Thesis topics – Laboratory of Genetics

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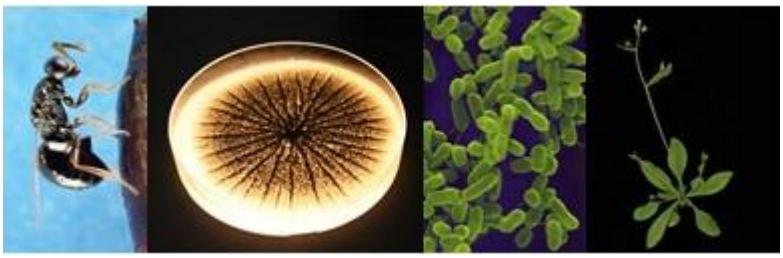

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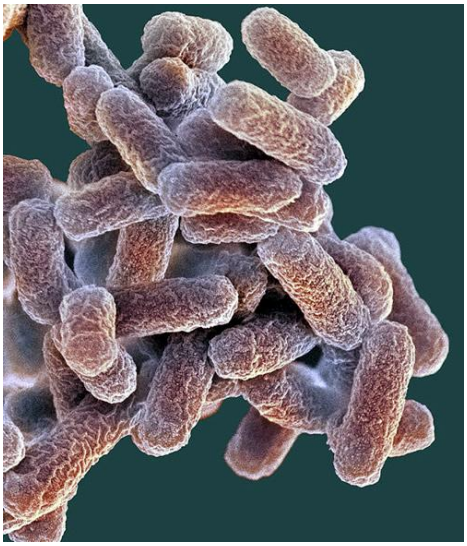
1. The predictability of microbial evolution

a. Question: Which factors determine the dynamics and repeatability of genetic and phenotypic evolution? Specifically, how does the shape of the fitness landscape and population dynamic parameters, as population size, mutation & recombination rate, affect the process of evolution?
b. Methods: Evolution experiments with the bacterium *E. coli* of varying population size, mutation & recombination rate and selective conditions; gene and genome sequencing, MIC assays, competition experiments, statistical analyses.
c. Organism: *Escherichia coli*



E. coli bacteria

2. Determinants of antibiotic resistance pathways

a. Question: Which environmental and genetic factors determine the mutational pathways to antibiotic resistance?
b. Methods: In vitro and in vivo evolution experiments with the enzyme TEM-1 beta-lactamase and its bacterial host *E. coli*, possibly using millifluidic technology; sequencing, expression assays, MIC assays, competition experiments.

Organism: *Escherichia coli*

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3. The evolution of niche construction

a. Question: How does evolutionary change affect future evolution via changes in environmental conditions?
b. Methods: In vivo evolution experiments with bacteria or fungi; strain construction; competition experiments and assays of fitness components under various environmental conditions.
c. Organism: *Aspergillus nidulans*, *E. coli*

Contact: arjan.devisser@wur.nl

What is the role of bacteria in fungal resistance to medical and agricultural azoles?

Research Questions:

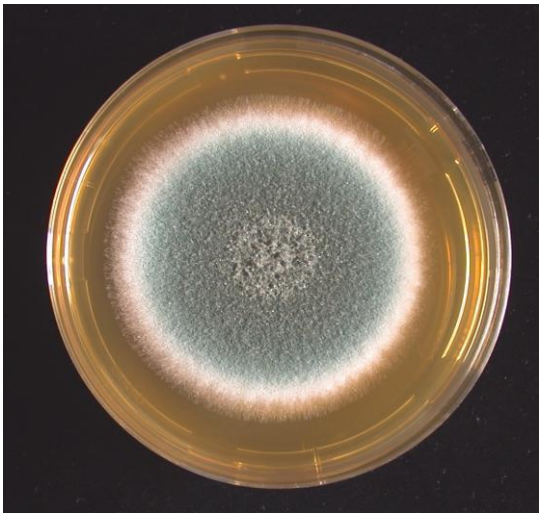
What is the cross resistance against medical and agricultural azoles and azole-like volatiles produced by bacteria?

Is the cyp51A mechanism involved in resistance to bacterial azole-like volatiles?

Techniques: MIC-assays, sequencing, ergosterol assays (HPLC)

Organism(s): *Aspergillus fumigatus*, azole-producing soil-bacteria

Contact: fons.debets@wur.nl



Aspergillus fumigatus fungus growing on a petri dish

On the role of heterokaryosis in the emergence of azole resistance in fungi

Research questions

What is the level of heterokaryon (in-)compatibility in A.fumigatus isolates from hospitals and the environment?

What is the occurrence and stability of natural/induced heterokaryons?

How does the nuclear ration of a heterokaryon change to the changing environment?

Techniques: growth experiments, sequencing/bioinformatics

Organism: *Aspergillus fumigatus*

Contact: fons.debets@wur.nl

Genetics of self/non-self recognition in button mushrooms (Champignon)

Research Question:

What is the interaction between compatible and incompatible varieties of *Agaricus bisporus.*

Which chromosomes of Agaricus bisporus contain *vic*-genes?

Techniques: Fluorescence microscopy of differentially labelled nuclei; mapping *vic*-genes by compatibility tests between chromosome substitution lines

Organism(s): *Agaricus bisporus* (button mushroom)

Supervisors: Anton Sonnenberg, Karin Scholtmeijer & Fons Debets

Contact: fons.debets@wur.nl

Evolutionary properties of mycotoxin production by filamentous fungi: field trips in Zambia and experiments in Wageningen

Filamentous fungi can contaminate maize and nuts, staple foods in Zambia. Contamination is through the production of mycotoxins: highly toxic compounds produced by Aspergilli. In this thesis project, you will travel to Zambia to do field sampling at markets and depots where maize and nuts are sold and stored. At the University of Zambia, you will isolate fungal cultures that you will further analyse for toxin production in Wageningen. Toxin production is coded for by a gene-cluster. You will study the presence and properties of this cluster. Toxins are thought to have a benefit for fungi in defending its ecological niche to other fungi. You can study whether this is indeed the case in short and long term ecological and evolutionary experiments.

Fitness effects and epistasis of beneficial mutations

Beneficial mutations are the driving force for evolution and have been subject to numerous theoretical studies. Since beneficial mutations are rare, they are difficult to study experimentally. In a thesis project, you can use the filamentous fungus Aspergillus nidulans in an environment that reduces its fitness to study beneficial mutations. The spatially structured growth of this fungus on a surface directly reveals the presence of beneficial mutations and allows an experimental study on the distribution of available beneficial mutations versus the distribution of mutations that actually

get fixed. Epistasis of beneficial mutations will be measured by combining several spontaneous beneficial mutations into one background.

Microbial ecology of Zambian fermented products: evolution of microbial communities

Analogous to well-known fermented products such as yoghurt, wine, beer and sauerkraut, Zambia has many endogenous fermented products. The fermentation processes are ancient and result in safe products with an increased nutritional value and an increased shelf life than the raw materials. The microbiology of fermentation in these traditional foods is largely unknown and represents an outstanding opportunity for addressing both fundamental and practical questions of wide concern and is very suitable for a thesis project. In particular, why are the microbial communities in these fermented products so stable and what mechanisms prevent the invasion of novel strains such as pathogenic bacteria. These questions represent long-standing issues in community ecology and evolutionary biology, namely, what makes natural microbial communities both diverse and stable over the long-term.

Evolution of antagonistic interactions, the evolution of bacteriocin production by Pseudomonas aeruginosa

Bacteriocins are compounds produced by bacteria and are directed to closely related species. It is known that these compounds have an important ecological function and influence many microbial interactions. Bacteriocins have been suggested as novel antibiotics in medicine. In a survey using 150 clinical isolates of the Pseudomonas aeruginosa, I found that the majority of these isolates can be inhibited by bacteriocin produces by laboratory isolates of P. aeruginosa. This inhibition peaks at intermediate levels of genetic similarity of the producing strain and the targeted strain. In a thesis, you could perform evolutionary experiments to examine to what extent the level of production of these bacteriocins can be a target of selection.

Contact: sijmen.schoustra@wur.nl

On interaction parameters for stable bacterial coexistence

Starter cultures for milk fermentation usually exist of 1 or 2 bacteria that acidify and thicken the milk while giving it a distinct flavour. In natural fermented products fermentation happens with many more bacteria that are stable over time. To understand how these bacterial communities can be so stable, we did experiments with multiple strain communities.

In these experiments we combined five bacteria in a community and transferred this community in 1) fresh milk, 2) milk with added glucose, and 3) milk with added yeast extract for 30 times to see the stability over time. Within five transfers one of the players was not detectable anymore in all three environments. Another player was below detection limits within ten transfers, but only in the environment with added yeast extract (Figure 1).

To have a better idea of why some bacteria were still in the community after 30 transfers while others were not detected anymore we can look at the role these bacteria play in the community and what niche they occupy. According to the niche exclusion principle, one niche can only be occupied by one bacteria.

The use of resources and production of metabolites gives all bacteria a certain "role" in the community (Figure 2). The current project will focus on making a detailed representation of the interactions in the constructed bacterial community. The metabolic network that is formed will be extended with factors like bacterial growth rates and fluxes of other metabolites. The results of the transfer experiments can be used understand dependencies of certain resources.

After that, the known parameters can be used to simulate bacterial growth by a computer model of the system. This simulations can test the hypotheses of the interactions, bacterial frequencies and community stability.

Are you interested in this project? Or do you want to know more? Contact me!
anneloes.groenenboom@wur.nl

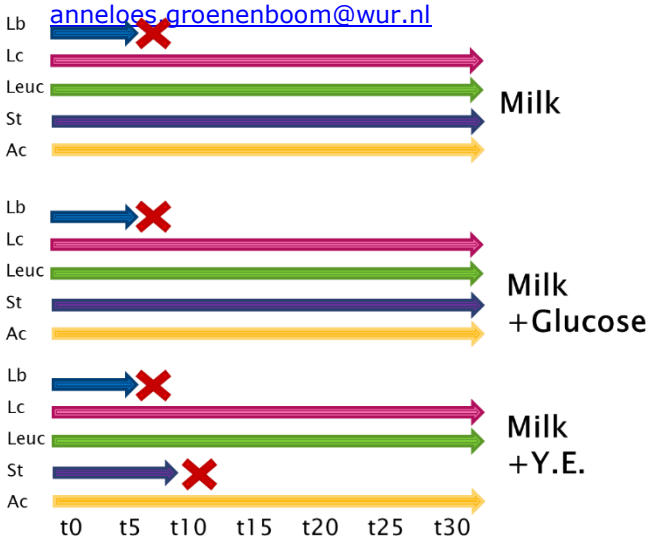


Figure 1 Bacterial presence during transfer experiment

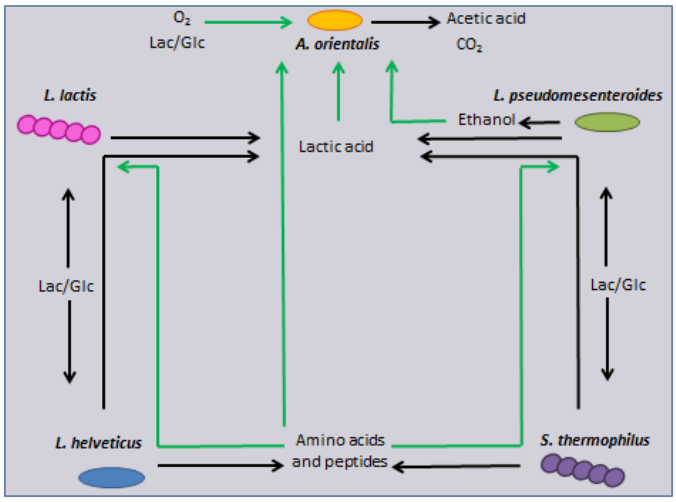


Figure 2 Metabolic network in community with 5 bacteria

On resilience against invasion of a pathogen in a microbial community

Resilience again invasions is an important characteristic of a microbial community. It can ensure the continued existence of all organisms and their functionality. This resilience can be obtained by for example the production of antimicrobial factors, but also the community structure can increase or decrease the success of an invader. A divers community structure, where most of the available niches are occupied, leaves less space for the invader to establish in the community. This phenomenon is called the niche exclusion principle, which says that one niche (constructed of all physiochemical characteristics) can only be occupied by one organism.

In our experiments we use natural communities that are obtained from a spontaneous fermented milk in Zambia, called Mabisi. During earlier experiments these communities were found to be very resilient against the invasion of an E. coli. We hypothesise this is due to both the diversity of the communities as well as their ability to acidify the milk quickly, resulting in an unflavoured environment by E. coli. In new experiments we want to use more acid resistant invaders and in the meantime decrease the diversity of the microbial communities to be invaded.

In this project you will work with both culture methods and molecular methods to analyse the bacterial community and the invader. Eventually we want to obtain insight in the causes of resilience in natural communities and the role of the niche exclusion principle in the success of invasion.

Are you interested in this project? Or do you want to know more? Contact me!
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Cultivating the termite fungus *Termitomyces*

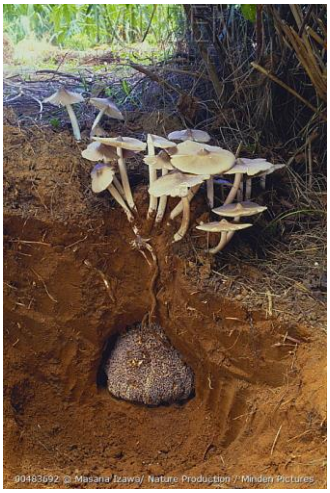
a. Question: Termites in Africa and Asia cultivate fungi of the genus Termitomyces. During millions of years the termites have cultivated their fungi and selected them to become domesticated crops. The fruiting bodies of these fungi, mushrooms, are also interesting for us humans. They are highly nutritious and relatively protein-rich mushrooms, which are collected by local people as an important food source. Unfortunately, we cannot cultivate those mushrooms artificially yet.
b. Methods: In this project you will study the techniques used in commercial mushroom cultivation in order to select, test and optimise a range of potential cultivation techniques for this fungus. Supervision of this project will be shared between the Laboratory of Genetics (Sabine Vreeburg) and Plant Breeding (Arend van Peer)
c. Organism: *Termitomyces spp.* associated with various species of fungus-growing termites.

Contact: sabine.vreeburg@wur.nl; duur.aanen@wur.nl

Ultra-selfish genetic elements in *Termitomyces* fungi: presence, fitness effect and transmission.

a. Question: We have recently discovered the presence of a range of mitochondrial plasmids. Mitochondria descend from once free-living bacteria and still have their own DNA. Additionally, fungal and plant mtDNA also can have linear plasmids, that have variable fitness effects by integrating into the mtDNA and sometimes disrupting mitochondrial functioning. The presence of such 'ultra-selfish' genetic elements in *Termitomyces* raises some interesting questions for the functioning of the agricultural mutualism of termites and *Termitomyces*. Do those elements reduce the fitness of the fungi? How are they transmitted? Can termites keep such genetic diseases under control?
b. Methods: This project will use microbial and DNA techniques (primer development, PCR, sequencing, transmission experiments).
c. Organism: *Termitomyces spp.* associated with various species of fungus-growing termites.

Contact: mathijs.nieuwenhuis@wur.nl; duur.aanen@wur.nl



Termitomyces fungus growing inside termite mound

Conflict and cooperation among fungal crops cultivated by termites

a. Question: Termites in Africa and Asia cultivate fungi of the genus *Termitomyces*. The fungus is actively farmed by the termites in a monoculture garden well protected in the termite nest. Interactions between different fungal clones and between stages in the fungal crop cycle may have important consequences for genetic exchange and thereby influence crop productivity. In this project, the details of interactions between the different stages of the fungal crops will be studied as well as the consequences for genetic exchange and productivity.
b. Methods: You will make use of the *Termitomyces* culture collection in Petri dish essays, and primarily use microscopic, microbial and DNA techniques.
c. Organism: *Termitomyces spp.* associated with various species of fungus-growing termites.

Contact: margo.wisselink@wur.nl; duur.aanen@wur.nl

The pros and cons of monoculture fungus farming in fungus-growing termites

a. Question: species of fungus-growing termites cultivate their fungus as a single-strain monoculture. On the short term monoculture maximizes the yield. In this project you will study if there are also possible disadvantages.
b. Methods: microbial techniques, pcr and sequencing.
c. Organism: *Termitomyces spp.* associated with various species of fungus-growing termites.

Contact: duur.aanen@wur.nl

Self-nonself recognition in mushrooms

a. Question: Genetic individuals of mushroom species generally are able to recognize genetically different individuals (self-nonself recognition or allorecognition). In some species, allorecognition is macroscopically visible by the formation of a zone of antagonism, called barrage, between two individuals. In this project, you will estimate the number of genes involved in allorecognition in the species *Trametes versicolor* ('elfenbankje'). In this project, you will do field work to collect mushr
b. Methods: field work, microbial techniques, designing primer sequences, pcr and sequencing.
c. Organism: *Trametes versicolor* (elfenbankje)

Contact: duur.aanen@wur.nl; ben.auxier@wur.nl

Trametes versicolor fungus

Inbreeding depression in mushrooms

a. Question: Mushroom-forming fungi have a life cycle that deviates from most other sexual organisms. First, upon mating, two haploid nuclei remain separate so that each cell contains two genetically different haploid nuclei. Second, mating does not occur between single-celled gametes, but between multicellular individuals that are being fertilised entirely. Third, they can live as a monokaryon and a dikaryon for extended periods of time, but the duration of the monokaryon stage in nature is unknown. The relative importance of the monokaryotic and dikaryotic stages influence the strength of selection against recessive deleterious mutations, the presence of which will be visible as inbreeding depression. In this project, you will determine inbreeding depression in the species *Trametes versicolor*, by comparing inbred and outbred dikaryons.

b. Methods: microbial techniques, pcr and sequencing.

c. Organism: *Trametes versicolor*.

Contact: duur.aanen@wur.nl

Cheating in fungi

a. In fungal individuals, selection within the individual is favoured by two characteristics: i) cells are either absent or leaky, meaning that nuclei and other organelles can move within the individual; ii) fungal individuals can fuse, meaning that one individual can exploit another one. In *Neurospora crassa* experimental evolution has demonstrated that cheating easily evolves. In this project you will study the mechanism of cheating/

b. Methods: microbial techniques, qpcr and microscopy

c. Organism: *Neurospora crassa*

Contact: duur.aanen@wur.nl; eric.bastiaans@wur.nl

Neurospora crassa

The effects of endosymbionts on the biological control of aphids in bell pepper greenhouses
In cooperation with project described below

Research questions:
- Do endosymbiont-carrying aphids perform better in greenhouses than uninfected aphids?
- What is the effect of different pest control strategies on aphid densities?
- Which parasitoid wasp species can be found in greenhouse aphid mummies?

Techniques:
- Fieldwork: Sampling in bell pepper greenhouses (You need to have a valid driver's license!)
- Insect identification and rearing
- Molecular techniques: DNA extraction, PCR

Organism:
- Aphids (*Myzus persicae*, *Aulacorthum solani*, *Macrosiphum euphorbiae*)
- Parasitoid wasps (*Aphidius ervi*)

Contact: helena.donner@wur.nl

The presence of protective endosymbionts in greenhouse aphids
In cooperation with project described above

Research questions:
- Which endosymbionts are present in greenhouse aphids?
- How do different pest control strategies affect endosymbiont frequencies in greenhouses?
- Are there genotype x genotype interactions between specific aphid and symbiont strains?

Techniques:
- Molecular techniques: DNA extraction, PCR, sequencing
- Fieldwork
- Possibilities for bioinformatics

Organism:
- Aphids (*Myzus persicae*, *Aulacorthum solani*, *Macrosiphum euphorbiae*)

Contact: mariska.beekman@wur.nl

Aphidius parasitoid attacking *Myzus* aphid

How endosymbionts affect aphid fitness and resistance against parasitoids

Research questions:

- What are the fitness effects of endosymbiont infections in greenhouse aphids?
- Do endosymbionts present in greenhouse aphids protect the aphids against parasitoid wasps?

Techniques:

- Creating novel aphid-endosymbiont interactions (microinjections, antibiotic treatment)
- Molecular techniques: DNA extraction, PCR
- Aphid fitness measurements
- Parasitism rate determination

Organism:

- Aphids (*Myzus persicae*, *Aulacorthum solani*, *Macrosiphum euphorbiae*), parasitoid wasps (*Aphidius ervi*, *Aphidius colemani*)

Contact: mariska.beekman@wur.nl

Quantitative genetics of life-history trade-offs in parasitoid wasps

Research questions:

- What is the heritability of life-history traits in parasitoid wasps?
- Is there evidence for genetic correlation and trade-offs between reproduction and survival in parasitoid wasps?

Techniques:

- physiological measurements (egg load and fat measurements)

Organism:

- *Nasonia vitripennis*, parasitoid wasps

Contact: bart.pannebakker@wur.nl

Functional analysis of ageing-related candidate genes in parasitoid wasps

Research questions:

- How do specific candidate genes relate to longevity and changes in larval diet?

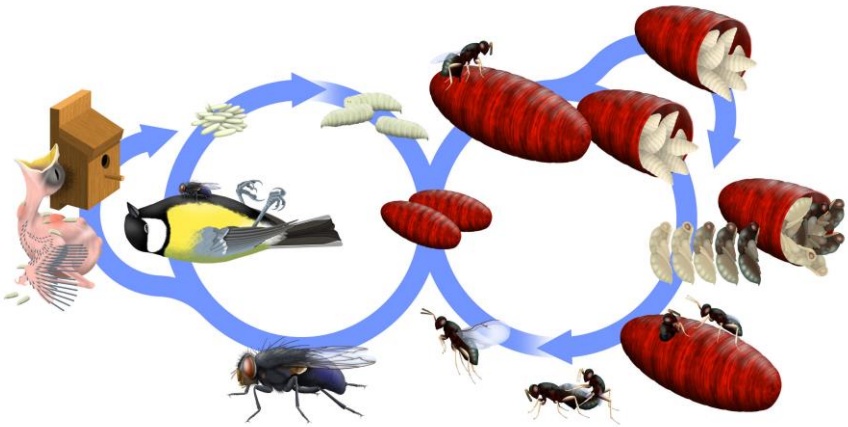
Techniques:

- DNA isolation, PCR, sequencing, gel electrophoresis, RNA extraction, cDNA library preparation, qPCR

Organism:

- *Nasonia vitripennis*, parasitoid wasps
-

Contact: bart.pannebakker@wur.nl



Lifecycle of the parasitoid wasp *Nasonia vitripennis*

Genome-wide association analysis for life-history traits in parasitoid wasps

Research questions:

- What is the genetic basis of life-history traits in parasitoid wasps?

Techniques:

- Physiological and behavioural measurements on a panel of inbred parasitoid lines, genome-wide association analysis, statistical analysis, bioinformatics

Organism:

- *Nasonia vitripennis*, parasitoid wasps

Contact: bart.pannebakker@wur.nl

Ovaries, testes and germline stem cells: reproductive physiology in parasitoid wasps.

Research questions:

- How does the investment in reproduction (i.e. reproductive organs) respond to changes in the environment?
- What is the role of reproductive organs in the life-history of wasps?
- What is the role of germline stem cells?

Techniques:

- Behavioural observations, dissection, microscopy, DNA isolation, PCR, sequencing, gel electrophoresis, RNA extraction, cDNA library preparation, qPCR

Organism:

- *Nasonia vitripennis*, parasitoid wasps

Contact: bart.pannebakker@wur.nl

Genetic variation for production parameters in the buffalo worm (*Alphitobius diaperinus*)

Research questions:

- What is the heritability of production parameters (body weight, protein and fat composition) of the buffalo worm?
- Does genetic variation limit the upscaling of production of buffalo worms for human food?

Techniques:

- physiological measurements (egg load and fat measurements)
- quantitative genetic modelling

Organism:

- *Alphitobius diaperinus*, buffalo worm

Contact: bart.pannebakker@wur.nl

Manipulating infectious parthenogenesis in parasitoid wasps used for biological control

Research questions:

- Determining the role of important insect symbiont *Wolbachia* in the parasitoid wasp *Trichogramma brassicae*, specifically if it can be transferred horizontally instead of the usual vertical route
- Decoupling the presence of *Wolbachia* from important life history traits, such as longevity, and implications for biocontrol
- This would partially be a replication study of Huigens et al 2000, "Infectious Parthenogenesis," (Nature) – but also going a step further to see if this method can be used in a different species of *Trichogramma.*

Techniques:

- Behavioural observations, microscopy, DNA extraction, PCR, sequencing, gel electrophoresis; data analysis

Organism:

- *Trichogramma brassicae*, parasitoid wasp

Contact: kim.ferguson@wur.nl
More information: https://goo.gl/bqGK9f

Assessing strain-specific molecular markers in parasitoid wasps used in biological control

Research questions:

- Is it possible to compare different populations of the same species based on molecular markers?
- How many microsatellites are enough to successfully differentiate between species? Populations?
- Is there a credible risk of intraguild-competition from introduced biocontrol agents on native populations of similar species (may not be able to test this directly, but can also be a thought experiment)

Techniques:

- DNA extraction, PCR, sequencing, gel electrophoresis; data analysis, light bioinformatics

Organism:

- *Trichogramma brassicae*, parasitoid wasp

Contact: kim.ferguson@wur.nl
More information: https://goo.gl/5PEDjL

Interactions between bacteria from urinary tract infections

Urinary tract infection bacteria on chromogenic agar

Urinary tract infections in the elderly people are often caused by more than one pathogenic bacterial species. We have recently discovered that these bacteria that cause urinary tract infections interact with each other, they 'help' each other to grow, or they 'fight' for food resources. In addition, these interactions seem to modulate the tolerance to clinically used antibiotics.

Research question1:
How do bacteria, isolated from healthy people, interact with pathogens that cause urinary tract infections?

Research question2:
Can bacterial interactions affect the acquisition of antibiotic resistance?

Methods:
By means of bacterial growth in the presence and absence of clinically used in the antibiotics, and molecular biological experiments, we will find out what some of these interactions are.

Organisms: *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella oxytoca, and more*

Contact: marjon.devos@wur.nl

Evolution and adaptation of *Aspergillus fumigatus* in Cystic Fibrosis patients

Cystic fibrosis (CF) patients are confronted with pulmonary infections and frequently *A. fumigatus* is cultured. CF patients receive for long periods of time antifungal treatment to prevent or cure infections. The fungus *A. fumigatus* is able to adapt and evolve in the lung environment of these patients.

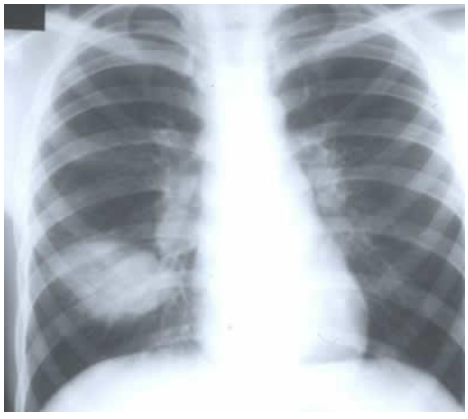
Research questions

CF patients are followed for many years and many *A. fumigatus* isolates are available from these patients. Are these isolates clonally related and how did they evolve or adapt to long periods of antifungal pressure?

Techniques: growth experiments, microsatellite analysis and sequencing/bioinformatics.

Organism: *Aspergillus fumigatus*

Contact: eveline.snelders@wur.nl



Pulmonary *Aspergillus* infection

Natural frequency of diploids of *Aspergillus fumigatus*.

A.fumigatus is an haploid fungus and has different cycles of reproduction; asexual, parasexual or sexual. Antifungal resistance is currently a problem in patients suffering infections from this fungus. Evidence shows that the antifungal pressure in the environment caused the resistance mechanism in this fungus but the type of reproduction of the fungus involved in this process is unknown. In nature only asexual reproduction has been observed but this doesn't explain the genetic variation of this fungus that we see by genetic analysis. In many different haploid fungi there is a low natural frequency of diploids in nature.

Research questions

Are diploid *A. fumigatus* naturally present and what is the frequency? What is the diploid frequency in clinical samples and among sexual progeny? Experimentally diploids can be constructed, how do the ancestral haploids segregate? And does antifungal pressure in our environment have any effect on this process? Are diploids involved in the emergence of antifungal resistance?

Techniques: growth experiments, construction of diploids, sexual crosses, antifungal evolution experiments, sequencing.

Organism: *Aspergillus fumigatus*

Contact: eveline.snelders@wur.nl

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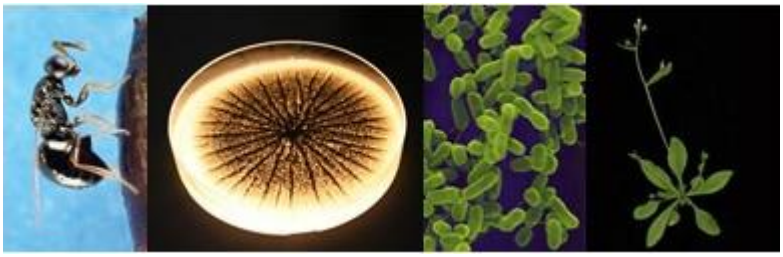
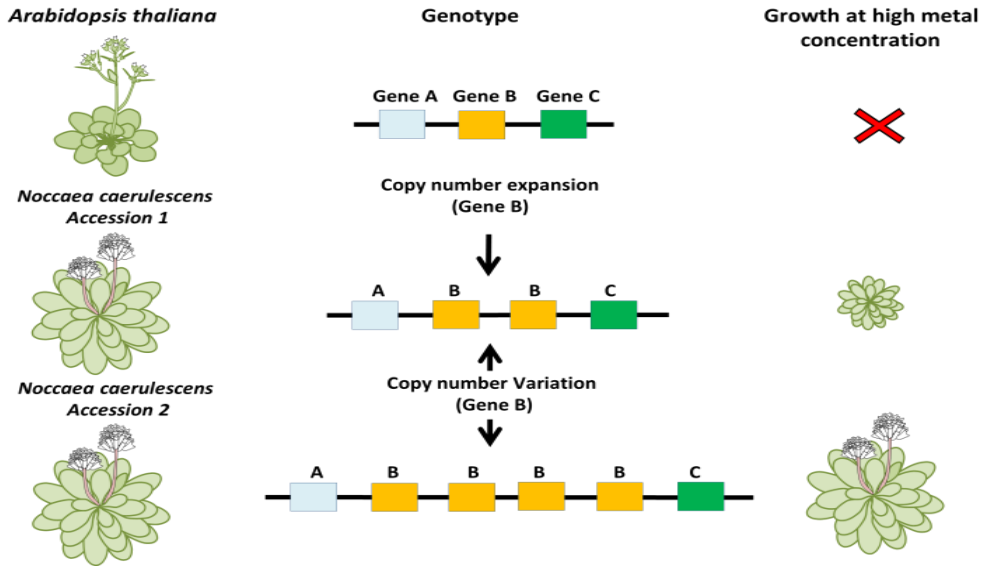


Plant Genetics

The role of gene duplications in the heavy metal adapted *Noccaea caerulescens*

Noccaea caurelescens is a heavy metal adapted and heavy metal hyperaccumulating species. Genome comparisons between *Noccaea* and the related non-accumulator species *Arabidopsis thaliana* revealed copy number expansion (gene duplication) of several metal homeostasis genes in *Noccaea*. Moreover, resequencing of several *Noccaea* accessions revealed CNV for some of these genes between these accessions. Among *Noccaea* accessions there is large variation in the tolerance to high metal concentrations.



Research question:

1. What are the contributions of these duplicated genes in relation to heavy metal adaptation?

Techniques: DNA isolation, RNA isolation, electrophoresis, PCR, gene expression assays, qPCR, physiological experiments.

Organism: *Noccaea caerulescens*

Supervisors: Mark Aarts and René Boesten

Contact: mark.aarts@wur.nl

Mapping and validation of a QTL for photosynthesis efficiency in potato

(collaboration between the Lab of Genetics and the Horticulture and Product Physiology group)

Background
Recent work (Prinzenberg et al., 2018) has shown the mapping of several quantitative trait loci (QTL) for potato seedling photosynthesis efficiency under control and different abiotic stress conditions. One of these QTLs, found under control conditions, maps to chromosome 5 of potato, close to marker SOT05-20265893. Very close to this marker resides a gene that appears to be a likely candidate to be involved in causing the QTL. This gene encodes a chloroplast located PROTEIN DISULFIDE ISOMERASE (*PDI6*; Wittenberg et al., 2014). Another locus, mapped under the same conditions, was found on chr. 3, mapping closely to marker SOT03-40197844. There is one candidate gene mapping in the vicinity, which is the *LPA2 (Low PSII accumulation 2)* gene. There is an F1 population available from a cross between two tetraploid parents, which will be segregating for both loci.

Approach
The potato population will be tested for confirmation of the segregating markers, in a small subset of ~20-50 plants. Once confirmed, the full potato population (~400 plants) will be grown in our photosynthesis efficiency phenotyper, the Phenovator (Flood et al., 2016) to examine segregation of the efficiency of photosynthesis (ΦPSII, the operating light use efficiency, F_v'/F_m'). At the same time, the genotypes of all seedlings will established (PCR-based KASP-assay) to determine which plants are expected to show the strongest and the weakest phenotype. From ~50 of these plants cuttings will be made. These plants should cover as much as possible the genetic spectrum for both loci (with 4 alleles of each locus segregating that is quite challenging), to produce clones that can be tested in further physiological experiments, to establish the consequence of the available allelic variation for the two loci

References
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Copy number variation in Dutch Arabidopsis thaliana accessions

The ever increasing volume of available plant genome information reveals evidence that gene copy number expansion and subsequent copy number variation (CNV; i.e. variation in the number of copies of a certain DNA fragment, such as whole genes, within one species) lie at the basis of plant adaptation to abiotic stress. A primary effect of gene copy number expansion is alteration of gene expression. On longer timescales, duplicated copies might evolve new functions through the accumulation of mutations.

A unique population consisting of many natural *A. thaliana* accessions, collected all across the Netherlands will be used to study local adaptation. The complete genomes of all these accessions will soon be available which will allow the identification of copy number variation. It will be very interesting to see how much CNV is present across such a small geographical distribution range, and how this relates to local adaptation.

Research question:

1. How is copy number variation related to local adaption in this population?
2. Can we use this population for Genome Wide Association studies?

Techniques: DNA isolation, RNA isolation, electrophoresis, PCR, gene expression assays, qPCR, physiological experiments.

Organism: *Arabidopsis thaliana*

Supervisors: Mark Aarts and René Boesten

Contact: mark.aarts@wur.nl

The genome of *Hirschfeldia incana*

Hirschfeldia incana (hoary mustard) is a member of the Brassicaceae plant family and occurs naturally in the Mediterranean climate area of Europe and Africa. It has become an invasive weed in the US, South America and Australia. In its native range, it grows as a winter annual species. It flowers abundantly in early spring and finishes its life cycle before the summer. A remarkable property of this species is that this species exhibits a very high rate of photosynthesis. Recently we have sequenced this plants genome.

Research question:

1. What is the genetic basis of species for its extreme photosynthesis capacity?
2. Is copy number variation related to its efficient photosynthesis ability?

Techniques: DNA isolation, RNA isolation, electrophoresis, PCR, gene expression assays, qPCR, physiological experiments, bioinformatics.

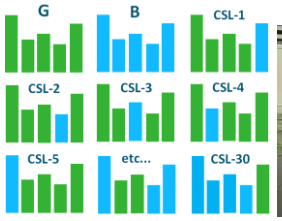
Organism: *Hirschfeldia incana*

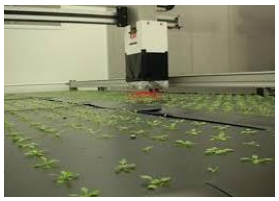
Supervisors: Mark Aarts and René Boesten

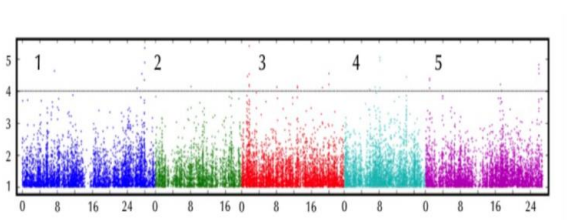
Contact: mark.aarts@wur.nl

Improving photosynthesis by using novel genetic resources and variation

With a projected growth of nearly 50% until 2050, human population increase paces faster than yearly yield improvements required to feed all of these people. Almost all major traits in our crop species had seen their potential being nearly maximized by past breeding efforts, except for one. Photosynthesis – the green engine of life itself – is the last major trait that can be significantly improved by breeding, but has never been selected for because of its daunting complexity and it is difficult to measure reliably. Fortunately, new phenotyping systems (Phenovator) and genetic resources (Chromosome substitution libraries (CSLs)) have been developed and explored that are able to unravel the complexity and genetic variation underlying traits related to photosynthesis. Epistasis in particular, the interaction between genes, is a promising type of genetic variation that scientists and breeders hardly ever look at. Understanding and improving photosynthesis in our major crop species will be key to overcome future discrepancies between population growth and crop production.







Possible projects:

- **Epistatic Genome-Wide Association Study (GWAS) analysis for photosyntetic traits**
  Statistics, experimental design

- **Analysis of possible candidate genes affecting photosynthesis efficiency.**
  qPCR, sequencing, bioinformatics

- **Applying next generation mutagenic tools (e.g. CRISPR-cas9) to prove causal genes.**
  Gene cloning, Genetic transformation, functional analysis

If interested, do not hesitate to contact! Combinations of the above are available, depending on the stage of the project and type of projects that are available.

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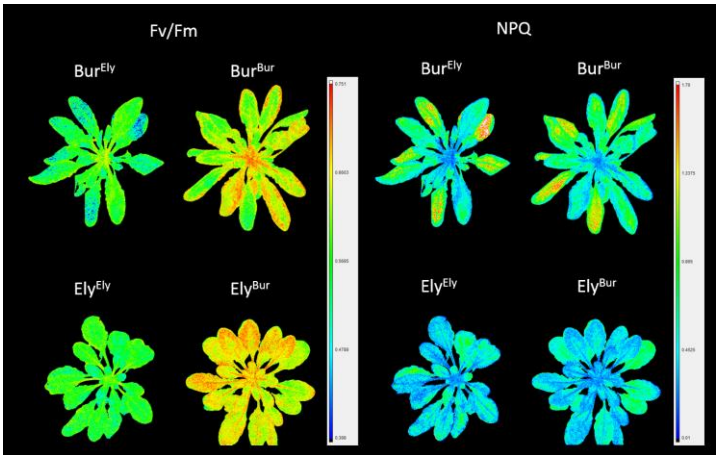
Exploring the unexplored: Unravelling genetic variation for the cyto-nuclear interaction in Arabidopsis thaliana

What if I told you that there is a source of variation found in every crop that plant breeders are not yet using. Modern plant breeding has almost exclusively focussed on the variation located in the nuclear genome (i.e. nucleome), and has largely neglected the small but significant genomes present in the chloroplasts and mitochondria (the plastome). These two organelles form the core of photosynthesis and energy balance of the cell. It has been shown that the nucleus and organelles communicate with each other to ensure proper functioning of the plants. The variation naturally available in the plastome has been shown to be of agricultural and evolutional relevance. The degree to which variation in the nuclear and cytoplasmic genomes influences the interaction is not well understood. A further understanding of this interaction will open new options for crop plant breeding and provide a new perspective on evolutionary ecology.

To do so we recently developed a powerful genetic toolkit to facilitate the rapid generation of cybrids in *Arabidopsis thaliana* (Flood, Theeuwen et al., in preparation). Cybrids are plants that contain the cytoplasm of one accession and the nucleus of another accession. This allowed us to produce cybrids between 7 different *A. thaliana* accessions. Each of the 7 nuclear genomes was combined with the plastome of the other 6 accessions, giving rise to a panel of 49 lines: the seven parental lines (wildtype plasmotype-nucleotype combinations) as well as 42 new plasmotype-nucleotype combinations. This panel has extensively been characterized for several phenotypic traits (e.g. plant growth and photosynthesis II efficiency), the whole primary metabolome (by mass spectrometry) and gene expression (RNA sequencing). From which we concluded that the cyto-nuclear effect is highly epistatic in nature, and therefor highly unpredictable (Flood, Theeuwen et al., in preparation). Moreover, significant differences between cybrids are relatively rare, as this panel consists of accessions that harbour relatively low genetic variation.



Fluorescence images for two photosynthetic parameters. Names of genotypes given as Nucleotype^{Plasmotype}. Fv/Fm is a parameter giving the maximum photosynthesis II efficiency, NPQ represents the amount of energy dissipated. In these genotypes Fv/Fm is plasmotype inherited, while NPQ is nucleotype inherited.

Following these key findings several projects are ongoing to explore the cyto-nuclear interaction in more detail. Amongst the projects are the following:

- **More genetic variation equals more phenotypic variation?**
  One key hypothesis that came out of the initial research was that more genetically distinct genotypes (for both the nucleus as well as the plasmotype) lead to more distinct phenotypes. We are building a new population to generate a snapshot of all genetic variation for the plasmotype available, which will be phenotyped using our Phenovator.
  Techniques: Population development, Marker assays, Phenotyping (automated phenotyping facilities), RNA-seq, Statistical analysis
- **Functionally validating genes underlying the cyto-nuclear interaction**
  From the current datasets we identified candidate genes, which are potentially involved with the cyto-nuclear interaction. These genes have not been shown to been involved with this process, so we are functionally validating these genes.
  Techniques: CRISPR-Cas9, Molecular techniques, RT-qPCR, Phenotyping

- **Can de nucleus adapt towards the plasmotype?**
  By generating cybrids we were able to separate the nucleus from it's "own" plasmotype, by doing so we reveal potential adaptation to a low photosynthetic performance. We are in the process of both physiologically and genetically unravelling this process.
  Techniques: High-throughput Phenotyping, Designing phenotyping protocols, Fine mapping, Hypothesis development, RNA-seq
- **Use GWAS to explore the cyto-nuclear interaction**
  Due to the huge efforts in sequencing and phenotyping, more and more data is available. This project aims at using these datasets to explore the cyto-nuclear interaction using GWAS in a way that has never been done before.
  Techniques: Statistical analysis, Method development, Phenotyping, Crossing genotypes

Contact: Tom Theeuwen (tom.theeuwen@wur.nl) and Prof. Mark Aarts (mark.aarts@wur.nl)

Exploring natural variation in Arabidopsis to reveal new genes involved in Zn homeostasis

In a recent Genome Wide Association Studies, several genes involved in regulation of plant growth under zinc stress have been identified.

Research questions:

1. Which of the genes show a mutant phenotype under different Zn conditions when knocked-out?
2. Previously several Arabidopsis genes, have been selected to be potentially involved in Zn tolerance, what is the biological function of (one of) these candidate genes?

Techniques: DNA isolation, RNA isolation, electrophoresis, PCR, gene expression assays, T-DNA knock-out line selection, physiological experiments.

Organism: *Arabidopsis thaliana*

Supervisors: Mark Aarts and René Boesten

Contact: mark.aarts@wur.nl

QTL mapping candidate genes involved in zinc tolerance

The genetics underlying tolerance to high zinc concentrations have not yet been studied. Quantitative trait locus (QTL) mapping can indicate regions in the genome which are associated with zinc tolerance. In this project, you can make use of a set of recombinant inbred lines (RILs) made between a zinc susceptible and a zinc tolerant *Arabidopsis thaliana* accession.

Research question:

1. Which genes are involved in zinc tolerance in *Arabidopsis thaliana*?

Techniques: mapping traits in RIL populations, DNA isolation, RNA isolation, T-DNA knock-out insertion line selection, T-DNA insertion line verification, physiological experiments.

Organism: *Arabidopsis thaliana*

Supervisors: Mark Aarts and René Boesten

Contact: mark.aarts@wur.nl

Is the development of a unique root barrier the reason *Noccaea caerulescens* plants are natural metal hyperaccumulators

Nutrients are transported across the root through the apoplastic, symplastic or transmembrane pathways. The regulation of such transport depends on the formation of specific barriers in the endodermis: a Casparian strip, composed of lignin, in young cells and the deposition of suberin in mature cells. This holds for most species. In the extreme Zn/Ni/Cd hypertolerant and hyperaccumulating species *Noccaea caerulescens,* this is different. *Noccaea caerulescens* roots have a different root radial pattern when compared to Arabidopsis. Arabidopsis roots have three cell layers (epidermis, cortex and endodermis), whereas *N. caerulescens* has an extra layer called peri-endodermis between the cortex and the endodermis. This cell layer contains an additional root barrier composed of cellulose and lignin, which suggests it may function as a nutrient barrier, similar to the Casparian strip (lignin) in the endodermis. We are interested in studying this mysterious cell layer and understand its function. To understand the role of this root barrier in the extreme adaptation of this species to heavy metal exposure, mutants of *N.* caerulescens have been identified with alterations in this peri-endodermal layer and barrier.

Project

The project will involve the further phenotypic and molecular characterization of three *N. caerulescens* root barrier mutants. Plants need to be grown on hydroponics and exposed to different concentrations of heavy metals to study the root phenotypes and the root and shoot ionome. The expression of genes known to be involved in lignin or cellulose deposition and mineral transport will be investigated by qRT-PCR or RNA-seq. Candidate genes can be tested for their role in root barrier formation in RNAi experiments generating transgenic plants.

Techniques: Hydroponic culture, ionome analysis, microscopy, gene expression analysis, RNAi silencing, *Agrobacterium rhizogenes*-mediated transformation.

Supervisors: Mark Aarts

Contact: mark.aarts@wur.nl

Know thy neighbour, kinship recognition in *Arabidopsis thaliana*
Research questions:

How do plants distinguish related from nonrelated individuals? Signalling and reception.

What is the effect of kin versus non-kin interaction on plant performance?

Techniques:

Plant growth interaction experiments. In vitro, green house and hydroponic systems.

Genetic mapping, metabolomics, physiological measurements.

Organism: *Arabidopsis thaliana*

Contact: joost.keurentjes@wur.nl

On the brink of being, genetic factors involved in speciation

Research Questions:

What are the genetic factors that determine incompatibility and diversification in plant species?

Techniques:

Crossing experiments, phenotyping, genotyping, genetic mapping

Organism(s): *Arabidopsis thaliana*

Contact: joost.keurentjes@wur.nl

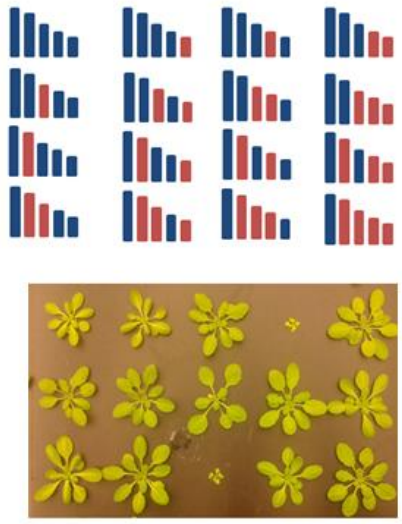
CREATION OF NOVEL GENETIC RESOURCES FOR MAPPING

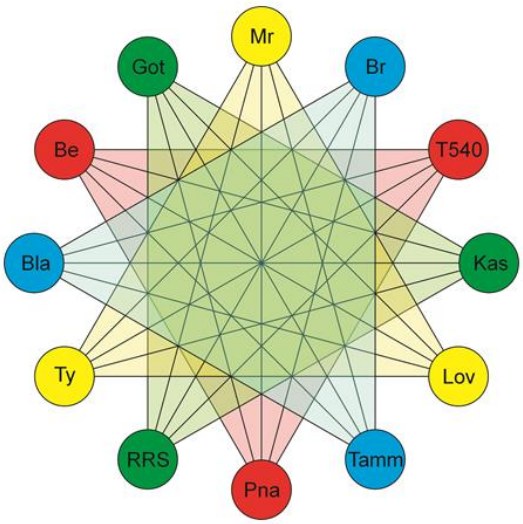
The Project

This project is focused on studying complex quantitative traits (*phenotypes*) and determining their genetic basis by studying the plants' genetic composition (*genotypes*) by using novel genetic resources. This allows a wide range of research paths to be taken, from natural variation studies to the creation of genetic resources for an in-depth study. Many of those genetic resources (populations) have already been created and helped elucidating the genetic mechanisms of important plant traits, their molecular basis, and their biological function. Most of our research is conducted on *Arabidopsis thaliana*, a model plant which can be easily cultivated and shows a relatively low genetic complexity. As a final goal, we want our results to be translated to cultivated crops, which should be the ones that finally benefit from the knowledge acquired.

The Project has been developed between Botanical Genetics and Biometris, and has been cofunded by STW and Rijk Zwaan, a plant breeding company. Currently we are working on creating different genetic resources, including highly complex population types, involving multiple parents; as well as reduced complexity populations. Each have different and often opposed characteristics, in terms of *how good* they are in associating a trait to a genetic region. Two examples of novel populations are a "**Complex Cross Design**", involving 30 interconnected populations form the 12 most diverse

parents of a worldwide set of; and a "**Chromosome Substitution Library**", a set of lines containing assemblies of intact chromosomes derived from two different parents.





This work can be divided in different parts which are usually developed simultaneously:

1) **Developing novel resources:** designing the populations genetic architecture, crossing, genotyping and selecting the desired plants.

2) **Trait Analysis:** define the optimum experimental design for the experiment. Grow the plants and score them for the phenotypes of study. Statistical analysis of the given values and QTL mapping.

3) **Efficiency improvement:** redefine the traits that are expected to answer the given biological question; optimise the technical steps detected as limiting (such as crossing, vernalisation requirements, pollen storage, etc.).

Thesis/Internship: Student who has interests in this topic please do not hesitate to contact us.

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