and numerous hypotheses have been suggested to explain the underlying mechanisms. Here, we tested whether and how natural genetic variation of the host species modulates these *Wolbachia*-mediated reproductive phenotypes. Using a single *Wolbachia* variant, a nuclear genotype reference panel of the spider mite *Tetranychus urticae* was created that consisted of *Wolbachia*-infected and cured near-isogenic lines. To unravel the role of host modulation in CI, we performed a highly replicated age-synchronized full diallel cross composed of incompatible and compatible control crosses. We observed striking variation in CI and uncovered that infected males modulate different features of CI compared to uninfected females. Both male and female modulation interacted with the genotype of the mating partner, suggesting that these modifier systems are underpinned by complex genetic architectures. To unravel the role of host modulation in sex allocation distortion, we first demonstrated that this *Wolbachia*-mediated reproductive phenotype is driven by increasing egg size, promoting egg fertilization. Using the nuclear genotype reference panel, we gathered evidence that host modulation was also an important determinant for the strength of sex allocation distortion and acted by regulating the egg size effect. Finally, we parametrized a deterministic model to simulate the invasion of the focal *Wolbachia* variant in different mite populations. Our simulations suggest that sex allocation distortion is sufficient to remove invasion thresholds, allowing CI to drive *Wolbachia* from low infection frequencies. Together, these findings underscore the importance of host genetics for *Wolbachia*-host interactions and help elucidate the mechanisms that underlie the widespread occurrence of maternally transmitted symbionts in haplodiploids.

A12: Impacts of the host extracellular microbiome on an animal-microbe endosymbiosis

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Animals live in a microbial world with a plethora of endo- and extracellular symbionts, yet disentangling the functions of endocellular symbionts from extracellular symbionts has not been interrogated. Fundamental questions include do endosymbionts function in the absence of extracellular symbionts? If so, do endosymbionts take advantage of the newfound host state? What changes in host biology occur in a strict state of one host, one endosymbiont, and no other microbiome? Here we cultivate germfree *Drosophila melanogaster* flies with and without their resident endosymbiotic *Wolbachia* that induces cytoplasmic incompatibility (CI), wherein embryos of *Wolbachia*-infected males and uninfected females are inviable. We report three central findings: (i) The endosymbiotic adaptation of CI is not contingent on the rest of the microbiome, though the presence of the extracellular symbionts significantly reduces CI penetrance; (ii) The endosymbiotic virulence varies during development of otherwise germfree-flies. While the endosymbiont slightly increases the embryo-to-larval survival, it reduces larval-to-pupal and pupal-to-adult survival. (iii) The loads of the endosymbionts and expression of CI factors *cifA* and *cifB* are under investigation and will be reported on at the conference. This study demonstrates, for the first time, the self-sufficiency of an intracellular endosymbiont in manipulating host reproduction and the role of the general microbiome in preventing endosymbionts from more fully hijacking the host.

A13: Cytological analysis of Wolbachia Cif proteins

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Wolbachia-mediated Cytoplasmic Incompatibility (CI) is a widespread sterility syndrome that is controlled by a pair of bacterial proteins collectively known as Cifs. In *Wolbachia* strains that infect *Culex pipiens* mosquitoes (*w*Pip), CifA and CifB (also known as CidA and CidB) function a type II toxin-antitoxin system, where the CidB toxin is neutralized by the antidote CidA by direct protein interaction. Using transgenic expression of these *w*Pip effectors in *Drosophila melanogaster*, we have established that CidB is escorted by CidA during spermatogenesis and is eventually loaded in sperm nuclei. At fertilization, CidB poisons DNA replication in the male pronucleus, resulting in the loss of paternal chromosomes at the first zygotic division (Horard *et al.*, *Curr Biol*, 2022). Intriguingly, recent cytological analyses of Cif effectors from *w*Mel revealed a different behavior of CifA and CifB in their host species (Kaur *et al.*, *PLoS Biology*, 2022), supporting a "host-modification" model of CI. To directly compare Cids and Cifs, we have established transgenic lines expressing tagged versions of *w*Mel CifA and CifB. We will present our latest results and discuss hypotheses regarding the impact of Cifs in *D. melanogaster* germ cells.

A14: CifA and CifB are both *in vitro* nucleases that promote spermatid DNA damage

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The worldwide endosymbiosis between arthropods and *Wolbachia* bacteria is an archetype for reproductive parasitism. This parasitic strategy rapidly increases the proportion of symbiont-transmitting mothers, and the most common form, cytoplasmic incompatibility (CI), impacts insect evolution and arboviral control strategies worldwide. During CI, sperm chromatin is modified according to the Host Modification model of CI via two nuclear-targeting proteins, CifA and CifB, that alter the evolutionary-conserved, histone-to-protamine transition in *Drosophila melanogaster* and *Aedes aegypti*. Here we hypothesize that Cif proteins metabolize nucleic acids of developing sperm to initiate chromatin integrity changes. Using *in vitro* and *in situ* transgenic, mutant, enzymatic, and cytochemical assays, we show that CifA is a previously-unrecognized DNase and RNase, and CifB is a DNase. *In vitro* nuclease activity translates to *in situ* spermatid DNA damage at the canoe stage of spermiogenesis. Evolution-guided mutations notably ablate Cif enzymatic activity. Nucleic acid metabolism of spermatids expands the biochemical breadth of activities associated with Cif functions in spermiogenesis and broaden our fundamental understanding of CI-associated sperm modifications.

A15: Male killing induced by maternally inherited viruses in arthropods

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Male killing (or early male killing), the selective death of males during embryonic or early larval stages, has been observed in various arthropod species and is considered as an adaptive or selfish strategy for cytoplasmic parasites. Many of the reported cases of male killing are induced by bacterial symbionts such as *Wolbachia* and *Spiroplasma*. Because such bacterial symbionts typically have 1000 or more genes in their genome, it is often challenging to isolate the gene responsible for male killing. Here, I will discuss recent discoveries of male-killing viruses from several insect species. In the fly *Drosophila biauraria*, a maternally inherited double-stranded RNA virus DbMKPV1 belonging to the family Partitiviridae was found to cause male killing during late embryonic development. Among the four dsRNAs possessed by DbMKPV1 (dsRNA1-dsRNA4), dsRNA4 was identified as the male-killing gene. None of the 27 dsRNAs possessed by a male-killing virus OGV (belonging to Partitiviridae) found from the moth *Homona magnanima* were homologous to dsRNA4 of DbMKPV1. We also found a male-killing virus SlMKV from the moth *Spodoptera litura*, but this virus belongs to a different taxonomic group. Similarities and differences between these viruses in terms of genome structure and host phenotype are discussed. Although mostly hidden, such viruses can be taxonomically widespread and cause male killing in various arthropods.

Other Symbionts (G)

G1: To Kill or not to Kill: Evolutionary long-term relationships with facultative Male-Killing *Spiroplasma* in Neotropical flies

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Spiroplasma are wall-less gamma proteobacteria found in many insects that can act as reproductive parasites via Male-Killing (MK), or to protect their hosts from other insect parasites like nematodes and wasps. Recent comparative genomic studies in the model system *D. melanogaster* have uncovered that *Spiroplasma* genomes evolve at an extremely high mutation rate and that their effector proteins causing these “Dr. Jekyll and Mr. Hide” phenotypes stem from phages that coevolve intimately with the bacterium.

MK-*Spiroplasma*, however, were initially discovered in different neotropical Drosophila populations belonging to the willistoni group species by Donald Poulson in the early 1960s as so-called Sex Ratio Organisms (SROs), but their prevalence in nature was low. Although intensively studied during this pre-genomics area in respect to their microbiology, trans-infectivity and phenotypic consequences by Poulson & Williamson in their native hosts (first-degree infections), these lines unfortunately got lost. Only the *Spiroplasma* strain NSRO of *D. nebulosa* that was transferred earlier into the heterologous host *D. melanogaster* (second-degree infection), is now available for comparative genomic and phenotypic studies on their core genomes but also their associated plasmids and phages to decipher their short- and long-term dynamics in *de novo* melanogaster as well as their ancestral neotropical reservoir hosts.

We hence have recently recollected neotropical Drosophila flies in Costa Rica and Ecuador and found by systematic PCR screening besides *Wolbachia* two different *Spiroplasma* infection types: one relatively rare *S. citri*-group member that causes partial MK under standard developmental temperatures as well as a second strain belonging to the *S. pousonii* group, which is at low titer close at their PCR-detection limits, but quite frequent in neotropical hosts. Under enhanced developmental temperatures however, *S. poulsonii* titers increase significantly and induce strong SR distortions in different neotropical host lines belonging to the willistoni as well as saltans group species. These data strongly imply that the *S. poulsonii* type infection is a conspecific long-term symbiont of neotropical fly hosts at almost fixation that is, however, asymptomatic at low titer under normal conditions, but can turn into an SRO upon stress like temperature shift or introgression.

G2: The role of the intracellular symbiont *Lariskella* in a leaf-footed bug

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We examined the role of an alphaproteobacterium, *Lariskella arthropodarum* in the leaf-footed bug *Leptoglossus zonatus*. *Lariskella* is found within a recently characterized family of

uncultivable intracellular symbionts in the *Rickettsiales, Midichloriaceae*. Members of this diverse family of bacteria infect a wide range of hosts that include terrestrial arthropods, aquatic invertebrates and protists, and have received considerably less attention compared to the other lineages of *Ricketsiales*. *Lariskella* has been found in a number of true bugs in the Pentatomomorpha (Hemiptera), and also in ticks and fleas, but its function in any system has not been determined. The bug *L. zonatus* also has an obligate nutritional symbiont, *Caballeronia* (in the Burkolderiaceae) that it acquires from the environment early in development each generation. We were able to cure *L. zonatus* of *Lariskella* with antibiotics early in development before the bugs were fed *Caballeronia* to initiate a *Lariskella*-free line. To examine the role of *Lariskella* on bug performance and reproduction as well as possible *Lariskella - Caballeronia* interactions, we reared bugs in a factorial experiment with both *Lariskella* and *Caballeronia* positive and negative treatments. While *Caballeronia* negative treatments showed the major fitness deficits we expected, bugs with and without *Lariskella* had equivalent performance and fecundity. In subsequent crossing experiments, however, we found that significantly fewer eggs hatched when *Lariskella*-negative females were mated with *Lariskella* positive males, consistent with a cytoplasmic incompatibility (CI) phenotype. Field surveys of *Lariskella* frequency in samples of *L. zonatus* in Arizona and California show high but variable rates of infection. *Lariskella* joins a select number of symbiotic bacteria that have been shown to cause CI, including other Alphaproteobacteria (*Wolbachia, Mesenet*), *Rickettsiella* (Gammaproteobacteria), *Cardinium* (Bacteroidota), and *Spiroplasma* (Mycoplasmatota).

G3: Genome dynamics across the evolutionary transition to endosymbiosis

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Endosymbiosis – where a microbe lives and replicates within a host cell – is an important contributor to organismal function that has accelerated evolutionary innovation and catalysed the emergence of complex life forms. However, the evolutionary processes and genomic adaptations associated with transitions to endosymbiosis are poorly understood and are largely inferred from comparisons between highly evolved obligate endosymbionts and their phylogenetically distant free-living relatives. Here, we use comparative genomic analysis of the bacterial genus *Arsenophonus* (Gammaproteobacteria, Enterobacterales) to reveal the complex processes involved in the evolution of persistent symbiosis. We compared the genomes of several *Arsenophonus* strains with different life histories, ranging from environmentally acquired to facultative and obligate insect endosymbionts. The initial transition from environmentally acquired infection to persistent symbiosis was associated with the loss of type VI secretion and CRISPR-Cas defence systems. The loss of CRISPR-Cas was associated with the acquisition of prophage elements, leading to significant genome expansion and the gain of diverse secreted effectors and toxins potentially associated with symbiosis. The subsequent transition to become a vertically inherited stable endosymbiont was associated with relaxed purifying selection, gene pseudogenisation, and eventual genome reduction and reduced %GC in the obligate endosymbiotic genomes. Our results suggest that the early stages of the evolution of an endosymbiotic lifestyle require rapid evolutionary innovation, involving the horizontal acquisition of new traits necessary to establish of host association, enabled in part by the loss of genome defence systems. Consequently, early-stage microbial symbionts may undergo rapid and extensive genome expansion prior to the processes of reductive evolution traditionally associated with adaptation to an intracellular lifestyle.

G4: How can *Wolbachia* transcriptomic methods be used to determine the role of *Midichloria Mitochondrii* in *Ixodes* ticks?

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Tick-borne diseases are considered a major emerging health problem around the world, affecting more than one billion people and accounting for over 700,000 deaths annually. Similarly to Wolbachia, *Midichloria mitochondrii* are a-proteobacterial symbionts of the Order Rickettsiales and infect host germline cells. *M. mitochondrii* are observed in high abundance in disease causing *Ixodes* ticks; *Ixodes ricinus* and *Ixodes holocyclus.* They are found throughout the tick body, with strong localisation in the ovaries, found in both the cytoplasm and within the inter-membrane space of the mitochondria. Although abundantly present, the biological role of *M. mitochondrii* is not well understood. To determine this, Wolbachia lateral gene transfer (LGT), metatranscriptomic and differential expression methods from Chung et. al 2020 were employed for the analysis of *M. mitochondrii.* This study identified a 1,015 nt long (88% identity) LGT event from *M. mitochondrii* to *I.ricinus*, which was confirmed using PCR and phylogenetics. This LGT event encodes a protein coding gene and while its function is unknown, it is transcribed by the tick host. After the detection of LGT events, *M. mitochondrii* transcripts from *I. ricinus* and *I. holocyclus* RNA sequencing data were classified by functional groups. Although the functional groups of *M. mitochondrii* reads varied between *I. ricinus* and *I. holocyclus*, these differences were not statistically significant, suggesting the endosymbiont may play similar roles in both hosts. Furthermore, differential expression analyses showed 51 differentially expressed (DE) *M. mitochondrii* genes between *I. holocyclus* adults and nymphs, as well as eight DE genes between *I. holocyclus* adult females and males. The data from the DE analysis will also be used to determine if any biological pathways are differentially expressed between the different groups.
This study reports the first instance of LGT from bacteria to *I. ricinus* ticks. The transcriptomic analyses additionally enhanced our understanding of the activity of *M. mitochondrii* and revealed that the symbionts' biological role is similar between *Ixodes* tick species. However, the differential
expression analysis showed some gene regulation between tick sexes and life stages. Better understanding the endosymbionts of disease-causing hosts, such as *Ixodes* ticks, is critical for the development of control mechanisms and reducing the spread of tick-borne diseases.

G5: Deleterious endosymbionts for aphid pest control

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Endosymbiotic bacteria that live inside the cells of insects are typically only transmitted maternally and can spread by increasing host fitness and/or modifying reproduction in sexual hosts. Transinfections of *Wolbachia* endosymbionts are now being used to introduce useful phenotypes into sexual host populations but there has been limited progress on applications

using other endosymbionts. Here we develop a novel pathway to application in aphids by transferring the endosymbiont *Rickettsiella viridis* to the major crop pest *Myzus persicae*. *Rickettsiella* infection greatly reduced aphid fecundity and modified aphid body color, from light to dark green. Despite inducing host fitness costs, *Rickettsiella* spread rapidly through caged aphid populations via plant-mediated horizontal transmission. The phenotypic effects of *Rickettsiella* were sensitive to temperature, with the loss of body color modification at high temperatures, a reduction in aphid heat tolerance, and temperature-dependent spread through populations. *Rickettsiella* shows potential to spread through natural *M. persicae* populations by vertical and horizontal transmission. Establishment of *Rickettsiella* could reduce crop damage by modifying population age structure, reducing population growth and providing context-dependent effects on host fitness. Our work highlights the importance of plant-mediated horizontal transmission and interactions with temperature as drivers of endosymbiont spread in asexual insect populations.

Molecular and Cellular Biology (B)

B1: *Wolbachia* supports development and buffers against nutritional stress

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Wolbachia's broad success as a vertically transmitted infection cannot be attributed to manipulations of host reproduction alone. Using the *Drosophila melanogaster* model, we show that *Wolbachia* infection supports fly development and buffers against nutritional stressors including reduced nutrition and removal of the gut microbiome. *Wolbachia* infection across several fly genotypes and a range of nutrient conditions resulted in reduced pupal mortality, increased adult emergence, and larger size. We determined that the exogenous supplementation of pyrimidines rescued these phenotypes in *Wolbachia*-free flies, suggesting *Wolbachia* plays a role in providing this metabolite that is normally limiting for fly growth. Additionally, *Wolbachia* was sensitive to host pyrimidine metabolism: *Wolbachia* titers increased upon transgenic knockdown of the *Drosophila de novo* pyrimidine synthesis pathway but not knockdown of the *de novo* purine synthesis pathway. Ongoing work leverages high throughout metabolomics and *Drosophila* genetics to define the metabolic landscape and regulation of host-*Wolbachia* nutritional interactions.

B2: *Wolbachia* and the transcriptional landscape of stress: A comparison between strains *w*MelPop-CLA and *w*AlbB at single-nucleotide resolution

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Symbiont-mediated control of arbovirus transmission entails the transfection of the alpha-proteobacterium *Wolbachia* into mosquito vectors, which greatly reduces vector competence. The choice of optimal *Wolbachia* strain for mosquito population replacement programmes needs to consider several factors, such as potential negative fitness effects on the mosquito host, the speed of replacement induced by cytoplasmic incompatibility, and the stability of the transinfection in the face of stressors such as heat. Mounting evidence indicates that different

Wolbachia strains exhibit profound differences in resilience to the high temperatures that are regularly experienced in dengue-endemic countries. For instance, *w*MelPop-CLA (originally isolated from *Drosophila melanogaster*) is more easily lost from transinfected *Aedes aegypti* at elevated temperatures than is *w*AlbB (native to *Aedes albopictus*). However, very little is understood about the regulatory response to stress in *Wolbachia* at the transcriptional level. Here, we apply Cappable-Seq to unveil the primary transcriptome of these *Wolbachia* strains under low, optimal and high temperatures during mosquito cell culture. Stark differences in temperature tolerance between *w*MelPop-CLA and *w*AlbB were confirmed *in vitro*. Transcriptional start sites (TSS) were mapped at single-nucleotide resolution for the first time in an endosymbiotic bacterium responding to stress, demonstrating that both *w*MelPop-CLA and *w*AlbB have the capacity to utilise all four known TSS types, including a high proportion of antisense TSS. Global TSS discovery also enabled the identification of TSS promoter motifs associated with the global regulator sigma-70. Interestingly, wMelPop-CLA exhibited very high levels of expression for prophage-related TSS, whereas insertion sequence-associated TSS were more dominant in *w*AlbB. Evidence is also provided for the ability of *Wolbachia* to express alternative transcriptional units/sub-operons that can independently regulate *wspB*. This study represents the first comparative transcriptomic analysis between *Wolbachia* strains in use for transinfected mosquito strategies and will facilitate experimentally guided strain choice in future.

B3: The taming of a mighty bug: how neotropical *Drosophila* flies restrict *Wolbachia* infection

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Wolbachia bacteria are maternally transmitted intracellular microbes that are not only restricted to the reproductive organs but often found in various somatic tissues of their hosts. The abundance of endosymbionts in the soma, a dead end for the bacteria, causes a multitude of effects on life history traits of their hosts, which are still not well understood. Thus, deciphering the host-symbiont interactions on a cellular level throughout the lifecycle of the host is of great importance to understand their homeostatic nature and spreading success of a microbe.
In the present study we uncovered high restriction of *Wolbachia* infection to embryonic primordial germline cells and to certain cells of the soma in several neotropical *Drosophila*. This unprecedented restriction is determined early in fly embryogenesis via selective autophagy and preserved through larval hatching and metamorphosis. We observed tight interactions of *Wolbachia* with membranes of the endoplasmic reticulum, which might play a scaffolding role for autophagosome formation and further elimination of the endosymbiont. Finally, by analyzing *Drosophila* transinfected with non-native *Wolbachia*, we uncovered that the host genetic background regulates tissue tropism of infection. Our data demonstrate a novel and peculiar mechanism to limit and spatially restrict bacterial infection to the future germline and also to some somatic stem cells already during early host embryogenesis at the maternal-to-zygotic transition.

B4: *Wolbachia* endosymbionts manipulate GSC self-renewal and differentiation to enhance host fertility

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The alphaproteobacterium Wolbachia pipientis infects arthropod and nematode species worldwide, making it a key target for host biological control. Wolbachia-driven host reproductive manipulations, such as cytoplasmic incompatibility (CI), are credited for catapulting these intracellular bacteria to high frequencies in host populations. Positive, perhaps mutualistic, reproductive manipulations also increase infection frequencies, but are not well understood. Here, we identify molecular and cellular mechanisms by which Wolbachia influences the molecularly distinct processes of GSC self renewal and differentiation. We demonstrate that wMel infection rescues the fertility of flies lacking the translational regulator mei-P26, and is sufficient to sustain infertile homozygous mei-P26-knockdown stocks indefinitely. Cytology revealed that wMel mitigates the impact of mei-P26 loss through restoring proper pMad, Bam, Sxl, and Orb expression. In wild-type individuals, wMel infection elevates lifetime egg lay and hatch rates. The beneficial fertility reinforcement mechanisms described here may promote the emergence of mutualism and the breakdown of CI.

B5: Co-transmitted *Wolbachia* exhibit distinct patterns of distribution in host ovarian tissue

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There is significant interest in the ability of some *Wolbachia* and other intracellular microbes to simultaneously colonize a host. Not only do such coinfections impact host biology and evolution, but they may provide opportunities for the management of insect pests. A naturally occurring, stably-transmitted *Wolbachia* coinfection is present in some *Drosophila simulans*, with *Wolbachia* strains "*w*Ha" and "*w*No". This system provides a tractable model to examine infection dynamics of cotransmitted *Wolbachia* and their ecology within the host. Our previous work demonstrated that the ratio of *w*Ha to *w*No in young embryos does not reflect those observed in the ovaries, suggesting that the two *Wolbachia* strains have different transmission dynamics. We developed strain-specific probes and used high resolution FiSH microscopy to examine the spatial and temporal changes in *w*Ha and *w*No titers within host ovary tissues in 3-D space. Our results indicate that these two infections exhibit distinct patterns of spatial distribution that differ between cell types and across egg development. These different distribution patterns may indicate that *w*Ha and *w*No are engaging in intracellular niche partitioning.

B6: Multiple *Wolbachia* subpopulations co-occur in single *Culex pipiens* mosquitoes

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Wolbachia is a maternally inherited intracellular bacterium that infects a wide range of arthropods including mosquitoes. The endosymbiont is widely used in biocontrol strategies due to its capacity to modulate the arthropod reproduction and to limit pathogen transmission.
While most studies assume that *Wolbachia* infections are mono-clonal within individual hosts, we show here that an ancestral *Wolbachia* can evolve to a set of closely related, but genetically distinct, subpopulations which coexist within the same hosts and are maternally transmitted to

host progeny. To this aim, we reconstructed *Wolbachia* Metagenome-Assembled Genomes in naturally-infected single individual *Culex pipiens* mosquitoes from both ovary and midgut metagenomes for the first time and afforded *Wolbachia* population genomic analyses within and between single individuals. We observed consistent single nucleotide and amnio acid variations across samples that we also confirmed in egg metagenomes from geographical distant locations. This study reveals the presence of multiple *Wolbachia* populations co-existing in individual mosquitoes that could have critical importance for the good use of *Wolbachia* in basic and applied research.

B7: pWCP is a widely distributed and highly conserved *Wolbachia* plasmid in *Culex pipiens* and *Culex quinquefasciatus* mosquitoes worldwide

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The widely distributed *Wolbachia* bacterium can block pathogen transmission and dramatically manipulate host reproduction, representing one of the most promising solutions for vector control. Yet, due to its endosymbiotic nature, *Wolbachia* is not easily amenable to genetic manipulation, which imposes strong constraints on dissecting its mechanisms of action. Recently, several *Wolbachia* plasmids, carrying phage WO-like genes and Insertion Sequences, have been discovered. Here, we investigated the diversity and biogeography of the first described plasmid of *Wolbachia* in *Culex pipiens* (pWCP) in several islands and continental countries around the world—including Cambodia, Guadeloupe, Martinique, Thailand, and Mexico—together with mosquito strains from colonies that evolved for 2 to 30 years in the laboratory. We used PCR to screen for the presence of pWCP in both the germline and somatic tissues of individual mosquitoes, and highly accurate Sanger sequencing to evaluate potential variations. Our data show that the presence of pWCP is not incidental and that it is remarkably stable across generations in very different environments and associated selective pressures. The architecture, gene content and the very sequence of pWCP is extremely conserved with only minor variations in hypervariable regions. This suggests a critical role for this mobile element in the endosymbiont biology and could support the development of a much-needed tool for *Wolbachia* genetics and engineering.

B8: Antisense nucleic acids as tools to explore *Wolbachia*-host interactions

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There are currently no methods and tools available to grow *Wolbachia* outside of host cells and genetically manipulate them. To enable gene studies in *Wolbachia* to investigate their interactions with the host, we developed a method to knockdown *Wolbachia* genes in the host cells using antisense nucleic acids. As an example, we knocked down the *Wolbachia*'s *RNase HI* gene using antisense RNA to study its role in virus blocking. *wRNase HI* is induced in mosquito cells within 30 min after infection with dengue virus (DENV) and is able to degrade viral RNA. We found higher RNase activity in the cytoplasm of *Wolbachia*-infected cells compared to uninfected mosquito cells against viral RNA, and overexpression of *w*RNase HI in mosquito cells in the absence of *Wolbachia* resulted in decreased DENV replication. In contrast, DENV replication increased in both *Wolbachia*-infected cell lines and mosquitoes when *wRNase HI* was silenced using antisense RNA. These results indicate a contribution of *w*RNase HI to virus blocking observed in the presence of *Wolbachia*. In addition to *wRNase HI*, we were able to knock down other *Wolbachia* genes, overall suggesting that antisense RNA can be used for gene silencing in *Wolbachia*, opening a new avenue to study *Wolbachia*-host interactions. This approach can also be extended to other non-*Wolbachia* endosymbionts with similar limitations. The presentation will also provide the latest follow-up results on optimizing and improving *Wolbachia* gene silencing.

B9: Isolation and propagation of novel *Wolbachia* strains in tick and insect cell lines from the Tick Cell Biobank

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Insect cell lines have been used for isolation and propagation of *Wolbachia* since the first mosquito cell line naturally infected with *w*AlbB was generated nearly 30 years ago. Since then, increasing numbers of continuous *in vitro* culture systems, either naturally or experimentally infected, have greatly expanded the scope of *Wolbachia* research. Recently, tick cell lines have also been found to be capable of supporting infection with some strains of *Wolbachia*. The Tick Cell Biobank houses the world's largest collection of tick cell lines, as well as a growing collection of new insect cell lines. This has allowed us to carry out studies on susceptibility of a range of arthropod cell lines to a panel of *Wolbachia* strains of diverse origins. Here we summarise how insect and tick primary cultures and cell lines have been used to isolate *w*Pip, *w*Pap and *w*Wil from, respectively, *Culex pipiens* mosquitoes, *Phlebotomus papatasi* sandflies and *Drosophila willistoni* fruit flies, and *w*CfeF and *w*CfeJ from *Ctenocephalides felis* fleas, thereby supporting symbiont genome sequencing efforts. Moreover, we report use of multiple arthropod cell lines derived from ticks, midges, mosquitoes and honeybees to separate a mixture of *w*CfeF and *w*CfeJ from an originally co-infected tick cell culture.

Experimental evolutionary approaches to symbiosis (H)

H1: Experimental evolutionary approaches to symbiosis

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In nature, symbiotic associations with microorganisms are ubiquitously found, which often play pivotal biological roles in animals, in plants and even in human. Now “symbiosis” and “microbiome” are among the important keywords not only in basic biology but also in agriculture and medical science. However, highly intimate symbiotic associations are difficult to work on experimentally, because the symbiotic partners are usually non-model organisms that are integrated into an almost inseparable biological entity. Here, the symbiotic microorganisms cannot survive outside the host and thus are mostly uncultivable. Such statements as “nobody has seen the process as to how intimate mutualistic associations have evolved” and “genetic engineering and functional analysis of uncultivable genome-reduced symbiotic bacteria are very difficult” plainly depict the situation under which understanding of symbiosis has been severely hindered.
Now we are aiming at bringing about breakthroughs to overcome these difficulties, by making use of the establishment of novel insect-*E. coli* and mammal-*E. coli* experimental symbiotic systems and the development of recent genome engineering technologies, thereby drastically promoting our understanding of symbiosis.
Here I present an overview of ERATO Fukatsu Evolving Symbiosis Project as an introduction to this session “Experimental evolutionary approaches to symbiosis”.

H2: The things have small beginnings: various single mutations could make *Escherichia coli* an insect mutualist

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"Insect essential symbiotic bacteria have evolved from free-living bacteria." – This is an insightful hypothesis advocated in the early days of symbiotic research. The hypothesis has been reinforced by incorporating the outcomes of molecular phylogeny and comparative genome analyses and has become generally accepted. However, the empirical research supporting this hypothesis are scarce. Moreover, it is an unsolved riddle what factors prompted bacteria to change their lifestyle from free-living to obligate symbiosis at a very early stage of the evolution of symbiotic relationships. In the recent paper entitled “a single mutations makes *Escherichia coli* an insect mutualist”, our ERATO project clearly shows that a single mutation occurred in the *E. coli* genome significantly improved the performance of host stink bugs and suggests this mutation represents an evolutionary entrance to establish mutualistic symbiosis. However, it can’t be that this mutation is the only such evolutionary entrance. There must be multiple and diverse possibilities. With such confidence, we launched a large-scale evolutionary experiment project discovering unexplored evolutionary pathways toward mutualism. In this talk, I will report the progress of this project and introduce evolutionary lines in which a novel mutation in *E. coli* genome likely improves host performance.

H3: Exploring the universality of symbiosis: constructive and quantitative approaches to symbiotic evolution

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Symbiotic relationships are ubiquitous in nature, but the questions of how such relationships emerge in evolution and what their universal properties remain unclear. In order to address these questions, it is crucial to conduct a detailed analysis of the symbiotic evolutionary process in the laboratory. Recently, we have developed an experimental symbiotic system between insects and *E. coli* (Koga et al., Nature Microb. 2022), which opens a door for quantitative and constructive approaches to symbiotic evolution. In this presentation, the following three topics based on the artificial symbiotic system will be discussed:
(1) Ex vivo reconstruction of symbiotic evolution. We are attempting to reconstruct the phenotypic changes that occurred in the insect-*E. coli* symbiotic evolutionary experiment by using experimental evolution in a liquid medium. The aim is to verify whether different phenotypic changes can establish a symbiotic relationship. To establish such a system, it is necessary to develop technology that can change the phenotype of *E. coli* in the desired direction through evolutionary experiments. In this study, using a laboratory automated system, we developed a method to achieve the evolutionary trajectories toward the target phenotype by incorporating feedback control into the selection environment. Using this experimental evolution system, we successfully achieved *E. coli* strains with a similar phenotype to the symbiotic *E. coli* strains that evolved with insects.
(2) Analysis of symbiotic population dynamics using DNA barcode technology. To capture the population dynamics of symbiotic bacteria in the insect gut, we constructed a library of symbionts with random barcode sequences in their genome and inoculated them into the host insect. The distribution of unique barcode sequences extracted from the insect's symbiotic organ provides valuable information about the growth and migration of symbionts in the insect gut. For example, we discovered a significant correlation between the barcode distributions in the posterior end of the symbiotic organ and the surface of eggs laid by the insect, indicating that symbiotic bacteria localized to the posterior end are inherited by the next generation. By utilizing this technology, we plan to clarify the population dynamics of symbionts in detail, including quantifying the effective population size, bottlenecks, and migrations in the host, which can serve as the foundation for a better understanding of symbiotic evolution.
(3) Construction of an IS-amplified *E. coli* strain to facilitate genome reduction. It is suggested that the significant increase in the number of insertion sequences (IS) in the genome will lead to genome reduction in symbiotic bacteria. To study this phenomenon, we constructed *E. coli* strains in which IS sequences were artificially amplified. Our approach involved designing an artificial IS that used *E. coli* IS1 as a backbone and carried the fluorescent protein mCherry. Then, we incorporated the artificial IS into *E. coli* and sorted cells with higher fluorescence intensity using FACS. Through iteration of the selection procedure, we successfully obtained an *E. coli* strain with a large number of artificial IS sequences in the genome. This IS-amplified strain is now being used for symbiotic evolutionary experiments with insects investigating the dynamic rearrangement of genome structure.

H4: Experimental coevolution in a defensive symbiosis community

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Defensive microbial symbionts can protect hosts against pathogen infection. When under attack from coevolving pathogens, will hosts respond by depending on their symbionts to greater degree, or by investing more in their own defence mechanisms? In turn, how will coevolving pathogens shape the evolutionary trajectories of defensive symbiont populations? We experimentally coevolved a tripartite community of genetically-diverse nematode hosts, virulent pathogens (*Staphylococcus aureus*), and defensive symbionts (*Enterococcus faecalis*). After 15 host generations, we detect signatures of host dependence emerging under pathogen coevolution. We show evidence of heightened host susceptibility to pathogens when coevolved defensive symbionts are removed. For defensive symbionts, coevolution with pathogens may also drive greater dependence on the host environment. Defensive symbionts that coevolved with pathogens show the most compromised growth rates when returned to a free-living environment. Finally, we characterize the effect of tripartite coevolution on the diversification of host and symbiont populations, and reveal the modes of selection operating. The data provides new insight into the evolutionary responses of defensive symbioses when faced with coevolving pathogens.

H5: Bacterial growth and competition in geometrically structured habitats

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Bacterial cells in the wild often reside in spatially-structured habitats. Habitat structures may impose constraints on population dynamics as physical boundary conditions and affect competition among bacterial organisms or strains. However, analyzing population dynamics of bacterial cells directly in their natural habitats such as soil and intestinal crypts is usually challenging due to their extreme structural diversity and uncertainties of biochemical conditions. To untangle habitats' geometrical influence on long-term population dynamics, we developed microfluidic devices with chambers of simple and complex geometries. We monitored 100-10000 bacterial cells in the chambers with defined geometries and medium conditions for over one week. We find that habitat structures can qualitatively alter the dynamics of two neutral bacterial strains between fixation of one strain and coexistence of the two strains. Furthermore, an *Escherichia coli* strain that acquired a symbiotic ability with a stinkbug *Plautia stali* in an experimental evolution [1] can coexist or outperform an ancestral strain in the structured habitats despite its lower growth rate in batch cultures. These results demonstrate that habitat structures can significantly affect bacterial population dynamics and imply discrepancies in bacterial fitness between structured and non-structured environments.

H6: Genetic mutation in *Escherichia coli* genome during adaptation to the murine intestine is optimized for the host diet

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Mammalian gut microbes colonize the intestinal tract of their host and adapt to establish a microbial ecosystem. The host diet changes the nutrient profile of the intestine and has a high impact on microbiota composition. Genetic mutations in *Escherichia coli*, a prevalent species in the human gut, allow for adaptation to the mammalian intestine, as reported in previous studies. However, the extent of intestinal fitness elevated by genetic mutation and the effects of diet change on these mutations in *E. coli* remain unclear. Here, we show that notable mutations in sugar metabolism-related genes (*gatC, araC*, and *malI*) were detected in the *E. coli* genome just two weeks after colonization. Deletion of these genes elevated *E. coli* fitness in the murine intestine, though the extent was dependent on the host diet. *In vitro* cultures of medium containing nutrients abundant in the intestine also showed increased *E. coli* fitness after deletion of the genes-of-interest associated with their metabolism. Furthermore, the host diet was found to influence the developmental trajectory of gene mutations in *E. coli*. Taken together, we suggest that genetic mutations in *E. coli* are selected in response to the intestinal environment, which facilitates efficient utilization of nutrients abundant in the intestine. Our study reveals some of the intestinal adaptation mechanisms of gut microbes as well as a potential means by which we can manipulate the microbiota.

H7: Whole genome cloning of genome-reduced insect symbionts

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Many symbiotic bacteria have undergone "reductive evolution" in which their genomes have shrunk in the process of evolution, since they have depended on their hosts for metabolites. In particular, many insect symbiotic bacteria have a mutualistic relationship with their host, so that many of them have an extremely tiny genome. These symbiotic bacteria do not have genes essential for bacterial growth, so they could no longer grow outside their hosts. While genome sequencing must be informative to understand these symbionts, functional analysis of their genes would be difficult because of lack of gene knocking-down or overexpression. In this study, as a first step to elucidate the gene function of insect symbiotic bacteria, we attempted whole-genome cloning of insect symbiotic bacteria with a relatively small genome. As a result, we succeeded in cloning the entire genomes of several symbiotic bacteria. These clones would also be valuable as bioresources for maintaining and distributing genomes of unculturable symbionts. In this presentation, I would like to discuss the usefulness and future prospects of our approaches.

H8: Specificity of an obligate leaf beetle-bacterial symbiosis

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Symbiont specificity is predicted to stabilize obligate insect-microbe symbioses. Despite the prevalence of nutritional and defensive symbioses in insects, mechanistic insights into the specificity of these interactions are often hampered by the limited ability to disrupt and experimentally exchange symbionts across different host species. Here, we leverage the tractability afforded by an extracellular symbiont transmission route in tortoise leaf beetles

(Coleoptera: Cassidinae) to quantify the specificity governing their obligate symbiosis with *Candidatus* Stammera capleta. Cassidines derive pectin-degrading enzymes from *Stammera*, allowing them to exploit a leafy diet rich in recalcitrant polysaccharides. *Stammera* is localized in specialized organs connected to the foregut and extracellularly transmitted from mother to progeny via egg caplets. Caplet removal disrupts symbiont transmission, enabling downstream manipulation and exchange of *Stammera* across different host lineages. We conducted experimental transfections in aposymbiotic eggs of the tortoise beetle, *Chelymorpha alternans*, using *Stammera* derived from three additional Cassidinae species. Symbiont transfection was successful across all treatments, highlighting that *Stammera* can be exchanged across host lineages. However, we observe differences in the efficacy of symbiont colonization depending on the genetic distance of nonnative *Stammera* strains relative to the native symbiont. Reflecting this, the fitness of aposymbiotic larvae is restored following symbiont transfection of *Stammera*, but only in treatments bearing strains that are closely related to the native symbiont. The positive correlation between symbiont genetic distance and host survivorship highlights the strong influence of symbiont genotype on host development. Overall, our findings confirm that the high degree of host-symbiont specificity plays a significant role in obligate symbiont maintenance over long evolutionary timescales.

H9: Parallel Evolution in *Wolbachia*-Infected Mosquito Cell Lines

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Wolbachia symbionts are widespread across arthropods where they cause reproductive manipulations and/or confer fitness benefits such as protection against viral pathogens. Their self-spreading ability coupled with their antiviral effect has been harnessed in health programmes to curb the transmission of mosquito-borne diseases such as dengue. These intracellular bacteria are genetically diverse and closely-related strains often vary in density within the host, a trait that broadly correlates with their ability to block viruses. Such diversity is a useful tool to dissect host-*Wolbachia* interactions at the cellular level and to understand how these are shaped by natural selection. Here we generated eight new mosquito cell lines by introducing a range of *Wolbachia* strains into the *Aedes albopictus* Aa23 cell line. As expected, we found that the symbiont density was overall strain-dependent, yet highly unstable with large fluctuations observed within short timescales. Importantly, following transinfection we identified *de novo* mutations in the *Wolbachia* genomes potentially involved in density regulation, some of them showing signs of parallel evolution across replicates. Altogether, our results demonstrate that the cell culture model is a valuable tool to study within-host selection and identify the genetic basis underlying variation in symbiont density.

Poster presentations:

Posters session I

Wolbachia in Nematodes (E)

E1: Analysis of antibiotic resistant clusters in filarial nematodes
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Filarial nematodes are human parasites that infect millions of people across the globe and cause debilitating diseases such as Elephantiasis and African River Blindness. These worms share a symbiotic relationship with *Wolbachia*, and rely on these bacteria for survival and proper embryogenesis. Taking advantage of this crucial symbiosis, efforts to find drugs to kill the adult worm by targeting *Wolbachia* have proven to be promising. Rifampicin, a common antibiotic, has been shown to significantly decrease *Wolbachia* levels in filarial nematodes. However, recent studies have discovered that *Wolbachia* rebound to normal levels after eight months post-Rifampicin treatment. Large *Wolbachia* clusters are present in the germline of the filarial nematodes, and their number, size, and density are resistant to conventional antibiotic treatment. We hypothesize that these clusters of *Wolbachia* provide the source of *Wolbachia* rebound. In an effort to develop a next-generation anti-*Wolbachia* therapy, we have characterized these clusters at a cellular level and screened for compounds that specifically target these clusters. These results of this analysis will be presented.

Ecology and Evolution (F)

F1: Discover the Microbes Within! The *Wolbachia* Project
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Discover the Microbes Within! The *Wolbachia* Project (wolbachiaproject.org) has facilitated real-world, scientific research opportunities to classrooms at the high school and undergraduate level for over 15 years. Increasingly embraced by global citizen scientists and research trainees new to the *Wolbachia* field, the project leads participants through a series of carefully curated and discovery-based labs to explore *Wolbachia* infection frequencies within local environments. Students gain state-of-the-art experience in molecular biology techniques such as DNA extraction, PCR, and gel electrophoresis, and they apply bioinformatics to explore Sanger sequencing, arthropod barcoding and phylogenetics. Observations uploaded to the *Wolbachia* Project Database (wolbachiaprojectdb.org), such as the diverse palearctic arthropods collected near Zurich, are sometimes novel to the field and can provide pilot data for exploring new symbiotic systems. Here we will present freely available resources that can be incorporated into broader impacts and community engagement activities. We will discuss a roadmap for collaborations that further amplify *Wolbachia* outreach to the international audience, currently reaching participants in over 19 countries and 44 US states, and provide opportunities for educational contributions.

F2: *Wolbachia* impacts vector ecology and the transmission of Banana bunchy top virus by the banana aphid, *Pentalonia nigronervosa*

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Microbial symbionts of several crop pests and disease vectors mediate interactions with ecological communities, shaping several components of world agriculture. Among the best-studied are aphids, which vector damaging plant pathogens while harboring several heritable symbionts conferring protection against ecological threats. The bacterial symbiont, Wolbachia, is found in many economically important arthropod species, and while among the best-studied, Wolbachia biology in aphids remains obscure. Our recent findings indicate that Wolbachia is widespread in the banana aphid, Pentalonia nigronervosa, the vector of Banana bunchy top virus (BBTV; Nanoviridae) a major pathogen of Musaceae. Since Wolbachia is particularly known for anti-viral phenotypes, we investigate whether Wolbachia infection impacts within-vector dynamics of BBTV. We conducted transmission assays using banana leaf disks to examine whether aphids with Wolbachia were capable of transmitting BBTV and determined that those with Wolbachia transmitted BBTV at a lower rate compared to those without. Through quantitative PCR we discovered that BBTV accumulation and retention, over time, were much lower in aphids with Wolbachia compared to those without suggesting Wolbachia disrupts the transmission of BBTV. We also compared aphid fitness between those with and without Wolbachia and that fed on either healthy or BBTV infected banana plants (W-/Healthy W-/BBTV+; W+/Healthy W+/BBTV+). We found that W+/Healthy aphids outperformed W-/Healthy aphids indicating that Wolbachia infection improves aphid fitness. We also found that the fitness of W-/BBTV+ aphids was comparable to W+/Healthy aphids indicating symbiont-free aphids benefited from feeding on BBTV infected plants. However, it was Wolbachia-infected aphids who benefited the most when feeding on BBTV infected plants; W+/BBTV+ aphids developed faster and were more fecund than all other combinations. Our findings provide further understanding of how heritable microbes may impact vector transmission of plant diseases.

F3: Metagenomic sequencing reveals multiple *Wolbachia* supergroups from *Ctenocephalides* spp. fleas

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Ctenocephalides spp. fleas, including the cosmopolitan cat (*Ctenocephalides felis*) and dog (*C. canis*) fleas, as well as *C. orientis* (restricted to tropical and sub-tropical Asia), are important vectors of multiple zoonotic diseases, namely cat scratch fever (caused by *Bartonella* spp.) and flea-borne spotted fever (caused by *Rickettsia felis*). Apart from bacterial pathogens, recent efforts to characterise the microbiome of *C. felis* from the US also revealed the occurrence of

two genetically divergent *Wolbachia* strains, with one, *w*CfeT, being proposed as a nutrient-provisioning symbiont, and the second, *w*CfeJ, as a potential reproductive parasite.
The current study presents the genomic assembly of a novel strain, *w*Cori, from *C. orientis*. We have also further isolated two *Wolbachia* strains, *w*CfeF and *w*CfeJ, from Malaysian *C. felis* into tick cell culture and sequenced their genomes. We demonstrate that the three *Wolbachia* strains are representatives of different supergroups, of which supergroup I (*w*Cori and *w*CfeT) and the newly designated supergroup V (*w*CfeJ) appear to be specific to fleas. These *Wolbachia* genomes exhibit unique combinations of features associated with reproductive parasitism or mutualism. The *w*CfeF genome from supergroup F was found to combine both features: having prophage WO, cytoplasmic incompatibility factors (*cif*)-like genes, and the biotin operon of obligate intracellular microbes. Moreover, the genome of *w*Cori, similar to the closely-related *w*CfeT, contained prophage WO and vitamin biosynthesis genes that are rare among the repertoire of *Wolbachia* accessory genes. The Malaysian *w*CfeJ genome was almost identical to its counterpart from the US, harbouring *cif*-like genes but no vitamin biosynthesis genes. These findings add to the complexity of flea-*Wolbachia* relationships, supporting the need for future work to unravel the driving forces underpinning the evolution and genomic divergence of these and other *Wolbachia* strains reported from *Ctenocephalides* spp. fleas.

F4: Transition of *Wolbachia* and other facultative bacterial endosymbionts towards co-obligate lifestyle in scale insects

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Symbiotic relationships with diverse microbes significantly contribute to the ecological and evolutionary success of insects. Bacterial symbionts can play a crucial role in providing them with essential nutrients, enhancing their immune systems, or defending them from natural enemies such as viruses and parasitoids. *Wolbachia*, the most abundant endosymbiotic bacterium in insects, is well-known for its various reproductive manipulations within hosts. However, it can also sometimes establish a mutualistic relationship with its host, for example by providing nutrients or protection from viruses. Scale insects are small sap-feeding hemipterans that harbor a huge diversity of bacterial endosymbionts. Unfortunately, the exact functional roles of these symbionts remain poorly studied. Using comparative genomics, we investigated the potential functional roles of *Wolbachia* and other facultative endosymbionts in several scale insect families. Our preliminary results suggested that several lineages of *Wolbachia* and other 'facultative endosymbionts' might be in fact transitioning towards a co-obligate lifestyle and provisioning essential nutrients to their scale insect host.

F5: Life in a changing world: complex effects of *Wolbachia* infection and temperature on fitness of wild-caught *Drosophila melanogaster*

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Wolbachia bacteria are common endosymbionts of many arthropods found in gonads and various somatic tissues. They manipulate host reproduction to enhance their transmission and confer complex effects on fitness-related traits. Some of these effects can serve to increase the survival and transmission efficiency of *Wolbachia* in a host population. The *Wolbachia-Drosophila melanogaster* association is an excellent model to study this question. In world-wide populations of *D. melanogaster* flies there was a recent replacement of the ancestral *w*MelCS *Wolbachia* variant by *w*Mel and the reason for this turnover is still unknown. It appears that *w*Mel strain provides an advantage for the infected host compared to uninfected flies or host carrying *w*MelCS. In our work, we investigated the influence of different *Wolbachia* strains on fitness-related host traits at developmental and adult stage, to test how *Wolbachia* infections and environmental variation affect host fitness. Freshly collected *D. melanogaster* flies from Portugal were used in our study to minimize the effects of lab adaptation, inbreeding, drift and resulting fitness artefacts. We uncovered complex temperature-specific effects of *Wolbachia* infection, suggesting that host-symbiont interactions in nature are strongly dependent on the genotypes of both partners and their thermal environment.

Other Symbionts (G)

G1: *Spiroplasma* in neotropical *Drosophila*; tracing hidden diversity of male-killing bacteria

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Spiroplasma is a gram-positive, cell wall-less, motile bacteria found in various organisms. In *Drosophila*, these maternally inheritable bacteria were first found in neotropical willistoni group samples in the 1960s with low prevalence as a sex-ratio disorder agent. Unfortunately, these strains were lost before being closely studied by molecular methods. Later, *Spiroplasma* has been found in different *Drosophila* species and is known to show various phenotypes ranging from male-killing to protection against parasitoids and nematodes. Still, far too little attention has been paid to *Spiroplasma* in neotropical flies to understand their long-term interactions with .their natural hosts and their associations with other symbionts

Here, we investigate both the natural prevalence of *Spiroplasma* and *Wolbachia* in their native reservoir host populations of neotropical *Drosophila* species and further their phenotypic consequences for the host. Firstly, we managed to detect asymptomatic *Spriroplasma* infections that seem almost fixed in natural populations of neotropical species of willistoni and saltans groups together and without *Wolbachia* indicating the early introduction of symbionts in their common ancestor flies. This systemic *Spiroplasma* infection belongs to the poulsonii clade and the detection by standard PCR is limited by temperature, i.e., infection only appears when flies are exposed to elevated temperatures and thereby express male-killing. Secondly, we found previously uncovered Sex-Ratio disorder strains belonging to the c*itri* group *Spiroplasma* which is in contrast to previously known poulsonii male-killing but at low prevalence in *D. paulistorum* .hosts in combination with *Wolbachia*

Our unexpected findings of systemic-asymptomatic and sporadic-pathogenic *Spiroplasma* strains in neotropical *Drosophila* hosts will open a new understanding of their long-term .coexistence and dynamics over evolutionary dimensions

G2: Experimental evolution of inherited symbionts in novel hosts

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Symbiosis is present in almost all animals, and endosymbiotic bacteria, such as *Spiroplasma*, are commonly found in terrestrial arthropods. These inherited symbionts are not necessary for host survival but are highly prevalent and exhibit various phenotypic traits, including "male killing," "feminization," or provide selective advantages to their hosts. Despite the stability and efficiency of host-symbiont associations passed from mothers to offspring, the lack of co-cladogenesis suggests that many symbiont-host associations are temporary over evolutionary time. Therefore, hereditary symbionts must regularly establish themselves in new hosts, a process known as "host shifting," to survive. Host shifts are significant evolutionary events for both hosts and symbiont. Our study aims to investigate the factors encoded by symbionts that are crucial for host shifts and how environmental interactions impact host shifts. We will use model host-symbiont systems, such as the *Drosophila/Spiroplasma* system, to conduct host-shifting experiments and characterize the molecular evolution of inherited symbionts in response to experimental host shifts. Furthermore, we plan to incorporate genomic features associated with successful host shifting in natural populations of inherited symbionts. This study will provide insight into how host shifting impacts symbiont evolution in novel hosts and explain the remarkable diversity and abundance of inherited symbionts in arthropods.

G3: Characterization of the bacteriome in natural populations of *Aedes albopictus from Greece*

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Mosquitoes (Diptera, Culicidae) are one of the most diverse arthropod groups in the world and their feeding behavior as hematophagous gives them the ability to transmit an enormous number of pathogens from one host to another. Symbiotic bacteria contribute to a multitude of important biological functions such as nutrition, reproduction, protection against pathogens and communication, affecting multiple physiological factors of their insect hosts, including fitness, longevity and survival. The composition of the mosquito microbiome varies across mosquito populations; however, the factors that contribute to this variation are poorly understood. Understanding the factors that shape the mosquito microbiome will inform how mosquito-borne disease transmission varies among environments and help to develop effective disease-mitigating strategies. In this study, we assessed the diversity and variation of the

microbiome of a medically important mosquito, *Aedes albopictus* from two geographical regions of Greece. PCR amplification of almost the full length 16S rRNA gene, and sequencing with the Oxford Nanopore Platform will enable us to characterize and assign taxonomy at species level in most of the cases, thus providing useful information on the bacterial profile of the Greek *Ae. albopictus* populations.

Molecular and Cellular Biology (B)

B1: Ins and Outs of Wolbachia: A Proteomics Approach

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The molecular means used by *Wolbachia pipientis* to facilitate infection of a host are not well defined, and continue to create scientific questions within the endosymbiont community. Using a proteomic approach with established infected insect cells, we used mass spectrometry to identify the intracellular *Wolbachia* proteins and compared these to the *Wolbachia* proteins from a 7-day incubation of extracellular *Wolbachia pipientis*. Using comparative phylogenetics and domain characterization, we worked to better understand the conservation and function of these *Wolbachia* proteins. These data informed our list of potential secreted effectors that are seemingly upregulated when outside of the host cell. Included among these effectors are WD0346 (known to contain a FIC domain) and WD0385 (an ankyrin repeat domain). Working with these effectors as tagged constructs in the *Drosophila melanogaster* model organism, we can express the proteins using the Gal4 system to identify the host protein interactions these effectors exploit to infect, maintain, and proliferate within the host, as well as discover novel phenotypes (of the fly or the *Wolbachia*) in the presence or absence of a *Wolbachia* infection.

Experimental evolutionary approaches to symbiosis (H)

H1: Spatiotemporal dynamics of insect symbionts in microfluidic devices

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Most animals have existed in symbiotic relationships with microbes. These microorganisms perform a variety of functions and are often essential to the host's survival and reproduction. In the case of the brown-winged green stinkbug *Plautia stali* (Hemiptera: In the Pentatomidae), symbiotic bacteria (total 6 species: Sym A~F) specialize in the M4 region of the midgut and remain there steadily throughout the life cycle of the host (1). In previous studies, the morphogenesis of the M4 region from newborn to adult and the symbiont colonization process were described in detail (2). In this study, M4-mimicking microfluidic devices have been

designed and fabricated to observe cell growth and biofilm-formation dynamics of culturable Sym C~F. Though Sym C~F shows similar growth curves in batch cultures, unique and reproducible spatiotemporal dynamics were observed for each species at the cell population level in structured crypt-like chambers in the microfluidic devices. The dynamics of Sym C accompanied prominent biofilm formation near the openings of the chambers. However, the experimental evolution of Sym C in batch cultures for 15 days changed its spatiotemporal dynamics significantly with the altered size and position of biofilms. When Sym C and Sym E are co-cultured in the device, Sym E can colonize the chambers more rapidly than Sym C initially but is eventually outperformed by Sym C through competition. These results suggest that spatiotemporal population dynamics in the microfluidic devices are sensitive signatures for different bacterial species and genotypes and may allow us to probe their colonization strategies for establishing symbiosis with the hosts.

H2: Consequences of insertion sequence-mediated genome reduction in endosymbiotic organisms -- experimental evolution and theory

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Diverse insects rely on essential nutrients from endosymbiotic bacteria. These endosymbiotic bacteria are characterized by their tiny genomes. Although bacteria in intermediate stages of genome reduction have been identified, it remains unclear what occurs during the intermediate steps of extreme genome reduction and the consequences of genome reduction for the establishment of endosymbiotic mutualism. In this study, we aim to complement previous genomic and genetic studies with experimental evolution and theoretical approaches.
Comparative genomics has revealed that transposons called insertion sequence is a major driver of extreme genome reduction in endosymbiotic bacteria. During our experimental attempts to reconstruct the evolutionary process of insertion-mediated genome reduction, we observed that insertion sequences could delete sequences adjacent to them. We hypothesized and experimentally verified that insertion sequences could delete sequences between two genes placed far apart, leading to the formation of operons. This might explain why some endosymbiont genomes are operon-rich.
Genome reduction can lead to a decrease in both metabolic and regulatory capacity due to gene loss and operon formation. How this decrease of two factors influences the evolution of endosymbiosis remains unclear. Using a computational model of symbionts, we found that genome reduction is not solely a consequence of symbionts relying on their hosts. Rather, we argue that genome reduction might also facilitate the formation of endosymbiotic mutualism by accelerating host-parasite coevolution.

H3: Molecular mechanism of vertical transmission in stinkbug

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Vertical transmission of beneficial microbial partners is a crucial system for sustainable host-symbiont relationships, for which many insects develop elaborate structural, physiological,

and biochemical mechanisms. Most plant sap-sucking stinkbugs possess their specific symbiotic bacteria in a posterior region of the midgut where hundreds of symbiont-harboring crypts are arranged in rows. In adult females of the stinkbug family Pentatomidae, the posteriormost crypts are morphologically and biochemically specialized, which have been suspected to be devoted to vertical symbiont transmission [1,2]. Here, we investigated the functional specialization of the female-specific posteriormost crypts, mainly using the brown-winged green stinkbug *Plautia stali*. We performed comparative transcriptomics of the normal crypts and the posteriormost crypts, thereby listing up the genes significantly upregulated in the posteriormost crypts. By RNAi knockdown screening of these genes, we identified two genes indispensable for vertical symbiont transmission. The first gene encoded a unique secretory protein with no predictable function based on similarity searches. An outstandingly high expression of the gene was restricted to the posteriormost crypts. RNAi knockdown of the gene expression eliminated the symbiotic bacteria from the posteriormost crypts, consequently resulting in disrupted vertical symbiont transmission to the offspring. Another gene encoded a transcription factor regulating the posteriormost cypt-specific genes including the above-mentioned secretory protein gene. Taken together, we uncovered the functional specificity and the regulatory mechanism of the female-specific crypts for vertical symbiont transmission.

H4: Histological survey of bacteriocytes for harboring *Blattabacterium* endosymbiont among diverse cockroaches

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The existence of the bacterial endosymbiont in cockroaches, named *Blattabacterium*, and the specialized cells for harboring them, called bacteriocytes, has been known since the late 19th century. Since then, many histological, morphological, and microbiological studies have been reported. Recently, a substantial body of molecular phylogenetic and microbial genomic work has been compiled mainly from genomic and transcriptomic approaches. However, developmental, physiological, other biological aspects of the cockroach-*Blattabacterium* endosymbiosis still have much room for detailed investigation. Here we report a comprehensive histological and morphological investigation of the *in vivo* distribution of *Blattabacterium*-harboring bacteriocytes among diverse cockroach species as well as the ovarial transmission of the endosymbiont and the embryonic development of the bacteriocytes in the German cockroach *Blattella germanica* using fluorescence *in situ* hybridization with *Blattabacterium*-specific probes. In addition, we are attempting to produce aposymbiotic cockroaches using antibiotics, which was reported in several early studies but has not been reproduced recently. Our data indicate that the *in vivo* distribution of the bacteriocytes is highly conserved across sexes, life stages, and different species of cockroaches, being scattered among the abdominal fat body cells. These results contribute to our understanding the endosymbiotic relationship in cockroaches, as well as aid in further investigating the evo-devo aspects of the insect-bacterium endosymbiosis.

H5: Polyploidy and insect-bacterium symbiosis: both symbionts and bacteriocytes exhibit extraordinarily high number of genomes per cell

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Many insects utilizing nutritionally unbalanced diets are in symbiotic association with microorganisms, thereby complementing the nutritional deficiency. The host insects often harbor bacterial endosymbionts in specialized cells called the bacteriocytes, where both the host cells and the symbiont cells tend to be polyploid. Considering the well-known effects of polyploidy represented by enlarged cell volume and upregulated gene expression, it is conceivable that polyploidization of both the symbiont cells and the host bacteriocytes may be somehow involved in endosymbiosis. Furthermore, in highly specialized bacterial symbionts with drastically reduced genomes, polyploidy may have significantly affected their evolutionary fate, although few studies have comprehensively investigated the symbiosis-associated polyploidy. In this study, we analyzed ploidy levels of insect bacteriocytes and bacterial symbionts to elucidate the patterns of polyploidization. As for the insect side, we examined the ploidy dynamics of the bacteriocytes in the pea aphid *Acyrthosiphon pisum*. The bacteriocytes were hyper-polyploid ranging from 16- to 256-ploidy throughout the lifecycle. Size of the bacteriocytes was strongly correlated to size of the nuclei, suggesting the possibility that cell enlargement by polyploidization may contribute to the aphid-*Buchnera* nutritional mutualism, wherein the effects of polyploidy on the gene expression patterns are to be scrutinized further. As for the bacterial side, we are now comprehensively analyzing the ploidy levels of a variety of insect symbionts including *Buc hnera*, and exploring the general evolutionary patterns across the diverse symbiotic bacteria. We will discuss the functional and evolutionary significance of polyploidy in insect bacteriocytes and bacterial symbionts, and the future directions of the polyploidy studies on insect-microbe symbiotic associations.

H6: Disruption of a tryptophan metabolism gene as evolutionary entry point toward insect-bacterium mutualism

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Using laboratory evolutionary experiments, we recently demonstrated that *Escherichia coli* can promptly evolve into an mutualistic symbiont of the stinkbug *Plautia stali* by single mutations: loss-of-function mutations in either *crp* or *cyaA*, which disrupt the carbon catabolite repression (CCR) global regulator system, can drive the evolution of insect-microbe mutualism (Koga et al. 2022 Nat Microbiol). Of hundreds of *E. coli* genes regulated by the CCR system, 55 genes were commonly downregulated in the evolved symbiotic *E. coli* strains. When *E. coli* mutant strains devoid of these genes were infected to aposymbiotic nymphs of *P. stali*, a deletion mutant of tryptophan metabolism-related gene, *tnaA,* significantly improved adult emergence rate and body color of the host insect, which was almost comparable to the effects of Δ*cyaA* and Δ*crp*

mutants. Quantitative analysis of free amino acids revealed that the tryptophan concentrations in the hemolymph and the symbiotic organ of the insects infected with Δ*cyaA*, Δ*crp* and Δ*tnaA* mutants were significantly, more than ten times, higher than those of the control insects, suggesting that tryptophan accumulation due to *tnaA* deletion may contribute to the host's fitness improvement. We found that, interestingly, neither the symbiotic bacteria naturally found in *P. stali* and other stinkbugs nor the soil-derived *Pantoea* isolates potentially capable of symbiosis with *P. stali* retained *tnaA* gene in their genomes. In contrast, when infected with *Pantoea* strains retaining *tnaA* gene, the host insect exhibited no fitness improvement. Based on these results, we propose the hypothesis that *tnaA* deficiency may be the starting point toward the evolution of stinkbug-*Pantoea* mutualistic association.

Posters session II

Pests and disease control (C)

C1: *Wolbachia*-mediated viral blocking in natural solitary bee populations

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Wolbachia are alphaproteobacterial endosymbionts, transmitted from mother to offspring. This inherited symbiont is mainly known for its wide distribution, around 80% of the known fauna, and its different fitness effects on various hosts. On this occasion, the bacteria draw our attention to their viral blocking phenotype. In strict laboratory conditions, it has been found that the bacteria can reduce viral infection. Despite this success, the evolutionary stability of viral blocking remains unknown. More importantly, it has never been tested in nature which is considerably more complex considering all the uncontrolled parameter involved. In this project, we try to demonstrate whether a *Wolbachia*-induced viral blocking mechanism occurs in natural populations of different solitary bee species in the UK or not. To this end, we will separate *Wolbachia* infected and *Wolbachia*-free bees and we will compare the viral titres of the most common bee viruses of those two groups. Generalized linear mixed models, assigning *Wolbachia* infection status as fixed variable, will be used to explore viral titres. In the hypothesis where *Wolbachia* blocks viruses in solitary bees, we would expect higher viral loads in *Wolbachia*-free bees. Furthermore, we would also expect a negative correlation between relative viral and *Wolbachia* titres.
This project is essential to understand how pathogens impact the evolutionary ecology of one the most common symbionts on earth. It is also crucial, to establish future *Wolbachia*-based strategies targeting arboviruses.

C2: The First Report for the Presence of *Spiroplasma* and *Rickettsia* in Red Palm Weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in Egypt

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The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier), (Coleoptera, Curculionidae, Rhynchophorinae), is an invasive pest of palm trees. RPW invaded Egypt and became a great threat to the Egyptian date palm *Phoenix dactylifera* L.. In this study, 36 RPW individuals were collected from 6 different locations in Egypt. The presences of endosymbionts in the RPW individuals were assayed. The phylogenetic analysis of the RPW inhabiting Egypt was conducted using Cytochrome c oxidase sub-unit 1 (*CO1*) gene. *Spiroplasma* was found, for the first time, in all individuals, while *Rickettsia* was found, for the first time, in individuals collected from only 3 of the 6 locations. Endosymbionts harbouring Egyptian RPW were closely related to those harbouring Diptera and\or Trombidiformes associated with palm trees. This may be due to horizontal transmission through palm sap or through ectoparasites living on the RPW. Finally, the phylogenetic analysis of the RPW inhabiting Egypt was conducted. The collected individuals were closely related to Saudi Arabia specimens collected from the eastern region. Thus, Saudi Arabia may be the origin of the RPW which invaded Egypt. Individuals from populations inhabiting same geographical locations were closely related. This may be due to secondary invasion incidents that may have taken place through transportation of infested date palm trees and offshoots from infected to uninfected locations. This study reports the first incidence for the presence and coexistence of *Spiroplasma* and *Rickettsia* in RPW collected from Egypt. Also, it was found that the collected individuals of RPW were closely related a Saudi haplotype.

C3: Evaluation of the interaction between *Wolbachia*, *Aedes aegypti* and insecticide exposure and resistance.

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The city of Rio de Janeiro/Brazil can be considered an epidemiological mosaic of arboviral diseases as there is co-circulation of dengue, Zika, and chikungunya. Moreover, *Aedes aegypti* mosquitoes with the endosymbiotic bacterium *Wolbachia* (*w*Mel strain) has already been disseminated in 29 neighborhoods, reaching an area of 95 km2 and 950,000 people. An important point related to *Ae. aegypti* control in Brazil is the continues and massive use of pyrethroid insecticides (PY) in the recent decades, resulting in native mosquito populations highly resistant to this compound. We have established colonies of *Ae. aegypti* with genetic background from Rio de Janeiro infected with wMel and the *w*AlbB strain, the latter particularly performs better in field than *w*Mel under specific conditions such as higher temperature. In this project, we aim to explore the interaction among *Aedes*, *Wolbachia*, and arboviruses in a field setting, focusing on how insecticide resistance in vectors with *w*Mel or *w*AlbB strains may

impact the long-term use of *Wolbachia* to mitigate arbovirus cases. For that, we will study *Ae. aegypti* mosquitoes from Rio de Janeiro with *w*Mel and *w*AlbB, and without *Wolbachia*, regarding the effects of non-lethal exposure to insecticides (PY) and environmental temperature, evaluating the impact of these factors on the vector competence and vectorial capacity of mosquitoes, such as arbovirus susceptibility, longevity, *Wolbachia* load, maternal transmission of the bacteria, etc. Afterwards, we will develop mathematical models using laboratory and field data to characterize the potential of invasion of the *w*Mel and *w*AlbB strains under different environmental conditions. Our results will contribute to a better understanding of the interaction between bacteria, mosquitoes, and insecticide resistance, and aid in ensuring the success of the of integrated control strategies in the field.

C4: Identification of host factors that regulate *Wolbachia* salivary gland titer

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Wolbachia, a maternally transmitted bacterial endosymbiont occurring in nearly half of all insect species, provides a model for studying the factors that regulate intracellular bacteria abundance. Identifying factors that regulate intracellular bacteria titer has significant biomedical implications because higher *Wolbachia* titers correlate with increased suppression of dengue viral replication in host mosquitos. Host factors that regulate *Wolbachia* titer in the salivary gland is of particular interest because the virus is directly transmitted from the salivary gland to its human host. Here, we use RNAi in *Drosophila melanogaster* to specifically knockdown candidate host genes in salivary glands and analyze the effect on *Wolbachia* titer and distribution. Because *Wolbachia* is encompassed by host membrane, we have focused on genes regulating the ER, Golgi, and endocytic pathways. The results of this analysis will be presented.

C5: Evaluation of the *Wolbachia*-harboring Medfly VIENNA 8 genetic sexing strains developed as a model for IIT-based control of agricultural pests

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Wolbachia pipientis is an obligate intracellular, maternally inherited bacterium belonging to gram-negative a-proteobacteria. It is found mainly in the reproductive tissues of arthropods and is responsible for the induction of several reproductive lesions, including feminization, parthenogenesis, male killing, and cytoplasmic incompatibility (CI). CI is probably the most common phenotype and can be either unidirectional or bidirectional.
The Mediterranean fly (Medfly) *Ceratitis capitata* (Diptera: Tephritidae) is a cosmopolitan agricultural pest of high economic importance. It has been a model for the development of pest control methodologies inducing sterility to natural populations, such as the sterile insect technique (SIT). The SIT is an environment-friendly control method, where overwhelming numbers of sterile males are released into the target area, to mate with the wild females. In Medfly, male-only releases are more effective, facilitated by the existence of genetic sexing strains (GSS), with the last generation of the VIENNA 7 and VIENNA 8 GSS being used in mass rearing facilities worldwide.

Medfly is not a typical host for *Wolbachia* so Incompatible Insect Technique (IIT) has been proposed against it and over the past two decades, *Wolbachia* strains have been transferred to Medfly (*wCer2* and *wCer4*) using *Rhagoletis cerasi* as a donor. In our study, VIENNA 8 GSS harbouring *Wolbachia* have been constructed, infected with either *wCer2* or *wCer4* through genetic crosses with previously developed Medfly *Wolbachia*-infected strains and have shown that the new strains a) exhibit the expected genetic sexing character in respect both to the pupae colour and the temperature sensitivity (production of only males, b) are genetically stable when filtered at each generation and any recombination individuals are removed, c) are uni- and bidirectionally incompatible, being suitable for IIT purposes and d) that the presence of *Wolbachia* is stable in the new strains for the generations studied. Therefore, to the existence of the GSSs, and the complete *Wolbachia*-delivered CI, Medfly can be also a model to comparatively study both benefits and restrictions of IIT or combined IIT/SIT approaches. Perfect sex separation is a greater necessity for IIT compared to SIT applications since accidental release of infected females could lead to their dispersal in the natural population and subsequent replacement with an infected natural population, no longer susceptible to IIT.
In the present study the following parameters of the VIENNA 8, *Wolbachia*-harboring GSSs were investigated: i) whether they could maintain their genetic stability for consecutive generations if not filtered, a prerequisite for the colony upscaling to produce males for release purposes, ii) whether could suppress cage populations under laboratory scale conditions and iii) the female productivity and male-induced CI throughout their lifespan.

C6: *Wolbachia*-mediated pathogen blocking is cell-autonomous

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Vector control agencies across the globe are releasing *Wolbachia*-infected mosquitoes to replace natural mosquito populations with those incapable of transmitting disease. These efforts have been highly successful, with field trials showing that areas with *Wolbachia*-infected mosquitoes have a significant reduction in dengue incidence and hospitalization compared to uninfected areas (O'Neill et al., 2018). Despite *Wolbachia's* prominence in disease control, the field has yet to resolve the mechanism by which *Wolbachia* inhibit virus replication. Preliminary data suggests that virus replication is markedly suppressed in populations of *w*Mel-infected JW18 *Drosophila melanogaster* cells, despite only about half of the cells being visibly infected with *Wolbachia* via DAPI stain. These results remain consistent even at a multiplicity of infection of up to 100 infectious virus particles per cell. It was this discrepancy that prompted investigations into whether *Wolbachia*-mediated pathogen blocking is truly cell autonomous, given that previous work has shown that *Wolbachia* do not confer antiviral protection to uninfected cells through a transwell (Nainu et al., 2019). Here we show that in a mixed population of *Wolbachia*-infected and uninfected cells, *Wolbachia*-infected cells do not protect uninfected cells from viral infection, supporting a cell-autonomous mechanism of *Wolbachia*-mediated pathogen blocking.

C7: Forward genetic screen of *Wolbachia* to uncover the genetic basis of titer regulation

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Wolbachia is a common endosymbiont of arthropods, present in upwards of 60% of insect species and filarial nematode species. This symbiont is known to establish several types of interactions with its hosts, ranging from mutualistic to parasitic. One such interaction is the *Wolbachia*-mediated protection against viral infection of insects, such as in flies and mosquitos. This is being applied to prevent the transmission of arboviruses in endemic regions. Viral protection correlates with the titers of the bacteria, where more proliferative *Wolbachia* provide stronger protective effects. However, the inability to culture *Wolbachia* in the laboratory has hindered the development of common molecular tools to further study the phenotype. Previously, we have successfully performed a forward genetic screen by means of random mutagenesis with the use of the chemical EMS. Through the selection of over proliferative mutagenized lines, we were able to establish two new mutant lines, *w*MelPop2 and *w*MelOctoless. These mutants are characterized only by the amplification or deletion of the Octomom region, respectively, which results in higher titers and protection in both instances. Now we are performing a similar screen with the use of gamma radiation, a highly-penetrating electromagnetic radiation, to both increase the throughput and ensure *Wolbachia* exposition to the mutagen. Through such a screen we will attempt to circumvent the limitations in the genetic studies of *Wolbachia* and identify genes and mechanisms involved in *Wolbachia* titer regulation and, potentially, in viral protection.

C8: Target identification of AWZ1066S, a *Wolbachia* specific drug for the treatment of filariasis

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Wolbachia is an obligate symbiont of medically important filarial nematodes and is required for normal worm development, growth and embryogenesis. *Wolbachia* depletion with doxycycline leads to immediate and permanent sterility of adult female worms and a reduced adult lifespan. Through large scale screening of over 2 million compounds, the anti-*Wolbachia* (A-WOL) consortium have identified several candidate compounds with superior anti-*Wolbachia* activity compared with doxycycline. Two of these, AWZ1066S and TylAMac (flubentylosin), have entered phase I and phase II clinical trials. AWZ1066S is highly specific to *Wolbachia*, which de-risks antimicrobial resistance development to off-target bacterial co-infection and dysbiosis of the host microbiome during treatment. AWZ1066S also has a more rapid kill profile than any existing anti-*Wolbachia* drugs and can achieve its maximum effect after only one day of treatment. Genetic approaches to determine the target are challenging due to the intractability of *Wolbachia*, however a chemical proteomic approach using photoreactive probes has identified potential drug targets in *Wolbachia*. In our published work (Hong *et al*. PNAS, 116(4):1414), using *Wolbachia* infected insect cell-lines, we were able to shortlist a small number of potential *Wolbachia* target proteins for further confirmation and validation through expression in model systems. More recently, we adapted and further optimised a method to enrich and purify *Wolbachia* extracellularly (Rasgon *et al*. 2006 Appl. Environ. Microbiol. 72(11):6934), from the host cells, and can obtain significant quantities of extracellular *Wolbachia* for target identification and additional chemical biology experiments. Based on this breakthrough, we will repeat early chemical proteomics experiments using purified *Wolbachia* as the starting point and compare outcomes to confirm protein targets of interest. In addition,

purified extracellular *Wolbachia* can also be used as the starting point in a forward genetics approach (Duarte *et al*. 2021 PLoS Genet. 17(6):e1009612) to generate a C6/36 line harbouring *Wolbachia* resistant to AWZ1066S enabling mode-of-action and mode-of-resistance studies. By combining complementary proteomic and genomic approaches we aim to elucidate the molecular target(s) of AWZ1066S desirable for regulatory approval as a novel anti-*Wolbachia* therapy in the treatment of filarial diseases.

C9: Using reverse genetics screening with ensemble machine learning to identify genetic variants affecting *Wolbachia* titres and antiviral protection

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Wolbachia, one of the most abundant intracellular obligatory bacteria, confers protection against viruses to its *Drosophila* host. The discovery of *Wolbachia*-induced antiviral protection in diverse hosts led to a direct impact on human welfare through the prevention of transmission of dengue, zika, and other arboviruses, by deploying *Wolbachia*-carrying mosquitoes. However, little is known about the genetic and mechanistic basis by which *Wolbachia* induce antiviral protection, largely because *Wolbachia* is not yet amenable to genetic manipulation.
To overcome this limitation, we are using a reverse genetics screening approach that combines the use of random mutagenesis with targeted pooled amplicon sequencing of several *Wolbachia* genes, including candidates to affect symbiont titres, DNA repair or interaction with its host. To identify low frequency mutations after mutagenesis from experimental errors (false positives) that occur during a complex sequencing process, is critical. Several bioinformatic tools, based on different statistical methods have been developed to identify different genetic variants. To improve sensitivity and specificity of detection of low frequency mutations, we will combine the predictions and best auxiliary features from various variant caller models and ensemble machine learning (ML) methods. This experimental approach will help us to identify genes affecting *Wolbachia* growth regulation and antiviral protection and, consequently, help to develop more efficient *Wolbachia*-based mosquito population replacement strategies.

C10: Emerging arboviruses in Europe: Is *Wolbachia* the answer?

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Many zoonotic, vector-borne diseases are emerging or reemerging across Europe. Viruses such as West Nile and Usutu are continually extending their range and increasing in incidence throughout the continent. New controls are needed to tackle these pathogens. *Wolbachia* has proven a successful control strategy in other parts of the world, both for mosquito control and virus-blocking. Despite this, little is known about *Wolbachia*-virus interactions in key European vectors, namely *Culex pipiens s.l* and *Aedes albopictus.* This study aims to understand the role that native *Wolbachia* infections play in Usutu and West Nile virus replication, and to characterise anti-viral effects of different *Wolbachia* strains in species-appropriate systems (i.e.,

live *Culex/Aedes* mosquitoes or *Culex/Aedes* derived cell lines). This work increases understanding of vector competence and West Nile/Usutu virus – Wolbachia interactions.

C11: The resilience of *Wolbachia* (and host) to evolutionary and environmental change

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Wolbachia-infected *Aedes* mosquitoes are increasingly being deployed around the world to control mosquito populations and the arboviral diseases they spread. These interventions have been enormously successful, but challenges and uncertainties remain. Can *Wolbachia* releases succeed in every environment? Can the desirable effects of *Wolbachia* (e.g. cytoplasmic incompatibility and viral blocking) withstand evolutionary forces? And will current interventions be resilient to future climate change? Here I highlight recent work from the Pest and Environmental Adaptation Research Group (PEARG) on natural and novel *Wolbachia* infections across mosquitoes and *Drosophila*. Our research over the last decade has shown that *Wolbachia* transinfections in mosquitoes are incredibly stable once established but remain vulnerable to environmental factors including high temperatures and egg quiescence. Our recent work extends these findings by identifying novel phenotypic effects of *Wolbachia*, tracking long-term evolutionary changes, developing new *Wolbachia* mosquito strains and demonstrating the robustness of *Wolbachia* infections to a variety of environmental stressors. Ongoing work explores how climate change will affect *Wolbachia* infections in both natural and novel hosts, the role of endosymbionts in climate adaptation, and tracking host genetic changes that suppress *Wolbachia* phenotypes.

C12: Viral symbionts of *Anagyrus vladimiri* (Hymenoptera: Encyrtidae) - localization and molecular-level effects

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Relatively to bacterial symbiosis very little is known about non-pathogenic viral symbionts. However, non-pathogenic viral symbionts have been shown to have diverse effects on their hosts. The system studied includes the parasitoid wasp *Anagyrus vladimiri* (Hymenoptera: Encyrtidae) a biocontrol agent, its Reovirus symbiont (AnvRV), and its host the citrus mealybug *Planococcus citri* (Hemiptera: Pseudococcidae), an agricultural pest. In vivo experiment show that *A. vladimiri* carrying AnvRV have significant reduction in encapsulation rate by *P. citri,* but no difference in fecundity. Transcriptome analysis of parasitized and unparasitized *P. citri*, as well as *P. citri* parasitized by AnvRV^{+} and AnvRV^{-} *A. vladimiri*, provides insight into the mechanisms by which AnvRV manipulates both *A. vladimiri* and *P. citri*.

Reproductive manipulation (A)

A1: Different Infection Patterns of the Symbiotic Microorganism *Wolbachia* in Male and Female of *Homona magnanima*

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Tea totorix *Homona magnanima* distributed in Japan harbors three strains of *Wolbachia*: *w*Hm-a strain with unknown function, *w*Hm-b strain causing cytoplasmic incompatibility, and *w*Hm-c strain increasing host female fitness. *Wolbachia* infection patterns in adult of *H. magnanima* collected across Japan were unbiased: *w*Hm-b strain infection rate was lower in males than in females, resulting in different infection patterns between males and females. Here, the objective of the study is to clarify how the unbiased male-female *Wolbachia* infection pattern in *H. magnanima* occurs. First, we examined the infection rates and the densities of *Wolbachia* at each growth stage to determine the timing of the differences in *w*Hm-b strain infection between host sexes. *w*Hm-b strain was vertically transmitted from mother to offspring and began to decrease during the fourth instar of males. In addition, we investigated the tissue specificity of each *Wolbachia* strain and revealed that each strain differed tissue specificity between host sexes. Based on the results, we consider that the *Wolbachia* density may be controlled differently among the tissues.

A2: Wolbachia symbiosis and its effect on host gene expression in the Mediterranean fruit fly, *Ceratitis capitata*

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Wolbachia are maternally inherited endosymbiotic α-proteobacteria that induce dramatic alterations on the reproductive biology of their hosts. In addition to their ability to manipulate their host reproduction, *Wolbachia* have also been proved to modify other biological functions including immunity, mating, learning and memory capacity, sleep, feeding, locomotory behavior, host protection against other pathogens, and olfaction response. The molecular mechanisms underlying these *Wolbachia*-host interactions are yet to be fully elucidated and have never been studied in the Mediterranean fruit fly (medfly), *Ceratitis capitata*. The major *Wolbachia*-induced reproductive phenotype in *C. capitata* is Cytoplasmic Incompatibility (CI). However, it has been demonstrated that the bacteria also affect medfly fitness traits such as adult lifespan, immature developmental duration, adult flight ability and response to thermal stress. In the current study, we used RNA-seq to investigate the impact of *Wolbachia* on the medfly gene expression profiles in different insect tissues. Our findings demonstrate that *Wolbachia* bacteria alter the expression profiles of many genes related to the reproductive and immune system of the fruit fly. A great number of the differentially expressed genes identified in this study are also known to be involved in other biological functions including carbohydrate and lipid metabolism, muscular function, and cytoskeleton composition. Our study sheds light on the complex mechanisms contributing to medfly-*Wolbachia* dynamics.

A3: A quantitative interaction among endosymbiotic bacteria in *Mermessus fradeorum* spiders

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The majority of arthropod species are infected by maternally-transmitted bacterial symbionts, of which *Wolbachia* is the most widespread. Co-infections by multiple maternally-transmitted endosymbionts that induce contrasting reproductive manipulations are common among arthropods. *Mermessus fradeorum* (Berland, 1932) from the USA are naturally co-infected with five strains of bacterial endosymbiont, of which three are *Wolbachia* strains and the other two are *Rickettsiella* and *Candidatus* Tisiphia. The emergent phenotype of the symbiotic consortium is bacterially induced feminization. The contributions of each symbiont toward this phenotype are not entirely clear, but current data suggest that *Wolbachia1* is a necessary component and that *Wolbachia3* also contributes to the phenotype. Separately, the *Rickettsiella* symbiont induces cytoplasmic incompatibility (CI).

We hypothesize that within-host symbiont community dynamics affect the extent of the reproductive phenotypes of the host and are mediated by bacterial titer. We further hypothesize that the stability of the consortium may be destabilized if *Rickettsiella's* putative necessity to rescue CI is removed. To test these hypotheses, we have continually mated feminized spider lineages (five-fold and four-fold infected) with either *Rickettsiella*-only infected males (R-treatment) or with uninfected males (C-treatment) for 12 generations, measuring offspring sex ratio, the titer of all five symbionts, and ability to rescue CI. Initial qPCR results showed that in the 5th generation, the dominant symbiont is *Rickettsiella*, followed by *Wolbachia1* and *Ca*. Tisiphia with *Wolbachia2* is the scarcest in feminized females in both mating treatments. However, the pattern changes in the 9th generation, where regardless of mating treatments, the most abundant bacterial symbiont is *Wolbachia1*, followed by *Ca*. Tisiphia, and *Wolbachia2* with *Rickettsiella* as the scarcest symbiont, indicating a significant increase and decrease in abundance of *Ca*. Tisiphia and *Rickettsiella*, respectively, in still feminized females.

To further understand the symbiont transmission, we measured the *Rickettsiella* titer of *Rickettsiella*-only infected males used in this experiment. Initial data showed that *Rickettsiella* abundance is halved in *Rickettsiella*-only infected males relative to singly-infected females, however five times higher than in five-fold infected females, indicating that host sex and co-infection status have a significant impact on the symbiont titer. Additionally, qPCR results implied that *Ca*. Tisiphia remained present with very low abundance in males from R-treatment and C-treatment despite attempts to generate *Ca*. Tisiphia-free lines, and that it also remained present with very low abundance across all female lineages in different assemblies. This may indicate a potential for re-infection from the environment, suggesting another factor affecting the dynamics of the within-host bacterial composition and subsequent phenotypes. To further understand the environmental effect, we will compare spiders' bacterial populations and the observational data as sex ratio and CI rescue from two different environments (US and Israel).

A4: Prophage characterisation in circularised genomes of *Wolbachia* strains *w*Stri and *w*Bol1-b

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Wolbachia's range of reproductive manipulations are made possible via a suite of host effectors encoded by genes associated with prophage and pseudo-prophage inserted in the bacterial genome. The abundance of mobile elements and repetitive sequences in this genome, combined with the challenges of DNA extraction from an obligate endosymbiont, have made complete *Wolbachia* genome assemblies difficult to produce until recently. The *Wolbachia* strains *w*Stri and *w*Bol1-b (derived from the planthopper *Laodelphax striatellus* and the butterfly *Hypolimnas bolina,* respectively) are classified in supergroup B, notable for infection and host manipulation across many different orders of arthropods. Here, we present complete and circularised genomes for these strains and use them to identify and characterise the prophage regions present within. Long-read sequencing was performed with Oxford Nanopore Technologies' MinION platform on DNA extracted directly from infected Sf9 and C6/36 cell cultures. Reads originating from *Wolbachia* were identified from these metagenomic samples, assembled *de novo*, and then corrected by Illumina sequencing performed on the same material This resulted in single-contig, circularised genomic assemblies for *w*Stri and *w*Bol1-b, 1.75 and 1.47 Mb in length, respectively, with BUSCO scores of 99.7%. Both represent an improvement over the most complete *w*Stri and *w*Bol1-b assemblies in the NCBI Genome database. The Prophage Hunter software tool was then used to identify potential prophage by similarity matching to a database of essential features. Fifty-eight candidate prophage regions were defined in *w*Stri, and 45 in *w*Bol1-b. Of these, two in *wStri* were classified as 'active'; *i.e.*, containing a full suite of genes necessary for entering the lytic pathway. Conversely, all putative prophage in *w*Bol1-b lacked critical features, indicating degraded pseudo-prophage. Ongoing work is characterising prophage regions of both strains through *Wolbachia* RNA-Seq and protein expression from transgenic *E. coli*. This study lays the foundations for the possible production of bacteriophage WO *in vitro* and the potential for genetic manipulation of *Wolbachia*.

A5: *Wolbachia* affects host egg-laying behavior in *Callosobruchus analis*

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Oviposition site selection by hosts affects the survivorship of host offspring when resource competition among larvae is severe. In *Callosobruchus* species of bean beetles, female adults lay their eggs on the bean surface. Since larvae complete their development within a single bean and cannot move outside, they compete for limited resources. Beetles have adapted to such a resource-limited environment through two competition strategies: contest and scramble. Larvae with contest competition monopolize the bean when multiple eggs are laid on a single bean, while larvae with scramble competition share a single bean and complete their development except when their intake is insufficient for development. Adult size is often larger in the contest type than that in the scramble type, but the number of emerging adults is larger in the scramble type. Thus, females of the contest type should disperse their eggs across multiple beans (*e.g.*, even distribution) to overwhelm the number of scramble-type individuals.
Callosobruchus analis (Coleoptera: Chrysomelidae) represents a contest-type competition. We investigated host egg distribution in *C. analis* infected with CI-inducing and non-CI-inducing

Wolbachia to examine whether the infection of CI-inducing *Wolbachia* causes the alteration of host behavior. *C. analis* has two infection statuses, either wCana1 singly or doubly with wCana1 and wCana2 strains of *Wolbachia*. CI was observed only in the doubly infected hosts. We found that female hosts infected with CI-inducing *Wolbachia* laid their eggs more randomly than females infected with non-CI-inducing *Wolbachia*. *C. analis* hosts with CI-inducing *Wolbachia* might be disadvantageous in the larval resource competition.

A6: The leaf miner *Liriomyza sativae* (Diptera: Agromyzidae) acquired CI- resistance by incorporating the *Wolbachia* genome

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Liriomyza trifolii, which was confirmed to have invaded Japan in 1990, is frequently infected by *Wolbachia* (*w*Ltri) and induces cytoplasmic incompatibility (CI). CI is explained by the modification of the lethal factor (cifB) in the sperm of infected males, which leads to embryonic lethality when mated with uninfected females, whereas the action of the rescue factor (cifA) in infected females promotes embryonic development. However, there have been no reports of infected L. sativae, a closely related species that can hybridize with L. trifolii, and it was confirmed as uninfected in 2000.
Therefore, considering the possibility that the effects of *Wolbachia* infection may differ between the two species, we conducted a crossbreeding experiment using four lines of *L. trifolii* (infected line, antibiotic treated uninfected line) and *L. sativae* (uninfected line, *w*Ltri injected infected line). The hatchability of uninfected *L. trifolii* female was decreased by mating with infected males. In contrast, uninfected *L. satiate* didn't decrease hatchability when mated with infected males of both species, suggesting the presence of CI-resistance in *L. sativae*. To investigate the cause of CI-resistance, we analyzed the *L. sativae* genome and confirmed the insertion of a genomic fragment, that is homologous to the *Wolbachia* prophage and contains sequences corresponding to cifA and cifB, into the nuclear genome. We are currently conducting an observation of the location of the genomic fragments of cif gene by FISH and a genomic analysis of the CI rescue factors that are likely to be involved in the acquisition of CI-resistance.

A7: The impact of *Wolbachia* infection, temperature, and host fruit on adult life history traits of the Mediterranean fruit fly

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Wolbachia pipientis is a maternally inherited endosymbiont, mostly known for its ability to manipulate reproduction of its insect hosts. The most common *Wolbachia*-induced reproductive phenotype, Cytoplasmic Incompatibility (CI), was demonstrated on a major pest of fruits and

vegetables, the Mediterranean fruit fly, *Ceratitis capitata*, using *Wolbachia* transinfected lines. CI causes embryonic lethality under specific crosses and has been proposed as an additional tool for environmentally friendly pest control methods (Incompatible Insect Technique, IIT), using *C. capitata* as a model system. Therefore, many studies have focused on *Wolbachia* effect on insect biological traits that are important for the application and success of pest control methods, such as immature developmental duration, adult longevity and flight ability, male mating competitiveness and female fecundity. However, the impact of *Wolbachia* in combination with temperature and nutrition conditions during immature development on the biological traits of emerging adults is yet to be investigated. In the current study, we determined the effect of three temperature regimes during immature development (15, 25, 30°C) and two host fruits (apples, bitter oranges) on adult survival and lifespan, and female fecundity of *Wolbachia*-infected and non-infected medfly lines. Our results demonstrate that survival and lifespan of *Wolbachia*-infected adults, and fecundity are negatively affected by immature development under higher temperature regimes but remain unaffected by the fruit host. We discuss the effect of *Wolbachia*, temperature and host fruit under the frame of the known sensitivity of the endosymbiont to high temperatures, the potential interactions of *Wolbachia* with gut symbiotic bacteria, and the potential variations of *Wolbachia* impact depending on the genetic background of both the insect host and the bacterium.

A8: The effect of *Wolbachia pipientis* on the Super Cooling Point of different developmental stages of the Mediterranean fruit fly

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The physiology, reproduction and overall fitness of insects are influenced by interactions with endosymbiotic communities of microbes. One of the most common insect endosymbionts is the maternally inherited *Wolbachia pipientis*, mostly known for its ability to manipulate its host reproduction. *Wolbachia* effect on insects is not restricted to reproductive alterations but extends to a wider range of biological functions related to immunity, mating, sleep, feeding, locomotory behavior, host protection against pathogens, and olfaction. Abiotic factors such as temperature and nutrient availability might alter *Wolbachia* influence on their host. Although wild populations of the Mediterranean fruit fly, *Ceratitis capitata*, are not infected with *Wolbachia*, there are transinfected lines that provide an interesting model system to study the effect of the endosymbiont on biological traits of its insect hosts. *Wolbachia* impact on *C. capitata,* includes the manipulation of its reproduction and may involve alterations in other fitness traits such as adult lifespan, immature developmental duration, adult flight ability and response to thermal stress. We studied, for the first time, the combined effect of *Wolbachia* infection, sex, age, adult diet, and cold acclimation during both the immature developmental and adult life stage, on the Super Cooling Point (SCP) of *C. capitata*. Our findings showed that *Wolbachia* effect on medfly SCP varied depending on the age/life stage and sex of the insect, and the protein availability of the adult diet. Also, cold acclimation affected the SCP of the infected adults, but had no effect on the SCP of infected pupae. We discuss the differential influence of *Wolbachia* under the frame of the known variations of *Wolbachia* impact depending on its genetic background, and the potential interaction with other symbionts of the insect gut that might affect the production of antifreeze or ice-nucleation agents.

A9: Dosage Sensitive Screens for Suppressors and Enhancers of CI

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Wolbachia is a maternally inherited endosymbiont that manipulates host reproduction to favor infected females. Cytoplasmic Incompatibility (CI) is the most common form of reproductive manipulation. Crosses between infected males and uninfected females result in dramatic reductions in egg hatch (CI) while crosses in which both parents are infected produce normal egg hatch rates (RESCUE). This provides a selective advantage to infected females. The Wolbachia factors that cause CI and Rescue, known as the cif genes, have been identified but their host targets remain unknown. To identify these host targets we have taken advantage of the wealth of genetic reagents in *D. melanogaster* to screen for dosage sensitive enhancers and suppressors of CI. The results of this analysis will be presented.

A10: Effect of Late Male-killing viruses infected in *Homona magnanima*

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Arthropods are infected with various selfish factors that disrupt the sex ratio, and one of the mechanisms is the "male-killing" phenomenon, which specifically kills only infected males. *Homona magnanima*, the oriental tea tortrix, is a major pest of tea plants, and Osugoroshi virus 1-3 (OGVs) of the family *Partitiviridae* have been identified as maternally inherited agents that cause the male-killing phenomenon. Three RNA-dependent RNA polymerase and 24 related genes have been detected in a virus-infected host, but their functions are still unknown. To analyze the characteristics of OGVs, we performed RNA-seq analysis of male and female tortrix at the lethal age of males in this study. The results showed that the abundance and abundance ratio of the virus differed between the sexes depending on the segment. LC-MS/MS analysis was also performed to investigate differently expressed proteins with OGVs infection. Total 54 differently expressed proteins were identified between infected and uninfected males. And the expressions related to immunity were increased in infected males compared to uninfected males. In addition, ribosomal proteins tended to be decreased in infected males.
Furthermore, we purified virus particles and analyzed the viral proteins contained in them. As a result, three OGVs-derived proteins were identified as candidate structural proteins.