





### COMPUTER-PROCESSED AND EDITED CONFOCAL MICROSCOPY IMAGES OF TWO DISTINCTLY SHAPED LEAVES AT AN EARLY STAGE OF DEVELOPMENT

Cell outlines can be easily resolved due to the expression of a transgenic fluorescent protein, enabling growth to be followed in great detail. These leaves, described as 'simple' or 'complex', represent related members of the mustard family, thale cress (Arabidopsis thaliana, right) and hairy bittercress (Cardamine hirsuta, left), respectively. Both plants serve as model species within the Department of Comparative Development and Genetics, where genetic and microscopic data are combined in computer models to help understand how such different shapes are generated in nature. The image is the result of joint research efforts within the Department of Comparative Development and Genetics and edited by Dr. Peter Huijser.

### SCIENTIFIC OVERVIEW 2020 MAX PLANCK INSTITUTE FOR PLANT BREEDING RESEARCH



MAX-PLANCK-INSTITUT

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## Welcome to our Institute



We have compiled this overview to provide an introduction to the research work of the Max Planck Institute for Plant Breeding Research. Here we summarise the Institute's scientific aims and organisation and outline the projects being pursued by each research group. We have tried to present our work in a style that will appeal to a general audience as well as to scientists, and we hope you will find the text both absorbing and informative. We foster a curiosity-driven approach to plant sciences that stimulates collaborations and yet provides the freedom to shape individual careers.

The report describes the work of 26 research groups. More than half of the Directors, Group Leaders and PhD students, and two-thirds of the postdocs, come from abroad, underlining the international character of the Institute. In addition to the departmental groups, the Institute houses independent research groups and service groups that focus on topics not covered by any of the four departments. We place great emphasis on the training and mentoring of PhD students. With over 70 members, this group represents a sizeable fraction of the 400 staff on the campus. Many of them participate in structured programmes such as the International Max Planck Research School (IMPRS), MPIPZ Graduate School or the Centre of Excellence on Plant Sciences (CEPLAS), a regional research and training initiative undertaken jointly with the universities of Düsseldorf and Cologne. Our Student Coordinator follows the progress of all students closely, offering supplementary courses and advice and organising retreats and annual student meetings. In addition, for more than 50 post-doctoral scientists, the Institute's cutting-edge infrastructure and intellectual environment provide advanced training and serve as a spring-board to a research career in academia or in the plant science industry.

Members of our Institute play important roles in plant science at both national and international level, and make our campus a premier site for basic research on plants in Europe. We have particularly close links with the regional universities of Cologne and Düsseldorf, which participate in our IMPRS and provide the academic framework for our PhD students. In addition, we collaborate with them in the context of four Collaborative Research Centers (Sonderforschungsbereiche) funded by the Deutsche Forschungsgemeinschaft (DFG) and CEPLAS. We are especially indebted to the Max Planck Society for the allocation of an annual core grant that enables us to carry out many of our scientific activities.

We hope that whatever your background, you will enjoy reading about our science in the following pages.

PAUL SCHULZE-LEFERT Managing Director

# Historical Background of the Institute

The Institute was originally founded in 1928 as part of the Kaiser-Willhelm-Gesellschaft, and at that time was located in Müncheberg (Brandenburg). The founding Director, Erwin Baur, initiated breeding programmes with fruits and berries, as well as basic research on Antirrhinum majus and the domestication of lupins. After the Second World War, the Institute moved west to Voldagsen (Niedersachsen), and was relocated to new buildings on the present site in Cologne in 1955. The modern era of the Institute began in 1978 with the appointment of Jeff Schell and the development of plant transformation technologies and plant molecular genetics. The focus on molecular genetics was extended in 1980 with the appointment of Heinz Saedler. The appointment in 1983 of Klaus Hahlbrock broadened the expertise of the Institute in the area of plant biochemistry, and the arrival of Francesco Salamini in 1985 added a focus on crop genetics. From 1978-1990, the Institute was greatly expanded and new buildings were constructed for the departments led by Schell, Hahlbrock and Salamini, in addition to a new lecture hall and the Max Delbrück Laboratory building that housed independent research groups over a period of 10 years.

After 2000, a new generation of Directors was appointed in view of the approaching retirements of Klaus Hahlbrock and Jeff Schell. Paul Schulze-Lefert and George Coupland were appointed in 2000 and 2001 respectively, and Maarten Koornneef arrived three years later upon the retirement of Francesco Salamini. Miltos Tsiantis began work on establishing a Department of comparative development and genetics in 2013 after the retirement of Heinz Saedler. Raphael Mercier, the latest arrival, established the Department of Chromosome Biology in 2018 following Maarten Koornneef's retirement. The new scientific departments brought a strong focus on utilising model species in order to understand the regulatory principles and molecular mechanisms underlying selected plant traits. The longer-term aim is to translate these discoveries into breeding programmes through the development of rational breeding concepts. The arrival of a new generation of Directors also required modernisation of the infrastructure. So far, this has involved the complete refurbishment of the building that houses the Plant Developmental Biology laboratory (2004), construction of a new guesthouse and library (2005), as well as new buildings for Stores, the Outreach Department and Workshops along with a new entrance gate (completed in spring 2009). The new laboratory building for the Koornneef Department (now the Department of Chromosome Biology) was completed in 2012, as was the communal building that links all four science departments, and which houses meeting rooms, offices and the bioinformatics groups. An extensive new space to accommodate the Genome centre's servers was opened in the spring of 2012, while a partial overhaul of the former Saedler department building (now the Department of Comparative Development and Genetics) was begun in mid-2013. Modernisation of the glasshouses, including a new glasshouse complex for three departments, was begun in December 2013 and was completed at the end of 2015.



### Directors at the Institute in Cologne (since 1955)

1936 - 1961	Wilhelm Rudorf
1961 – 1979	Josef Straub
1967 – 1978	Wilhelm Menke
1978 – 2000	Jeff Schell
1980 – 2009	Heinz Saedler
1983 - 2002	Klaus Hahlbrock
1985 - 2004	Francesco Salamini
2000 – present	Paul Schulze-Lefert
2001 – present	George Coupland
2004 - 2016	Maarten Koornneef
2012 – present	Miltos Tsiantis
2018 – present	Raphael Mercier

# Organization and Governance of the Institute

The Institute comprises four scientific departments, seven independent research groups, three scientific service groups, the greenhouse service group, an outreach department that presents plant science to the public and press, and the administrative department, which includes the technical workshops and library.

The Board of Directors is responsible for the management of the Institute. The Board is made up of the Directors of the four scientific departments and the Head of Administration. It meets once a month under the chairmanship of the Managing Director. The Board of Directors takes decisions on matters such as how the budget of the Institute should be allocated, recruitments, promotions and the purchase of major equipment. The Board frequently invites senior scientists and/or service managers to these meetings for consultation on these issues and to provide a broader basis for decision making.

BOARD OF TRUSTEES	BOARD OF DIRECTORS Managing Director: Paul Schulze-Lefert	$\longleftrightarrow$	SCIENTIFIC ADVISORY BOARD
SCIENTIFIC COORDINATOR			HEAD OF ADMINISTRATION
PLANT DEVELOPMENTAL BIOLOGY Director: George Coupland Reproductive Development and the Evolution of Perennial Life History George Coupland Evolutionary and Computational Genomics of Matural Variation in Plants Andrea Fulgione Quantitative Approaches to Multicellular Dynamics in Plant Development Pau Formosa-Jordan Epigenetic Regulation of Plant Development Franziska Turck	PLANT-MICROBE INTERACTIONS         Director: Paul Schulze-Lefert         Integrative Bioinformatics         Ruben Garrido-Oter         Multi-Kingdom Interactions in the Plant Microbiome         Stephan Hacquard         Resistance Pathway Dynamics in Plant Immunity         Jane Parker         Recognition and Signalling in Plant Innate Immunity         Paul Schulze-Lefert         Structure and Dynamics of the Plant Immune Network         Kenichi Tsuda	INDEPENDENT RESEARCH GROUPS Functional Genetics of Barley Stamen Maturation Ivan Acosta The Evolution of Annual and Perennial Life Histories Maria Albani Rot Communication at the Single Cell Level Tonni Grube Andersen Molecular Basis of Adaptive Evolution Angela Hancock Structural Biology Ujie Chai Transport Processes in Crop Plants Wolf Frommer	ADMINISTRATIVE OFFICES Administration Equal Opportunities Greenhouses Health and Safety Office International Office IT Services Library Public Relations Technical Services
Director: Raphael Mercier Evolution of Meiosis André Marques Molecular Mechanisms of Meiosis Raphael Mercier Genomics of Extreme Adaption in Polyploids Polina Novikova Genome Plasticity and Computational Genetics Korbinian Schneeberger Meiosis in Crops Charles Underwood	GENETICS Director: Millos Tsiantis Bioinformatics and Comparative Genomics Xiangchao Gan Comparative Genetics of SBP-Box genes and Genome Editing Peter Huijser The Genomic Consequences of Breeding System Evolution Stefan Laurent Plant Development and Diversity Miltos Tsiantis Genetic Basis of Phenotypic Evolution Angela Hay   Associated Independent Research Group	SCIENTIFIC SERVICE GROUPS The Max Planck-Genome-Centre Cologne Bruno Huitel Central Microscopy (CeMie) Ton Timmers Protein Mass Spectrometry Service and Basic Immune System of Plants Hirofurni Nakagami	PHD OFFICE International Max Planck Research School (IMPRS) MPIPZ Graduate School POSTDOC OFFICE Postdoc Programme Fellowship Programme

DEPARTMENTS AND GROUP LEADERS WITHIN THE DEPARTMENTS

INDEPENDENT GROUP LEADERS

ADMINISTRATION AND TRAINING



Each Director is also head of a scientific department and is responsible for the scientific programme, budget and personnel of that department. Each department comprises several research groups that are led by research scientists, who in turn are responsible for the scientific programme, personnel and budget of their research groups.

The service groups provide support in technical areas that underpin the work of the scientific departments. Each service group is managed by a service facility leader, who is responsible for the services provided and the management of staff within the group. Each service group consults with a Users' Committee comprising a scientist from each scientific department and the head of the service group. Each of these groups is chaired by a Director.

The Head of Administration is responsible for managing the administration department, workshops, library and security. The administration department manages issues such as appointment contracts, the budget of the Institute, the building programme, the Institute canteen, Institute housing and maintenance of the Institute grounds.

The Institute Scientific Coordinator assists primarily the Managing Director but also all other Directors in directing ongoing scientific matters of the Institute relating to their respective area of responsibility. The PhD Coordinator, who manages the Graduate Schools and who is responsible for student welfare, is formally attached to the administrative department.

The Postdoc Coordinator, who is also attached to the administration, supports MPIPZ postdocs in their professional training and development, and offers guidance and resources on all postdoc matters.

The Faculty of the Institute comprises the Directors of the Institute, all group leaders from each department, heads of service groups and independent research group leaders. This committee meets monthly to discuss issues raised by its members. Major issues discussed have been future scientific strategy, particularly how to incorporate crop-plant research within the Institute, purchase of large pieces of equipment, and new recruitments. This committee has proven to be an important conduit for channelling the views of research scientists to the Board of Directors, and has helped create a more horizontal management structure within the Institute.

The scientific programme of the Institute is assessed every three years by the Scientific Advisory Board, which reports to the President of the Max Planck Society.

The Board of Trustees meets annually and oversees the management of the Institute. The Board also provides important links with local and national organisations within the sphere of activity of the Institute.

# Research Objectives and Major Emphases

#### **BACKGROUND AND PRESENT STATUS**

"Can plant breeding be transformed into a rational, predictive science?" This question motivates all of the research programmes at the Institute. We conduct basic molecular biology and genetics research on plants with the goal of developing more efficient breeding techniques and environmentally sound plant protection strategies for crops.

The last 15 years have seen a tremendous increase in our knowledge of the molecular mechanisms underlying plant biology. This progress is largely based on studies on model species, principally Arabidopsis thaliana. However, the deeper knowledge of the regulatory components and mechanisms controlling plant traits that has resulted from these studies has not had a sustained impact on plant breeding. The work of the Institute aims to test whether a deeper understanding of regulatory mechanisms obtained in model species will allow rational approaches to making desirable changes in selected traits in crop plants. This also requires the study of biological processes in crop plants, with particular emphasis on understanding the variation present within each species. Genetic variation is the raw material with which plant breeders work. Greater knowledge of the processes and genes that control trait variation will allow much more efficient breeding, using either marker-assisted selection or the transfer of genes to crop plants.

Even in Arabidopsis, our understanding of the regulatory mechanisms that control plant traits is limited to a patchwork of individual genes, and the connections between the proteins they encode are often poorly understood. Therefore, focused programmes have been established within the Institute to elucidate the molecular mechanisms controlling traits of agronomic importance. These programmes investigate plant-pathogen interactions and the plant microbiota (Schulze-Lefert), flowering time control (Coupland) how biological forms develop and diversify (Tsiantis) and how genetic recombination, which is at the heart of heredity, can benefit plant breeding (Mercier). All of these traits are studied within a genetic framework. In addition to intensive studies based on induced mutations and reverse genetics, natural genetic variation is exploited to provide an understanding of the natural plasticity of complex traits under the influence of quantitative genetic variation. To study this variation in more detail, molecular population genetics and genomics has become an important tool in addition to quantitative genetics. Comparative studies of the function of similar genes in different species and computational modelling help us to understand how genetic variation between species can alter conserved regulatory networks to create new structures or responses to the environment. Model plants are carefully chosen to provide meaningful comparisons with Arabidopsis, on the basis of their relatedness (Arabis alpina and Cardamine hirsuta) or because they display specific genetic complexities or properties. It is hoped that a better understanding of this Darwinian variation will teach us how known regulatory networks can be manipulated in order to create traits not normally found in a particular species. Furthermore, we have increasingly complemented our genetic approaches with biochemistry and cell biology. These methods both identify proteins that are refractory to genetic approaches and reveal further layers of regulation beyond transcriptional control. We believe that integrated approaches that bridge the boundaries



between traditional research disciplines such as genetics, molecular biology, biochemistry, cell biology and computational biology are crucial for developing a multi-scale understanding of selected plant traits.

Together, these approaches allow us to ask detailed questions. How many components contribute to a particular trait and how are their functions inter-related? How many components within the network can be used to create variation in the trait? Which of these components vary in nature and how many of them can be changed without pleiotropic effects? Can directed genetic alterations be made in crop plants to create desirable phenotypic changes in selected traits?

The Institute has developed an extensive technological infrastructure to help answer such questions. Since 2010, an integrated 'Max Planck Genome Centre' (MP-GC) has been located on our campus which provides state-of-the-art next generation DNAand RNA-sequencing services. The MP-GC provides next generation sequencing services to three core Max Planck Institutes located in Cologne, Bremen and Plön. The MP-GC continuously develops new protocols and integrates the most recent developments in genome/bioinformatics technologies. The Centre generates large-scale raw data sets for subsequent genome annotation of plant or microbial genomes and comparative genome analysis, including regulation of genome activity, by individual research scientists. In addition, the Institute has significantly enhanced its resources in the area of bioinformatics and modelling, by recruiting several group leaders with expertise on computational biology and regulatory networks.

Our technology platform in protein mass spectrometry has been greatly improved over the past few years, and in 2016 we appointed Hirofumi Nakagami as the new head of research and service group, whose research aims to deepen our understanding of plant-microbe interactions by combining advanced proteomics technologies and evolutionary biology. In recent years, we have significantly invested in both confocal light and electron microscopy in order to visualize dynamic processes at subcellular resolution or at tissue/organ level for the computational modelling of morphogenesis. Continuous development of this complex technological infrastructure is vital for a rational understanding of plant traits. This infrastructure is heavily utilised by all four departments and provides attractive training opportunities for students, most of whom will only have encountered the traditional research disciplines at their university.

### **FUTURE ORIENTATION**

The Institute's mission requires coordinated efforts to balance research in model systems and crop plants. The long-term goal of our research is to use the knowledge gained for breeding programmes and new methods in plant breeding. We have begun to extend our research activities on the reference plant Arabidopsis thaliana to its relatives, including Cardamine hirsuta and Arabis alpina. In addition, research is being conducted with Rhynchospora pubera as a representative of a species with holocentric chromosomes and the liverwort Marchantia polymorpha representing one of the earliest land-plant lineages. This enables comparative approaches that are driven by evolutionary trait analysis in a phylogenetic framework to reveal mechanisms underlying inter-species trait variation and the origin of evolutionary novelties. Across all departments, the Institute focuses its crop-related research on barley, by exploring how knowledge gained on fundamental traits and trait variation in model plants can be transferred to a crop context. An additional crop used for the transfer of insights gained from model plants and for studying crop-related traits is the tomato. We believe that future trait modelling, based on guantitative interactions of its genetic components, will expedite the transition from empirical to rational, predictive plant breeding. Widening our research to evolutionary trait analysis with phylogenetically related reference plants also opens up opportunities to understand the molecular basis of their ecological adaptations to different natural environments. Furthermore, the plant microbiota has emerged as a key player of plant-soil feedback, leaving a species-specific microbial footprint in soil substrate over generations. This footprint alters soil properties that influence the performance of seedlings, with consequent effects not only in natural plant populations and communities, but also in agricultural contexts. Together, this could provide valuable clues towards the development of novel concepts for knowledge-based agroecological plant production forms, including intercropping and crop rotation.

# Co-operation and Communication within the Institute and with Cologne University

#### INTERACTIONS WITHIN THE INSTITUTE

Numerous collaborative research projects are ongoing, between individual research groups both within and between departments. These research collaborations are vital in order to test new ideas at the interface between seemingly distinct plant processes or to enable the dissection of complex traits using different methods. Both the International Max Planck Research School (IMPRS) and the Cluster of Excellence on Plant Sciences (CEPLAS), a joint regional research and training activity together with the Universities of Düsseldorf and Cologne, provide incentives for joint PhD research projects between research groups that have complementary skills and expertise. The mission of CEPLAS is to contribute new paradigms in order to solve urgent problems in plant performance and production through exploitation of natural variation and biodiversity. A combination of evolutionary analysis and synthetic biology is applied for interdisciplinary research on four major themes: plant performance, by mapping the interface between development and metabolism, plant microbiota metabolic networks and edaphic adaptation, synthetic and reconstruction biology, and data science and data management.

### CO-OPERATION WITH DÜSSELDORF AND COLOGNE UNIVERSITIES

Groups from the Institute are participating in a Collaborative Research Centre (CRC/SFB) programme with the University of Cologne, which is funded by the Deutsche Forschungsgemeinschaft (DFG). The SFB 1403 on Cell Death in Immunity, Inflammation and Disease in plants and animals began in 2020. Several MPIPZ groups also contribute to the DFGfunded priority programmes DECRyPT (Deconstruction and Reconstruction of the Plant Microbiota) and MAdLand (Molecular Adaptation to Land). In total, these programmes fund twelve research projects in the Institute.

The Cluster of Excellence in Plant Science (CEPLAS) is a major focus of collaboration with the universities of Cologne and Düsseldorf. It incorporates retreats and monthly science meetings as well as funding professors, post-docs, students and technical assistants at MPIPZ.

The Institute's Graduate School is run in close collaboration with the regional Universities of Cologne and Düsseldorf. The International Max Planck Research School (IMPRS) includes faculties from both the Institute and the University. It provides a forum for scientific communication and improves contacts and collaborations between plant science groups in both organisations on a daily basis, giving a thorough overview of all plant research activities at the annual IMPRS retreats involving both faculties and students.

#### PH.D. PROGRAMME AND EDUCATION

Providing high-quality education for young researchers is of particular concern to the MPIPZ. We support the future of plant science by creating an environment that provides a solid base for best possible education outcomes of young scientists. This





includes the opportunity to test new ideas, novel concepts and unconventional approaches, as well as fostering creativity and scientific curiosity. We promote multilateral collaboration in an increasingly complex scientific network. Here, young scientists from all over the world with diverse scientific backgrounds find a research environment that supports their development as researchers. The Institute's research would be unthinkable without the contribution of our students. Moreover, the international character of the Institute offers valuable insights into intercultural differences that allow the students to reconsider their position in a globalised world and to prepare for the international challenges to come.

The doctoral programmes, especially the IMPRS graduate programme, also promote scientific collaboration among European institutions. Training in modern plant sciences thus contributes to the future of the whole continent. Our Research School helps to curb the loss of scientific talent and counteract the diminishing interest in plant science as a career path. This interdisciplinary approach ensures that the students not only obtain a Ph.D. degree, but also have the opportunity to learn complementary skills that will be recognised as a valuable career qualification. Throughout the entire graduate programme, each student receives scientific support from a Ph.D. Thesis Advisory Committee (TAC), as well as general support from fellow students and scientists from neighbouring disciplines. The demands of the modern scientific community are met through complementary training, including training in the communication of science and preparation for an increasingly dynamic and flexible global job market.

The statistical data for the past several years reveal a continuously high level of interest in joining our research programmes among applicants from all parts of the world. This clearly demonstrates that the Ph.D. education provided at the Max-Planck Institute for Plant Breeding Research is internationally recognised, and underlines the reputation of the MPIPZ as an attractive institution for a qualified Ph.D. education.

### DEPARTMENT OF PLANT DEVELOPMENTAL BIOLOGY

Director: George Coupland



Plant growth and development respond to a wide variety of environmental cues. This versatility enables plants to succeed in diverse environments. To control these responses, plants continuously monitor environmental parameters such as light, temperature, and nutrient availability. We study the genetic, molecular, and cellular mechanisms that control plant reproductive development in response to environmental signals. Many plants flower in response to environmental cues. This allows them to successfully reproduce in diverse environments. The evolution of different environmental responses also affects the geographical range and life history of closely related species. These effects are utilised by plant breeders to maximise crop yields in different environments.

We use computational, molecular-genetic, biochemical, and cell biology-based methods in the crucifer family in order to study reproductive development and its evolution between annual and perennial species. We aim to identify molecular mechanisms by which these plants respond to environmental cues to regulate cellular behaviour at the shoot apex during the switch to the reproductive state. We use quantitative approaches and modelling to understand the contributions of different regulatory pathways. By using population genetics methods, we study adaptation and evolution of plants across their wide geographical range. Our joint activities result in a collaborative, multi-disciplinary environment in which we can study plant reproductive development.

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### George Coupland

PERENNIAL A. ALPINA EXPRESSES TWO REGULATORY SYSTEMS THAT LIMIT REPRODUCTION IN TIME AND SPACE

### Reproductive Development and the Evolution of Perennial Life History

In all living organisms, reproduction is strictly controlled. In higher plants, reproduction starts with the formation of flowers. This transition from vegetative growth to flowering is controlled by environmental cues such as seasonal changes in daylight or temperature. The interaction between environment and floral development ensures that plants flower at the appropriate age, on specific branches, and at the optimal time of year. These responses optimise fitness in nature and are utilised in agriculture to ensure maximal yield. We study flowering in model annual and perennial species. Annual plants live for less than one year, flower profusely, and die after flowering. By contrast, perennials live for many years and flower several times during their lives. We use a combination of genetics, molecular biology, biochemistry, and imaging to understand how floral transition is controlled and changes during evolution.

### FLORAL TRANSITION IN THE MODEL ANNUAL SPECIES ARABIDOPSIS THALIANA

A. thaliana is used as a model annual plant to study the mechanisms controlling floral transition. During long summer days, it flowers a few days after germination. By contrast, during short winter days, it takes around six weeks to flower. We have defined a regulatory pathway that triggers rapid flowering under long days, as well as a second default pathway responsible for flowering under short days. The long-day pathway acts as a bi-stable switch that shifts the plant from the vegetative state (in which it forms only leaves) to a reproductive state (in which it stably forms flowers). This developmental transition occurs at the growing tip of the plant, the shoot apical meristem. On exposure to long days, the flowering pathway rapidly induces changes in the size and shape of the shoot apical meristem. Before flowering, the normally flat meristem becomes tall and domed (Figure 1). This alteration in shape involves rapid increases in cell number and size induced by components of the flowering pathway as well as dynamic changes in the expression of enzymes responsible for the biosynthesis of gibberellin (a phytohormone) in the meristem. Shortly after doming, the first floral primordia are formed. The meristem will continue to form flowers even if transferred to an environment with non-inductive short days. At the regulatory level, the switch from the vegetative state to reproduction involves removal of flowering repressors. One class of repressors includes the APETALA2 (AP2) transcription factor and its close relatives. The expression of these AP2 genes is repressed by microRNA172. Based on gene editing and confocal microscopy analyses of the gene family encoding this non-coding RNA, we recently proposed that the stable repression of AP2 expression contributes to the bi-stable switch to flowering and meristem doming. We are currently exploring how the activities of components of this switch are influenced by environmental cues to alter cellular behaviour at the shoot apex.



#### Figure 1:

Change in size and shape of the shoot meristem as it transitions from vegetative to reproductive development. Meristems of plants grown in short days for 2 weeks (left), in short days for 2 weeks and then exposed to 3 long days (middle) and exposed to a further 2 long days (right). Colour denotes cell area (red: large cells; blue: small cells). Images: Atsuko Kinoshita and Alice Vayssières.

### COMPARATIVE ANALYSIS OF PERENNIAL ARABIS ALPINA

We developed *A. alpina*, a close relative of *A. thaliana*, as a model system to study perennialism. *A. alpina* displays classical features of perennials. For example, in contrast to annuals, it restricts the duration and extent of flowering. We recently showed that *A. alpina* expresses two systems that limit reproduction in time and space and differ from those in *A. thaliana*. The first involves the MADS-box transcription factor PER-PETUAL FLOWERING 1 (PEP1), which is repressed in extended cold periods (vernalisation) to allow flowering to proceed and then reactivated to return the

plant to vegetative growth. The second system, which is based on microRNA156, acts in young meristems to prevent the flowering response to vernalisation. However, in older meristems, the transcriptional repression of genes encoding microRNA156 allows the vernalisation response to proceed. This occurs because the transcription factor SQUAMOSA BINDING PROTEIN LIKE 15 (SPL15), a target of microRNA156, is expressed and promotes the transition of older meristems to the flowering state. We aim to gain a better understanding of the function of the microRNA156/ SPL module in floral transition and how it is regulated by the age of the meristem.

#### SELECTED PUBLICATIONS

Kinoshita, A., Vayssières, A., Richter, R., Sang, Q., Roggen, A., van Driel, A.D., Smith, R.S. and Coupland, G. **Regulation of shoot meristem shape by photoperiodic signaling and phytohormones during floral induction of Arabidopsis.** eLife 2020. 9: e6066. (2020) Hyun Y., Vincent C., Tilmes V., Bergonzi S., Kiefer C., Richter R., Martinez-Gallegos R., Severing E. and Coupland G. **A** regulatory circuit conferring varied flowering response to cold in annual and perennial plants. Science 363, 409-412 (2019) Hayama R., Sarid-Krebs L., Richter R., Fernández V., Jang S. and Coupland G. **PSEUDO RESPONSE REGULATORs stabilise CONSTANS protein to promote flowering in response to day length.** EMBO Journal 36(7), 904– 918 (2017) Mateos J. L., Tilmes V., Madrigal P., Severing E., Richter R., Rijkenberg C. W. M., Krajewski P. and Coupland G. Divergence of regulatory networks governed by the orthologous transcription factors FLC and PEP1 in Brassicaceae species. Proceedings of the National Academy of Sciences of the United States of America 114(51): E11037-E11046. (2017) Hyun Y., Richter R., Vincent C., Martinez-Gallegos R., Porri A. and Coupland G. **Multi-layered Regulation of SPL15 and Cooperation with SOC1 Integrate Endogenous Flowering Pathways at the Arabidopsis Shoot Meristem.** Developmental Cell 37, 254–266 (2016)



### Pau Formosa-Jordan

WE COMBINE MATHEMATICAL MODELLING, TIME-LAPSE MICROSCOPY, AND QUANTITATIVE IMAGE ANALYSIS TO UNDERSTAND HOW COM-PLEX MULTICELLULAR DYNAMICS ARE ORCHES-TRATED IN DEVELOPING PLANT TISSUES

### Quantitative Approaches to Multicellular Dynamics in Plant Development

Throughout plant development, cells grow, divide, and become different from one another to form tissues and organs that perform specific functions. This process results from the interplay of cell signalling (mediated by gene regulatory networks and hormonal transport) and mechanical cues, together with the integration of environmental signals (e.g. temperature and light). We still have a limited quantitative understanding of the complex multicellular dynamics that occur in developing plant tissues as well as how such dynamics result in reproducible developmental outcomes.

At the MPIPZ, we study the multicellular dynamics of different plant developmental processes using a combination of mathematical modelling, time-lapse microscopy, and quantitative image analysis.

### DYNAMICS OF CELLULAR PATTERNING

In developing tissues, initially equivalent cells become different from one another and form tissues containing different cell types. The arrangement of different cell types is referred to a cellular pattern. In our group, we study how complex cellular patterns are formed in different developmental contexts as a result of the interplay between cell signalling and growth. In one collaboration with the Locke and Jönsson labs (University of Cambridge) and the Roeder Lab (University of Cornell), we found that temporal fluctuations in the cellular concentration of a transcription factor contribute to patterning the epidermis of the *Arabidopsis* sepal. Our work suggests that noise in gene expression is one factor in creating cellular patterns.

After recently arriving at the MPIPZ, we continue to investigate how patterning is generated in the epidermis. In the leaf epidermis, a combination of cellular patterns arises. Trichomes (protective hair cells), stomata (gas exchange cells), and giant cells (large cells that have been related to organ curvature control) appear scattered across the blade and are interspersed between undulated pavement cells. In each of these different patterns, we are evaluating how important noise in gene expression is for initiating patterning as well as how cell-to-cell interactions, cell growth, and cell division affect the patterning process itself. Our final goal is to understand how specific combinations of the different patterns arise.



#### Figure 1:

Microscopy image showing the arrangement of different cell types in the Arabidopsis leaf epidermis. Cell outlines are shown in yellow, and cell nuclei are shown in magenta. In this image, the stomata cells appear scattered throughout the epidermis (small yellow ellipsoid-shaped cell pairs), and a large trichome emerging of the epidermis is visible. The undulated cells are pavement cells. In our group, we study the dynamical principles driving the formation of these cellular patterns.

### DYNAMICS OF DEVELOPMENTAL TRANSITIONS

Plants undergo striking developmental transitions such as seed germination or floral induction. These processes can be understood as rich multicellular dynamical systems that are continuously modulated by environmental cues. Little is known about the dynamics of the key regulators of these complex developmental processes at the single cell and multicellular levels.

In a recent collaboration with the Leyser and Locke groups (University of Cambridge), we modelled the dynamics of the gene regulatory network underlying seed germination in order to understand why seeds exposed to the same environmental conditions germinated at different times. Our results indicate that variability in seed germination times can be understood as an emergent property of noise in gene expression and that the variability range can be modulated by the properties of the underlying genetic network for seed germination.

In collaboration with the Coupland group at the MPIPZ, we will explore how spatio-temporal patterns of gene expression at both the single cell and tissue levels drive the initiation of flowering. Our group will generate multicellular models that incorporate the interplay of the environmental cues with the molecular pathways that drive the flowering process.

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### Andrea Fulgione

WE AIM TO DECIPHER THE COMPLEX FEEDBACK BETWEEN DEVELOPMENTAL, ECOLOGICAL, AND EVOLUTIONARY PROCESSES IN SHAPING GENOMIC VARIATION IN NATURAL PLANT POPULATIONS

# Evolutionary and Computational Genomics of Natural Variation in Plants

Natural plant populations have large amounts of genomic variation that can be used as a tool to link biochemical or developmental perturbations to phenotype and organismal fitness. The configuration of variants across the genome also retains information on the origin and age of the species, the biogeographic dynamics of migration, and adaptation to the biotic and abiotic environment. We use computational and population genetics approaches to integrate molecular function, phenotype, and fitness into a systemic view of the evolution of plant genomes. We use the perennial, alpine plant Arabis alpina as a model species and the annual, cosmopolitan Arabidopsis thaliana for a contrast in life-history strategies. Understanding natural adaptive dynamics, for example in relationship to drought and the developmental response to environmental cues, can help forecast evolutionary responses to climate change and reveal variants of agricultural interest.

#### **EVOLUTIONARY GENOMICS OF A. ALPINA**

Thanks to the tools developed in recent years (e.g. the genome sequence and mutant screens for functional genes), *A. alpina* has become an excellent model perennial species for evolutionary genomics. We capitalise on these resources by re-sequencing large numbers of individual plants from populations collected in diverse environments. We use computational and population genetics approaches applied to our new population-level genomic data set in order to reconstruct adaptive and demographic processes in this species. We integrate these analyses with phenotypic data, growth chamber experiments, and the molecular validation of candidate functional variants. We aim to decipher how natural selection shapes genomic and phenotypic variation in natural populations of *A. alpina* and to identify gene variants important in adaptation to extreme environments.

### FLOWERING BEHAVIOUR ACROSS EUROPEAN POPULATIONS OF A. ALPINA

Flowering behaviour is a crucial component of plant fitness that is tuned to local environments by cues such as daylength and winter temperatures. Although flowering behaviour has been extensively studied in the model plant *A. thaliana*, perennial plants may differ in the complexity of the trait as well as in the evolutionary forces that influence it. In this collaborative project within the department, we characterise flowering behaviour across the European range of the perennial herb *A. alpina* in controlled conditions, in experimental plantations at native sites, and in natural populations.



Figure 1:

Arabis alpina growing in its natural environment. Tuning flowering behaviour to the local environment is a crucial component of plant fitness.

This comprehensive map of flowering behaviour across the European range reveals wide phenotypic variance within most populations. The southernmost populations are notable exceptions; these require prolonged cold winter temperatures in order to flower (vernalisation). Our results suggest that the adaptive significance of flowering behaviour in these populations likely stems from selection on a drought-avoidance strategy.

### EVOLUTIONARY GENOMICS OF FLOWERING BEHAVIOUR IN A. ALPINA

A fitness trade-off between the time of flowering in response to environment and the size of the plant at reproduction is expected to result in stabilising selection within populations. However, empirical observations frequently reveal directional selection favouring early flowering. Here, we address this long-standing paradox by focusing on A. alpina populations under selection for the late-flowering strategy and comparing these with other populations in which early flowering occurs. We use computational and population genomics approaches to reveal adaptive dynamics in known flowering genes and identify new players in this process. Performing these studies in the perennial A. alpina is expected to reveal factors that contribute to the complexity in flowering behaviour and its evolutionary consequences that are not detected in the annual model A. thaliana. Characterising these intriguing examples of adaptation towards an unusual phenotypic optimum will shed light on the evolutionary forces that regulate flowering behaviour in natural populations.

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### Franziska Turck

### THE BEST DECISIONS ARE BASED ON EXPERIENCE!

### Epigenetic Regulation of Plant Development

Plant genes can acquire a memory, which defines their expression state based on past experience. By stabilising an expression state for future reference, this epigenetic memory determines how strongly (if at all) genes will respond to current environmental and developmental cues. Gene memories are mechanistically linked to the packaging of nuclear DNA into a protein-containing chromatin structure. In particular, tightly packed chromatin will freeze the underlying genes in a repressed state.

The Polycomb Group (PcG) pathway creates locally restricted tight chromatin packaging. A PcG-mediated memory is metastable and autonomous at each locus. This means that each locus in each cell can acquire or lose the PcG-mediated epigenetic memory independently. Our group aims to better understand PcG-dependent gene regulation in plants. We are particularly interested in understanding how PcG proteins choose their target regions and how PcG regulation is integrated into plant development.

### EPIGENETIC MEMORY FACTOR -HOW TO KNOW WHERE TO GO

In recent years, substantial progress has been made in understanding how PcG protein complexes recognise their target genes. Epigenetic regulation is underpinned by a genetic code consisting of several distinct *cis*-elements. These recruit sequence-specific transcription factors that in turn act as PcG recruiters. These *cis*-elements are scattered across several kilobase-pair long regions and act in a combinatorial way. This individually modulates the extent of PcG recruitment in different cell types for each locus (Figure 1).

In particular, we have demonstrated the importance of one cis-element and its cognate transcription factors. We discovered that Telomere Repeat Binding Factors (TRBs), which bind to telobox motifs, directly recruit Polycomb Repressive Complex 2 to target regions. Polycomb Repressive Complex 2 is a subunit of the PcG pathway. As their name suggests, TRBs were originally described by their propensity to associate with the telomeric repeats at the ends of chromosomes. Telomeric repeats consist of direct repeats of several hundred teloboxes. There could thus be a competition between telomeres and PcG target genes for the binding of TRBs. In the coming years, we will follow up on the hypothesis that telomere dynamics influence PcG-mediated memory states.

### INTEGRATION OF INFORMATION AT THE FLOWERING LOCUS T LOCUS

Among functionally related gene groups highly enriched for PcG targets are those that promote reproductive development. The induction of flowering is one of the most crucial steps in a plant's life cycle and therefore requires integration of environmental and developmental signals into a complex genetic network. PcG targets that promote flowering are transcriptionally repressed during vegetative development. Other targets that repress flow-



ering acquire a PcG-repressed state in response to environmental or developmental cues. The Turck group has selected *FLOWERING LOCUS T (FT)* for a detailed study of transcriptional regulation. *FT* and *FT*-like genes encode mobile proteins that act as a flowering hormone in many plant species. We showed that *FT* transcriptional regulation in response to photoperiod in leaves is dependent on two distal enhancers representing accessible chromatin islands within PcG-repressed chromatin. A novel enhancer we are currently characterising is also responsible for expressing *FT* in developing siliques. We hope to elucidate how distinct distal enhancers can dynamically shift their contribution to *FT* expression dependent on tissue type and the perception of different external cues.

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#### Figure 1:

A schematic representation of the memory path of an expressed gene as it becomes epigenetically repressed. Top: an expressed gene (line) wrapped around nucleosomes (blue) that is predisposed towards stable epigenetic repression by the presence of PcG recruiting cis-motifs (telobox as red line, RY motifs as yellow line). The expressed state is in equilibrium with a non-expressed state (middle); transition between the states is regulated by the availability of activating (TF, blue circle) and repressing (TRBs, red; VALs, yellow) transcription factors (middle). If the non-expressed state persists, PcG-associated telobox and RY motifs recruit their binding factors, TRB- and VAL-family proteins, respectively. TRB and VAL-family proteins, in turn, recruit POLYCOMB REPRESSIVE COMPLEXES 2 and 1 (complex pictograms), which carry out histone modifications such as the tri-methylation of lysine 27 of histone H3 (red circle, labelled Me) and the mono-ubiquitination of lysine 121 of histone H2A (yellow circle, labelled Ub)). These modifications and protein-protein interactions between PRCs lead to a fully compacted state that is much less reversible (bottom)

### DEPARTMENT OF CHROMOSOME BIOLOGY

Director: Raphael Mercier



24 RESEARCH DEPARTMENTS

The capacity to reproduce and transmit genetic information is one of the few key features that define life. Both natural selection and breeding can optimise genetic information. They enable plants to adapt to the environment and confer beneficial characteristics for agriculture. The Department of Chromosome Biology at the MPIPZ aims to understand how genetic information is transmitted and modified over generations. The essence of heredity is meiosis, the special form of cell division that shuffles genetic information at each generation and drives the evolution of all eukaryotes - from animals to plants. The major focuses of the Department of Chromosome Biology are meiosis and meiotic recombination. However, we also explore other key sources of genomic

variation such as mutations, genome rearrangements, and polyploidisation. We develop multiple-scale approaches from molecules and cells to species and populations - and use cutting-edge technologies in microscopy, cell biology, genetics, and genomics to address questions such as: How is meiotic recombination regulated in terms of the number and localisation of genetic exchanges? How are chromosomes faithfully transmitted to gametes? How does meiosis evolve in response to changes in chromosome structure or whole genome duplication? Conversely, how do meiosis and genome reorganisation shape adaptation? Finally, we explore the possibility opened by a better understanding of meiosis and heredity to develop transformative innovations for plant breeding.

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### André Marques

THE DIVERSITY OF LIFE CAN BE FOUND AT ALL ORGANISATIONAL LEVELS OF LIVING BEINGS. UNDERSTANDING THE DIFFERENT WAYS THROUGH WHICH LIFE MANIFESTS ITSELF IS FASCINATING

### **Evolution of Meiosis**

Based on centromere organisation, chromosomes are essentially classified into two main types:

- monocentric chromosomes one centromere domain per chromosome
- holocentric chromosomes multiple centromere domains distributed genome-wide (Figure 1).

This kind of chromosomal organisation evolved several times independently in animals and plants with specific adaptations. Most of what we know about adaptations to deal with holocentric chromosomes is derived from the animal model *Caenorhabditis elegans*. A holocentric plant model is necessary; because this kind of chromosomal organisation evolved independently in different lineages, all adaptations to deal with it are also expected to be different. In my team, we aim to understand the effect of this unique chromosome structure on the genome evolution and meiotic adaptations of holocentric plants from the genus *Rhynchospora* (Cyperaceae) (Figure 2).

### GENOME ORGANISATION AND EVOLUTION OF HOLOCENTRIC PLANTS

In order to establish a holocentric plant model, we propose to sequence the genome of *R. pubera* (our model) and related *Rhynchospora* species with different genome features. With the recent advance of long-read sequencing technologies, high-quality genome assemblies of non-model species became feasible. This is because the repetitive regions of this genome can be more easily resolved. Having access



#### Figure 1:

Differences between monocentric and holocentric chromosomes, and recombination hotspots localisation.



Figure 2: Rhynchospora plants growing in the MPIPZ greenhouse.

to the genomes of these holocentric plants will not only pave the way to establishing a new model organism but also increase our understanding of how holocentricity evolved in land plants. How does chromosome structure influence genome evolution in this highly diverse group? Does meiotic recombination occur in or around centromeric regions?

### MEIOTIC RECOMBINATION AND INVERTED MEIOSIS IN HOLOCENTRIC PLANTS RECOMBINATION IN HOLOCENTRIC PLANTS

Monocentric organisms show restricted meiotic recombination at centromeres (cold regions). We therefore aim to better understand how meiotic recombination is regulated in plants with holocentric chromosomes. However, to date, no detailed studies related to this topic have been conducted in plants (Figure 1). Using cutting-edge technologies, we perform several analyses with the aim of characterising meiotic recombination rates and identifying the meiotic proteins involved in the evolution of meiotic adaptations in these organisms. Using holocentric plants as a model to understand how meiotic recombination is regulated at centromeric regions should unveil new strategies to address recombination issues in monocentric organisms.

### **INVERTED MEIOSIS**

Holocentric plants also show an interesting meiotic division with the occurrence of inverted meiosis in which sister chromatids segregate early at the end of first meiotic division. In the genus *Rhynchospora*, both chiasmatic (*R. pubera*) and achiasmatic (*R. tenuis*) inverted meiosis are observed. Cohesion, condensation, and chiasmata related genes may play an important role in the structural changes associated with inverted meiosis. Special attention will be given to these classes of genes. They will first be selected for further characterisation.

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\*equal contribution

#### DEPARTMENT OF CHROMOSOME BIOLOGY



### **Raphael Mercier**

MEIOSIS MAKES EACH EUKARYOTE – INCLUDING YOU AND THE PLANTS YOU EAT – GENETICALLY UNIQUE

### Molecular Mechanisms of Meiosis

Meiosis has two key functions in the life cycle of eukaryotes:

- it halves the number of chromosomes in the gametes
- it creates new combinations of alleles on which natural or artificial selection can act

Increasing our knowledge on meiosis has important implications in medicine. This is because meiotic errors are astonishingly frequent in human, being the major cause of trisomy and pregnancy loss. Deciphering the how and why of meiotic recombination is also crucial in our understanding of the evolution of eukaryotes. Finally, gaining control over the process of meiosis and recombination is important for plant breeders.

Four unique features define meiosis:

- · chromosome pairing
- crossovers that generate genetic exchanges
- modified kinetochore orientation to segregate homologous chromosomes
- altered cell cycle machinery allowing two successive divisions

Our group aims to elucidate the mechanisms of meiosis using *Arabidopsis* as the main model system and combining the power of genetic screening, molecular genetics, genomics, and advanced microscopy.

### MECHANISMS AND REGULATION OF MEIOTIC RECOMBINATION

Crossovers enhance genetic diversity and are essential for a proper chromosome distribution in most eukaryotes. The number and distribution of crossovers along the chromosomes are tightly regulated. There is at least one crossover per chromosome, rarely more than three, some very hot genome spots, and other regions (e.g. peri-centromeric regions) completely lacking crossovers. We use diverse genetic screens to understand the mechanisms of crossover formation and regulation. We decipher both pro- and anti-crossover mechanisms and have identified and characterised three mechanisms that greatly limit crossovers (FANCM, RECQ4, FIGL1). We now aim to identify additional mechanisms that promote or prevent crossover - notably at peri-centromeres - and which control crossover distribution among and along the chromosomes.

### **DIVERSITY OF MEIOTIC RECOMBINATION**

Beyond the analysis of recombination mechanisms in a few laboratory strains, we aim to explore the extent of the diversity of crossover frequencies in the wild as well as the underlying genetic determinants. Recent advances in DNA sequencing now make it possible to analyse recombination in multiple individuals. Because of its large geographic distribution, *Arabidopsis* is a highly suitable model for exploring the question of recombination diversity and its potential adaptive value.



#### Figure 1:

**Arabidopsis** chromosomes in meiotic prophase. Each chromosome is arranged along an axis (purple, REC8). Pairs of homologous chromosomes are tightly associated along their length and connected by the transverse element ZYP1, the C-terminal end (Green) of which is associated with the axial element. Genetic exchanges occur in this highly organised structure. STED microscopy.

#### MODIFICATION OF CELL CYCLE AND CHROMOSOME DISTRIBU-TION DURING MEIOSIS

In this project, we analyse two key features that allow ploidy reduction at meiosis:

- the control of the number of cell divisions, which must be exactly two
- the control of chromosome distribution, which separates homologues at the first division and sisters at the second division

Dedicated genetic screens and downstream functional analyses allow us to decipher mechanisms that prevent meiocytes from exiting meiosis after a single division or prevent entry into a third division. We are also exploring what regulates kinetochore orientation and sister chromatid cohesion during meiosis I and II.

### EXPANDING THE TOOL BOX FOR PLANT BREEDING

A better understanding of meiosis allows us to propose disruptive innovations for plant breeding. Through international collaborations, we have shown that crossovers can be massively increased in various crop such as pea, tomato, and rice. We also took advantage of accumulated knowledge on meiosis to engineer clonal reproduction through seeds in *Arabidopsis* and rice.

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### Polina Yu. Novikova

POLYPLOIDS ARE ABLE TO ADAPT TO BOTH EXTREME INTERNAL CHANGES AND THE EXTERNAL ENVIRONMENT

# Genomics of Extreme Adaptation in Polyploids

Polyploid organisms have more than two sets of chromosomes as a result of a whole-genome duplication or a whole-genome hybridisation. Polyploidy is often associated with extreme environments, where it can be triggered by external stress. If successfully established, it can provide an adaptive advantage in harsh conditions. The establishment of polyploid populations also entails cellular adaptation to another extreme condition - polyploidy itself. We study the interplay between adaptation and polyploidy in order to understand the molecular mechanisms of adaptation to doubled genomes as well as genomic and organismal consequences of polyploidisation and its ecological importance. In order to achieve a comprehensive understanding of polyploidy, we focus on multiple different systems including model genus Arabidopsis, popular crop species Fragaria, aquatic flowering plants Potamogeton and even an animal system - Neobatrachus.

### GENOMICS OF THE ESTABLISHMENT OF TETRAPLOID SIBERIAN ARABIDOPSIS LYRATA POPULATIONS

A. *lyrata* is a member of the genus *Arabidopsis* and has a wider geographical distribution than the model *A. thaliana* although it is shifted towards boreal regions. We explore the natural variation of ploidy in *A. lyrata* as well as the mechanisms of establishing new polyploid populations in the face of environmental challenges. The difficulty in establishing polyploids is adapting meiotic regulation to provide the faithful chromosome segregation after the whole-genome duplication – often in conditions of reduced effective population sizes, harsh environments, and competition with more common diploids. In contrast to the European tetraploid *A. lyrata* populations, the establishment of which was facilitated by the introgression of already adapted to tetraploidy *A*.

**Figure 1:** Geographical distribution of **A. lyrata** herbarium.





#### Figure 2:

Schematic representation of the reticulated structure of the Australian burrowing frogs Neobatrachus with three independent origins of the polyploid species. The tetraploid species (in green) occupy wider and more arid zones than the sister diploids (in purple).

arenosa alleles, the formation of the Siberian tetraploid *A. lyrata* is independent. This provides a rare opportunity to study parallel adaptation to whole-genome duplication in closely related populations and cross-species comparisons.

### ADAPTATION TO POLYPLOIDY ACROSS KINGDOMS: FROM PLANTS TO ANIMALS

Whole-genome duplications are characteristic for plants but are recognised as important hallmarks in the evolutionary history of all life forms, including vertebrates. In order to understand complex adaptation patterns to external and internal challenges, we apply the population genomics approach to different plant and animal systems with varying ploidy and a wide geographical spread. One such system is a diploid-tetraploid species complex of Australian burrowing frogs *Neobatrachus*, amphibians living in a desert. *Neobatrachus* is among the few exceptions of sexually reproducing polyploid animals with tetrasomic and mixed inheritance. Sexual reproduction is more frequent in animals than in plants and often requires dosage compensation, which can be disturbed by whole-genome duplication. This partially explains the over-representation of polyploidy in plants. Like in plants, the sex chromosomes of amphibians are often undifferentiated and do not require dosage compensation. This allows these animals to tolerate genome doubling more easily. Although not sessile, ectothermic animals are also susceptible to environmental stress. This can trigger the production of unreduced gametes and lead to striking associations between instances of polyploidy and harsh environments. In collaboration with the South Australian Museum, we sequence genomes of Neobatrachus frogs to assess genetic variation and identify potentially selected regions and adaptive changes in tetraploid frogs compared with diploid frogs. The aim of the project is to unravel the mechanism of polyploidy-driven adaptation in these amphibians and whether plants and animals use the same strategy to adapt their cell cycle machinery to the strong selection pressure imposed by polyploidy.

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### Korbinian Schneeberger

WE DEVELOP METHODS TO RECONSTRUCT GENOMES, FIND DIFFERENCES BETWEEN THEM AND UNDERSTAND HOW THEY CONTRIBUTE TO PHENOTYPIC DIFFERENCES

### Genome Plasticity and Computational Genetics

Since the advent of next-generation sequencing (NGS) more than a decade ago, scientists all over the world have been reconstructing the genomes of a steadily growing number of plants. In addition to model species, the genomes of most plants that are eaten, used as fodder, or are used for other purposes (e.g. oil production or human health products) have already been sequenced. Clearly, the main challenge in genomics has shifted from data generation to the reconstruction and interpretation of genomic sequences. Efficient data-handling methods must therefore be developed in order to keep up with the constantly evolving NGS technologies. Our group is focussed on advancing NGS-based analyses by addressing questions that could not be resolved previously. After all, NGS can do more than simply assemble genome sequences. We use whole-genome sequencing-based methods to:

- drive forward genetics by directly linking mutant phenotypes to genetic changes
- understand how genomes change over time (e.g. because of controlled mechanisms like recombination during meiosis).

### HAPLOTYPE-RESOLVED GENOME ASSEMBLIES

The assembly of heterozygous genomes (i.e. genomes that have differences in the maternally and paternally inherited chromosomes) remains a challenge. We have developed a method in which we use single single-cell sequencing of haploid gamete (pollen) genomes to reconstruct the sequences of the highly similar – but not identical – maternal and paternal chromosomes. After independently assembling each haplotype, the genome assembly is completed using a genetic map derived from the recombination patterns within the same gamete genomes (Figure 1). After developing this concept, we can now apply it to more complex cases like reconstructing the many genomes of polyploid plants.

### MEIOTIC RECOMBINATION

A key ingredient in linking phenotypes to their causal genetic variation is meiotic recombination. In order to understand the exact make-up of meiotic recombination, we develop methods to analyse recombinant genomes (including machine learning-based methods and single-cell sequencing) and apply these methods to understand the natural variation of meiotic recombination across populations and species.



#### Figure 1:

Overview of single-cell sequencing-based genome reconstruction (Figure taken from Campoy, Sun et al, 2020). a. Extraction of gamete nuclei. b. Single-cell genome sequencing of haploid gametes and haplotype phasing. c. Genetic map construction based on the recombination patterns in the gamete genomes. d. Long-read sequencing of somatic material. e. Separation of long reads based on genetic linkage groups using phased alleles. f. Independent assembly of each haplotype of each linkage group. g. Scaffolding assemblies to chromosome-level using the gamete-derived genetic map.

#### SOMATIC MUTATIONS

Each eukaryotic individual consists of more than one cell and thus carries more than one genome. In fact, the individuals of some species, including fruit trees, can consist of billions of cells. During the development of a tree, the genomes of some cells can undergo mutations. This implies that these individuals do not carry only one genome. We use single-cell genome reconstruction of tissue species to understand the differences in the genomes of individual trees. A better understanding of these differences (and their underlying mechanisms) can help elucidate the genetic basis of bud sport mutations, which describe the sudden phenotypic change in parts of a plant. Occasionally, such bud sports can introduce highly beneficial traits and lead to the development of novel cultivars. Knowing the genetic basis underlying bud sports can guide breeding efforts and broaden our understanding of the extensive phenotypic variation that can be observed in nature.

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#### DEPARTMENT OF CHROMOSOME BIOLOGY



### Charlie Underwood

EXPLORING MEIOSIS AND PLANT REPRODUCTION IN A WIDER NUMBER OF SPECIES WILL ALLOW US TO BETTER UNDERSTAND HOW NATURAL AND ARTIFICIAL SELECTION HAVE SHAPED THESE PROCESSES

### Meiosis in Crops

Meiosis and meiotic recombination play a key role in the generation and exploration of genetic diversity. By studying meiosis in diverse model systems, we are beginning to understand how meiosis has been shaped and modified over evolutionary time as well as the influence this has on genome structure and transmission. Previous studies of plant meiosis have largely focused on *Arabidopsis thaliana* complemented by the major grass crop species, including maize, rice, and wheat. Tomato (*Solanum lycopersicum*) is known to be a powerful system for meiotic cytology (Figure 1). Yet molecular genetic approaches remain to be fully utilised.

We are establishing tomato as an alternative dicotyledonous plant model species for studying meiosis. In recent years, tomato has re-emerged as a popular model plant species. This is partly due to the many high-quality reference genomes, efficient genome editing, and the capacity to perform comparative studies in the many wild species of Solanum section Lycopersicon. We expect dwarf rapid-cycling tomato varieties will be powerful models for performing forward genetic screens because tomato has a different evolutionary trajectory to *A. thaliana*. Genetic redundancy is thus not likely to be shared. Through a combination of reverse and forward genetics, hybrid population generation, genomics, and super resolution microscopy, we aim to better understand the molecular mechanisms of plant meiosis.

### MODULATING MEIOTIC RECOMBINATION IN HYBRID TOMATO PLANTS

The vast diversity of wild tomato species (Solanum section Lycopersicon) adds to the appeal of using tomato for meiosis research (Figure 2). Wild species are important genetic resources because they are sources of resistance to biotic and abiotic stresses in crop improvement. Over five million years of diver-



Figure 1:

An atlas of tomato meiosis by staining the DNA of spread chromosomes A) Interphase, B) Leptotene, C) Zygotene, D) Pachytene, E) Diplotene, F) Diakinesis, G) Metaphase I, H) Anaphase I, I) Dyad, J) Metaphase II, K) Anaphase II, L) Tetrad


### Figure 2:

Natural variation of leaf shape in cultivated and wild tomato plants. Top row, from left to right, S. lycopersicum cv. Moneyberg-TMV, S. pimpinellifolium (LA1478), S. pimpinellifolium (LA1547), and S. cheesmaniae (LA1039) Bottom row, from left to right, S. cheesmaniae (LA1407), S. galapagense (LA0317), S. galapagense (LA0438), and S. pennellii (LA0716)

gent evolution has separated the cultivated tomato from its distant wild relative *S. lycopersicoides* (a distance similar to that between humans and chimpanzees). Strikingly, hybrids between the cultivated tomato and *S. lycopersicoides* are viable. However, recombination suppression between homeologous chromosomes is a major post-zygotic barrier to genetic exchange between related species. We are working on tomato interspecific hybrids of varying evolutionary distances in which the parents diverged from 200,000 years up to 5 million years ago. Through this work, we will explore how varying genetic polymorphism levels between homologous chromosomes alter meiosis and the genome-wide distribution of meiotic recombination.

The modulation of meiotic pathways could be of great use in crop breeding. In our group we are interested in how the mutation of meiotic genes in hybrid tomato plants can lead to increased crossover, which can speed up gene introgression and mapping, and the total absence of crossover, which can be used to produce clonal offspring.

### NATURAL VARIATION IN MEIOTIC RECOMBI-NATION RATE IN WILD TOMATO SPECIES

Natural genetic loci can control meiotic recombination at the level of individuals and populations. In humans, genetic variation in two genes, *RNF212* and *PRDM9*, controls meiotic recombination rate and position, respectively. In my previous work, I contributed to mapping the first such locus in a plant species. We discovered that natural variation in the *HEI10* gene controls recombination rate in various *A. thaliana* accessions. Intriguingly, both *RNF212* and *HEI10* come from the same family of genes (meiotic E3 ligases), suggesting that variation in this gene family controls meiotic recombination rate in species that are separated by over one billion years of evolution.

Natural genetic loci likely also play an important role in controlling meiotic crossover rate in the tomato family. Using the wild tomato species *S. pimpinellifolium*, which is the closest related species to the domesticated tomato, we explore natural variation in meiotic recombination rate at the species level.

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# DEPARTMENT OF PLANT-MICROBE INTERACTIONS

Director: Paul Schulze-Lefert



Research in the Department is aimed at understanding how plants interact with microorganisms, and specifically how they protect themselves from pathogenic microbes while harnessing the useful properties of beneficial microorganisms. One major research theme focuses on the interplay between plant immune systems and harmful microbial invaders. By studying instances in which immunity fails and infection ensues, we aim to identify key regulatory steps in plant immunity that can be manipulated to boost plant growth and health. Understanding the establishment and functions of microbial communities associated with healthy

plants, called the plant microbiota, defines a second major research theme. We seek to understand the plant and microbial factors governing microbiota formation as well as how plants take advantage of the microbiota to adapt to nutrient-poor environments and to protect themselves from microbial pathogens. These insights are a prerequisite for the rational development of probiotics to improve plant performance. As well as genetics, molecular biology and biochemistry, we take full advantage of the power of bioinformatics to study the structures and functions of plant-associated bacterial communities.

INTEGRATIVE BIOINFORMATICS Ruben Garrido-Oter

MULTITROPHIC PLANT-MICROBE INTERACTIONS Stéphane Hacquard

RESISTANCE PATHWAY DYNAMICS IN PLANT IMMUNITY Jane Parker

INNATE IMMUNITY AND THE PLANT MICROBIOTA Paul Schulze-Lefert

STRUCTURE AND DYNAMICS OF THE PLANT IMMUNE NETWORK Kenichi Tsuda



### Ruben Garrido-Oter

WE AIM AT DEVELOPING AND APPLYING COMPUTATIONAL TOOLS TO UNDERSTAND THE EVOLUTIONARY AND ECOLOGICAL PRINCIPLES THAT GOVERN THE STRUCTURE AND DYNAMICS OF COMPLEX MICROBIAL COMMUNITIES

# **Integrative Bioinformatics**

Microorganisms often form diverse communities of interacting species, some of which also associate with eukaryotic hosts such as humans or plants. These associations are based on metabolic exchanges and can provide the host with beneficial functions, such as nutrient mobilization or pathogen protection. However, the ecological forces and molecular mechanisms that govern microbiota assembly and stability are not well understood. The ability to predict the structure and function of these complex microbial communities is critical to understanding them and harnessing their full potential. Unfortunately, microbiota research has, until recently, been limited to descriptive analyses of observations obtained from natural communities, which are constrained in their ability to directly test hypotheses and determine causality from correlations. To overcome these limitations, we focus on developing bottom-up experimental approaches and computational tools that allow for reproducible conditions and controlled perturbation of specific factors. We believe that these approaches, which enable us to design and build synthetic communities and ecosystems of reduced complexity, will provide novel insights into the genetic, molecular and ecological mechanisms that drive microbiota dynamics and functions.

### ANCESTRAL PLANT-MICROBIOTA INTERACTIONS

Amplicon sequencing of microbial communities associated to a variety of multicellular plants, ranging from ferns to flowering plants, has revealed the existence of a conserved set of microbial taxa invariably found in all host species. Recently, we have shown that this core microbiota is also assembled from the surrounding soil by unicellular algae, resulting in stable microbial consortia governed by mutualistic interactions. Employing the model algae Chlamydomonas reinhardtii in gnotobiotic and mesocosm experiments with soil- and root-derived bacteria, we show that there is a common principle driving the assembly of the core plant microbiota across host species. Our model posits that the provision of organic carbon in the form of photosynthates, which is the main limiting factor for microbial growth in soil, is a key and possibly ancestral feature that allows photosynthetic organisms, from green algae to land plants, to recruit a healthy commensal microbiota. Our observation that Chlamydomonas is capable of recruiting from a set of bacterial taxa from soil that almost perfectly overlaps with the root core microbiota, supports our model and indicates that we can use this unicellular organism as a proxy to understand first principles governing plant-microbiota interactions.

Characterization of complex microbial consortia requires the development of real-time monitoring technologies at multiple scales. By using microscopic algae as photosynthetic hosts, we have successfully managed to build simple synthetic ecosystems that show stability over long periods of time (thousands of generations). In addition, these experimental systems allow for monitoring of community structure (using amplicon sequencing) and functions (using transcriptomic and metabolomic profiling) at a very high resolution. We believe that we can employ these approaches to explore the molecular and genomic mechanisms underpinning ecosystem stability.



### Figure 1:

Conservation of the core microbiota across diverse species of plants, including the models Arabidopsis thaliana, Lotus japonicus and the green algae Chlamydomonas reinhardtii. Phylogeny inferred from aligned sequences of the rbcL gene, encoding the large subunit of RuBisCO using a Bayesian method with a strict molecular clock model. Bar plots represent relative abundances of each of the six identified core taxonomic groups across host species.

### COMPUTATIONAL TOOLS FOR THE ANALYSIS OF SYNTHETIC MICROBIAL COMMUNITIES

Reconstitution microbiota experiments have been key to a number of recent advances in the field, including insights into how the plant innate immune system interacts with commensal microbes, or how the bacterial microbiota provides the host with pathogen protection and helps mobilize nutrients from the surrounding soil. These experiments, which employ synthetic communities of microbes that have been characterized and sequenced in isolation, generate data that differs in several key factors from that produced by sampling of natural communities. Unfortunately, state-of-the-art computational approaches have not been designed to address some of these differences, creating a number of problems and preventing researchers from fully utilizing their data. In parallel with our experimental research lines, our group also focuses on the development of novel computational tools specifically designed for processing and analysing high-throughput data obtained from microbiota reconstitution experiments and large microbial genome collections. Examples of these tools, currently under development, include algorithms for error correction and high-accuracy classification of amplicon data from synthetic communities, analysis of multi-species meta-transcriptome datasets, fast de novo orthology prediction, or microbial co-occurrence network inference and analysis.

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### DEPARTMENT OF PLANT-MICROBE INTERACTIONS



### Stéphane Hacquard

PLANTS RELY ON THEIR RESIDENT BACTERIA TO PROTECT THEM FROM HARMFUL MICROBES

# **Multitrophic Plant-Microbe Interactions**

Since they first colonized land some 450 million years ago, plants have hosted bacteria and filamentous eukaryotes such as fungi and oomycetes on their roots. These microbes from different kingdoms of life are collectively known as the root microbiota and play fundamental roles in plant health. However, the extent to which interactions among these microbes modulate host fitness remains largely unknown. We aim to bridge this gap by linking bacterial, fungal, and oomycetal community interactions and testing the impact of mixed synthetic microbial communities (SynComs) on plant health.

# A FEW WIDESPREAD CORE TAXA DOMINATE THE ROOT MICROBIOTA IN NATURE

We have profiled the multi-kingdom microbial communities in roots and surrounding soil across 17 natural populations of the model plant *Arabidopsis thaliana* in Europe. We found that the local environment shapes the composition of microbial communities in soil more extensively than in roots. Variation in fungal and oomycetal communities in roots is primarily explained by differing climates between Northern and Southern Europe, in contrast with the variation in bacterial communities that is primarily explained by differences in soil conditions.



### Figure 1:

Scanning electron microscopy picture of A. thaliana root surface colonized by a large diversity of different microbes.



Figure 2:

Impact of multi-kingdom microbial consortia on plant health. Plants recolonized with filamentous eukarvotes (fungi, oomycetes) show reduced growth and survival compared to those recolonized with root bacteria. Co-inoculation of bacteria and filamentous eukaryotes promotes plant growth, illustrating that modulation of filamentous eukaryote growth in plant roots is key for plant productivity.

We observed strong local adaptation between A. thaliana populations but unexpectedly, across large distances, differences in soil properties and microbes contribute only marginally to host adaptive divergence. In contrast, a difference in climate between sites contributes strongly to local adaptation in A. thaliana. Despite major differences in soil conditions and climate between these diverse European habitats, we also observed that roots of A. thaliana often associate with the same small group of highly abundant microorganisms. We are now using these data as a basis for the rational design of low-complexity, yet representative, SynComs to dissect how host-microbe and microbe-microbe interactions modulate plant health.

### MICROBIAL INTER-KINGDOM INTERACTIONS IN ROOTS PROMOTE PLANT SURVIVAL

Having characterized the associations between healthy A. thaliana and bacteria, fungi, and oomycetes across large geographic distances, we then sought to determine whether interactions among these microbes can modulate their ability to colonize roots and promote plant health. In reconstitution experiments using germ-free A. thaliana inoculated with mono- or multi-kingdom SynComs consisting of bacteria, fungi, oomycetes, or their combinations, we observed that plant survival in the presence of fungi and oomycetes was dependent on the simultaneous presence of bacteria (Figure 1). Thus, we have discovered that a primary function of the bacterial root microbiota is to protect plants

from excessive colonization by filamentous eukaryotes. We therefore propose that the plant immune system is insufficient for effective protection against filamentous eukaryotes, and that root bacteria provide extended immune function, which is needed for plant survival in nature. Notably, plants simultaneously re-colonized with bacteria and filamentous eukaryotes showed enhanced growth and survival, illustrating that coexistence and maintenance of multi-kingdom microbial consortia in roots is likely key for plant productivity. We are now aiming to identify the bacterial genes that modulate fungal growth in plant roots, either directly through competition or indirectly through modulation of the host immune system.

### **FUTURE CHALLENGES**

Using synthetic ecological systems, we attempt to break down complex ecological processes into testable hypotheses. Particularly, we are interested in how synthetic multi-kingdom root consortia promote plant growth in the context of environmental stresses, how the immune system modulates microbial load in roots, which microbial genes are activated upon host contact and needed for host colonization, and how cooperation and competition among microbiota members contribute to maintaining balanced microbial diversity in roots. Our major long-term goal is to identify key regulatory circuits in plant-associated microbes that modulate community assembly and functions, thereby affecting host performance.

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### Jane Parker

ARABIDOPSIS PROVIDES A SPRINGBOARD TO DETERMINE HOW IMMUNITY NETWORKS VARY BETWEEN SEED PLANT LINEAGES

# Resistance Pathway Dynamics in Plant Immunity

My group is studying induced mechanisms of plant innate immunity against host-adapted pathogens. We aim to understand how plants regulate their immune responses, which pathways and molecules stop a pathogen from growing, and to what extent immunity barriers impact the establishment and activities of plant-associated beneficial microbes. Using Arabidopsis thaliana as a model host for (hemi-) biotrophic pathogens and endophytes, we're building a protein functional network for basal immunity - a broadly effective, low-level host response which slows virulent pathogen growth, and for effector-triggered immunity - an acute reaction mediated by intracellular nucleotide-binding/leucine-rich repeat (NLR) receptors recognizing pathogen disturbance. Our analysis of defence control and dynamics ranges from receptor homeostasis/activation to signalling and resistance execution. Arabidopsis provides a springboard to interrogate immunity network architectures in other seed plant groups, such as solanaceae (e.g., tomato and tobacco) and monocots (e.g., rice and barley).

### NLR IMMUNITY AND HOST CELL DEATH PATHWAYS

There are major gaps in our knowledge of how NLR receptors activate downstream pathways. Using Arabidopsis and tobacco (Nicotiana benthamiana), we discovered that a sub-family of NLR receptors (the TNLs) uses a coevolved module of EDS1-family (non-NLR) proteins with certain Helo-domain-containing signalling NLRs to confer host cell death and resistance. In Arabidopsis, this TNL 'cell death' branch is genetically and molecularly distinct from another transcriptional branch mediating basal and systemic immunity around host cell death sites (see Fig. 1). Using phylogenomics, CRISPR-mediated knockouts of gene families and in vivo assays, we are reconstituting operational resistance modules. The results are important for assembling functional innate immunity pathways and for transferring potentially useful resistance traits to a crop species, such as monocot rice. To this end, we have started to interrogate rice (Oryza sativa var. Kitaake) immunity networks with colleagues in France (Montpellier) and China (Fujian).



### Figure 1:

The eudicot model Arabidopsis and unrelated monocots have different NLR receptor panels and usage of immunity pathways, mediated by EDS1-family complexes (EDS1-SAG101 or EDS1-PAD4) with particular Helo-domain signalling NLRs (ADR1 and NRG1). These features raise interesting questions about variation in defence network architectures across seed plant groups.

### BIOCHEMISTRY OF IMMUNITY PROTEIN COMPLEXES

In parallel with the *in vivo* studies, we're working with Jijie Chai's group from the University of Cologne to obtain biochemical insights to immunity protein complexes and their molecular dynamics, by expressing and characterizing recombinant proteins. This analysis works towards a structural (atomic-level) determination of immunity modules. The experiments will provide crucial insights to how plant proteins regulate and execute immune responses.

### IMPACT OF DEFENCE PATHWAYS ON ROOT FUNGAL ENDOPHYTE ACCOMMODATION

Here, we investigate how plants discriminate between beneficial, neutral and harmful (pathogenic) microbes in the soil. In collaboration with Alga Zuccaro (Uni. Cologne), we're examining a root accommodation programme between *Arabidopsis* and fungal endophyte strains, which the host is normally able to contain and benefit from in terms of resilience to biotic and abiotic stresses. We're testing effects of different immunity/biotic stress sectors and nutrient conditions on plant-endophyte associations and outcomes. Our aim is to identify cues from the environment, plant and/or fungus which are key to accommodation versus defence decisions.

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### Paul Schulze-Lefert

UNDERSTANDING HOW PLANTS INTERACT WITH BOTH HARMFUL AND BENEFICIAL MICROORGANISMS WILL FACILITATE THE DEVELOPMENT OF SUSTAINABLE STRATEGIES TO IMPROVE PLANT HEALTH AND GROWTH

# Innate Immunity and the Plant Microbiota

Healthy plants in nature host a great diversity of commensal and mutualistic microorganisms, termed the plant microbiota. In the past several years, we have contributed to establishing plant microbiota science as a novel research field. Building on foundational work describing the composition of plant-associated microbial communities, we are now starting to learn about the principles underlying microbiota establishment and maintenance as well as the functions of these microbial assemblages. Plants have evolved elaborate immune systems that recognize the presence of potential pathogens and mount powerful immune responses that stave off infection by microbial intruders. We aim to elucidate the molecular mechanisms of plant immunity against pathogenic microbes. The question of how plants can tolerate intimate coexistence with a variety of commensal microorganisms, while maintaining effective resistance to potential pathogens, defines an important goal of our research.

# STRUCTURE AND FUNCTIONS OF THE BACTERIAL ROOT MICROBIOTA

We have shown that soil type is a major driver of the composition of root-inhabiting bacterial communities and that membership of the root microbiota is limited to only a fraction of the large diversity of microbes found in soil. We have also succeeded in isolating and culturing microorganisms from the majority of bacterial and fungal groups of the leaf and root microbiota, which has allowed us to reconstitute synthetic microbial communities that are representative of the diversity found in nature. Introduction of these synthetic communities to germ-free plants then allows us to disentangle the impact of different microbes on plant physiology. One recent breakthrough has been our discovery that the bacterial root microbiota is essential for plant survival in soil and protects plants against root-associated fungi and oomycetes. We also demonstrated an essential function of the bac-



### Figure 1:

### Microscopy image

Colonization of the Arabidopsis thaliana root surface by commensal soil-derived rhizobacteria. Arabidopsis thaliana plants expressing the fluorescent YFP protein localized at the plasma membrane (green-colored) were inoculated with a commensal rhizobacterium constitutively expressing the fluorescent BFP protein (magenta-colored). Bacterial colonization of the primary root and root hair surfaces was visualised by confocal laser scanning microscopy and is shown as a 3D projection.



### Figure 2:

Graphical abstract.

The plant-associated microbiota plays important roles in alleviating plant nutrient deficiency. In response to a shortage of available iron, plants produce and secrete coumarins, secondary metabolites that both directly promote mobilisation of iron from the surrounding soil and induce the plant's microbiota to mobilise iron for plant uptake.

terial root microbiota in improving iron nutrition of plants in iron-limiting soils.

We hypothesize that the plant immune system plays an important role in microbiota establishment and we aim to identify the underlying molecules and pathways. To understand the inner workings of the microbial assemblages, we explore metabolic diversity and metabolic interdependencies among commensal members and with the plant host as potential determinants of community stability and microbial services. These community services (traits) include indirect pathogen protection, mineral nutrient mobilization, and abiotic stress tolerance. The insights that we gain may enable the development of rational probiotics for low-input agricultural ecosystems with reduced utilization of fertilizers and pesticides.

### RECOGNITION OF PATHOGENIC MICROBES BY PLANT IMMUNE RECEPTORS

We investigate how immune receptors that reside inside plant cells detect the presence of pathogen-delivered molecules called effectors and activate powerful immune responses that terminate pathogen growth. The immune receptors are encoded by plant disease resistance (R) genes that are often used by plant breeders to select resistant crop varieties. Immunity mediated by these receptors is typically associated with host cell death at sites of attempted pathogen invasion. The diversified receptors in plants comprise a protein family, designated Nucleotide-Binding Domain and Leucine-Rich Repeat containing proteins (NLRs). Intriguingly, plant NLRs are structurally related to intracellular receptors of the innate immune system in animals and humans.

We primarily use the barley crop and the model plant Arabidopsis thaliana to address the co-evolutionary dynamics between host and pathogen at the level of populations and conservation of receptor function across different plant lineages. A particular highlight has been our recent finding that highly sequence-related MLA receptor variants in barley directly detect sequence-unrelated pathogen effectors of the powdery mildew fungus, a widespread cereal pathogen. This suggests that it may be feasible to rationally design synthetic receptors to detect pathogen effectors that escape surveillance by the plant immune system.

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### Kenichi Tsuda

UNRAVELLING GLOBAL GENE EXPRESSION CHANGES IN BOTH PLANTS AND MICROBES IS CRUCIAL FOR UNDERSTANDING THE INTERACTIONS BETWEEN THEM

# Structure and Dynamics of the Plant Immune Network

Plants associate with microbial communities called the microbiota, which contribute to plant health. However, at the same time, they come under attack from harmful microbes. Problematically for plants, these microbial friends and foes are often alike. Thus, a fundamental question is how plants discriminate between harmful and beneficial microbes in order to survive and reproduce in nature. Our major goal is to understand the interaction between plants and bacteria at the molecular and global levels.

### **BUILDING THE PLANT NETWORK**

Measuring changes in gene and protein expression is a powerful tool for disentangling complex biological processes and can point to how an organism is responding to a given condition. Global views of gene and protein expression are known, respectively, as transcriptomes and proteomes. We have investigated the transcriptomes of various plant variants compromised either in individual or multiple immune signalling pathways during infection with bacterial pathogens. Our results have shown that multiple signalling pathways together contribute to the rapid and robust changes in gene expression that are necessary for effective immunity.

### **BUILDING THE BACTERIAL NETWORK**

Knowledge on the impact of plant immunity on bacterial metabolism is rather limited. We have analysed the transcriptomes and proteomes of bacterial pathogens in planta in different host variants. The work has revealed hundreds of bacterial genes and proteins to be influenced by plant immunity. For instance, the bacterial iron acquisition pathway as well as the needle-like structure that bacteria use to inject virulence molecules into plant cells are both targeted by plant immunity to restrict pathogen growth.

# DIRECT SUPPRESSION OF BACTERIAL GROWTH BY PLANT IMMUNITY

Plants can inhibit the growth of invading bacteria, but the mechanism remains obscure. Furthermore, such defence often comes at the expense of plant growth. We discovered that an evolutionarily conserved plant protein serves as 'molecular scissors' that cut a highly conserved bacterial protein important for virulence, thereby directly suppressing bacterial growth. Artificially boosting expression of the molecular scissors increased plant resistance but did not trigger immune activation associated with plant growth retardation. Thus, our finding suggests an approach for increasing pathogen resistance without compromising plant yield.



### Figure 1:

Integration of plant and bacterial transcriptomes. Correlations between the expression of each plant and bacterial gene were calculated and visualized. The figure shows how the expression of individual plant and bacterial genes is linked.

### MAPK-MEDIATED SYSTEMIC ACQUIRED RESISTANCE

In response to local pathogen infection, plants are capable of increasing disease resistance in distant leaves, a phenomenon termed systemic acquired resistance (SAR). We have uncovered a positive regulatory loop that is critical for regulation of SAR induction. This loop consists of signalling molecules called mitogen-activated protein kinases (MAPKs), stress response transcription factors, enzymes and regulatory metabolites.

### ABIOTIC AND BIOTIC STRESS CROSSTALK

Trade-offs between stress responses to physical and biological factors are thought to contribute to prioritizing responses to one stress over another thereby increasing plant fitness in response to individual stresses. However, this does not explain if and how this crosstalk is beneficial under conditions where a plant would encounter both types of stress simultaneously, a situation which is frequent in nature. We found that the effects of immune signalling activated by physical stresses are dependent on leaf age in *A. thaliana*; this causes disease susceptibility in old leaves while young leaves are protected from these effects. We found that this leaf-age dependent crosstalk is crucial for maintaining plant fitness during combined stresses.

### **FUTURE CHALLENGES**

To understand the factors that determine the consequences of plant and bacterial interactions as well as plant and plant microbiota interactions, we are simultaneously investigating gene expression changes in both plants and their microbiota (Fig. 1).

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# DEPARTMENT OF COMPARATIVE DEVELOPMENT AND GENETICS

Director: Miltos Tsiantis



Plants show considerable morphological variation in organ shape, size, and number. The work in our department is aimed at elucidating the basis for such variation. We address two fundamental questions:

- How do plants develop and grow into complex organisms starting from a fertilised egg?
- 2. How did plant form diversify through evolution?

To answer these questions, we work at the interface of developmental genetics, evolutionary biology, population genetics, and biomechanics. This combination of approaches helps us to identify genetic networks that underpin the different aspects of morphology and understand how the balance between conservation and divergence of these networks generates diversity during evolution. One key challenge arises from the fact that an organism's form is determined by a cascade of processes that take place at different levels of organisation. The final form is the result of complex feedback loops of genetic regulation, signalling, cell proliferation patterns, and tissue growth. It thus becomes increasingly difficult to conceptualise how the processes that influence growth and patterning are combined and integrated to produce organismal form. To resolve these issues and consolidate our biological findings, we use computational approaches to help reveal fundamental principles that regulate the development and diversity of plant form. By building a predictive framework that conceptualises how biological forms develop and diversify, we gain a clearer understanding of the natural world and improve the knowledge base that underpins plant breeding.

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### Xiangchao Gan

THE CURRENT FLOOD OF RAW SEQUENCING DATA AND AGGREGATE BIOLOGICAL INFORMATION ARISING FROM THE STUDY OF THE GENOME AND ITS MANIFESTATION MAKES IT A GOLDEN AGE FOR DATA INTEGRATION AND DISCOVERY

# Bioinformatics and Comparative Genomics

A key goal in biology is understanding the genetic basis for phenotypic diversity. High-throughput sequencing increasingly enables the detection of genetic differences between individuals. Nevertheless, identifying causal relationships between genotypic and phenotypic variation remains a key focus for evolutionary biology, human genetics, and plant breeding. We are interested in developing and applying new statistical models and algorithms to genome-wide inter- and intraspecific genome comparisons in order to investigate the causal genes/loci underlying trait diversity.

### GAP-FREE CHROMOSOME-SCALE GENOME ASSEMBLY

High-quality genome assembly has wide applications in genetics and medical studies. However, achieving gap-free chromosome-scale assemblies using highthroughput sequencing can still be a challenge. We implemented GALA (Gap-free long-read assembler), a statistical framework for genome assembly through a multi-layer computer graph that identifies mis-assemblies within preliminary assemblies or chimeric raw reads and partitions the data into chromo-



### Figure 1:

Integrating the effect of SNPs, INDELs, and SVs for burden analysis leads to the identification of a novel association locus around FRI for phenotypes related to flowering time.



### Figure 2:

GALA implements a statistical framework for chromosome-by-chromosome assembly. The preliminary assemblies and raw reads are aligned against each other and encoded into a multi-layer graph. The conflicting alignments, indicated in red, are then removed iteratively by splitting the nodes involved. New edges are assigned accordingly. Nodes are then clustered into different linkage groups for independent assembly.

> some-scale linkage groups. The subsequent independent assembly of each linkage group generates a gap-free assembly free from the mis-assembly errors that usually impede existing workflows. This flexible framework also allows us to integrate data from various technologies such as Hi-C, genetic maps, and even motif analyses for *de novo* or reference-guided assemblies. GALA is also the first practical computational framework on chromosome-by-chromosome assembly.

# STRUCTURAL VARIATION STUDIES

Many structural variants are associated with trait diversity. However, the genome-wide study is still limited because of computational challenges in their reliable identification. The long-term commitment of the group to genome assembly provides us with a competitive edge in this field. For example, IMR/DENOM developed in the

group can reliably detect insertions, deletions (INDEL), and structural variants (SV) such as those two variants in FRI, which are regarded as challenging to most algorithms. This allows us to perform genome-wide analysis in order to investigate the role of INDELs and SVs. Our study showed that a multi-allelic artefact caused by inconsistent alignments was a key obstacle for testing the association of insertion and deletion polymorphisms (INDELs) as well as for integrated association methods such as burden testing. To address this problem, we developed the Irisas software. This synchronises variants and integrates the impact of SNPs, INDELs, and structural variants for burden testing. We also identified novel trait loci that previous SNPbased association studies failed to map and which contain established candidate genes. We are also applying the method to explore the influence of structural variation on the genetic architecture of trait diversity.

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### Stefan Laurent

STUDYING THE GENOMIC DIVERSITY OF NATURAL POPULATIONS IS KEY TO UNDERSTANDING BIOLOGICAL EVOLUTION

# The Genomic Consequences of Breeding System Evolution

The sequencing of the full genomes of model and non-model organisms has now become routine. We are currently witnessing how modern highthroughput technologies are revealing what is commonly referred to as the substrate of evolution: inheritable natural genetic variation. Whether those variants are single nucleotide polymorphisms, small insertions or deletions, copy number, or larger structural variants, they raise questions about their effect on phenotypic and fitness differences between organisms as well as their origin and the nature of the evolutionary processes that determined their history. Questions about the relation between natural genetic variation and the respective contributions of selection, migration, genetic drift, mutation, and meiotic recombination, are central to our group's projects. We develop and use statistical methods to summarise and interpret large-scale genomic dataset in the light of population genetics theory. We also apply our knowledge in statistics and computational biology to several research projects (e.g. developmental transcriptomics) carried out in the department of Professor Tsiantis.

### THE EVOLUTION OF BREEDING SYSTEMS

The evolution of breeding systems is of great importance for the ecology and evolution of natural organisms. In plants, variation in mating systems is often associated with different features of the life history or demography of populations. Yet reproductive strategies themselves can evolve within a single



Figure 1: The mosaic nature of genomic information The genome of sexually reproducing organisms is actually composed of a mosaic of DNA segments with very diverse ancestries. In our group, we study the properties of these DNA segments and use them to describe and understand the evolutionary history of wild plant populations. This figure is based on genetic simulations and shows the fundamental relation between the age and the length of such DNA segments, thereby highlighting the effect of meiotic recombination on genomic variability at evolutionary time-scales. The x-axis shows the length (in base pairs) of non-recombining DNA segments. The y-axis shows the age of the earliest common ancestor (in generations). Older fragments are shorter because of the cumulative effect of recombination events.



Figure 2: Deciphering cellular identity and activity using single-cell transcriptome data. Clustering analyses of single-cell transcriptome datasets based on matrix factorisation techniques can be used to study patterns of cell identity and activity in developing plant organs (here the leaf). Such analyses allow the identification of different cell types as well as the genes responsible for cellular differentiation.

species, and transitions of breeding systems are commonly observed at the phylogenetic level. In flowering plants, the most common transition is the one from outcrossing to self-fertilisation. A rich body of empirical and theoretical literature describing the selective processes behind these transitions has accumulated. Shifts to pre-dominant self-fertilisation have a profound impact on the developmental, demographic, genomic, and ecological features of a species. However, there is still no statistical method that allows us to infer the age of shifts in mating systems using genome-wide polymorphism data. In this project, we are developing a model-based approach with the aim of jointly estimating changes in rates of self-fertilisation and fluctuations in population sizes. The precise dating of transitions to self-fertilisation will allow us to better understand the climatic conditions in which these major evolutionary changes occur and to evaluate how long self-fertilising species manage to maintain themselves in fluctuating environments.

### DEVELOPMENTAL TRANSCRIPTOMICS: STATISTICAL ANALYSES OF TIME-LAPSE AND SINGLE-CELL TRANSCRIPTOME DATA

Temporal and spatial analysis of transcriptome data is a powerful approach to studying how gene regulatory networks control organ development. Transcriptomics data obtained from time-lapse and single-cell experiments thus represent an exciting opportunity to monitor early developmental pathways at the genetic level and the establishment of cellular identities. In our group, we develop statistical and computational tools for analysing time-series bulk-RNAseg and single-cell RNAseq (scRNAseq). Our current work is focused on identifying developmental-stage and cell-type specific genes as well as on the comparative analyses of developmental transcriptome data between different species. In a collaboration with the group of Prof. Miltos Tsiantis, we apply the methods developed in our lab to empirical data on the comparative analysis of leaf development between different plant species.

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### **Miltos Tsiantis**

### WE SEEK TO UNDERSTAND THE GENETIC BASIS FOR MORPHOLOGICAL EVOLUTION

# Plant Development and Diversity

# WE SEEK TO ADDRESS TWO FUNDAMENTAL QUESTIONS IN BIOLOGY:

How do biological forms develop, and what is the basis for their diversity? To address these questions, we first aim to elucidate how genotypes are translated into organismal forms through the process of morphogenesis. Second, we seek to conceptualise how the balance between conservation and divergence in morphogenetic regulatory networks yield different organismal forms during evolution. We approach these problems using genetics as well as biological imaging, genomics, and computational modelling. We believe that working at the interface of these areas will allow us to attain a predictive understanding of how biological forms develop and diversify (Figure 1).

Our research programme is empowered by the use of *Cardamine hirsuta* (hairy bittercress), a common weed we developed as a model system for studies in the evolution of development. *C. hirsuta* is related to the reference plant *Arabidopsis thaliana* (thale cress) and is also amenable to both forward and reverse genetics approaches, including efficient transgenesis. Despite their common attributes, *C. hirsuta* and A. thaliana differ in key morphological traits, including leaf shape, shoot branching, floral structure, and fruit development. Comparative studies between the two species can thus greatly enrich our knowledge of the molecular mechanisms that drive the evolution of form. The analysis of both induced and natural variation within this comparative framework coupled with broader, phylogenetically informed studies across seed plants will help us understand the genetic basis for evolutionary change.

# MORPHOGENESIS AND THE CONTROL OF FORM

Can we conceptualise morphogenesis in a predictive manner? The form of an organism is determined by a cascade of developmental processes that take place at different levels of organisation. The final form is the result of complex feedback loops of genetic regulation, signalling, and tissue growth. We aim to delineate such interactions and develop predictive models that conceptualise the process of development. We study leaf morphogenesis and patterning as well as cell fate delimitation during embryo, shoot, and root development.



### Figure 1:

Confocal laser scanning micrograph of a Cardamine hirsuta developing leaf primordium stained with propidium iodide and visualised using MorphoGraphX software.

### THE MECHANISTIC BASIS FOR MORPHOLOGICAL DIVERSITY

Is morphological diversity between species generated by a large number of small-effect genetic differences or by a small number of large-effect genetic changes? Are a handful of key genes responsible for the evolution of multiple morphological traits? How are genes that drive diversification positioned within the genetic regulatory networks that influence form? Which specific genes have changed to produce the vast degree of morphological diversity seen in nature? And how do these genes change? For example, are mutations that result in stable morphological change more likely to be found in the coding or regulatory segments of genes? Do they behave in a dominant or recessive fashion? Are they already present in populations or does their sudden appearance generate diversity?

### PATHS OF EVOLUTIONARY CHANGE

How repeatable is evolution? Does diversity in the same types of genes or pathways underlie variation in the same trait in different instances? Do inter- and intra-specific variation in morphology of the same traits arise via equivalent morphogenetic pathways? How prevalent is the role of positive selection in sculpting diverse plant forms? Can the agents of selection be identified?

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### Angela Hay



MY RESEARCH INVOLVES UNDERSTANDING HOW AND WHY SPECIES LOOK DIFFERENT FROM EACH ANOTHER. THIS IS A COMMON GOAL OF EVOLUTIONARY AND DEVELOPMENTAL BIOLOGY AND SHAPES HOW WE VIEW THE NATURAL WORLD.

# **Genetic Basis of Phenotypic Evolution**

We use two related plant species – *Cardamine hirsuta* and *Arabidopsis thaliana* – in order to study the processes by which the genetic networks underlying complex traits produce variation in those traits and diverge over time. We focus on two traits: the gain of explosive seed dispersal and the loss of petal number robustness in *C. hirsuta.* We focus on these complex traits because the direction of evolutionary change is known, and they are likely to have adaptive value for seed dispersal and pollination strategies.

### **EXPLOSIVE SEED DISPERSAL**

Plants use many different strategies to disperse their seeds. Among the most fascinating are exploding seed pods. Explosive pod shatter in C. hirsuta involves very rapid coiling of the fruit valves to launch the seeds. This movement is so fast that high-speed cameras are needed to even see the explosion. (How fast? Seeds are accelerated from zero to ten metres per second in about half a millisecond!) To gain a comprehensive understanding of this process, we needed to relate observations at the plant scale all the way down to the cellular and genetic scales and systematically link each scale with mathematical modelling. We found that at the organ scale, tension within the fruit valve generates the elastic energy required for explosion. This tension is produced by differential contraction of valve tissues through an active contraction of epidermal cell length. The explosive release of this tension is controlled at the cellular scale by asymmetric lignin deposition within endocarp b cells. This striking pattern is associated with explosive pod shatter across the Brassicaceae plant family. By bridging these different scales, we



### Figure 1:

The violent explosion of seed pods is one of the fastest movements in the plant kingdom. A multi-scale model uses interactions between cell and tissue-level processes to reproduce the explosive seed dispersal found in popping cress (Cardamine hirsuta). The background of this image shows consecutive simulations from a model of coiling fruit valves.



### Figure 2:

Live imaging and projection of APETALA1 signal onto the curved surface of **C. hirsuta** flowers shows that the **Arabidopsis** protein (top) accumulates in more cells than the **C. hirsuta** protein (bottom) in the part of the flower where petals form (dashed circle).

revealed an integrated mechanism for explosive seed dispersal that links evolutionary novelty with complex trait innovation.

### PETAL NUMBER ROBUSTNESS

Robustness is a fundamental property of developmental systems. That is, these systems are tolerant (rather than sensitive) to normal environmental and genetic challenges. For many plants, including the model species Arabidopsis, petal number is a robust trait. Cardamine hirsuta is an exception to this rule. In this plant, petal number varies between each flower. We wanted to find out what genetic changes are responsible for this difference in robustness between species. We found that evolutionary change in a single transcription factor, APETALA1, led to loss of robustness in C. hirsuta. We showed that having the Arabidopsis copy of this gene makes petal number robust. In contrast, having the C. hirsuta copy of this

gene makes petal number variable. This also holds true when we swap the gene copies between species. We explained these results using quantitative trait locus analysis. We used this method to identify the action, interaction, number, and location of regions in the genome that control petal number in C. hirsuta. When we introduced the Arabidopsis copy of APETALA1 in this context, it suppressed the action of all other genetic variants that cause petal number to vary. This genetic interaction, called epistasis, is what confers robustness. Other examples in which the action of genetic variants is context-dependent include the incidence of common human diseases and the ability of organisms to cope with climate change. In both cases, genetic differences only matter in the context of changing lifestyle or environment. Our fundamental findings about the genetics of robustness may eventually help to increase our understanding of these other processes.

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# INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS



Giving talented young scientists from diverse backgrounds the opportunity to prove themselves as leaders of independent research groups complements and expands the focus of the departments. The groups directed by these younger scientists operate outside the departmental structure and can pursue their own research topics for a period of up to five years.

Currently, six independent research groups supply expertise in functional genetics of barley stamen maturation (Ivan Acosta), the evolution of annual and perennial life histories (Maria Albani), root communication (Tonni Grube Andersen), structural biology of receptor complexes (Jijie Chai), molecular basis of adaptive evolution (Angela Hancock), genetic basis of phenotypic evolution (Angela Hay). Service groups are also independent of the departments and are headed by tenured scientists who perform research tasks, in addition to service duties which they carry out in collaboration with groups inside and outside the Institute. Ton Timmer's group manages our imaging facilities comprising a wide variety of confocal light and electron microscopic instrumentation, Hirofumi Nakagami's group provides a service on advanced protein mass spectrometry analysis, and Bruno Hüttel is head of the above-mentioned Max Planck Genome Centre (MP-GC), a core facility providing cutting edge technologies in next generation DNA- and RNA-sequence analysis.

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### INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS



### Ivan Acosta

UNRAVELLING THE MOLECULAR MECHANISMS OF STAMEN MATURATION IN CEREALS IS NECESSARY TO ADVANCE CROP BREEDING

# Functional Genetics of Barley Stamen Maturation

Anther dehiscence (i.e. opening) to allow pollen release is one of the terminal functions of plant stamens. It is a prerequisite for the timely fertilisation of male and female gametes. Therefore, it is indispensable for successful plant reproduction and critical for crop yield. Anther opening requires various events in specific cell types. These include:

- a. the separation or break down of the septum and stomium
- b. the modification of endothecium cell walls with secondary thickenings, which create forces necessary for pollen release

How these essential processes are regulated and carried out at the cellular and molecular levels is not completely defined. The current knowledge is mostly limited to the model plant *Arabidopsis* in which specific transcription factors and the hormones auxin and jasmonate are involved. A fundamental understanding of the molecular basis of anther opening should provide targets to effectively control male fertility in crops. This remains an unfulfilled need of crop breeders.

We study the cellular and molecular events leading to the maturation and dehiscence of barley anthers as a model for flowering plants in general and temperate cereals in particular. To this end, we take advantage of the historic collection of barley *male*  sterile genetic (msg) mutants to identify factors necessary for anther opening or late stamen development (Figure 1). We use microscopy to characterise the morphology, histology, and cell (ultra) structure of these mutants. We also use genetic and genomic approaches to identify the corresponding MSG genes. Functional genetic analyses include gene editing via CRISPR-Cas9 and MSG protein localisation in anthers with fluorescent reporter lines. For this purpose, we have successfully established barley transformation in an improved version of the Golden Promise cultivar. This removes all bottlenecks traditionally associated with the transformation technology. The Golden Promise line grows more robustly, provides material to study stamen maturation within 6-7 weeks, and completes its life cycle in 3 months

### MALE FERTILITY GENES IN BARLEY

*msg32* mutants undergo post-meiotic pollen degeneration associated to defects in pollen wall development. This is likely due to impaired secretion of nutrients and wall materials from the "pollen-feeding" tapetum cells. The MSG32 protein is a putative mitochondrial aldehyde dehydrogenase localised in the endothecium, middle layer, and tapetum. We propose that it is indispensable for maintaining mitochondria function under the unusually high energy demands of the tapetum. *MSG32* orthologues in *Arabidopsis* and maize are not essential for male fertility. This high-

# Wild typemsgImage: the second second

### Figure 1:

Anther dehiscence in barley. Apical pore openings are visible in wild-type (red arrow) but absent in mutant anthers (yellow arrow). Scale bars = 0.5 mm.

lights the importance of cross-species studies for a broader picture of the mechanisms of stamen development and function in flowering plants.

In another *msg* mutant, anthers fail to dehisce, and pollen grains do not accumulate starch nor swell during stamen maturation. We therefore propose that pollen expansion contributes to anther opening. The corresponding *MSG* protein participates in auxin biosynthesis and localises exclusively in pollen grains. This suggests that they are autonomous for auxin production and that auxin is essential for their maturation.

We have recently characterised two other anther dehiscence *msg* mutants affected in the separation of septum and stomium cells. The mutated *MSG* proteins are putative cell wall and pectin-degrading enzymes. At least one of the genes is specifically expressed in the septum and stomium regions. This supports the hypothesis that anther opening requires active cell wall and cell junction disassembly. We are currently identifying the gene affected in yet another *msg* mutant impaired in the accumulation of secondary thickenings in the endothecium layer.

### STRESS BIOLOGY OF STAMEN MATURATION IN BARLEY

In response to the current challenges of climate change, we are studying the effects of short-term heat or drought on stamen maturation. We plan to search for drought tolerance loci in a library of wild-barley introgression lines in the Scarlett cultivar. The heat-stress project is part of GENDIBAR, a larger consortium using the genetic diversity of Mediterranean barley to understand and utilise its adaptation to harsh environments. We are thus looking for heat tolerance loci in a set of Mediterranean barley landraces.

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### INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS



### Maria Albani

### SEVERAL TRAITS UNDERLINE THE DIFFERENCES BETWEEN ANNUAL AND PERENNIAL PLANTS.

# The Evolution of Annual and Perennial Life Histories

Most temperate and alpine species are perennials and live for several years. In my group, we use *Arabis alpina* as a model to understand the regulation of adaptive traits that contribute to the perennial life history. *A. alpina* is an alpine Brassicaceae species and, most importantly, a close relative of the annual model *Arabidopsis thaliana*. The close phylogenetic relationship between these two models allows us to address at the molecular level the regulation of traits in closely related annual and perennial species.

Annual and perennial species show differences in shoot architecture as well as patterns of reproductive development. The molecular mechanisms regulating both traits in perennials are complex and under-explored at the molecular level.

### PLANT ARCHITECTURE IN ARABIS ALPINA

We have described shoot architecture in *A. alpina* and have shown that it is organised in zones of differential bud activity and fate, including a zone of buds that remain dormant for multiple seasons. We

have shown that flowering in *A. alpina* – and specifically the temporal separation of the different steps of flowering (floral induction, flower bud initiation and anthesis) – shapes shoot architecture and ensures the maintenance of dormant buds. Exposure to prolonged cold treatment also regulates the TCP transcription factor BRANCHED 1, which functions in bud dormancy.

### THE ROLE OF FLOWERING TIME REGULATORS IN PERENNIAL TRAITS AND SHOOT ARCHITECTURE

Previous studies have indicated that flowering time regulators in *A. alpina* have acquired additional roles that contribute to the perennial life cycle. One characteristic example is the MADS box transcription factor FLOWERING LOCUS C (FLC), a key floral repressor in the vernalisation pathway. When *A. thaliana* is exposed to prolonged cold, *FLC* mRNA levels are stably silenced, thus plants can initiate flowering. The *FLC* orthologue in *A. alpina*, *PERPETUAL FLOWERING1* (*PEP1*) has acquired additional roles that contribute



### Figure 1: Shoot architecture of a flowering A. alpina plant

to shoot architecture and repress flowering in subapical axillary branches. We have further characterised the role of AaFLC/PEP1 in shoot architecture. We have also demonstrated that genotypes that lack an active *PEP1* allele show higher mortality in field conditions as well as modified seed traits such as seed dormancy and longevity. In order to identify additional flowering time regulators that regulate perennial traits, we performed an enhancer mutagenesis screen of the *pep1* mutant. Characterisation of enhancer mutants highlighted unique roles of two transcription factors that regulate flowering in *A. thaliana*, APETALA2 and TARGET OF EAT2 (TOE2). The *A. alpina* AP2 (PERPETUAL FLOWERING2) feeds into the vernalisation pathway by upregulating *PEP1* mRNA levels in axillary branches, thereby ensuring that they will continue vegetative development. The *A. alpina* TOE2 represses the rate of axillary meristem initiation and regulates the number of axillary branches available to perceive environmental cues required for flowering.

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### INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS



### Tonni Grube Andersen

THE UNDERGROUND PARTS OF PLANTS AND THEIR DYNAMIC ASSOCIATION WITH THE ENVIRONMENT IS A TREASURE CHEST OF AGRICULTURALLY RELEVANT FEATURES YET TO BE DISCOVERED.

# Root Communication at the Single Cell Level

Because roots must provide nutrients and water for the plant while also responding to potentially harmful microbes, they continually integrate diverse signals to ensure plant survival. Not only does the plant need to elicit the right response at a cellular level but the intensity of stress also varies dramatically across different parts of the root system which makes decision making extremely complex. Although we have some understanding of how plants respond and integrate such stresses, we have yet to find out how this is coordinated at the level of individual cells. Intriguingly, certain cells in the endodermis, the boundary that separates the inner vasculature from the outer layer of the root, specifically respond to certain stress situations such as low nutrient availability. This suggests that these cells, termed "passage cells", might serve as communication hubs between the root and the outer environment (Fig 1). We are trying to elucidate the function of these passage cells and use them as a model to study development and communication between the root and their physical and biological environments. To achieve this we use state-of-the-art fluorescence-based microscopy with near-native physiological set-ups that involve precise control of nutrient and light availability. We employ this with single-cell gene expression analysis and plant-microbe community studies. Combined, these approaches allow us to investigate how specialised communication occurs between plants, the soil and microbes. We mainly work with the model





plant *Arabidopsis thaliana* for which there is a highly developed genetic toolbox. However, because part of our research is focussed on how microbial associations are connected to plant transport systems, we also work with the legume *Lotus japonicus*, which forms highly specialised, mutually beneficial associations with bacteria that, in exchange for sugar, provide the plant with nitrogen.



### Figure 2:

Our vertically-oriented confocal microscope for the long-term high-resolution imaging of plant-microbe interactions

### WHAT DETERMINES PASSAGE CELL IDENTITY AND FUNCTION?

We use transcriptional and translational genetic approaches as well as state-of-the-art single-cell techniques to investigate gene expression at the level of individual cells. This will allow us to gain insight into what defines the passage cells early during their differentiation from stem cells. A similar approach applied to the fully differentiated root parts is used to determine the genes expressed in established passage cells and thereby identify which function they have in the root.

### A PLATFORM FOR HIGH-RESOLUTION, LONG-TERM IMAGING OF COMMUNICATION BETWEEN PLANT ROOTS AND THE ENVIRONMENT

The analysis of root behaviour and communication using standard protocols involves trade-offs that affect the ability to measure precise and minute changes. This complicates interpretation. Specifically, for microscopy

analysis, plants are manipulated (e.g. moved to microscopy slides), which induces stress. This results in unpredictable cellular and developmental responses. Moreover, as roots respond to gravity, an important aspect to consider is that when plants are mounted in a typical horizontally oriented microscope, the roots will try to grow downwards and after a few hours will hinder imaging. An elegant and simple solution to overcome this is to "flip" the microscope at a 90° angle (Fig. 2) and thereby allow unrestrained growth along the vertical axis. When equipped with LED light illumination and fluidic media exchange systems, such a set-up supports long-term experiments with several days to weeks of continuous imaging. We can thus assess physiological plant responses and associations between roots and individual members of the microbiome over a hitherto unachievable spatiotemporal scale.

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### INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS

### Jijie Chai



MY LABORATORY FOCUSES ON THE STRUCTURAL AND BIOCHEMICAL INVESTIGATIONS OF NUCLEOTIDE-BINDING DOMAIN (NBD) AND LEUCINE-RICH REPEAT (LRR) CONTAINING (NLR) PROTEINS WITH THE AIM OF ELUCIDATING THEIR SIGN ALLING MECHANISMS

# Structural and Biochemical Studies of NLR Proteins

NLR proteins are a large family of intracellular immune receptors conserved in both animals and plants. NLRs in animals function as pattern recognition receptors (PRRs) that detect pathogen-associated molecules patterns (PAMPs) or host-derived danger signals (DAMPs) in the cytosol. Ligand perception induces oligomerisation of NLRs. This results in the formation of cytosolic multiprotein complexes called inflammasomes that mediate caspase-1 activation. Active caspase-1 promotes proteolytic cleavage of the gasdermin D (GSDMD) substrate, thus inducing the pore-forming activity of the N-terminal domain (GSDMD-N). Plant NLRs confer specific recognition of pathogen-derived effectors and initiate effector-triggered immunity (ETI) characterised by localised cell death at the site of infection. This is known as hypersensitive response (HR). NLRs in plants are classified into two main groups based on their different N-terminal domains: a coiled-coil (CC) domain in CC-NLRs (CNLs) and a Toll-Interleukin1-receptor (TIR) domain in TIR-NLRs (TNLs). Both CC and TIR domains act as a signalling module for NLR-mediated ETI. However, CNLs and TNLs vary in their mechanisms of activating resistance. Ligand

recognition of plant NLRs is diverse and includes direct and indirect recognition as well as recognition through paired NLRs and integrated domains. Regardless of the recognition mechanisms, ligand binding is believed to induce conformational changes in NLRs and consequently promote oligomerisation.

### PROJECT 1 ACTIVATION OF A PLANT NLR RESISTOSOME

A recent NLR project involved activating CNL ZAR1 in *Arabidopsis*. ZAR1 forms a constitutive complex with RKS1 to recognise the effector AvrAC. AvrAC uridy-lylates PBL2 and thus enables the modified PBL2 (PBL2UMP) to interact with RKS1 in the preformed complex to activate ZAR1-mediated immunity. Our recent cryo-EM studies revealed ligand recognition and activation mechanisms of ZAR1, thereby providing a template for understanding plant CNLs. The activated ZAR1 forms a higher-order complex referred to as a 'resistosome'. Its structure is comparable to those of the NLRC4 inflammasome and the Apaf-1 apoptosome. In contrast with the disor-



### Figure 1:

### Structure of the ZAR1 resistosome

Overall structure of the ZAR1 resistosome in two orientations. ZAR1, RKS1, and PBL2 are shown in cyan, light blue, and deep salmon, respectively. The N-terminal  $\alpha$ -helices of ZAR1 in the resistosome shown in purple play an essential role in ZAR1-mediated immune signalling – likely by forming a channel or pore in the plasma membrane.

dered CARDs in the latter two large protein complexes, the CC domains in the ZAR1 resistosome pack against each other and form an  $\alpha$ -helical barrel. This suggests that ZAR1 and animal NLRs may have distinct signal-ling mechanisms. Indeed, biochemical and functional data suggest that a funnel-shaped structure formed by the N-terminal  $\alpha$ -helix can function as a channel or pore to mediate AvrAC-in-

duced defence responses. A more recent study by Sophien Kamoun and colleagues showed that such a mechanism is likely conserved in many CNLs. This mechanism is conceptually analogous to that used by the NLR inflammasomes for activation of the poreforming protein GSDMD.

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### INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS



### Angela Hancock

THE OVER-ARCHING GOAL OF OUR RESEARCH IS TO DISCOVER THE MECHANISMS THAT UNDERLIE ADAPTATION TO CHALLENGING ENVIRONMENTS

# Molecular Basis of Adaptive Evolution

Understanding how plants adapt to extreme climatic and edaphic factors provides knowledge that can be applied toward achieving conservation goals and increasing agricultural productivity. Our research focuses on *A. thaliana* and related species living in extreme environments. The specific environmental selection pressures and traits we study are diverse. These plants can be found in arid, edaphic, and altitudinal extremes, and traits can include drought tolerance, timing of life cycle events, photosynthetic efficiency, metal transport, stature, and regulation of genome stability and gene expression.

### **AFRICAN ARABIDOPSIS GENOMES PROJECT**

While Arabidopsis thaliana grows primarily in human-mediated environments, populations also grow naturally in some alpine environments. While 'relict' populations in Eurasia have largely been replaced by recent expansion of a weedy clade (1001 Genomes Consortium, 2016), we recently found that populations from mountain ranges in Africa and the offshore islands are native and represent ancient stable lineages (Durvasula, et al., 2017; Fulgione and Hancock 2018; Fulgione et al., 2018). We then teamed



### Figure 1:

Map of new African Genomes Project populations (red) relative to previously sequenced **A. thaliana** populations (blue: 1001 Genomes Project, yellow: Chinese Genomes).



### Figure 2:

### Arabidopsis populations in Cape Verde

**A. thaliana** populations in A) Santo Antão and B) Fogo and c) a Neighbour-joining tree showing the genetic clustering of samples from the two islands.

up with collaborators to sample new A. thaliana populations from some of its most extreme environments, including the Cape Verde Islands, Afroalpine East Africa, the Fynbos of South Africa, Saudi Arabia, Israel, and Jordan. We are leading this collaborative project to identify the genetic basis for trait variation and adaptation in a new and extended set of globally distributed samples.

# ADAPTATION IN CAPE VERDE ISLANDS ARABIDOPSIS

Cape Verde is a geographic and climatic outlier relative to Eurasian populations of *Arabidopsis* (Hancock et al., 2011). Plants in Cape Verde experience a long dry season with limited and highly variable rainfall. We collected *A. thaliana* from various locations on the islands Santo Antão and Fogo. We are now using these accessions together with Moroccan accessions and Canary Islands accessions in order to reconstruct the demographic and adaptive histories of these populations. This ERC-funded project aims to reconstruct broad-scale patterns of adaptive evolution as well as spatially and temporally varying selection within the islands.

### ADAPTATION TO TROPICAL ALPINE ENVIRONMENTS

Plants in tropical alpine environments are faced with stress from multiple environmental factors, including cold temperatures, high UV, low CO2 partial pressure, and wind. The botanist Olav Hedberg famously described the alpine tropical environment as "summer every day and winter every night". Because temperature varies diurnally rather than seasonally in this environment, it is an especially important selective pressure in this environment. This DFG-funded project makes use of repeated adaptive evolution across altitudinal gradients to identify the genetic basis of phenotypic convergence and to compare the genetic architecture across mountain ranges.

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### INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS





THE MAX PLANCK-GENOME-CENTRE COLOGNE OFFERS STATE-OF-THE-ART SEQUENCING TECHNOLOGIES AND CUSTOMISED SOLUTIONS FOR RESEARCHERS OF THE MAX PLANCK SOCIETY.

# The Max Planck-Genome-Centre Cologne (MP-GC)

The Max Planck-Genome-centre Cologne (MP-GC) was founded in 2009 as a core facility for Next-Generation Sequencing (NGS). It is open for all scientists of the Max Planck Society and provides sequencing service for different NGS technologies.

The current MP-GC services cover DNA and RNA extraction with different protocols, sample quality control, sample clean-up, sample sizing, sample fragmentation, amplicon generation, DNase/RNase digestion, rRNA depletion, library preparation for over 20 different protocols, NGS sequencing, and a wide range of bioinformatic services on regular basis. Individual requests that require manual or automated processing are also handled.

Sequencing is currently performed on several different NGS platforms. The work volume covers the whole spectrum - viruses, bacteria, fungi, plants, and animals as well as metagenomic and environmental samples (e.g. prepared from water and soil). Short-read sequencing - which enables the generation of large amounts of data at considerably lower costs - is performed on Illumina sequencing platforms. Long-read sequencing data is generated on PacBio platforms or via Nanopore sequencing. Long sequencing reads are highly advantageous when genomic sequences are assembled. They can capture short and medium length repeats without gaps, which normally prevent the correct assembly with other NGS technologies. In transcriptomics, long reads enable full-length cDNA sequencing, which facilitates the detection of splicing variants.



### Figure 1:

NGS library preparation by robotics. High-throughput library preparation for NGS at MP-GC is routinely performed by robotic systems such as the Biomek i7 in a 96-well scale to minimise hands-on time and reduce the variation associated with liquid handling. The MP-GC tailors existing protocols and designs new ones if required.

The MP-GC applies a special protocol that targets the transcriptional start site of genes (Cartolano, Huettel et al. 2016). More recently, the MP-GC started working on single cell genomics and transcriptomics by combining tools for cell sorting (FACSAria, BD) and microfluidics (Chromium box of 10x Genomics) to obtain sequencing libraries for single cells.

Because of the large amount of data generated on these NGS platforms, the MP-GC computing facility is equipped with high-performance servers, an infrastruc-


#### Figure 2:

Taxonomic classification of sequencing data of an environmental microbial sample. Sequencing reads were assigned to different species, and the results were drawn proportionally on a circle with different taxonomic ranks ('Krona' plot). A high diversity of organisms can be covered by new sequencing technologies and identified by comparing against the growing large-scale databases. Unculturable bacteria as well as viruses or even eukaryotes can be studied from such meta-genomes.

ture for data management (including an Isilon storage system), and a tape-based data archive system.

The MP-GC also includes a robotics section, which covers a range of instruments that allow highthroughput sample processing and lab automation. Robotic tasks such as advanced sample pooling, automated nucleic acid extraction, and automated NGS library preparation are routinely performed at the MP-GC. More sophisticated workflows can also be automated upon request.

A major feature that distinguishes the MP-GC from commercial sequencing providers is the flexible planning and processing of any sequencing request according to the specific needs of a scientist. In addition to applying the most recent NGS and bioinformatics technologies, this entails individual guidance and support from template generation to data analysis. Stringent quality checks of input materials and subsequent consultation for recommended procedures ensure high quality results. The MP-GC also develops and adapts library preparation protocols and automation procedures according to specific requirements. Especially when dealing with low quality/quantity samples, the MP-GC has extensive experience and knowledge. In terms of sequencing, small-scale and large-scale projects can both be realised. There are almost no limits regarding sequencing amounts. With regard to these fundamentals, the MP-GC now supports scientists from MPI for Plant Breeding Research, the MPI for Marine Microbiology in Bremen, the MPI for Evolutionary Biology in Plön and the MPI for Chemical Ecology in Jena in successfully implementing their individual NGS projects.

MPI scientists are welcome to visit the MP-GC in order to learn the methods needed to prepare samples for sequencing and use the infrastructure of the MP-GC to carry out their research projects.

## For further information, please visit our website (http://mpgc.mpipz.mpg.de)

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#### INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS



### Hirofumi Nakagami

COMBINING ADVANCED PROTEOMICS TECHNOLOGIES WITH EVOLUTIONARY BIOLOGY WILL LEAD TO A DEEPER UNDERSTANDING OF PLANT-MICROBE INTERACTIONS

# Protein Mass Spectrometry Service and Basic Immune System of Plants

Proteotype is an influential layer to explain genotype-phenotype relationships. This is because proteins are responsible for orchestrating a whole range of cell functions. Protein levels do not necessarily correlate with transcript levels. Protein functions are also regulated by post-translational modifications and protein-protein interactions, which cannot be read from genome information. It is therefore important to determine and monitor protein abundance, proteoforms, and complex formation in order to identify regulatory components and understand the molecular mechanisms controlling plant traits.

The protein mass spectrometry service unit is equipped with state-of-the-art liquid chromatography-mass spectrometry (LC-MS) systems and has been expanding its repertoire in order to offer up-todate service for measuring protein status. Recent research outcomes achieved together with a number of research groups confirm the power of proteomics for understanding plant systems.

My research group is studying evolutionary conservation and diversification of protein networks that regulate complex immune systems in plants. For this, we apply proteomic technologies to describe cell-surface receptor-mediated signalling cascades. We have also established the liverwort *Marchantia polymorpha* as a new plant model for studying plant-microbe interactions.

### PROTEIN MASS SPECTROMETRY SERVICE

Recent technological advances have allowed the identification/quantification of thousands of proteins from complex samples in a single LC-MS run. Our service unit has been establishing methods and pipelines that can routinely analyse plant materials. We are now able to identify and quantify over 5000 proteins in an untargeted manner. This enables the discovery of proteins that regulate plant traits. In combination with highly efficient phosphopeptide enrichment method, we can routinely monitor phosphorylation status of thousands of proteins. Phos-



**Figure 1:** LC-MS image

Figure 2: Marchantia polymorpha infected with microbes



phoproteomics is a powerful approach for uncovering signalling systems regulating plant responses to changing environments. We have also established workflows for a targeted quantification, parallel reaction monitoring (PRM), which allows the sensitive detection and accurate quantification of peptides/ proteins of interest in complex samples.

### MONITORING PROTEOME DYNAMICS TO UNDERSTAND PLANT-MICROBE INTERACTIONS

The apoplastic space is one of the major battlegrounds for plant-microbe interactions. However, little is understood about substance of extracellular defence mechanisms. Our study identifying proteome dynamics of *Arabidopsis* leaf apoplastic fluid after the plant was inoculated with a nonadapted powdery mildew fungus provided new insights into the mechanism by which plants cope with fungal challenges. Other studies profiling the proteome of a bacterial pathogen in *Arabidopsis* leaves allowed us to determine bacterial proteins targeted by the salicylic acid (SA)-mediated immune system. The phosphoproteomic study of microbe-associated molecular pattern-triggered immunity revealed a phosphorylation-dependent mechanism by which cysteine-rich receptor-like protein kinase 2 regulates the activity of NADPH oxidase RbohD and thereby plant immunity.

#### IMMUNE SYSTEM OF THE LIVERWORT MARCHANTIA POLYMORPHA

Extant bryophytes (liverworts, hornworts, and mosses) are the earliest diverging lineages of land plants and represent key species for understanding the origin and evolution of plant systems. The liverwort M. polymorpha is an emerging bryophyte model. We succeeded for the first time in carrying out (phospho-)proteomic analysis of *M. polymorpha*. Studying plant-microbe interactions requires the establishment of proper and diverse pathosystems. However, information on pathogenic microbes that cause disease in M. polymorpha remains very limited. We therefore isolated fungal strains from diseased M. polymorpha. By using a newly established pathosystem, we provided the first evidence that salicylic acid and the jasmonate dn-OPDA play roles in resistance against fungal pathogens of M. polymorpha.

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#### INDEPENDENT RESEARCH GROUPS AND RESEARCH OF SERVICE GROUPS



### **Ton Timmers**

PLANT MICROSCOPY: OBSERVING DETAIL TO UNDERSTAND THE WHOLE

# Central Microscopy (CeMic)

The task of the Central Microscopy service group is to consult and advise researchers on imaging methodology as well as image processing and analysis. CeMic manages the microscope equipment of the MPIPZ and trains and supervises researchers in the operation of the instruments – either individually or in the form of practical courses and workshops on topics of general interest. The imaging requirements range from the whole plant level (with objects as large as several centimetres) to molecular details (like protein complexes at the nanoscale level). To cover this size range, the entire spectrum of imaging technologies – from a simple stereomicroscope to a sophisticated super-resolution light microscope and electron microscope – is available.

### FLUORESCENCE MICROSCOPY

The fluorescent tagging of proteins has revolutionised the field of live imaging. We can now visualise cellular processes in living organisms. This allows us to monitor gene expression and protein abundance and distribution as well as interaction with other cellular components. For this, the MPIPZ has modern fluorescence microscopes with both widefield and confocal systems. For whole plant organ imaging, clearing techniques are used in combination with multiphoton fluorescence microscopy and 3D imaging processing and analysis. Fast fluorescence life time (FLIM) analysis is used to differentiate between specific and non-specific fluorescence and



Figure 1: Confocal laser scanning microscopy of cleared samples.

- A. Distribution of membrane-bound green fluorescent protein in green in the shoot apical meristem of Arabidopsis thaliana.
- B: Distribution of nuclear-localised green fluorescent protein in the flower bud of Arabidopsis thaliana.
- C: Distribution of nuclear-localised yellow fluorescent protein in red in the flower pistil of Arabidopsis thaliana.
- D. Bright field image of C.



*Figure 2: Scanning and transmission electron microscopy.* A. Barely shoot apical meristem: developing inflorescence with numerous flower primordia (SEM).

*B. Detail of barley microspore cytoplasm showing the large vegetative nucleus, one of the two generative cells, and numerous starch-containing plastids (TEM).* 

C. Prominent nucleus and vacuoles of an endothecium cell in the anther wall of barley (TEM).

D. Detail of barley microspore showing parts of the cytoplasm with starch-filled plastids, Golgi stacks, and mitochondria as well as the peculiar architecture of the different cell wall layers (TEM).

monitor molecular interactions in vivo by measuring fluorescence resonance energy transfer (FRET).

The MPIPZ has recently started to develop a light sheet microscope system that will be adapted to image living whole plant organs over a period of several days without the need for dissection. The natural state of the plant can therefore be preserved during the observations.

Two technically different systems for super resolution are available at the institute: an Airy scan confocal microscope and a STED (Stimulated Emission Depletion) microscope.

### **ELECTRON MICROSCOPY**

In microscopy, the highest magnification and resolution is achieved with transmission electron microscopy (TEM). Subtle changes in fine structure within cells or in the morphology of the cell wall can be identified and cellular constituents can be precisely localised with immuno-gold antibody labelling technology. During sample preparation, the fine cellular ultrastructure close to the native state is preserved through the use of ultra-rapid cryofixation (high-pressure freezing) in combination with freeze-substitution.

Scanning electron microscopy (SEM) is used when fine detail in the morphology of plant organs needs to be analysed. The institute has an instrument that reaches a high resolution and magnification at low voltage and which can also be operated in cryo-mode.

### RESEARCH

The principal research projects are conducted in collaboration with the research groups at the institute. External collaborations include a project on the effects of heavy metals in lime trees with the group of Magdalena Krzeslowska from Poznan in Poland and a recent collaboration on nodulation in legumes with the group of Clare Gough from Toulouse in France. In the framework of CEPLAS, collaborations with the groups of Alga Zuccaro and Gunther Döhlemann from the University of Cologne investigated plant-fungus interactions at the TEM level.

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# SERVICE AND FACILITIES: INTERNATIONAL MAX PLANCK RESEARCH SCHOOL (IMPRS)

PhD coordinator: Stephan Wagner



Attracting and educating early career researchers in the plant sciences is a key interest of the MPIPZ. Together with the universities in Cologne and Düsseldorf, the institute has been successfully running one of over 60 International Max Planck Research Schools (IMPRS) for nearly two decades. The IMPRS on Understanding Complex Plant Traits using Computational and Evolutionary Approaches recruits outstanding students from all over the world and offers various interdisciplinary and collaborative graduate projects in a stimulating environment with access to the state-of-the-art infrastructure available at all three IMPRS partner institutions. All doctoral candidates regularly meet an advisory committee of experienced experts to constructively discuss the scientific progress made in the doctoral project. This ensures a timely completion of doctoral projects and promotes networking with the committee members that typically come from various institutions. Joint IMPRS activities such as scientific retreats further strengthen the interaction between doctoral researchers and their supervisors. The IMPRS organises training in core scientific skills such as writing, presenting, and publishing and supports doctoral researchers in attending international meetings. Upon completion of the doctorate, our early career researchers are accordingly anchored in the scientific community and equipped with the necessary skills for a successful career in the plant sciences.

The IMPRS programme also provides training in transferable skills that have become increasingly relevant in the job market. Courses and workshops organised at the MPIPZ are complemented with a large number of offerings from the partner universities. Both the University of Cologne and the Heinrich Heine University of Düsseldorf have established and professionalised cross-faculty- and more specialised graduate programmes to which all of our doctoral researchers have access. Several hundred international students, including those from high profile institutions, apply to the IMPRS programme each year, thereby demonstrating the MPIPZ's reputation as an attractive institute at which to obtain a doctorate.

# SERVICE AND FACILITIES: POSTDOCTORAL OFFICE

Post-doc coordinator: Elisa Garcia Garcia

The Max Planck Institute for Plant Breeding Research is an attractive place for post-doctoral researchers to advance their careers. Here, they can acquire stateof-the art knowledge, be trained in cutting-edge technologies, collaborate with international and high creative scientists, and be part of innovative projects.

Post-doctoral researchers are an essential community for the scientific advancement of the MPIPZ and central to promoting its interactive and high-quality scientific achievements. By creating the Post-doctoral Office in April 2008, the Institute continued its efforts to become an even more attractive place for these researchers. Its main aim is to support MPIPZ post-docs in their professional training and development and to offer guidance and resources to the MPIPZ on all matters related to the post-docs.

Since April 2019, all post-doctoral researchers at the MPIPZ have been able to benefit from a Career Development Programme (CDP). This programme serves as a structured tool to facilitate communication between post-docs and supervisors, strengthens the career awareness and career management skills of MPIPZ post-docs, and provides training opportunities for post-docs.

By going through the CDP, post-doctoral researchers get conscious knowledge of the needed skills for future career success. The Post-doctoral Office provides the opportunity to fill these needs and receive the desired training. Post-docs can also benefit for the over 20 courses that the Max Plank Society organises each year.



The Post-doctoral Office organises events and outreach to increase the visibility of post-docs, provide networking opportunities, and establish a community. The events – for example, the annual MPIPZ Post-doc Symposium – deal with career topics, scientific exchange, or community building opportunities. The MPIPZ also participates in and co-organises the CRAG-JIC-MPIPZ Early Career Researchers Conference every two years. This event is part of a commitment, signed by three institutes, to promote and support the careers of post-docs and doctoral candidates in their final year.

To increase the institutional career support for postdocs, the Post-doc Coordinator offers one-on-one career counselling services. By using them, post-doctoral researchers can obtain personalised information, assistance in in making an informed career decision, and useful advice for career planning.

# SERVICE AND FACILITIES: PHD REPRESENTATIVES AT THE MPIPZ

Once a year, the PhD students at the MPIPZ elect representatives to act as spokespersons for the PhD community. There is one internal representative per department as well as one each from the associated universities. An external PhD representative is also elected. This person is the main link between the MPIPZ and the Max Planck Society PhDNet, a network of all MPS PhD students. The PhD representatives act at the interface between the PhD community, the administration, and the directors of the Institute. This group of students works closely with the PhD coordinator and is charged with bringing issues of concern from and to PhD students. Communication between the PhD representatives of different institutes within the MPS is strongly supported by the PhDNet.

Within the past two years, several improvements for PhD students have been implemented. These mainly concern equality of payment between stipend and contract holders. One big achievement was designing and circulating an MPIPZ-internal PhD Survey in the past two consecutive years. This allows the PhD students to paint a picture of their respective situations. Among other things, the survey revealed a discrepancy in number of holiday days between different contract holders. In 2019, PhDNet successfully negotiated with the General Administration after identifying the same issue at the MPS level. This resulted in ensuring 30 days of holiday for every PhD contract holder. Just recently in early 2021, in a joint effort of the PhDNet, this increase of annual holidays was followed by a raise of MPG PhD-contracts from 50% to 65% E13 salary. Another important achievement made in close collaboration with the PhD coordinator was establishing funding possibilities for online courses and workshops, thereby providing an even greater pool of high-guality workshops beyond the ones organised on site. PhD representatives were also highly involved in organising the first Mental Health Awareness Week at the MPIPZ. This included several engaging lectures and workshops. Parallel to scientific interests, PhD representatives also support the social aspect of PhD community life by organising and supporting joint PhD meetings and social activities such as barbecues, sporting events, and others. Sometimes scientific and social engagement of the PhD representatives go hand in hand, as they helped to initiate a MPIPZ wide Interdepartmental Researchers Seminar (MIRS) series in early 2021. The MIRS aims at providing a relaxed environment for the employees of the institute to discuss science and to connect. As highlighted by these examples, the PhD representatives actively work as a team to promote the interests of the PhD community about the community and individual level at the MPIPZ.



# SERVICE AND FACILITIES: PIM – POST-DOC INITIATIVE AT THE MPIPZ



The PostDoc Initiative at the MPIPZ (PIM) is a group of post-docs that represents the interests of post-docs at the institute as well as within the Max Planck Society. Its main aim is to offer information and arrange courses intended to further the career development of the young scientists working at the MPIPZ. We also discuss topics related to equal opportunities and interactions between post-docs and their principal investigators. All post-docs of the MPIPZ are encouraged to share their ideas, concerns, and suggestions with the PIM. The PIM was founded in November 2007 and provides a platform for the integration, education, and networking of post-docs. We receive full support and financial aid from the MPIPZ. This allows us to sustain a large variety of activities. We also collaborate with the post-doc coordinator who was appointed in 2018. This allows us to efficiently organise larger events and more diverse courses. The PIM regularly organises classes and workshops on statistics, figure design, project

management, scientific writing, and many other topics. We invite and host speakers for the institute's weekly seminar series in order to offer opportunities for scientific discussions with international experts. We also initiate collaborations with labs of interest from other countries. The PIM also organises an annual post-doc symposium, where post-docs can meet, network, and initiate collaborative projects across departments. In addition to scientific activities, the PIM regularly arranges get-togethers and barbecues in order to facilitate the integration of new arrivals.

## SERVICE AND FACILITIES: WISSENSCHAFTSSCHEUNE PRESS AND PUBLIC RELATIONS

Wolfgang Schuchert



The WissenschaftsScheune is a project of the MPIPZ public relations. The aim of the WissenschaftsScheune is to spark curiosity about plant science. It gives the public an opportunity to experience many aspects of plant science and offers a broad spectrum of issues from basic research to sustainable agriculture. Its consists of a farm building and the demonstration garden. The science barn (500 m2) is located on the farm that adjoins the institute and provides space for presentations, exhibitions, and interactive displays with a replicated laboratory area. In the demonstration garden (6000 m2), more than 100 crops and wild forms are cultivated in small plots surrounded by biotopic structures: a sandy viewing hill, dry stone walls, flower strips, nesting aids for wild bees, bee hives, and an orchard with old varieties. The barn and garden are the central part of an agricultural park, the Landschaftspark Belvedere, initiated by the Cologne City Council and the state of NRW together with the MPIPZ.

The WissenschaftsScheune offers special programmes for primary schools, secondary schools, and adults (students, retired persons, associations, and journalists). We combine indoor and outdoor activities and use various media and tools designed for the various target groups. Special brochures and films of our series "WiS Begierig" are available for critical reflection after visiting the Wissenschafts-Scheune.

#### PROGRAMMES FOR PRIMARY SCHOOLS INCLUDE:

- Our daily bread food crops
- Plants used for things other than food
- Potatoes and their uses
- · Cabbage and its relatives
- What causes diseases in plants
- On the Amerindian Trail
- · Flowery meadow and wild bees
- Life in our soil

### FOR THE SECONDARY SCHOOLS, TOPICS INCLUDE:

- DNA-Isolation from plants
- Origin of our crop plants
- Biodiversity of the crops
- The role of photosynthesis
- The Mendelian Laws of Heredity

# GROUPS OF ADULTS ARE MAINLY INTERESTED IN:

- The research at the MPIPZ
- The Future of genetic engineering
- Evolution and domestication
- Guided tours of the institute and the science barn with discussion of current topics concerning research, agriculture, and the environment

With our new project "Agrobiodiversity – what role does the soil play?", we intend to communicate the importance of the soil for a sustainable agriculture in connection with the existing biodiversity stations with the focus of biodiversity above the soil surface. Taking into consideration the growing importance of the research of plant microbe interactions – one of the topics of the institute – we aim to explain the soil structure, making the diversity of soil life tangible and illustrating the influence of soil fertility on plants. For preparation and follow up a short film, and a brochure on the soil project are available. Visits can be booked online on our homepage (www. wissenschaftsscheune.de). A visit usually lasts up for up to three hours, deals with two selected plant topics, and includes an introduction to the MPS for adults. In 2018 and 2019, roughly 3000 people visited the WissenschaftsScheune and the MPIPZ as part of special events like Girls' Day, Cologne Children's University, and the open house.

When it comes to general PR activities the institute cooperates with various committees and organisations. It is represented in relevant working groups such as the Kölner Transferrunde der Industrieund Handelskammer (Cologne Exchange Forum of the Chambers of Industry and Commerce) and the Kölner Wissenschaftsrunde (Cologne Science Circle). We are also actively involved in training courses for group leaders and biosafety representatives arranged by the University of Cologne. On the Institute's homepage, press releases documenting scientific highlights are listed chronologically so that journalists can use these for reports. Special events like the weekly TATA Bar get-togethers organised by our PhD students, the Summer Party, or sporting events such as the institute's Run promote internal communication between staff and students and contribute to a pleasant working atmosphere. One highlight was the Open Day in summer 2018.



# SERVICE AND FACILITIES: IT SERVICES

Manager: Winfried Maus



The IT Services group focuses on customer-oriented services and currently consists of five IT platform and network specialists, three IT system integration trainees, and the head of the group. The MPIPZ hosts a vast and diverse networked IT infrastructure, including:

- a hybrid storage cluster consisting of redundant disk arrays and a robotic tape library
- a High-Performance Compute (HPC) cluster
- multiple clusters of virtualisation servers for hosting various services:
- · email and collaboration services
- custom web services
- · fax, print, and file sharing servers
- electronic laboratory notebooks (ELN)
- · archives of microscopy images
- · remote access facilities

Modern high-performance microscopes come with directly attached computer workstations and large local storage systems that necessitate the transfer an immense amount of microscopy data to the central storage system of the institute. The microscopy workstations also require constant special technical support and maintenance. The IT team assists the central microscopy group with this. The buildings of the campus use many computer based, networked control systems. These are operated by the building technicians. The IT team also works closely with the building technicians, provides IT-related support, discusses further developments, and provides technical input for the IT requirements of new construction projects.

The IT Services group offers consultation on the choice of computing equipment, assists with all procurement procedures, and provides all levels of technical support to the scientists and staff of the MPIPZ.

The further development of the campus IT infrastructure is planned in close cooperation with the Board of Directors, the Bioinformatics Steering Committee (BISCOM), the Sustainable Research Initiative, the Senior Scientific Research Council (SSRC), and the greenhouse group. The group is also connected to and in constant exchange with other Max Planck Institutes. The group actively helps with individual scientific IT projects on request. Notable examples include the Greenhouse management software for ordering space for plant trays and pots, the fertility management system (using plant sensors), and IT-related aspects of new high-performance microscopes and their extended storage and network requirements.

# SERVICE AND FACILITIES: LIBRARY

Librarian and Web Content Manager: Britta Hoffmann

The Library of the Max-Planck-Institute for Plant Breeding Research provides literature, electronic media, and information services to its scientific staff and guests. The collection is focussed on the fields of research covered by the departments and the research groups at the institute.

The MPIPZ-Library, together with the Max-Planck-Digital Library of the Max-Planck-Society, provides a broad selection of electronic journals and other scientific information resources such as e-books and databases. The institute pays for this service with 0.9% of its budget. A scientific committee supports the library in questions related to the research needs.

In 2020 the library holdings comprise approx. 23,000 printed journal-volumes and 5,500 monographs. The printed holdings can be searched in the online catalogue. At the moment, around 36,000 journals are available electronically. The library offers an entry point to all licensed and free e-journals. In addition to this the library can obtain all literature not available on site either electronically or as a hard copy. For this, users can fill out a form available via the Intranet. The request is then forwarded directly to the original paper or, if not available, to an ordering database.

The library supports scientists in all aspects of information retrieval and open access publishing. It is the interface to the Max Planck Digital Library, which negotiates central contracts with publishers. These contracts enable the scientists of the Max Planck Society to publish in open access journals free of charge. This is currently possible in over 8000 journals.



The library also collects all institute publications for institutional self-archiving of research output on the publication repository of the Max Planck Society (MPG. PuRe) and makes them available on the web site.

### **TECHNICAL EQUIPMENT:**

The 14 computer workstations have been updated in 2020. There are also eight additional working places for private laptops and two separate rooms equipped with computers available for temporary staff.

# SERVICE AND FACILITIES: GREENHOUSE MANAGEMENT

Manager: Aristeidis Stamatakis



The greenhouse team cultivates several plant species (model plants, rice, maize, tomatoes) for scientific experiments in close cooperation with the scientists. The staff includes nine gardeners, the technical supervisor, and the greenhouse manager and handles around 3500 culture orders per year.

### THE FACILITIES INCLUDE:

- New greenhouses: The total area is 4,000 m<sup>2</sup>. There are 24 8 × 8 cabins with cooled conditions as well as 10 8 × 8 and four 8 × 16 cabins with uncooled conditions.
- Basta greenhouse: Three 9 × 9 cabins with cooled conditions, each one used mainly for herbicide applications.
- Old greenhouse: Five cabins with a total area of 400 m<sup>2</sup> (under renovation).
- Two Saran greenhouses (total area 300 m<sup>2</sup>) in which plants can be grown under natural conditions over a prolonged period.
- There are also 21 x 15 m<sup>2</sup> cold frames and a field with automatic watering that are used for small outdoor experiments.
- 70 growth chambers and 17 walk-in chambers are distributed in four halls. Some of them are equipped with new-generation LEDs.

In order to more efficiently support scientists, a quality assurance/quality control system have been implemented. For this purpose, a special greenhouse management software has been developed. Using bar codes, the cultivation period as well as the exact position of each tray in the cultivation facilities can be traced. In addition, all plant treatments are recorded, and scientists are informed online about them.

During the last years, special attention has been given to the biological control of pests and disease as sustainable forms of irrigation and nutrition management are applied. Beneficial insects are released periodically. Ricinus communis plants are also cultivated in several greenhouse cabins in order to promote the accumulation of natural enemies. Cork is applied to the top of the pots to prevent egg laying by fungus gnats and other pests. In order to provide the roots with ideal moisture content, irrigation is carried out by means of capillary mats. Nutrients are fully controlled by specific formulas for every species and plant development stage. During the last months, a special sensor project has been developed to record light intensity, soil moisture, and soil electrical conductivity in order to maintain ideal root conditions and minimise stress on the plants.

For the cultivation in trays and various pots, approx. 400 m<sup>3</sup> of standardised soil substrate are needed per year. To fulfil the high legal safety requirements, transgenic plants (including seeds and soil material) are subjected to hot damp sterilisation. This sterilisation as well as the cleaning of planting trays after experiments and the autoclaving of greenhouse consumables is carried out in a new separate building.



# DIRECTIONS AND CONTACT: HOW TO GET TO THE MPIPZ

#### **BY CAR**

- Highway A1 (north): Take the Bocklemünd exit (# 102), turn left at crossroads, follow Venloer Straße, direction Köln-Zentrum. After approx. 2 km turn right at crossroads, follow "Militärring". After approx. 1 km turn right and follow the signs to the Max Planck Institute.
- Highway A1 (south): Take the Lövenich exit (# 103), turn right at crossroads, follow Aachener Straße, direction Köln-Zentrum.
- After about 1 km turn right towards A1 (north), A57 (north), Ossendorf, (Militärring).
- Turn left at next intersection (T crossing), take the third exit and follow the signs to the Max Planck Institute.
- If you are using your navigation system, please note that to reach the Institute from the right direction you must take the approach via "Militärring" and "Gregor Mendel Ring". Only this route allows you to turn on Belvederestraße to reach the Institute.

#### **BY TRAIN**

- Arrival at Cologne main train station (Köln Hauptbahnhof)
- Take Underground #5 (Ossendorf) to Subbelrather Straße/Gürtel
- Then take bus #141 (Vogelsang) or bus #143 (Bocklemünd) from the stop on Subbelrather Straße (on the other side of the intersection) to stop Goldammerweg.
- Walk (for approx. 15 min.) straight on Vogelsanger Straße and Carlvon-Linné-Weg (cross railway land motorway, pass farm on the righthand side, cross Belvederestr.).

#### **BY PLANE**

- Cologne/Bonn Airport: Take S-Bahn S13 or train (Regionalbahn RE8) to Cologne main station (Köln Hauptbahnhof). Proceed as described above.
- Düsseldorf Airport: Take S-Bahn S7 to Düsseldorf main station (Düsseldorf Hauptbahnhof), then train (IC, ICE, RE, RB) to Cologne main station. Proceed as described above.

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The MPIPZ is continually growing and evolving. For up-to-date information please see our website: www.mpipz.mpg.de

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