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**Centre for Ecological and Evolutionary Studies (CEES)**

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http://www.rug.nl/research/cees/

**Board:**
- Prof. dr. H. Olff, Director
- Prof. dr. J.L. Olsen, Chairman
- Prof. dr. J. Komdeur
- Prof. dr. ir. J.D. van Elsas

**Members:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Tel. numbers</th>
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</thead>
<tbody>
<tr>
<td>Bakker, Prof. dr. J.P.</td>
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<td>Beukeboom, Prof. dr. L.W.</td>
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<tr>
<td>Bijlsma, Prof. dr. R.</td>
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<td>Both, Prof. dr. C.</td>
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<td>Dietz, Dr. M.W.</td>
<td>3632044</td>
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<tr>
<td>Elsas, Prof. dr. ir. J.D. van</td>
<td>3632151</td>
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<tr>
<td>Elzenga, Prof. dr. J.T.M.</td>
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<td>Eriksson, Dr. B.D.H.K.</td>
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<td>Falcao Salles, Dr. J.</td>
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<td>Hemelrijk, Prof. dr. C.K.</td>
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<td>Kok, Dr. L.J. de</td>
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<td>Zande, Dr. L.P.W.G.M. van de</td>
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Highlights

Jeanine Olsen and Wytze Stam (MarBEE) win Award of Excellence for Lifetime Achievement from the Phycological Society of America

The award recognizes their pioneering work in algal evolution, phylogeography and invasive species biology. It was the first time that the award has been awarded jointly. Jeanine brought early attention to the role of climate change on phylogeographic distributions of coastal, habitat building species of algae (and also seagrasses). Her current research is focused on the genomic basis of rapid adaptation in the intertidal. Wytze was one of the very first researchers to apply scDNA-DNA hybridization kinetics in algae in the early days of molecular phylogenetics in the 1970s. He served as Subject Editor for the Society’s Journal of Phycology for 11 years. Together they have also worked extensively on invasive, tropical algae and were instrumental in international legislation to ban internet sales of aquarium species such as Caulerpa taxifolia.

PhD student Marloes Poortvliet (MarBEE) wins Best Student Paper Award at the 16th Annual Meeting of the European Elasmobranch Association’s Conference in Milan

Rays of in the genus Mobula (Mobulidae), like other elasmobranchs of the open ocean, are subject to high mortality from bycatch and targeted fisheries. Development of a realistic management program is difficult because little is known about their natural history. Using a set of nuclear and mt-loci it was shown that the giant manta ray, Manta birostris, belongs to the genus Mobula (and its eight species).

Mobula japonica is one of the most common pelagic species and dominant throughout the tropical Pacific. Using twelve nuclear microsatellite loci and the entire mitochondrial genome, genetic structure was surveyed across the Pacific Ocean. No significant population structure for either mitochondrial or microsatellite data was found. Likewise a population genomic approach utilizing the entire mitochondrial genome revealed an absence of structure. These results provide a compelling argument for development of broad international approaches for management and conservation of this charismatic species.
Dissertations granted by CEES in 2012

N.D.P. Bhola  The interplay between African protected savannas and their surrounding pastoral rangelands
Promotor: Prof. dr. H. Olff

A. Duarte  Evolution of Self-Organized Division of Labor in Social Insects
Promotores: Prof. dr. F.J. Weissing, Prof. dr. I.R. Pen

E. Folmer  Self-organization on mudflats
Promotores: Prof. dr. T. Piersma, Prof. dr. H. Olff

A. Hegemann  The skylark’s ecology and physiology in an annual-cycle perspective
Promotor: Prof. dr. B.I. Tieleman; Copromotor: Dr. K.D. Matson

N.P.C. Horrocks  The role of disease risk and life history in the immune function of larks from different environments
Promotor: Prof. dr. B.I. Tieleman

A.B.F. Ivens  The evolutionary ecology of mutualism
Promotores: Prof. dr. F.J. Weissing, Prof. dr. J.J. Boomsma, Prof. dr. I.R. Pen

T. Koevoets  Deadly combinations: hybrid incompatibilities in the parasitic wasp genus *Nasonia*
Promotor: Prof. dr. L.W. Beukeboom; Copromotor: Dr. L. van de Zande

R. Nazir  The ecological succes of *Burkholderia terrae BS001* and related strains in the mycosphere
Promotor: Prof. dr. J.D. van Elsas

S.A. Santos  Patterns of distribution, dynamics and genetic variation in the peppery furrow shell *Scrobicularia plana*
Promotor: Prof. dr. J.L. Olsen; Copromotores: Dr. ir. P.C. Luttikhuizen, Dr. ir. H.W. van der Veer

M. Schrama  The assembly of a saltmarsh ecosystem - The interplay of green and brown webs
Promotor: Prof. dr. H. Olff; Copromotor: Dr. M.P. Berg

E. Schut  Sexual selection in the blue tit: the role of the MHC and post-copulatory effects
Promotor: Prof. dr. ir. J. Komdeur; Copromotor: Dr. M.J.L. Magrath

M.A. Versteegh  Physiology in a life history perspective: stonechats’ adaptations to different environments
Promotor: Prof. dr. B.I. Tieleman

A. Zipperle  Clonal and sexy: The dynamics of sexual and asexual reproduction in dwarf eelgrass, *Zostera noltii* Hornemann in the northern Wadden Sea
Promotores: Prof. dr. J.L. Olsen, Prof. dr. W.T. Stam
## CEES PhD students in 2012

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<tr>
<th>Name</th>
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<tr>
<td>T. Aghajanzadeh</td>
<td>Ecophysiology of Plants / Razi Med. Diag. Lab Iran</td>
<td>Volatile sulfur compounds released by Brassica species as biofungicides in crop production</td>
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<tr>
<td>S.M. Amini Nasab</td>
<td>BESO / Univ. Behbahan, Iran</td>
<td>Individual variation in parent and offspring fitness in blue tits: the role of parental effects and environmental variables</td>
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<td>P. van den Berg</td>
<td>Theoretical Biology / NWO-ALW</td>
<td>Evolutionary causes and consequences of personality differences in cooperative behaviour</td>
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<tr>
<td>A.I. Bijleveld</td>
<td>Animal Ecology / NIOZ</td>
<td>Cost-benefit analyses of flocking in shorebirds, with a focus on information exchange</td>
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<td>R. Bom</td>
<td>Animal Ecology / NIOZ</td>
<td>Cascading predator-prey effects in a pristine seagrass-based food web</td>
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<td>V.S. Brauer</td>
<td>Theoretical Biology / NWO-ALW</td>
<td>Effect of temperature on the dominance of N-fixing cyanobacteria</td>
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<td>C. Burger</td>
<td>Animal Ecology / RUG-FWN</td>
<td>Latitudinal dispersal as solution to insufficient adaptation to climate change</td>
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<td>A.A. Cabrera</td>
<td>MarECon</td>
<td>Evolutionary Ecology of Marine Mammals</td>
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<tr>
<td>M. de Cássia Pereira e Silva</td>
<td>Microbial Ecology / NWO</td>
<td>Assessment of the baseline of soil microbial functioning across a range of soils</td>
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<td>M.S. Cretoiu</td>
<td>Microbial Ecology / EU</td>
<td>Meta genomics-based exploration of soils and aquatic samples</td>
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<td>W. Diao</td>
<td>Evolutionary Genetics</td>
<td>Genetics and genomics of presygotic isolation in Nasonia</td>
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<td>F. Dini Andreote</td>
<td>Microbial Ecology / NWO</td>
<td>Microbial succession in salt marsh: The ecological and functional properties of communities</td>
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<tr>
<td>S. Donadi</td>
<td>Marine Benthic Ecology and Evolution</td>
<td>Interactions between benthic communities and local resource conditions: bivalves, polychaetes and sediment stability</td>
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X. Du  
*Community and Conservation Ecology / Grant Chinese Academy of Sciences*
Progress in neutral theory for ecological communities

S. Duijns  
*Animal Ecology / NIOZ*
Foraging ecology of the Bar-tailed Godwit, *Limosa lapponica*

K. Elschot  
*Community and Conservation Ecology / OIO / NWO-ALW*
Biotically created vegetation heterogeneity during early primary succession on salt marshes

F.A. Encinas Viso  
*Community and Conservation Ecology / UE-Bursary*
Theoretical study of mutualistic interaction webs

R. Fokkema  
*Animal Ecology / NWO*
Family planning under social competition

W. Fokkema  
*Community and Conservation Ecology / Metawad, Waddenfonds*
Brent geese in the Waddensea: coupling individuals’ habitat use to species influence on (meta)ecosystem functioning

J. de Fouw  
*Animal Ecology / NIOZ / NWO-VIDI Van Gils*
Pervasive impact of intertidal migrant top predators on species diversity and size structure of its prey may lead to spatial knock-on effects between ecosystems

M. van der Geest  
*Animal Ecology / NIOZ / NWO-WOTRO (Integrated Programme)*
Fragile biodiversity linkages: production and consumption in a nutrient-poor seagrass-dominated intertidal ecosystem

S. Gerritsma  
*Evolutionary Genetics*
Identifying genomic variation among natural *Drosophila melanogaster* populations and its association to immunological resistance against parasitoids

J. van Gestel  
*Theoretical Biology / NWO*
The eco-evo-devo of biofilm formation

E. Geuverink  
*Evolutionary Genetics*
Genetics of sex determination in haplodiploid wasps

M.C.W.G. Giesbers  
*Evolutionary Genetics*
Genetics of adaptation: mating behaviour and reproductive strategies in Nasonia wasps

M.K. Gopala Krishnan  
*Microbial Ecology / NWO*
Studying the diversity of plant-associated bacterial communities in pioneer arcto-alpine plants
S. Grizard - Animal Ecology / RUG-FWN
How microbes shape bird eggs

M. Hammers - BESO / NWO / ALW
Individual model based analysis of the causes of senescence in a wild living cooperative breeding vertebrate species

I.U. Haq - Microbial Ecology / EU
Bio-indicators for soil health

R.A. Howison - Community and Conservation Ecology / UE-bursary
Plant-plant interactions along biotic & abiotic stress-gradients at salt marshes

O.J. Ivanov - Theoretical Biology
Network design and the response of complex systems to stress and deterioration

K.M. Jalvingh - Evolutionary Genetics
Genetic stress responses of Drosophila species in relation to their geographic distribution and population dynamics

D.J. Jimenez Avella - Microbial Ecology / BE-Basic
Metagenomics in the degradation of lignocellulosic substrates

B. de Jong - BESO / IWT / RUG
Influence of testosterone on female life history traits and fitness in great and blue tits

J. Jouta - Animal Ecology / NIOZ
Food webs of the Wadden Sea

S. Jurburg - Microbial Ecology / EU
Bio-indicators for soil health

R. Kentie - Animal Ecology / LNV / RUG-FWN
Spatial demography of Black-tailed Godwits: Metapopulation dynamics in a fragmented agricultural landscape

R. van Klink - Community and Conservation Ecology / Waddenfonds / VROM
Biodiversity and nature management on mainland saltmarshes

M. Langenhof - BESO / RUG - UE bursary
Surviving in a changing world: The effects of early-life conditions on determining an individual's behavior and adaptive response later in life

W.J. Ma - Evolutionary Genetics
Ecology and mechanisms of sex determination in the asobara genus (hymenoptera: braconidae)
Understanding the effects of bacterial diversity on community functioning and stability

Biodiversity and nature management on mainland saltmarshes

Strategies for improved bioprospection

Evolutionaire veranderingen in de biodiversiteit van Nederlandse insecten in respons op invasieve planten en insecten

Human cooperation - the role of prosocial norms

Potential mechanisms making bacteria fit in fungal-associated soil environmental niches

Seasonal dynamics of reproductive strategies and disease in tropical grassland birds living in habitats highly threatened by land use change

Biodiversity and nature management on mainland saltmarshes

Earthworm availability as key for the presence of wintering, migrating and breeding meadow birds

Pervasive impact of intertidal migrant top predators on species diversity and size structure of its prey may lead to spatial knock-on effects between ecosystems

Possibilities and constraints in life-cycle adaptation to climate change

The genetic basis of variation in reproductive diapause in Nasonia wasps

Species assembly at different trophic levels over environmental gradients in a savanna ecosystem
M. Poortvliet  
*Marine Benthic Ecology and Evolution*  
Population connectivity and dispersal in devil rays in the tropical Pacific

P.E. Puentes Téllez  
*Microbial Ecology / NWO*  
Adaptation of *E. coli* to heterogeneous conditions

I. Puga-Gonzalez  
*BESO / Theoretical Biology / RUG bursary*  
Self-organization of social behavior in primates

A.E. Quiñones Paredes  
*Theoretical Biology / UE bursary*  
The evolution of eusociality

M. Reich  
*Ecophysiology of Plants*  
Metabolic control of nutrient use efficiency at a whole plant perspective in a changing global atmosphere

J.L. Ruifrok  
*Community and Conservation Ecology / SBB*  
How herbivores create landscape mosaics of different plant functional types in temperate ecosystems

L. Salazar Jaramillo  
*Evolutionary Genetics*  
Comparative genomics of parasitoid resistance in *Drosophila* spp.

L. Schmaltz  
*Animal Ecology / RUG / Provincie Friesland*  
Comparative demography of Ruff and Golden Plover

J. Spoelder  
*HLB Laboratory / Ecophysiology of Plants*  
Endophyte communities in potato and their relationship with the host plant

E. van Velzen  
*Community and Conservation Ecology / UE-bursary*  
The interplay between speciation, dispersal and adaptation to local conditions in a heterogeneous environment, and its effect on food web/community structure

L. van Walraven  
*Animal Ecology / NIOZ*  
Natural and anthropogenic impact on North Sea gelatinous zooplankton population dynamics: implications for ecosystem structure and functioning

J. van der Woude  
*BESO / NWO - VICI*  
Fitness consequences of intra-specific competition and social stress in the Seychelles warbler

H.-Y. Yang  
*Animal Ecology / NIOZ / Beijing Normal University*  
Foraging ecology of red knots staging in Bohai Bay China
N. Yu  
Ecophysiology of Plants  
The role of receptor-like kinases in salt-induced changes or root architecture in Arabidopsis thaliana

E. van der Zee  
Animal Ecology / NIOZ / NWO-ZKO (WaddenEngine)  
A regime shift in bentic organisms in the Waddensea: causes and consequences for higher trophic levels

M. Zhang  
Microbial Ecology / CSC  
Bacterial-fungal interactions in soil and the role of plasmids

PhD Projects – Theses in preparation

Y. Afeworki  
WOTRO / Marine Biology (Ocean Ecosystems)  
Seasonal dynamics in the trophic ecology and energetics of an important grazer (the parrot fish Scarus ferrugineus) on the coral reefs of Eritrea in the Southern Red Sea

N.D.P. Bhola  
Community and Conservation Ecology / UE-bursary  
Characterization of the scale of spatial heterogeneity in savanna systems as novel approach to improving conservation planning

C.J. Camphuysen  
NIOZ / Animal Ecology  
Population Biology of lesser black-backed gulls in The Netherlands

J. van de Crommenacker  
BESO / WOTRO  
Stress and fitness in small bottlenecked populations of the tropical Seychelles warbler

A.L.F. Duarte  
Theoretical Biology / RUG bursary  
Division of labor in insect societies – integrating self-organization and evolutionary theory

E.O. Folmer  
Animal Ecology / Community and Conservation Ecology  
Patchiness of food and the structure of shorebird communities

C.X. Garzon López  
Community and Conservation Ecology / UE-bursary  
Determinants and consequences of the spatial arrangement of trees in the tropical rainforest of Barro Colorado, Panama

C.G. Gosling  
NWO-ALW Pionier bursary / Community and Conservation Ecology  
Biotic determinants of functional heterogeneity in a tropical savanna

A. Hegemann  
Animal Ecology / Vogelbescherming Nederland  
Strive to survive. The skylark’s ecology and physiology in an annual-cycle perspective
S.M.A.C. van Heuven  
**EU Carbo Ocean / Marine Biology**  
Meridional carbon transport in the North Atlantic Ocean

N.P.C. Horrocks  
**Animal Ecology / ALW VENI**  
The role of disease risk and life history in the immune function of larks in different environments

A.B.F. Ivens  
**Theoretical Biology / RUG bursary**  
Evolutionary ecology of the mutualism between yellow meadow ants and root aphids

T. Koevoets  
**Evolutionary Genetics**  
The genetics of postzygotic isolation in the haplodiploid sister species complex of *Nasonia*

A.L.W. Kuijper  
**Theoretical Biology / RUG bursary**  
Genetic conflict and the evolutionary causes and consequences of sex determination systems

T. Lok  
**Animal Ecology / NWO - ALW**  
Comparative demography of migratory and resident spoonbill populations with overlapping ranges

I.A. Neven  
**Ecophysiology of Plants / NWO-ALW**  
Inorganic carbon uptake of Southern Ocean Pacific phytoplankton in a high-CO₂ world

S. Perez Vila  
**Evolutionary Genetics / RUG Bursary**  
Ecology and evolution of fly parasitoid communities

S. Santos  
**Marine Benthic Ecology and Evolution / NIOZ**  
Patterns of distribution, dynamics and genetic variation in the peppery furrow shell *Scrobicularia plana*

M.J.J. Schrama  
**Community and Conservation Ecology / NWO Pionier bursary**  
Food web assembly in a dynamic salt marsh

E. Schut  
**BESO / RUG-FWN**  
Mhc based sperm competition in the Seychelles warbler and the blue tit

K. Sieben  
**Marine Benthic Ecology and Evolution / NWO-ALW**  
Marine trophic cascades - effects of fish diversity on coastal production

M.W. Smith-Kleefsmann  
**Evolutionary Genetics**  
Determinants of metapopulation dynamics: stochastic processes versus adaptation

E.E. van der Vaart  
**BESO / Theoretical Biology / NWO-TopTalent**  
Theory of mind in corvids: Insights from modeling
M.A. Versteegh  
*Animal Ecology / NOW-VENI*
Physiology in a life history perspective. Stonechat’s adaptations to different environments

A.M. Zipperle  
*Ubbo Emmius Bursary / Marine Biology (MarBEE)*
Inter-relationships between reproductive ecology, genetic structure and fitness in populations of the seagrass *Zostera noltii* in the Wadden Sea – North Sea
Animal Ecology
http://www.rug.nl/research/animal-ecology/

Group leader
Prof. dr. T. Piersma; from 01-06-2012 Prof. dr. ir. C. Both

Composition of the group in 2012:

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<th>Tenured staff</th>
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<td>Dr. J.W.H. Reneerkens</td>
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(part-time) field assistant

| N. Bot | RUG/Min. EZ | |
| Y.K. Galama | RUG/Min. EZ | |
| M. van der Geest | RUG/Metawad (Waddenfonds) | |
| N.M. Groen | RUG/Min. EZ | |
| R.J. Hibma | RUG/Min. EZ | |
H. Valkema
RUG/Min. EZ

R.P.W.M. Voesten
NWO-VIDI Tieleman

E. van der Velde
RUG/Min. EZ

S.J. Wouda
RUG/Min. EZ

R. van der Zee
RUG/Min. EZ

Other PhD students

Drs. A.I. Bijleveld
NIOZ
1.0 01-01-2010 - 01-01-2014

Drs. R. Bom
NIOZ
1.0 01-04-2011 - 01-04-2015

C.J. Camphuysen
NIOZ
1.0

Drs. S. Duijns
NIOZ
1.0 01-10-2009 - 01-10-2013

Drs. J. de Fouw
NIOZ
0.8 01-04-2010 - 01-04-2015

Drs. M. van der Geest
NIOZ
1.0 01-10-2006 - 01-02-2012

Drs. A. Hegemann

Drs. N.P.C. Horrocks

Drs. J. Jouta
NIOZ
1.0 01-01-2011 - 31-04-2015

Drs. T. Oudman
NIOZ
1.0 01-11-2010 - 01-11-2015

Drs. L. Walraven
NIOZ
1.0 01-09-2010 - 01-09-2014

Drs. E. van der Zee
NIOZ
1.0 01-03-2009 - 01-03-2013

Drs. M.A. Versteegh

Drs. Yang Hang-Yan
Beijing Normal Uni.-NIOZ
1.0
Introduction

All organisms are wonderfully adapted to the environments they live in. Their physiology, morphology and behaviour are continuously shaped by the forces of natural selection to make best use of the available resources and minimize the risks of diseases, predation or starvation. The Animal Ecology Group aims at fundamentally understanding how animals cope with their natural environments. This endeavor is studied at the levels of physiology, individuals, populations and foodwebs. Most of our research is carried out on wild birds in relation to their physical environments, and their food supplies, predators and diseases. Understanding questions about adaptation requires long-term investment in individually-marked populations, and most of our work has a firm ecological background in long-term population studies. These studies address questions on variation in reproductive success and survival, population numbers and how organisms can adapt when environments change. Our research is often experimental, both in the field and in the laboratory, to better understand how variation in individual performance is caused by their physiology, behaviour and social context. Our fundamental studies are often used in conservation efforts, and some of our studies are focused to understand how populations of endangered birds could be maintained. Our research projects span most of the globe: from the mudflats of Australia and Mauretania, the savannas of Africa, to the Dutch forests and the Arctic tundra of Greenland. Our long-term studies are mostly based in the Northern Netherlands where we study populations both in agricultural and more natural environments.

In 2012 the Animal Ecology group saw some major changes. Theunis Piersma stepped down as chair to fill the endowed chair in Global Flyway Ecology and in this new role he remains an active member of the group. Christiaan Both was appointed as the new chair, strengthening the group’s position in the fields of population ecology and evolutionary ecology towards global change.

In this annual report we give some of the recent research developments, spanning from new molecular work including microbes, experimental work on the cost of reproduction, and the applied angle of our long-term studies on the black-tailed godwit.

Overview of academic results

How do microorganisms shape eggs: microbial communities of eggshells and antimicrobial properties of albumen.
Stéphanie Grizard1,2, Joana F. Salles2 and B. Irene Tieleman1
1 Animal Ecology – 2 Microbial Ecology

Organisms must continuously deal with a plethora of microbes in their environment, and little is known how variation in microbial pressures impacts individual birds in the wild. In close collaboration with the Microbial Ecology Group we have started to illuminate how microbial pressures posed by the environment impact on the avian immune system. Because it is likely that immune system architecture varies between different environmental conditions – i.e. climate or seasons – it is important to further develop an independent measure of these microbial communities in the surrounding environment of birds. Establishing a link between immune functioning and the microbial community is an important new step in ecological immunology studies.

We have recruited eggs into this endeavor because they provide a simplified model system to study immune defenses. It is well known that microorganisms present on eggshells are susceptible to
invade the egg content and consequently act as a selective force on avian hatching success. In this earliest stage of life, microbes might be harmful because of their potential pathogenicity. Understanding which factors drive microbial communities on eggshells may lead to a better comprehension of evolutionary strategies that improve embryo survival, and the immune properties of eggs. Indeed, the albumen has antimicrobial properties relying on diverse proteins – lysozyme, avidin and, ovotransferrin – with bacteriostatic/bactericidal properties. Acting as a chemical barrier, it will help to shed light on the evolutionary ecological protection against microbial invasions. Our overall aim is to investigate the match between antimicrobial properties and microbial communities on eggshells, by focusing on the lark family. We sampled eggs of four different lark species over a range of ecological conditions: the Red-capped lark (*Calandrella cinerea*) and the Rufous-naped lark (*Mirafra africana*) in Kenya and, the Skylark (*Alauda arvensis*) and the Woodlark (*Lullula arborea*) in the Netherlands (fig. 1).

Our first challenge is describing these microbial communities on eggshells to fully comprehend their variation throughout embryo development and their potential implications for avian ecology. Although the effects of incubation on microbial growth have been studied extensively, most studies relied on culture-based methodology which covers only a minor (0.1-10%) and skewed part of the entire microbial community. Instead, we applied molecular techniques to characterize both bacterial and fungal communities in terms of structure (by PCR-DGGE (Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis)), abundance (by quantitative PCR) and, composition (clone library sequencing) on homing pigeons (*Columba livia*) eggshells. We showed, amongst others, that the structure of both bacterial (fig. 2) and fungal communities on eggshells changed between the first and the eighteenth day of incubation. Developing a suitable methodology to characterize the microbial communities on eggshells has now been taken as a first step, which we aim to apply in our field studies on avian ecological immunology.

![Figure 1: Red-capped lark (*Calandrella cinerea*) eggs in nest, in Kenya.](image)

![Figure 2: Non-metric multi-dimensional scaling (NMDS) plot of bacterial communities on pigeon eggs shows that freshly laid eggs cluster differently than incubated eggs, indicating that they harbor different bacterial communities. Freshly laid eggs, annotated Pi number are represented by black dots; incubated eggs, annotated Pi letter are represented by grey dots. The NMDS representation is based on a Bray-Curtis similarity matrix. A one-way Analysis of Similarity (ANOSIM) test determined that freshly laid eggs and incubated eggs were significantly different (P=0.02, R=0.92, stress=0.09).](image)
The demographic environment and the cost of reproduction
Rienk Fokkema, Richard Ubels and Joost Tinbergen

The cost of reproduction is essential to life-history theory, but empirically not well understood. In recent years we found support for a social cost of reproduction, by showing that the survival cost of raising a larger family was most prevalent in a local environment with high competition (Nicolaus et al, 2012). In our current project we aim at testing this in an experimental setting: pairs are forced to invest differentially in their current brood by giving them smaller or larger broods, and in the next season we provide nest boxes of different quality. If the cost of reproduction has an important social component, we expect that individuals that have worked harder will loose competition for the most preferred nest boxes over individuals that have raised an experimentally reduced family.

Nest box quality was manipulated by making them shallower, which potentially raises predation risk. Per study plot of 50 nest boxes, 10 remained their normal depth (deep), in 20 the depth was reduced by 4.5 cm (medium treatment) and in the remaining 20 the depth was reduced by 9 cm (shallow). Great tits bred mostly in the deep boxes, and almost refrained from breeding in the shallow boxes, thus showing a clear preference (fig. 3).

Figure 3: The fraction of nest boxes occupied as a function of the number of blocks in the nestbox in 2011. Deep boxes (0 blocks) are preferred by both species, great tits win competition for deep boxes from blue tits.

In contrast, blue tits took the medium depth boxes more often than the deep boxes. This is most likely explained by interspecific competition between both species for deep boxes (van Balen, 1984), although the possibility exists that species have a different preferred depth.

Why prefer deep boxes?
In the years 2011 and 2012 we measured breeding success in relation to nest box depth to find the rationale behind this choice. Over different breeding parameters, we only found that breeding success was slightly higher in deep boxes. This effect could be due to effects of nest box depth per se, but also result from quality differences between birds. We will elaborate on these effects in the future.

The main reason why the birds preferred deep boxes became apparent: while checking nest boxes every week we recorded cases of nest predation. Using an automatic camera we were able to ‘catch’ one responsible predator in the act. It was a pine marten! He/she approached from above and grabbed through the nest entrance in the box, sometimes successful, sometimes not (see fig. 4). Likely such predators are the main cause that tits prefer deep boxes because the risk of predation
was about four times higher in the shallow nest boxes (fig 4).

Figure 4: Pine marten depredating a nest box with two blocks. The nest was abandoned and as far as we know the incubating mother blue tit did not survive this attack. B) The probability of predation as a function of the number of blocks in the nest (2011).

Did parents raising small broods get deeper nestboxes in the next spring?
Yes, be it only under certain circumstances. Parents from reduced broods were very successful in getting deeper boxes when there was more choice of boxes in their direct surroundings, but this effect was not apparent if there was less choice available (fig. 5).

Figure 5: The fraction of parent birds in deep boxes as a function of the manipulation of their brood size in the previous year (data from 2011 and 2012). The effects differ with the availability of different types of boxes in the environment. When there is more local choice between nestboxes of different types the effect is evident (fig 3 A). For further explanation see text.

Although we do not fully understand the environmental effect, we consider this an important result because it demonstrates that competitive ability of parents is negatively affected by the brood size they raise, resulting in lower quality breeding sites in the subsequent year. As a result, selection will reduce optimal family size in a competitive environment as compared to a non-competitive environment, which could explain why birds in general lay smaller clutches at higher densities. Moreover, the best decision on family size in a competitive environment will also depend on the family size chosen by competing individuals, leading to the conclusion that decisions on family size
are frequency dependent. Although theoretically well established, empirical proof for the general principle that social factors affect optimal behaviour is still rare, and we will continue exploring these mechanisms in more detail.


**Conservation ecology of meadow birds**

Roos Kentie, Jos Hooijmeijer, Krijn Trimbos, Niko Groen and Theunis Piersma

In a paper in the Journal of Applied Ecology (2013), Kentie *et al*. showed that the most often applied agri-environmental schemes to protect breeding meadowbirds in The Netherlands (i.e. payments per clutch found, and postponed mowing), are not effective. Agricultural intensification of grasslands has been linked to the declines of many farmland bird species. In this study of over 1200 chicks from the 8500 ha study area in southwest Friesland in 2007-2010 we try to explain why black-tailed godwits (*Limosa limosa limosa*) are still in decline despite these agri-environmental schemes. We compare the growth and survival of chicks of black-tailed godwits from well-drained grassland monocultures managed for maximal dairy production (80% of the area) with those of chicks from herb-rich meadows with high water tables often managed in traditional ways for grassland birds (20%). Hatchling mass did not differ between herb-rich meadows and grassland monocultures, but chicks hatched on monocultures were on average 14-16% lighter at fledging and they had 4% smaller bills than chicks hatched on herb-rich meadows (fig. 6). Apparent survival during the first year of life for chicks hatched on herb-rich meadows was 2.5 times higher than that of chicks hatched on monocultures. That chicks hatched on monocultures had lower growth and survival rates than chicks on meadows indicate that these chicks suffer a higher risk of starvation and/or predation. The apparent survival on herb-rich meadows appears sufficient for a stable population. Conservation efforts should focus on the provision of herb-rich meadows with high water tables rather than the postponing of mowing only.

Figure 6: Body mass growth of known-age black-tailed godwit chicks hatched on either meadows or monocultures in 2004–2010. The curve is not fitted to these data but represents the Gompertz curve of Beintema & Visser (1989) for chicks caught in 1976–1985

Publications 2012

Doctorate granted by the institution, prepared within the institution

Hegemann, A. 2012 Strive to survive: The Skylark’s ecology and physiology in an annual-cycle perspective. Promotor: Prof. dr. B.I. Tieleman
Horrocks, N.P.C. 2012. The role of disease risk and life history in the immune function of larks in different environments. Promotor: Prof. dr. B.I. Tieleman

Publications in journals


Hegemann, A., Matson, K.D., Both, C. & Tieleman, B.I. 2012. Immune function in a free-living bird varies over the annual cycle, but seasonal patterns differ between years. *Decoelologia* **170**: 605-618


Versteegh, M. A., Helm, B., Gwinner, E., & Tieleman, B. I. 2012. Annual cycles of metabolic rate are genetically determined but can be shifted by phenotypic flexibility. *Journal of Experimental Biology*, **215**: 3459–3466


Professional publications


Books & Book Chapters


**Behavioural Ecology and Self-organization**  
http://www.rug.nl/research/behavioural-ecology-and-self-organization/

**Group leader**  
Prof. dr. J. Komdeur

Composition of the group in 2012:

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**Post-docs**

| Dr. H.L. Dugdale            | NWO-visitor's grant     | 0.5 | 01-07-2011 - 31-03-2012    |
| Dr. H. Hildenbrandt         | Agentschap NL           | 0.8 | 01-12-2011 - 15-10-2012    |
| Dr. P. Korsten             | NWO-ALW                 | 1.0 | 01-11-2011 - 01-11-2014    |
| Dr. L. Spurgin             | NWO-Rubicon             | 1.0 | 01-09-2012 - 31-08-2014    |

**PhD-students**

| Drs. S.M. Amini Nasab       | Univ. Behbahan, Iran-RUG| 1.0 | 01-08-2011 - 01-08-2015    |
| Drs. M. Hammers            | NWO-ALW                 | 1.0 | 01-10-2008 - 30-09-2012    |
| Drs. B. de Jong            | IWT-RUG                 | 1.0 | 01-01-2010 - 01-01-2013    |
| Drs. M.B.W. Langenhof      | RUG-UBbo Emmius         | 0.9 | 01-12-2009 - 01-06-2014    |
| Drs. I. Puga-Gonzalez      | RUG                     | 0.5 | 01-09-2007 - 01-06-2012    |
| Drs. J. van der Woude      | NWO-VICI                | 0.6 | 01-10-2008 - 31-03-2014    |

**Technical/Administrative Staff**

| secretary                  | RUG                     | 0.3 |                            |
| technical assistent        |                         |     |                            |
| M. van der Velde           | RuG                     | 0.4 |                            |
| Dr. E. Schut               | EU-CIG                  | 0.8 | 01-05-2012 - 31-10-2012    |
| IT-assistent               |                         |     |                            |
| Dr. J. Bakker              | RuG                     | 0.2 |                            |
| Dierverzorging             |                         | 0.1 |                            |

**Other PhD-students**

| Drs. R. Buij               | CML (Leiden)/RUG        | 1.0 | 01-01-2007 - 31-12-2012    |
| Drs. E. Schut              | RuG                     |     |                            |
| Drs. E. van der Vaart      | NWO-TOP                 |     |                            |

**Guests/Detachment**

<p>| Prof. Dr. S. Amador Kane   | NWO visitor grant       | 1.0 | 29-07-2012 - 14-08-2012    |
| Dr. E. Barrett            | NERC (UK)               | 1.0 | 01-08-2009 - 01-08-2012    |
| Drs. A. Braun             | Konrad Lorenz Research Station (Austria) |     |                            |
| Prof. Dr. M. Butovskaya   | NWO visitor grant       | 1.0 | 20-01-2012 - 20-02-2012    |
| Dr. H.L. Dugdale          |                         |     |                            |
| Drs. H. Edwards           | University of Sheffield (UK) | 1.0 | 01-10-2012 - 01-10-2015    |
| Drs. D. Gilroy            | University East Anglia (UK) | 1.0 | 01-10-2011 - 01-10-2014    |
| Dr. S.A. Kingma           | NWO-Rubicon             | 1.0 | 01-08-2011 - 01-08-2013    |
| Dr. I. Lesna              | UVA                     |     |                            |
| Drs. U. Lopez             | Université Paul Sabatier (Fr) | 1.0 | 10-11-2012 - 15-12-2012    |</p>
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<td>Drs. D.J. Wright</td>
<td>University East Anglia (UK)</td>
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Introduction

The Behavioral Ecology and Self-organization group focuses on understanding the self-organization and evolution of social behaviour in animals by combining theoretical, empirical and laboratory approaches. The strength of our group lies in our multifaceted approach of studying social behaviour. Apart from approaching social behaviour empirically and theoretically, we also study it from two perspectives: behavioural ecology (Komdeur) and self-organization and emergence (Hemelrijk).

**Behavioural Ecology:** Our research aim is to understand the evolution of social behaviour and the way in which this affects population dynamics. A social system defines how individuals in a population interact and includes all relationships of a sexual, agonistic and/or aid-related nature. All group-dwelling animals are influenced by their social environment to some degree, through processes of social stress, phenotypic plasticity, and learning. This can be a great advantage, especially in fast-changing environments, as individuals can benefit from strategies learned and adaptations gained by their parents. It may, however, also present a limiting factor when individuals are shaped by the previous generation to a situation that is no longer present. Consequently, individuals must constantly balance the costs and benefits of social life. Therefore, individuals in social species cannot be understood via their adaptations to the physical and ecological environments alone; their social dimension also needs to be considered. In empirical studies, we aim to understand how the social environment in which individuals live, and the degree of social interactions between individuals affect individual phenotypes (behaviours), and the consequences this has for fitness. We also aim to identify the genes important for adaptation and contemporary fitness in individuals and populations. In theoretical studies, we aim to unravel the importance of how changing social environments may bias evolutionary predictions in natural systems. This will have direct applications for evolutionary studies wishing to understand how environmental and social factors influence the evolution of phenotypes in field settings.

Our powerful combination of developing (experimental) field studies, molecular ecology, immunology, endocrinology, theoretical modelling and quantitative genetics will provide novel insights into social evolution, which can be used to inform conservation programs. We focus on natural systems for which we have comprehensive genetic parentage relationships and long-term data. Our main research themes are: (1) Dynamics of parental behaviour; (2) Early-life environment, social stress and individual behaviour; (3) Physiology of life histories and senescence; and, (4) Conserving threatened species;

**Self-organization:** The study of self-organization investigates how complexity at a higher level emerges from the interactions among simple units at a lower level. For instance, schooling behaviour of fish and flocking of birds may emerge from simple interactions among nearby group members. Using computer models we apply this process-oriented approach to uncover emergent phenomena of animal behaviour and their social organization, and to generate insights into how natural selection operates at several levels of complexity (e.g., individual, group, and community). Our specific aims are to understand the formation of social structure of fish schools, bird flocks and primate societies, and how the specifics of locomotion, aero- and hydrodynamics, individual personalities, memories, and social cognition affect these social structures. By integrating behaviour, interactions with others, aero- and hydrodynamics, and spatial structure in these simulations, our approach delivers new hypotheses that can be tested in natural settings. We test these model-based predictions by closely cooperating with empirical scientists. Our research themes are understanding of (1) attack strategies of aerial raptors on solitary birds and on flocks of birds; (2) complexity of bird flocks and fish schools; (3) hydrodynamics of fish schooling; (4) emergence of complex social
behaviour of cognitively simple local rules in primates and corvids; and, (5) evolution of complex social systems via selection at multiple scales.

**Highlights of BESO**

Elske van der Vaart (PhD student, with promotors Charlotte Hemelrijk and Rineke Verbrugge) received the New Investigator Award for outstanding paper from the Division of Experimental Psychology of the American Psychological Association for her paper on “Corvid caching: Insights from a cognitive model”. In this paper a computational model is presented of the memory dynamics of corvids during caching food and recovering it. It delivers two new hypotheses: 1) The decrease of recovery of caches over time is a consequence of random effects rather than of differential memory of different cache sites and 2) the choice of cache sites is based on positive, rather than negative, reinforcement.

Our group organized the first symposium day of Groningen Center for Social Complexity, Groningen, June 2012 (Charlotte Hemelrijk is one of the founders). Jan Komdeur received a TOP grant and an ALW grant from the Netherlands Organisation for Scientific Research (NWO) in collaboration with Hannah Dugdale and Franjo Weissing (Theoretical Biology, CEES, University of Groningen) to continue research on the Seychelles warbler. He also received an ALW-NWO grant to investigate the effect of metal contamination on stress response modulation and stress coping abilities in barnacle geese on Ny-Ålesund.

**Overview of academic results**

**Ovariance between offspring sex, paternity and laying order in blue tits**

Oscar Vedder, Michael Magrath, Marco van der Velde and Jan Komdeur

Variance in male reproductive success is typically higher than that of females, which in socially monogamous species is caused by extra-pair paternity as an additional means of increasing male reproductive success. Hence, if male success at acquiring extra-pair paternity is heritable and increases reproductive success (an assumption of ‘good genes’ explanations for extra-pair paternity) parents will increase their fitness when biasing the sex ratio of extra-pair offspring (EPO) towards sons.

Empirical evidence for this prediction is equivocal. A study in house wrens (*Troglodytes aedon*), with one of the greatest sample sizes, found a male bias among EPO, but the majority of others studies did not. A possible explanation for the absence of a male bias among EPO is the inability of females to precisely allocate paternity to a specific ovum. This can potentially be solved by biasing the whole litter or brood towards sons when a female has engaged in extra-pair copulation, which, at the population level, may also lead to a male bias among EPO. However, if females are more prone to engage in extra-pair copulation when mated to a male with heritable low fitness, it may be maladaptive to also bias the sex ratio of within-pair offspring towards sons. Another solution that does enable females to bias the sex ratio of EPO within broods, is to vary both offspring sex and paternity systematically with ovulation order. We explored this option in a wild population of blue tits (*Cyanistes caeruleus*)

During three breeding seasons, in a nest-box population at ‘De Vosbergen’ estate, we collected complete clutches one or two days prior to hatching, replaced them with model eggs to prevent desertion, and hatched all eggs in separate compartments in incubators. This enabled us to link hatchlings to the specific egg they hatched from. Within three days of hatching a small blood sample (ca. 10 μl) was collected from the nestlings. With molecular sexing and parentage analyses we were subsequently able to assess whether EPO were more likely to be male, and whether this was
achieved by a non-random distribution of both offspring sex and paternity over the laying order of eggs in a clutch.

We found that in one year (2009) EPO were indeed significantly more likely to be male (Fig. 1a), which was caused by a parallel decrease in the proportion of EPO and male offspring with laying order (Fig. 1b). However, in two other years (2006 and 2007) only the proportion of EPO declined with laying order, while the sex ratio of offspring did not change systematically over the laying order. Consequently, in those years EPO were not significantly more likely to be male.

The result from 2009 is the first evidence for animals to bias the sex ratio of EPO towards males by varying both in concert with ovulation order. This mechanism may be responsible for all reported male biases among EPO, as both species in which a male bias among EPO has been reported (house wrens and blue tits) also show a decline in EPO with laying order. However, our results suggest that we should not treat male bias within EPO as support for the more general hypothesis that parents increase their fitness if they bias the sex ratio of extra-pair offspring towards males. If there is a large fitness benefit of biasing the sex ratio of EPO towards males, it would be expected that this would be observed in all years. Hence, the fact that we did not observe this in two other years, despite the evidence for a mechanism to achieve this, does not suggest strong selection for a male bias among EPO.

The decrease in proportion of males with laying order that we only observed in 2009, and caused the male bias among EPO, may instead have been an adaptation to unknown year-specific environmental conditions. The fact that this may occasionally result in a male bias among EPO, in species with a declining proportion of EPO with laying order, may explain why reported associations between offspring sex and paternity have been inconsistent, both between and within species.

Testosterone reduces promiscuity but not attractiveness in female blue tits; an experimental study
Berber de Jong, Luc Lens, Marco van der Velde, Ton Groothuis and Jan Komdeur

In many animal species extra-pair copulations (EPC) are common and can increase fitness of males and females. In males, EPCs can increase their reproductive success, whereas in females the benefits of extra-pair copulations can be more indirect through improving the quality of the offspring. In
numerous male vertebrate species the steroid hormone testosterone (T) plays an important role in mating behaviour by increasing, amongst other things, their EPC rate. This is also stressed by an increase in mating behaviour and a peak in natural T levels at the beginning of the breeding season. Females of different vertebrate species also show an increase in natural T levels at the beginning of their breeding season, although in lower levels compared males. In contrast to what is known about the role of T in male mating behaviour, the role of T in female mating behaviour remains unclear.

To study the role of T in female mating behaviour, we created three groups of female blue tits implanted with T, flutamide (androgen receptor blocker) and empty implants before egg laying under field conditions in 2010 and 2011 (Fig. 2). We then scored the number of extra pair offspring (EPO) at day three during the years the females were manipulated and the subsequent breeding season (i.e. 2011 and 2012). Additionally, we tested under laboratory conditions whether males preferred to mate up with T- or flutamide-implanted females.

The number of nests with at least one EPO was lower for T implanted group, whereas flutamide had no effect relative to controls (Fig. 3). There was an overall treatment effect on the number of broods that contained at least one EPO ($\chi^2 = 7.25$, df = 2, $P = 0.027$). The T-group had significantly less broods with EPO (13%; 4/31) compared to the control-group (38% (10/26, Fisher’s exact test: $P = 0.03$) and the flutamide-group (40%; 12/30, Fisher’s exact test: $P = 0.02$). The control- and the flutamide-groups had similar percentage of broods containing at least one extra-pair offspring (Fisher’s exact test: $P = 1.0$). In the subsequent breeding season the difference between the three treatment groups disappeared. There was no significant long-term effect of the treatments on the number of nests with EPO (Fig. 3). The effect of T implants was not due to lower female attractiveness, because males did not show a clear preference for flutamide or T females during a mate choice test. Thus, increasing the likelihood that elevated T levels negatively influenced EPC rate by, for example, affecting mating behaviour.

Although the functional significance of female EPC is not completely clear, there is evidence in some species that having EPC can indirectly increase female fitness. Thus a reduction of EPO by experimentally elevating T might lower female fitness which is in contrast to what has been found in males. Our findings improves our understanding of the role of T in female mating behaviour and increases our knowledge on the evolution of natural peak T levels at the beginning of the breeding season in males and females.

![Figure 2a: Blood sampling a three-day old nestling for paternity analysis.](image-url)
Unhatched eggs are not caused by sperm depletion in blue tits
Elske Schut, Oscar Vedder, Jan Komdeur, Michael J. L. Magrath.

In bird nests, researchers commonly encounter eggs that fail to hatch after the normal incubation period has passed. In our study population of blue tits in the Vosbergen, the percentage of eggs that failed to hatch ranged between 2.5 and 4.5% in 2006, 2007 and 2009 (with the percentage of nests containing unhatched eggs ranging between 17 and 26.5%). Unhatched eggs can be caused by insufficient incubation, problems during embryo development or the eggs being unfertilized. To investigate how likely female blue tits are to lay unfertilized eggs, we studied the number of sperm on freshly laid blue tit eggs. Freshly laid eggs were collected during 2007 and 2009. We dissected the thin layers around the yolk (perivitelline layers) to count sperm. During the formation of the egg and immediately after fertilization, an extra layer is laid around the ovum, to prevent further sperm from penetrating. Sperm present at the site of fertilization are trapped between the layers and can be
visualised by staining them with a fluorescent dye (Figure 4). Previous studies demonstrated that sperm counts obtained from these layers are positively correlated with the number of sperm inseminated.

Copulation behaviour is likely to affect the amount of sperm that females have available for fertilization. Detailed information on the copulation behaviour of blue tits in relation to the laying of their clutch is unavailable in the literature. In most passerines, copulations cease soon after the first egg is laid. We therefore hypothesized that female blue tits run out of sperm towards the end of their clutch. This seems especially likely since blue tits lay an exceptionally large clutch (average 12.2 eggs in our study years; ranging between 5 and 19). We further investigated whether hatching success and the number of sperm on eggs belonging to that clutch were correlated.

Eggs contained on average 238 ±18 (SE) sperm and few eggs contained very low numbers of sperm (only three eggs contained <30 sperm) (Figure 5). Consistent with this finding, we found no evidence that sperm numbers decline towards the end of the laying sequence (p=0.67). This could mean that copulations in blue tits continue until the end of egg laying. An alternative explanation is that females laying a large clutch store more sperm already before the start of laying. We found no evidence that the number of sperm stored was correlated with clutch size, however (p=0.80). Sperm number was repeatable between eggs laid by the same female (r=0.61 ± 0.12 SE). This allowed us to use the sperm count of eggs sampled from a clutch as an indication of sperm count in other eggs from the same clutch. These eggs were left in the nest to determine their hatching success. No correlation between sperm numbers and hatching success was found (p=0.50). We conclude that, contrary to our expectations, female blue tits have sufficient sperm available to fertilize their entire clutch. Eggs that remain unfertilized are therefore unlikely to be the main cause for the occurrence of unhatched eggs in our blue tit population.
Effects of land use change in Sudano-Sahelian West Africa on the diet and growth of an avian predator
Ralph Buij, Ingrid Folkertsma, Kim Kortekaas, Hans de Iongh and Jan Komdeur

Anthropogenic changes in land use can influence raptor population dynamics, often resulting in population declines along gradients of intensified land use. Apart from the frequently examined and more obvious impact on productivity, changes in diet composition and prey quality induced by habitat alteration might also impair nestling growth rate and development. As a result, nestling condition at fledging may be affected, an aspect that has received scarce attention, despite the potentially important consequences for population persistence. In an effort to address this topic, we studied the diet and growth of Grasshopper Buzzard (*Butastur rufipennis*) nestlings in natural and transformed habitats of the Sudano-Sahel region of Cameroon (Fig. 6). Land conversion for cultivation, woodcutting and heavy grazing pressure affect large parts of its breeding range, depressing prey populations, a threat aggravated by the effect of changing rainfall patterns.

We examined the influence of land use and rainfall on growth parameters of 50 male and 36 female nestlings (68 first-born or single nestlings, 18 second-born hatchlings) measured from hatching to fledging, to understand the importance of changing environmental conditions on nestling condition at fledging. Our results showed that Grasshopper Buzzard diet composition differed between natural and transformed habitats: grasshoppers and small mammals were more frequently taken in natural
habitat, whereas lizards were most frequently taken in transformed habitat, reflecting differences in prey availability around nests in natural and transformed habitats. We found that nestling growth parameters, including asymptotic weight, were significantly affected by land use (Fig. 7): estimated asymptotic weight and weight gain from day 10 were higher in Grasshopper Buzzard nestlings in natural compared to those in transformed habitats. This effect was unrelated to other potentially confounding variables such as hatching order (Fig. 8), gender, hatching date, rainfall, or the presence of siblings, and suggests that fledglings in natural habitat attained a superior condition compared to those from transformed habitat. Although nestling growth is typically affected by a variety of environmental factors, including productivity and land use, to our knowledge this is the first study to report an effect of land use on raptor growth patterns in Africa, and suggests that nestling condition could be viewed as a potential tool to measure effects of land transformation on raptors in this important region. In another study of the same population, we found that productivity and nest success were unaffected by land use in Cameroon, suggesting that Grasshopper Buzzards cope fairly well with habitat changes. The opportunistic exploitation of a wide spectrum of prey and a diet shift in response to habitat degradation further support these conclusions. Despite such apparent adaptability to habitat change, nestling condition at fledging may be compromised under the changing conditions that affect much of this raptors’ distribution range, thereby possibly affecting the potential for survival and recruitment of remaining populations in a rather subtle way.

Figure 7. The a) asymptotic body mass and b) weight gain (±SE) for days 10 to 20, and days 20 to 30, for 50 male and 36 female nestlings (68 first-born or single nestlings, 18 second-born hatchlings) measured from hatching to fledging, in natural and transformed habitats. Land-use significantly influenced asymptotic weight and growth rates from day 10, after confounding covariates such as nestling sex and hatch order had been taken into consideration.
Museum DNA reveals a drastic, recent bottleneck in the Seychelles warbler

Lewis Spurgin, David Wright, Marco van der Velde, Nigel Collar, Jan Komdeur, Terry Burke and David Richardson

Piecing together the history of wild populations is not only interesting in its own right, but also helps researchers make sense of present-day behavioural and ecological processes. Historical population bottlenecks, in particular, can affect patterns of individual survival, reproduction and mating behaviour for many subsequent generations, even if the population subsequently recovers. Understanding the demographic history of populations is also of conservation importance; populations that have been small for a long time may be in less danger of extinction than populations that have undergone recent, drastic reductions in population size.

We used a powerful combination of museum specimens, molecular markers and simulations to examine 140 years of genetic diversity change in the endangered Seychelles warbler (*Acrocephalus sechellensis*). This species was discovered in the late 19th century on the tiny islands of Cousin and Marianne (Fig. 9), where birds were shot and museum specimens obtained (Fig. 10). By the 1960s the global Seychelles warbler population was reduced to less than 30 on Cousin. The population has now recovered thanks to conservation efforts, and has become a “model species” for ecology, evolution and conservation. However, we do not know whether the Seychelles warbler has ever been widespread and abundant, or when it went through a bottleneck.

Figure 8. Apart from a significant effect of hatch order (left: first-hatched nestling) on development illustrated here, nestling growth is impaired under anthropogenic habitat use.

Figure 9. Population history of the Seychelles warbler (pictured inset). Dates represent first and last known dates that Seychelles warblers were present on individual islands. Populations on Cousine, Aride, Denis and Frégate were established by translocations.
Across museum and contemporary samples, we found a 25% reduction in genetic diversity and strong genetic structure (Fig. 11). Our simulations showed that the warbler was bottlenecked from a large, outbred population – an ancestral $N_e$ of over 2,000 individuals was reduced to less than 50 individuals within the last century. This suggests that the Seychelles warbler was once widespread across the Seychelles, and that its population size has been drastically reduced by human disturbance. Our results have important implications for the conservation of the Seychelles warbler and other endangered island species.

**Telomere length and dynamics predict mortality in a wild bird population**

Emma Barrett, Terry Burke, Martijn Hammers, Jan Komdeur and David Richardson

Recently there has been much interest in the physiological mechanisms underlying senescent declines in performance in wild animals. Several molecular, cellular and physiological mechanisms
have been identified, including late-life changes in oxidative stress levels, hormone signalling and immune function. Such age-dependent changes are likely to influence both reproductive success and survival of elderly individuals and will therefore contribute to late-life declines in reproduction and survival, for example if elderly individuals become more susceptible to parasites and diseases than prime-aged individuals.

A mechanism that has received much attention because of its tight link with senescence is telomere shortening. Telomeres are repetitive nucleotide sequences that are located at the ends of chromatids. Without the protection of telomeres, or when telomeres reach a critical length, cells die or stop replicating, ultimately leading to organ failure and death. Understanding the age-dependence of telomere dynamics may significantly contribute to our understanding of senescence and provide us with the opportunity to test whether telomere dynamics might be used as an indicator of “biological age” (an individual’s physical state).

Using samples taken from a closed population of Seychelles warblers studied for over 20 years, we investigated life-long adult telomere dynamics and their relationship to mortality in natural conditions. We found that telomeres shortened with increasing age (Fig. 12) and body mass, and that individuals with shorter telomeres and greater rates of telomere shortening were less likely to survive until the next year (Fig. 13).
Our results provided the first clear evidence of an association between telomere length and mortality in the wild, and substantiated the prediction that telomere length and shortening rate may act as an indicator of biological age further to chronological age when exploring life-history questions in natural conditions. However, although we found an association between telomere length and mortality, whether telomere shortening causes the death of an individual or whether an individual’s death is caused by another factor that is linked to telomere length remains to be investigated. For example, an individual may die as a result of disease, but this disease may, at the same time, have caused telomere shortening. Further, long-term survival declined with age, but was not associated significantly with telomere length. This might suggest that short telomeres are associated with mortality in individuals of all ages, rather than in elderly individuals only. Future studies may investigate whether telomeres are a better predictor of mortality in elderly individuals than in young or mid-aged individuals. Future studies may also investigate how telomere dynamics are related to different ecological parameters (e.g. food availability, environmental conditions) and life-history characteristics (e.g. previous reproductive investment).

Understanding corvid cognition through models
Elske van der Vaart, Rineke Verbrugge and Charlotte Hemelrijk,

Corvids, the extended family of crows, have long enjoyed a reputation for being clever, a reputation increasingly supported by research. Interestingly, some of the most impressive results have come from experiments conducted within a single context: That of burying food to save it for future consumption. Such ‘caching’ has been used to study many different aspects of corvid cognition, ranging from memory to landmark use, and from future planning to the ability to socially outsmart competitors. However, often the interpretation of such experiments is difficult. Therefore, in our research, we have been studying corvid cognition with a new approach. We have built a kind of ‘virtual bird’, based on a number of basic assumptions about corvid memory and learning, and then exposed it to simulated versions of real experiments. By comparing model outcomes to empirical data, we have derived new explanations for the behavior of real corvids. This technique is often used to study humans, but is rarely applied to other species. Here, we give two examples of the insights generated by our ‘virtual bird’, on memory errors in Clark’s nutcrackers (*Nucifraga columbiana*) and on social cognition in Western scrub jays (*Aphelocoma californica*).

In the wild, Clark’s nutcrackers have been shown to accurately recover thousands of caches, months after burying them. In the laboratory, each bird caches in an experimental room until it has hidden a fixed number of items; then, it is given several ‘recovery sessions’, where it can recover more and more of its caches. Surprisingly, the birds seem to become significantly less accurate as recovery sessions progress, despite the fact that the intervals between sessions are too brief to affect their memory. This has led to the idea that Clark’s nutcrackers may remember some items better than others, and then recover their better-remembered items first. This hypothesis has been tested by giving the birds four recovery sessions, in two conditions. In the ‘free condition’, the full room was always available for recovery; in the ‘quarters condition’, only one-quarter was accessible at a time. If the birds were recovering the best-remembered items first, accuracy should decline in the ‘free condition’, while staying constant in the ‘quarters condition’, as the best-remembered items should be distributed equally across the room. Indeed, this is exactly what occurred (Fig. 14). However, attempts to discover what might make some caches more memorable have so far been unsuccessful.
Therefore, we attempted to simulate this experiment with our virtual bird. Inspired by ACT-R, a well-established model of human cognition, the virtual bird stores its caching and recovery events in chunks, whose activation depends on recency and frequency of use. Thus, the more often, and the more recently, it has cached in a particular location, the stronger its memory thereof. Surprisingly, this basic model already replicated the behavior of the real birds, despite the fact that it remembers all cache sites equally. This is due to chance effects. When the full room is available for recovery, the birds’ a priori odds of successfully recovering decline across sessions, while in the ‘quarters’ condition, these odds stay the same. The noise in the model’s memory system magnifies these chance effects, giving rise to the pattern seen in Fig. 14.

As another example, when Western scrub jays cache, they seem to employ many sophisticated tactics to confuse potential thieves. This has led some scientists to speculate that these birds may have a ‘theory of mind’, or the ability to think about the mental states of others. For instance, when they cache in one tray while watched, and in another tray while in private, they later re-cache more of the items cached in front of the other bird (Fig. 15), which may require the cacher to think about what the competitor wants and what it has seen.

However, with our virtual bird, we have discovered an alternative hypothesis. Western scrub jays also re-cache more while they are watched; although this is typically interpreted as an effort to confuse the onlooker, it might also be the result of a desire to cache more, brought on by the stress of another bird’s presence. When we put this repeated re-caching into our existing virtual bird, this causes it to experience more memory errors at recovery. If we assume that these memory errors also lead to stress – the stress of caches that seem to be missing – and that this stress again leads to a desire to cache more, which, due to the experimental setup can only be satisfied by rehiding items previously hidden, then the virtual bird also re-caches more of the items cached while the conspecific was watching. This provides another example of how this type of model can lead to new hypotheses, which can subsequently be tested by empirical researchers.
Consolation without empathy: an individual-based model and empirical data
Ivan Puga-Gonzalez, Marina Butovskaya, Bernard Thierry and Charlotte Hemelrijk

Friendly contacts between bystanders and former opponents of a fight have always puzzled primatologists. Specifically, the affiliative interaction between a bystander and a former victim has attracted much attention. This interaction is thought to relieve the distress of the victim and thus it has been called “consolation” (Fig. 16). However, whether consolation really reduces the victim’s tension has seldom been investigated. Only two studies, performed in chimpanzees, have tested this hypothesis finding contradicting results. Whereas one study did not find any evidence of stress reduction, the other one did. Further, because empathy was supposed to underlie consolation and consolation was found only in apes and not in monkeys, this has long been seen as evidence that apes were the only non-human primates capable of expressing empathy. However, consolation has recently been confirmed also in wolves, dogs and two species of monkeys; taxa that have lower cognitive capacities than apes. Thus, whether empathy necessarily underlies ‘consolation’ is questionable. Therefore, in the present study, we investigated the minimum cognitive abilities needed to display consolation. We do so by analyzing the occurrence of consolation in a monkey species, the Tonkean macaque and in an individual-based model (GrooFiWorld) in which individuals lack empathy and behave according to cognitively simple rules. In the model, individuals tend to remain in a group and when they are close to each other they may fight if their chance of winning is high; otherwise, they may groom their partner, particularly when they are anxious (Pu

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<td></td>
<td>Aggressor</td>
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<td>B) GrooFiWorld Model</td>
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Table 1. Percentage of conflicts followed by consolation in empirical data and the GrooFiWorld model.
Publications 2012

Doctoral Dissertations


Publications in journals


**Books & Book Chapters**


**Professional Publications**

Community and Conservation Ecology
http://www.rug.nl/research/community-and-conservation-ecology/

**Group leader**  
Prof. dr. H. Olff

Composition of the group in 2012:

### Tenured Staff

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### Technical and Administrative staff

**secretary**  
I.C. Jansen | RUG | 0.5 |

**chemical analist**  
N.D. Eck | RUG | 1.0 |

**botanical analist**  
J.J. Hogendorf | RUG | 1.0 |

**IT-assistent**  
ing. G.J. van den Burg | RUG | 0.8 |

**Guests**

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Introduction

The Community and Conservation Ecology group studies the interactions in ecological communities, to better understand the determinants of biodiversity, and to provide insight in how such communities can best be managed, restored and protected.

Species interact in ecosystems with other species and with abiotic factors through various types of interactions, often forming complex networks. Possible interactions include those between consumers and their resources, between organisms and abiotic (non-resource) conditions, between organisms and detritus compartments through excretion and mortality, non–trophic interactions between organisms (e.g. pollination or mutualism with microbes) and spatial interactions between neighbouring ecosystems through dispersal and nutrient flow. We therefore study organisms on different trophic levels (soil biota, plants, herbivores, predators) with emphasis on understanding the consequences of these interactions for the structure of ecological communities and the functioning of ecosystems. We do this by studying the key processes that determine the abundance and distribution of species in ecological communities, such as competition, facilitation, mutualism, plant-herbivore interactions and predator-prey interactions. In theoretical studies, we aim at unravelling the relative importance of stochastic and deterministic processes in structuring communities, and study the interplay of ecological and evolutionary processes.

We work both in the tropics and in the temperate zone to get a broad overview of the main processes that determine community structure and ecosystem functioning. In this, we try to combine theoretical, experimental and observational approaches. Our main study sites are located in the Netherlands (Wadden Sea, Oostvaardersplassen, Overijsselse Vecht), Tanzania (Serengeti) and South Africa (Hluwe-iMfolozi). Particularly our research in the Wadden Sea area has significantly expanded since last year, with special focus on the role of ecosystem engineers (sea grasses, mussels, lugworms) on community and ecosystem dynamics.

Highlights 2012:

On September 27th 2012 The Heineken Young Scientist Award 2012 for Environmental Sciences was awarded to the Cees postdoc dr. Tjisse van der Heide for his research on the role of seagrasses and other similar ecosystem engineers in the marine intertidal area.

The following sections contain specific areas of progress of our research during 2012.

Overview of academic results

Mesoherbivores, but not rhinos shape grasshopper communities in a rhino dominated savannah park

Fons van der Plas

African savannahs are among the few places on earth where one can still find more or less pristine, diverse communities of large grazer species. Large populations of zebras, impalas, wildebeests, warthogs, buffalos and rhinos co-occur in many reserves. It is often suggested that these grazers are essential in maintaining the high diversity that is associated with African savannahs. The largest grazer species in particular, the white rhino, is assumed to be play an extremely important ‘keystone species’ role, by creating patches of short vegetation and by facilitating all other herbivore species. However, whether it is really true that (i) grazers in general and (ii) white rhinos in particular are indeed so important for the diversity of other groups of organisms has rarely been tested. Especially their effects on the diverse group or organisms: insects have never been well studied. Due to the reduction in population sizes of many grazer species and especially due to recent poaching threats
on white rhino populations, it is crucial to understand the role of these herbivores for maintaining savannah biodiversity.

In this study, using ten year old exclosures, we investigated the effects of both white rhinos and all other large (i.e. larger than hares) herbivores on the diversity of grasses and grasshoppers. At eight sites in Hluhluwe-iMfolozi Park (South Africa), two types of fenced plots were set up ten years before data collection. The first type of fences excluded white rhinos from entering the associated ‘no rhino’ plot, while the second type of fences additionally excluded all other larger herbivores from the associated ‘no grazers’ plot. We also identified a third plot at each site: a ‘control’ plot, that was unfenced and therefore accessible for all grazer species. At each site and at each plot (nested within site), we identified all present grasses and grasshoppers and we measured their abundance.

It appeared that surprisingly, white rhinos had very little influence on either grass or grasshopper communities: in control plots, the same species of grasses and grasshoppers were found as in ‘no rhino’ plots (Fig 1 & 2). Our study area, Hluhluwe-iMfolozi, is known as a ‘rhino hotspot’ with possibly the highest density of white rhinos on earth, so one might expect very large effects of these animals on their surroundings, but we did not observe these. Contrary to this, other grazers did appear to affect both grass and grasshopper communities: while there were no differences in grasshopper species richness between ‘no rhino’ and ‘no grazer’ plots, ‘no rhino’ plots contained more species of grasses and very different (but not more species rich) communities of grasshoppers (Fig 1 & 2). This suggests that unlike white rhinos, all other large grazer combined do affect community assembly of grasses and grasshoppers, and are therefore important in maintaining savannah biodiversity.

An interesting question arising from these results is why white rhinos, which have the reputation of being ‘ecosystem engineers’ and ‘keystone species’, creating patches of low vegetation and facilitating other herbivores, did not appear to affect community assembly of other organisms in our study. Previous studies have suggested that white rhino’s have large impacts on their surroundings, but these studies were mostly performed around ‘middens’: places where rhinos regularly return to, to defecate. In savannahs, these places are among the most intensively used by rhinos, while in our study, we investigated effects of rhinos in ‘random’ savannah locations. Probably, white rhinos thus do act as ‘keystone species’, but only at very specific locations in the landscape. In most areas of the savannah, the combined effects of other herbivore species are much more important in explaining community assembly of plants and insects.
Landscapes with distinct vegetation mosaics often support high floral and faunal diversity and positively affect many ecosystems functions and services, due to their high structural diversity of the vegetation. For example, vegetation mosaics often contain a relative high plant, bird and mammal diversity (Harris 1988; Best et al. 1990; Kollmann and Schneider 1999; Magura et al. 2001; Fischer and Lindenmayer 2002; Ries et al. 2004). Furthermore, the presence of vegetation mosaics positively affects many ecosystems services, such as recreational values and protection against invasive species or against the spread of disturbances such as fire and pathogens (Turner 1989; Adler et al. 2001; Díaz and Cabido 2001; Woodcock et al. 2011). Consequently, for optimal use of our ecosystems as biodiversity refuges and for providing stakeholders of ecosystems with ecological and socioeconomic valuable ecosystem services, more knowledge about how vegetation mosaics are generated and maintained is needed.

Large herbivores can play a key role in generating and maintain structural diversity (Olff et al. 1999). However, they not always do so (Adler et al. 2001). A potential predictor for whether large herbivores may generate and maintain vegetation structural diversity may be the interaction with other drivers of structural diversity, such as anthropogenic and abiotic sources. For example, humans may interact with large herbivore-based processes by affecting herbivore density by herding, culling or by removing or introducing predators (Beschta and Ripple 2009). Large herbivores may interact with abiotic heterogeneity, e.g. by grazing mostly in more fertile soil patches (McNaughton 1984). The goal of my study is to gain more insight in how large herbivores can play a role in generating and maintaining structural diversity in vegetation in interaction with abiotic heterogeneity and different types of herbivore density control.

The findings of this study suggest that the potential of herbivores to generate structural diversity in the vegetation depends strongly how herbivore density is regulated, often a direct (i.e. culling of herding) or indirect anthropogenic effect (introduction or removal of predators). When
herbivore density is kept low by artificial means (i.e. herding or culling) structurally diverse landscapes, also at small spatial scales, may occur under a more relaxed set of conditions than when herbivore densities are regulated naturally (i.e. bottom-up regulation, or top-down regulation by predators).

When herbivore density is kept low abiotic variation seems to be an important predictor whether or not herbivores successfully generate and maintain a heterogeneous vegetation mosaic of grassland and woodland. When herbivore densities are bottom up regulated heterogeneous grassland-woodland mosaics are not so easily generated. Grazing refuges, such as woody debris, seem essential for tree recruitment. An alternative potential mechanism that would increase recruitment of woody species is fluctuating herbivore densities, e.g. due to very harsh winters or predation. This will have strong positive effect on tree recruitment, as at periods of high herbivore density lawns are generated, in which tree sapling survival is enhanced during periods of low herbivore density.

References

Pollinator population structure creates potential for sudden collapse of pollination service
Francisco Encinas-Viso, Tomás Revilla and Rampal S. Etienne

Plant-pollinator interactions are essential for ecosystem functioning and the maintenance of biodiversity. Many angiosperm plants depend on the service provided by pollinators to reproduce. Empirical studies of this type of mutualistic interaction are abundant. However, theoretical studies of plant-pollinator interactions are relatively scarce, originally focusing on very specific systems (e.g. fig-fig wasp) but more recently on mutualistic community dynamics. Some studies have proposed to
study plant-pollinator systems, and other types of mutualism (e.g. plant-mycorrhiza), in terms of consumer-resource interactions to develop more mechanistic models of mutualism. The theory of plant-pollinator interactions is progressing, but a crucial component of this interaction is missing in many theoretical studies: the consideration of population structure. Many pollinators are insects with complex life-cycles, i.e. they have several life-stages (e.g. egg, larva, pupa, adult) and each life-stage is subject to different selective pressures and can have multiple indirect effects on their mutualistic partners (i.e. plants).

Here, we study a facultative-obligate plant-pollinator system with pollinator population structure and based on consumer-resource interactions. This simple model assumes a more mechanistic plant-pollinator interaction than Lotka-Volterra models of mutualism by explicitly describing the resource and consumer dynamics between plants and pollinators, where there is an exchange of resources (i.e. nectar) for an ecological service (i.e. pollination). Our results indicate that population structure is highly important for the stability of plant-pollinator interactions and the management of pollination service.

Previous models have ignored pollinator population structure as a crucial component of the stability of plant-pollinator interactions. Our model shows that pollinator population structure is important for the stability and conservation of plant-pollinator interactions. Specifically, we find that decreases in larval maturation rate relative to adult mortality shifts the pollinator population towards a larvae dominated population decreasing the pollination service and jeopardizing the interaction (Figure 1). Pesticides are among the most important causes, slowing the larval maturation rate and increasing the adult mortality rate, particularly in Hymenopteran pollinators. Our model predicts that these effects of pesticides can produce a shift in the pollinator population structure to higher larva to adult ratios and decrease the population growth putting the pollinators in the Allee effect region (i.e. bistability region).

![Figure 1. The effect of pollinator population structure on the plant equilibrium. Changes along the y/δA axis represent a shift in pollinator population structure. If y/δA < 1, the pollinator population is dominated by larvae, however if y/δA > 1, the pollinator population structure is dominated by adults. The increase of plant equilibrium densities depends on the population structure and the plant's mutualistic offset (ω) (i.e. relative contribution of reproductive growth due to pollination against the maximum rate of vegetative growth). The sudden jump in the figure starting when ω > 1 and y/δA <1 shows the critical transition between alternative stable states (i.e. bistability) of the Allee effect region. y is the larva maturation rate and δA is the adult pollinator mortality rate.](image-url)
Publications 2012

Doctoral dissertations


Publications in journals


Etienne, R.S. & Haegeman, B. A conceptual and statistical framework for adaptive radiations with a key role for diversity-dependence. American Naturalist 180: E75-E89.

Etienne, R.S. & Rosindell, J. Prolonging the past counteracts the pull of the present: protracted speciation can explain observed slow downs in diversification. Systematic Biology 61: 204–213.

Etienne, R.S. & Rosindell, J. Comment on “Global correlations in tropical tree species richness and abundance reject neutrality”. Science 336: 1639.


Mahdavi, P., Akhani, H. & Maarel, E. van der. Species Diversity and Life-Form Patterns in Steppe Vegetation along a 3000 m Altitudinal Gradient in the Alborz Mountains, Iran. *Folia Geobotanica*. DOI 10.1007/s12224-012-9133-1

McInerny, G.J. & Etienne, R.S. Ditch the niche - is the niche a useful concept in ecology or species distribution modelling? *Journal of Biogeography* 39: 2096-2102.

McInerny, G.J. & Etienne, R.S. Pitch the niche - taking responsibility for the concepts we use in ecology and species distribution modelling. *Journal of Biogeography* 39: 2112-2118.

McInerny, G.J. & Etienne, R.S. Stitch the niche - a practical philosophy and visual schematic for the niche concept. *Journal of Biogeography* 39: 2103-2111.

Ordoñez, A. & Olff, H. Do alien plant species profit more from high resource supply than natives? A trait-based analysis. Global Ecology and Biogeography


Rosindell, J., Jansen, P. A. & Etienne, R. S. Age structure in neutral theory resolves inconsistencies related to reproductive-size threshold Journal of Plant Ecology volume 5, number 1: 64–71


Books/chapter in books


Chapters in this book:


Professional publications


Ecophysiology of Plants
http://www.rug.nl/research/plant-ecophysiology/

Group leader
Prof. dr. J.T.M. Elzenga

Composition of the group in 2012:

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| Guest                        |                 |
| L.A. Villerius               | Rijkswaterstaat |
Overview of academic results

Movement of water, nutrients and organic compounds in and out of plants or algae, between plant organs and between the cell wall compartment and the cytoplasm of a cell, are processes that define the functioning of an organism. The affinity of the uptake system for a limiting nutrient from the soil determines the outcome of the competition between species; the signalling molecules released by the roots shape the composition of the microbial community around the roots and facilitate the establishment of the symbiosis with beneficial bacteria and with mycorrhizal fungi; pathogenic bacteria trigger ion movements across the cell membrane, involved in the anti-bacterial response of the plant determining the susceptibility of a plant to the pathogen; the release of organic sulfur compounds by algae affect temperature on a global scale. With modern biophysical techniques the fluxes of organic compounds and ions can be quantified. The Ecophysiology of Plants group develops new techniques that enable us to measure with higher precision the fluxes of nutrient ions and climate active compounds, on physiological and ecological relevant scales. Although the measurements are sometimes carried out on a scale as small as a single vacuole or a root tip, or involve samples collected during an expedition to Antarctica, the main goal always remains to understand the functioning of the whole organism in its, often variable, environment. Plants are capable to respond to changes in their environment by changing growth pattern, adjust their physiology, resorb and form roots, accumulate defensive compounds or osmotically active, compatible solutes. This adaptive behaviour of algae and plants in turn can affect their environment, sometimes on a global scale.

Our approach, using advanced biophysical techniques to measure processes that are essential to plant functioning in a changing environment, and interpreting these in a whole plant context, is applied in two areas of interest:

1. Determining the interaction between global change factors and plant/algal performance: DMS release by marine algae in a changing polar environment, the effect of CO$_2$ levels on Sphagnum growth, algal photosynthesis and nutrient uptake efficiency in Brassica species, and the impact of atmospheric sulfur compounds on plant performance. In these projects effects of climate change and changes in the atmospheric composition on ecologically important processes are studied. Changes in plant growth due to increased CO$_2$ levels and the effects of atmospheric sulfur and nitrogen compounds on plant nutrient demand are themes that have a long history in the Plant Ecophysiology group. DMS is one of the so-called negative greenhouse gases: it can function as a condensation nucleus, inducing cloud formation that has a cooling effect on a global scale. The production, release and subsequent hydrolysis of DMSP by algae, is the main source of DMS. Sea-ice habitats and polar surface oceans are environments where prolific DMS production is observed. Quantification of the ecosystem processes that control the flux of DMS forms the basis of biochemistry modules of climate models. Since Sphagnum bogs are the only terrestrial ecosystem that can function as a carbon-sink over geological periods, the establishment and/or restoration of these bogs can ameliorate the CO$_2$ production due to burning of fossil fuel.

2. Describing, understanding and possibly optimizing the potential of plants to adjust its architecture, metabolism and physiology to suit the changing requirements of growing crop plants. Examples of such projects are: plant performance under suboptimal temperature, mechanisms of salt-tolerance in Arabidopsis, molecular basis of root architecture and uptake characteristics, and effects of plant-microbe interaction on plant physiology and development. These projects are characterized by a strong interest of commercial partners. By focussing the physiological research on crop plants the combination of challenging scientific questions and practice-oriented solutions has proved to be inspiring and productive.
In 2012, Ecophysiology of Plants received a financial boost with the granting of an ALW-investment grant (k€ 399). With the grant a new, state-of-the-art Time-Of-Flight mass spectrometer and incubation cabinet could be installed in the new Dutch Science Facility at the Antarctic research base Rothera. The new equipment will allow us to do experiments with stable isotopes, with which biological process rates of climate relevant compounds can be measured. The data will serve to develop algorithms for sea ice-ocean-climate models.

The preparations and opening of the labs in Antarctica received much attention in the Dutch press: on television, radio and in national and local newspapers. Our project Temporal dynamics of the climate gas dimethyl sulphide (DMS) and related compounds in a changing Antarctic sea-ice environment is one of the five Dutch projects at Rothera in the coming 3 years. Three of our staff members, Jacqueline Stefels, Maria van Leeuwe and Desiree den Os, will be working on year-round measurements of climate gases. The base is situated at the west side of the Antarctic Peninsula, where currently large effects of global warming are apparent.

In 2012 an international working group on 'Biogeochemical Exchange Processes at the Sea-Ice Interfaces (BEPSII)' was granted under the auspices of the Scientific Committee on Oceanic research (SCOR). Dr Jacqueline Stefels is co-chair of this working group and dr Maria van Leeuwe associate member. The group brings together sea-ice modellers and observationalists who will collaborate to develop state-of-the-art sea-ice-ocean-climate models. [http://www.scor-int.org/Working_Groups/wg140.htm](http://www.scor-int.org/Working_Groups/wg140.htm)
COST Action to improve fruit yield and quality

In 2012 the COST action “Grafting to improve yield and fruit quality under biotic and abiotic stress (FA 1204)” officially started. On behalf of The Netherlands, Dr. Jan Henk Venema is taking part in the Management Committee of this action. In addition, he is appointed as leader of the Working Group “Rootstock-Scion interactions and graft compatibility”.

http://www.cost.eu/domains_actions/FA/Actions/FA1204?parties
http://www.vegetablegrafting.unitus.it/

Rootstock selection to improve nutrient-use efficiency and abiotic stress tolerance in tomato
Jan Henk Venema, Leen Villerius, Tatsiana T. Charnikhova*, Harro J. Bouwmeester* and J. Theo M. Elzenga
* Laboratory of Plant Physiology, Wageningen University.

With the research program ‘Green Breeding’ the Dutch ministry of Economic Affairs supports research projects that are aimed at breeding crops with improved biotic stress resistance, nutrient-use efficiency and adaptability to changing climatic conditions. The development of such ‘robust crops’ may improve the sustainability of agriculture and horticulture. Green Breeding is a collaboration of Wageningen University, University of Groningen, the Louis Bolk Institute and several private breeding companies. The cooperation of private and public researchers guarantees an efficient incorporation of knowledge and results into breeding programs.

The project within Green Breeding led by our group explores the possibilities to use rootstocks as a tool to improve the nutrient-use efficiency and abiotic stress tolerance of tomato. Breeding rootstocks for these complex traits is hampered by the lack of practical selection tools like genetic markers and knowledge about the physiology behind a successful rootstock (root-shoot interaction). This project, therefore, aims to identify the key-physiological characteristics that reflect the complex underlying genetic make-up of desired root-derived traits. The generated knowledge will finally be used to develop a screening method, which supports breeders with the selection of vigorous rootstocks tolerant to abiotic stresses.

Within this project a set of inter-specific hybrids was generated after selection of wild tomato accessions, which contrast in vigour, low-temperature and salt tolerance. In grafting studies, the performance of these hybrids as rootstock is currently tested at the level of the root (morphology and architecture, hydraulic conductance, water and nutrient uptake), root-shoot signalling (xylem sap composition and pH) and scion (leaf morphology, development, growth, photosynthesis, stomatal conductance and fruit production) under different growth conditions. One of the main targets is to unravel the underlying hormonal regulations. For this purpose, a very sensitive method was developed in 2012 for the targeted analysis of seven different plant hormones, i.e. auxin, cytokinin, ABA, ACC (precursor of ethylene), GAs, JA, SA and their derivates by using ultra-performance liquid chromatography equipped with an electrospray ionization source coupled to tandem mass spectrometry (UPLC-ESI-MS/MS). This method gives us now the possibility to determine hormone profiles and to compare rootstock performance under different abiotic stress conditions. These analyses will hopefully elucidate the hormones or ratio’s which play a key role in root(stock) functioning and its interaction with the shoot (scion) under a particular growth condition.

In 2012, from February until November, a rootstock trial was executed at an organic tomato grower (BioVerbeek B.V., Velden, The Netherlands). The six tomato hybrids generated in this project were tested as rootstock and grafted with tomato cv. Cappricia as scion. Two commercial rootstocks, i.e. Maxiort and Big Power, were used as references. All eight rootstocks contained the same resistances against soil-borne pathogens. No significant differences in total yield, total fruit number or overall average fruit weight were observed between the eight rootstocks (Figure 1A). Instead, the more vigorous rootstocks produced a significant higher number and total weight of side shoots (Figure 1B).
Figure 1. Cumulative fruit yield (A) and side shoot weight production (B) of tomato (cv. Cappricia) grafted onto 8 different rootstock hybrids. Tomatoes were grown in soil under organic growth conditions. Shown are the means of 40 plants growing at 4 different plots in the greenhouse.

This trial indicates that in Dutch organic growing systems there may not be an advantage to use a vigorous rootstock bred to maximise fruit yield. Rather rootstocks can be selected that have strong (a)biotic stress resistances in combination with less excessive side shoot formation. In a subsequent trial in 2013, the same set of rootstocks will be compared under conventional growth conditions in Southern France. It is expected that under the more generative growth condition in Southern France significant differences in fruit yield will appear between the rootstocks. Running both trials, several physiological parameters as well as hormone profiles are measured to elucidate the physiological mechanisms underlying the differences in rootstock performance. The knowledge generated in this project will finally be used to develop a reliable high-throughput screening method, which supports the selection of vigorous robust rootstocks for tomato.

Copper toxicity and sulfur metabolism in Chinese cabbage are affected by UV radiation
M. Shahbaz, S. Parmar, C.E.E. Stuiver, M.J. Hawkesford & L.J. De Kok

Copper levels in agricultural soil may be strongly enhanced as a consequence of anthropogenic activities through the application of organic fertilizers, use of sewage sludge as a fertilizer, application of sewage water for crop production and the use of Cu-containing fungicides. Copper (Cu) is an essential redox-active transition metal for normal plant growth and development and a cofactor in many metalloproteins, but elevated Cu concentrations in the root environment (≥ 5 µM) may rapidly become phytotoxic. Cu has the potential to accelerate the formation of reactive oxygen species in plant tissues and the high light intensities which enhanced the toxicity of Cu to chloroplast functioning was presumably due to an enhanced production of hydroxyl radicals. Exposure of plants to elevated Cu concentrations generally results in leaf chlorosis, a loss of photosynthetic activity and in a reduced plant biomass production. It has been predicted that there will be an increase in UV radiation on the earth surface due to ozone depletion in the stratosphere and reduction of aerosols and clouds UV-B (280-320 nm) is the most harmful part of the UV spectrum for plants reaching the surface of the earth. UV-B absorption by foliage may result in the formation of reactive oxygen species, especially in chloroplasts. Consequently, high UV-B levels may alter thylakoid integrity, induce pigment degradation and substantially affect plant performance.

Biomass production, dry matter content, specific leaf area and pigment content of Chinese cabbage were all quite similar, whether plants were grown in the absence or presence of UV-A+B (2.2 mW cm⁻²). Elevated Cu⁺⁺ concentrations (2-10 µM) in the root environment and UV radiation had negative synergistic effects for Chinese cabbage and resulted in a more rapid and stronger decrease in plant biomass production and pigment content (Figure 2).
The quantum yield of photosystem II photochemistry (Fv/Fm) was only decreased at ≥ 5 μM Cu²⁺ in the presence of UV radiation, when leaf tissue started to become necrotic (Figure 2). The enhanced Cu toxicity in the presence of UV was largely due to a UV-induced enhanced accumulation of Cu content in both roots and shoots. An enhanced Cu content strongly affected the uptake and assimilation of sulfur in plants. The total sulfur content of the root increased at ≥ 2 μM Cu²⁺ in presence of UV and at ≥ 10 μM Cu²⁺ in absence of UV and that of the shoot increased at ≥ 2 μM Cu²⁺ in presence of UV and at ≥ 5 μM Cu²⁺ in absence of UV. In the shoot it could be attributed mainly to an increase in sulfate content. Moreover, there was a strong increase in the water-soluble non-protein thiol content upon Cu²⁺ exposure in the root and, to a lesser extent in the shoot, both in the presence and absence of UV.

The regulation of the uptake of sulfate responded to the occurrence of Cu toxicity directly, since it was more rapidly affected in the presence than in the absence of UV radiation (Figure 3).
For instance, the expression and activity of the high affinity sulfate transporter, Sultr1;2, were enhanced at ≥ 2 µM in the presence of UV, and at ≥ 5 µM Cu²⁺ in the absence of UV. In the shoot, the expression of the vacuolar sulfate transporter, Sultr4;1, was upregulated at ≥ 5 µM Cu²⁺ in the presence and absence of UV whilst the expression of a second vacuolar sulfate transporter, Sultr4;2, was upregulated at 10 µM Cu²⁺ in the presence of UV. It is suggested that high Cu tissue levels may interfere/react with the signal compounds involved in the regulation of expression and activity of sulfate transporters. The expression of adenosine S’ phosphosulfate reductase in the root was hardly affected and was slightly down-regulated at 2 µM in the presence of UV and at 10 µM in the absence of UV.

Copper toxicity in Chinese cabbage is not influenced by plant sulfur status, but interferes with correlations between sulfur metabolism-related gene expression and the suggested regulatory metabolites

M. Shahbaz, C.E.E. Stuiver, F.S. Posthumus, S. Parmar⁵, M.J. Hawkesford⁶ and L.J. De Kok

⁵Plant Science Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ. U.K.

Exposure of plants to elevated levels of metals may affect the uptake of sulfate by the roots as the consequence of a higher sulfur demand for the synthesis of sulfur-rich metal-chelating compounds, e.g. phytochelatins and metallothionins, the interference of the metals with the sulfate transporters directly and/or their reaction with the putative regulatory signal compounds. However, toxicity of Cu for Chinese cabbage was hardly affected by the sulfur nutritional status, despite the occurrence of slight differences in Cu contents of the tissues, e.g. upon H₂S exposure. The impact of elevated Cu²⁺ concentrations on biomass production, dry matter content and pigment content was quite similar for sulfate-sufficient plants, both in the presence and in the absence of H₂S, and for plants grown with H₂S as the sole sulfur source. Despite the observed differences in shoot to root ratios between sulfate-sufficient and sulfate-deprived plants, Cu exposure hardly affected shoot to root biomass partitioning. H₂S exposure of sulfate-sufficient and sulfate-deprived plants resulted in considerable differences in total sulfur, sulfate and water-soluble non-protein thiol contents, and the level of activity and expression of the sulfate transporters and of APS reductase, however it hardly affected the development of Cu toxicity symptoms.

H₂S exposure hardly affected the total sulfur content of roots and shoots of Chinese cabbage, since it resulted in downregulation of the sulfate uptake capacity of roots. The H₂S-induced partial downregulation of the sulfate uptake capacity occurred at all Cu²⁺ concentrations, despite the occurrence of an upregulation of the sulfate uptake capacity with increasing Cu content (Figure 4).
Figure 4. Impact of Cu\(^{2+}\) and H\(_2\)S exposure and sulfate deprivation on sulfate uptake capacity of Chinese cabbage. 10-day-old seedlings of Chinese cabbage were grown on a 25% Hoagland solution containing 500 µM sulfate (+S, light grey bars), fumigated with 0.2 µl l\(^{-1}\) H\(_2\)S (+S, H\(_2\)S, black bars) or 0 µM sulfate (-S, white bars), fumigated with 0.2 µl l\(^{-1}\) H\(_2\)S (-S, H\(_2\)S, dark grey bars) for 11 days. Plants were exposed to 0 to 15 µM Cu\(^{2+}\) in the root environment. Data on sulfate uptake capacity (µmol g\(^{-1}\) FW root h\(^{-1}\)) represent the mean of two independent experiments with 3 measurements with 3 plants each (± SD). Means with different letters are significantly different at p ≤ 0.01 (Student’s t-test).

H\(_2\)S exposure resulted in a decreased expression of Sultr1;2 and Sultr4;1 in the root and depressed the Cu-induced upregulation of these transporters. Sulfate deprivation resulted in an upregulated sulfate uptake capacity of the roots, and strongly enhanced expression of the Group 1, 2 and 4 sulfate transporters and APS reductase in roots and shoots (Figure 5).
H₂S exposure suppressed the upregulation of expression of sulfate transporters and APS reductase in the shoot upon sulfate deprivation. However, the strongly enhanced expression of sulfate transporters in the roots upon sulfate deprivation remained unaffected by H₂S exposure, whereas the sulfate uptake capacity increased even further (Figure 4 and 5). The shoot to root interaction between sulfur assimilation and the expression and activity of the sulfate transporters was ineffective in the absence of sulfate in the root environment, even though the atmospheric sulfur supply was sufficient to cover the plant sulfur demand for growth.

High Cu tissue levels removed correlations between sulfur metabolism-related gene expression and the suggested regulatory metabolites; apparently the signal transduction pathway of the sulfate transporters and APS reductase were overruled at high Cu tissue levels. When Chinese cabbage was exposed to elevated Cu²⁺ concentrations, the expression and activity of Sultr1;2 in roots and the expression of Sultr4;1 and Sultr4;2, and of APS reductase in both root and shoot were upregulated, even with a high sulfate content in roots and shoots (Figure 5). Moreover at elevated Cu²⁺ concentrations, there was hardly any relation between abundant water-soluble non-protein thiol levels (for the greater part reduced glutathione and for a lesser part phytochelatins and
cysteine) and the expression and activity of the sulfate transporters at sulfate-sufficient and at sulfate-deprived conditions, both in presence and absence of H$_2$S.

The presumed signal transduction pathway in the regulation of expression and the activity of the sulfate transporters and APS reductase (sulfate and water-soluble non-protein thiols) was bypassed or overruled, if Chinese cabbage was exposed to elevated Cu$^{2+}$ in the root environment. A key unresolved issue is the signal transduction between the sulfate reduction pathway in the chloroplasts/plastids and the transcription of sulfate transporters/sulfate reducing enzymes in the nucleus. It has been suggested that H$_2$S, as the first product of the sulfate reduction pathway, might function in plants in the cross-talk between the sulfate reduction pathway in chloroplast/plastid and the transcription of sulfate transporters/sulfate reducing enzymes in the nucleus. H$_2$S reacts rapidly with free Cu$^{2+}$ ions resulting in the formation of CuS as a precipitate. If H$_2$S would function as an endogenous gaseous transmitter in the cross-talk between the sulfate reduction pathway in chloroplast/plastid and the transcription of sulfate transporters/sulfate reducing enzymes in the nucleus, a Cu-induced decrease in H$_2$S concentration might be responsible for the observed upregulation of the expression and activity of the sulfate transporters and the expression of APS reductase.

**Metabolic control of nutrient use efficiency at a whole plant perspective in a changing global atmosphere**

M. Reich, M. Staal, J.T.M. Elzenga & L.J. De Kok

This project deals with the question how plants are able to fine-tune their nutrient uptake with the internal demand and the outside nutrient concentrations. Biochemical signalling involved in the control of nutrient uptake still widely remains a puzzle. The efficient up- and down-regulation of nutrient uptake by plant roots is a crucial process for plants. Up-regulation is needed to cover an increasing demand of the plant or to adapt to decreasing nutrient concentrations in the rhizosphere, while down-regulation on the other hand is needed to avoid an over-accumulation of nutrients but also to save energy, as nutrient uptake from the soil is highly energy demanding. But what is the chemical signal that “tells” the plant that either up- or down-regulation of the uptake system is required? How does the plant sense that it is sufficient or deficient of a certain nutrient? Furthermore plants that face a longer period of deprivation seem to change more than only the gene expression of the proteins which are responsible for the uptake: If plants are e.g. deprived from sulfate (an important macro-nutrient for plants) the uptake system of the plant is up-regulated within the first 24 hours. If plants are re-supplied with sulfate, the uptake system is depressed again quickly. However, if deprivation lasts for a longer time, this down-regulation does not set in anymore so promptly. Of what kind this adaptation to a low availability of nutrients exactly is, remains unknown until now.

To find out more about the regulation of sulfate uptake and to identify potential signal compounds, an ion-selective microelectrode (MIFE) is applied, for the first time to study sulfate uptake. The possibility to measure the fluxes of protons into intact plant roots (which are directly linked to the uptake of sulfate, as sulfate is imported via a sulfate/proton-symport), offers the chance to observe changes in sulfate uptake in a much higher spatial and temporal resolution than it could be done with methods based on radioactive labels or gene expression of sulfate transporters. In a first set of experiments it could be shown that proton fluxes in response to sulfate addition can indeed be used as a measure for sulfate uptake. *Brassica* plants that were deprived from sulfate for several days showed an influx of protons at the roots in response to a very low (70 µM) and a rather high (5 mM) concentration of sulfate, while roots of plants that were grown with sufficient sulfate showed this effect to a much lower extend or not at all (Figure 6).
Figure 6. Proton fluxes and pH at roots of *Brassica pekinensis* plants that were grown under sufficient conditions or sulfur deprived for 5 days. Bolts mark the time of addition of 70 µM and 5 mM MgSO₄. For a positive charged ion such as protons, a positive flux is per definition an influx. With this method, sulfate influx into plant roots can be estimated, as sulfate is imported via a sulfate/proton-symport.

This shows that especially the high-affinity sulfate uptake system is up-regulated in plants grown without sulfate, and that this fact can be measured using MIFE. In the field of sulfur research, which is currently dominated by molecular methods, our electrophysiological approach will deliver valuable contributions. E.g. we will now be able to test different potential signal compounds for their effect on the sulfate transport.

An additional aspect of this project is the effect of expected elevated levels of carbon dioxide (eCO₂) in a future atmosphere on the regulation of sulfate uptake and the sulfate use efficiency of plants. Experiments with plants under eCO₂ have shown an enhanced biomass production during the exponential growth phase and a decreased sulfur content in the leaves of these plants. This decrease recovered when plants left the exponential growth phase. It will furthermore be interesting if plants respond adequately to sulfur deficiency when they are exposed to eCO₂. It was found that plants, which were sulfate deprived for 6 days did not benefit from eCO₂ in form of an increase in structural biomass but accumulated a surplus of dry matter content in the shoot when they were simultaneously exposed to eCO₂, which was not the case for the roots (Figure 7).
Furthermore it is a well-known response of plants to eCO₂ to reduce the leaf area relatively to the plant biomass. Or in other words, the expansion of the leaf area did not keep pace with the increase in biomass. Under sulfur deficiency the same is usually observed in a much higher extent (Figure 7). The combination of both treatments, however, led to an even lower specific leaf area (SLA, leaf area per gram biomass). As the SLA is a proxy of the ratio of assimilating to respiring tissue, it is generally seen as an indicator for the plants future potential for growth. It is intriguing to know how plants that experienced a period of sulfur deficiency under eCO₂ will react to a re-supply with sufficient sulfate. Will they be able to make use of the additional, non-structural biomass that was stored during the time of starvation or will this high accumulation of carbohydrates disturb biomass and nutrient allocation? And might the decreased SLA due to eCO₂ be unbenefficial for continuing
maximum growth after re-supply? Answering these questions will help to estimate the consequences of eCO\textsubscript{2} due to anthropogenic climate change relation to sulfur supply and contribute to future projections concerning food security.

The project is embedded in the Marie-Curie Initial Training Network “BIONUT” (Biochemical and Genetic Dissection of Control of Plant Mineral Nutrition; www.bionut-itn.eu).

**Primary and secondary sulfur metabolism in Brassica species is affected by different sulfur sources**

Tahereh Aghajanzadeh, Malcolm J. Hawkesford\textsuperscript{a}, Sujeeth Neerakkal\textsuperscript{b}, Anna Koprivova\textsuperscript{c}, Stanislav Kopriva\textsuperscript{c}, Luit J. De Kok

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\textsuperscript{b}BioAtlantis Ltd., Kerry Technology Park, Tralee, Co. Kerry, Ireland
\textsuperscript{c}Department of Metabolic Biology, John Innes Centre, Norwich Research Park, Norwich

Sulfur is an essential macronutrient for plants, which is taken up as inorganic sulfate by the root. It is reduced and incorporated into cysteine, an amino acid at the crossroads of primary and secondary sulfur metabolism. Low molecular thiol compounds like glutathione are primary sulfur-containing compounds and are involved in defence against oxidative stress, detoxification of heavy metals or xenobiotics, and also in biotic interactions. Capparales contain secondary sulfur compounds, viz. glucosinolates are the best-known secondary sulfur compounds, which also may have significance in plant-pathogen interactions. In addition to sulfate, plants are able to take up atmospheric sulfur gases (H\textsubscript{2}S or SO\textsubscript{2}) via the leaves. Foliarly absorbed H\textsubscript{2}S may be directly metabolized into cysteine and subsequently into other organic sulfur compounds. Absorbed SO\textsubscript{2} may enter the sulfur reduction pathway as sulfite or as sulfate and is subsequently incorporated into cysteine and other organic sulfur compound. Excessive absorbed SO\textsubscript{2} may be transferred into the vacuole as sulfate. A 10 days exposure of Brassica species (Brassica juncea var. rugosa and Brassica. rapa var. komatsuna) to 0.25 µl l\textsuperscript{-1} H\textsubscript{2}S or SO\textsubscript{2} showed that both species were able to directly utilize foliarly absorbed atmospheric sulfur gases as sulfur source for growth. Exposure of both species to H\textsubscript{2}S or SO\textsubscript{2} hardly affected total plant sulfur content, whereas the sulfate uptake capacity was decreased at sulfate-sufficient condition, which indicated that plants had partially transferred from sulfate to atmospheric sulfur gasses as sulfur source. The water-soluble non-protein thiol content of the shoot of B. juncea and B. rapa was enhanced at 0.25 µl l\textsuperscript{-1} H\textsubscript{2}S and of B. rapa at 0.25 µl l\textsuperscript{-1} SO\textsubscript{2} (Figure 8).

![Figure 8](image-url)
Sulfate deprivation resulted in a decreased biomass production of both species and strongly decreased total sulfur and thiol content in both shoot and root. After exposure of plants to H₂S or SO₂, plant biomass production was restored; however, the total sulfur was still lower than that of sulfate-sufficient plants. There was a substantial increase in thiol content of shoot of B. *rapa* even more than sulfate-sufficient plants but thiol content in the shoot of B. *juncea* remained lower than that of sulfate-sufficient plants.

At an ample sulfate supply, exposure of both species to H₂S and SO₂ hardly affected content and composition of glucosinolate in both shoot and root, which was similar to total sulfur content (Figure 9).

![Figure 9](image.png)

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Upon sulfate deprivation, aliphatic, indol and total glucosinolate content of the shoot and root of *B. juncea* severely decreased whereas these were not affected in *B. rapa*, especially in the shoot. Exposure of *B. juncea* to H₂S and SO₂ partially increased individual and total glucosinolate content but in *B. rapa* it remained low and was similar to that in sulfate-deprived plants.

**Adaptive nitrogen uptake in peat-forming *Sphagnum magellanicum* mosses – a delicate trade-off between saturation and leaching**

Christian Fritz, Leon van den Berg, Leon Lamers, Muhammed Riaz, Theo Elzenga

Depleting the nitrogen supply available to roots of vascular plants in the soil by efficiently scavenging deposited nitrogen enables *Sphagnum* mosses to dominate in ombrotrophic conditions. Under elevated nitrogen (N) deposition nutrient sequestration is hampered and peat-forming *Sphagnum* mosses become outcompeted by vascular plants. N-uptake kinetics of mosses plays a major role in...
regulating N-availability to vascular plants and consequently plant competition. Factors reducing moss N-uptake are poorly understood.

In order to predict the efficiency of Sphagnum mosses to prevent accumulation of inorganic N in the soil we investigated the effects of concentration (1, 5, 10, 50, 100 and 500 µM), N-form (¹⁵N ammonium and nitrate) and exposure time (0.5, 2, 72 hours) on the uptake kinetics in Sphagnum magellanicum from a pristine bog in Patagonia, Argentina. Uptake rates of the pristine Sphagnum mosses were compared with rates of Sphagnum mosses derived from a Dutch bog exposed to decades of N-pollution. During the first 0.5 h, N-uptake followed saturation kinetics revealing a high affinity (Kₘ 11 µM). Uptake rates decreased drastically with increasing exposure times, which implies that earlier short-term N-uptake experiments may have overestimated long-term uptake rates and ecosystem retention. Sphagnum from the polluted site (~ long-term N exposure) showed lower uptake rates than mosses from the pristine site.

In a model, the conceptualized relationship between nitrogen concentration in rain and exposure time a living Sphagnum layer would need to retain some 90% of the nitrogen load is calculated (Figure 10).

Figure 10. Conceptualized relationship (log-log) between nitrogen concentration in rain and exposure time a living Sphagnum layer would need to retain some 90% of the nitrogen load. The shaded area (average residence time of rain) indicates increasing potential of leaching through the living layer of mosses. Maximal uptake rates Vₘₐₓ differ between scenarios: closed red squares ‘ammonium_pulse’ 35 µmol N gDW⁻¹ h⁻¹, open squares ‘ammonium_long’ 3.4 µmol N gDW⁻¹ h⁻¹, closed circles ‘nitrate_pulse’ 3.7 µmol N gDW⁻¹ h⁻¹, open circles ‘nitrate_long’ 1.0 µmol N gDW⁻¹ h⁻¹, and 1.0 µmol N gDW⁻¹ h⁻¹ open triangles ‘nitrate_low affinity’ respectively. A rain event of 5 l was simulated. The model was parameterised with a Kₘ of 11 µmol l⁻¹ for all simulations except ‘nitrate_low affinity’ where a Kₘ of 50 µmol l⁻¹ was assumed. We also indicate residence time of rain on the y-axis. Soft rainfall may have an average residence time of 10 to 30 min and heavy rainfall may remain only 0.5 to 5 min in the Sphagnum layer. Average nitrogen concentrations in rain (µmol N l⁻¹) relate to yearly wet deposition of nitrogen (kg ha⁻¹) by factor 0.105 assuming a rainfall of 750 mm.

Long term exposure to rain with N concentrations that are normally found in the Netherlands will eventually lead to increased nitrogen availability in the soil and thus to an opportunity for vascular plants to invade a bog.
Sphagnum seems highly efficient in using short N pulses (e.g. rainfall in pristine areas). This strategy has important ecological and evolutionary consequences: risk of N-toxicity seems to be reduced at the expenses of long-term filter capacity and, therefore, competitive advantage over vascular plants.

**DMSP-production in sea-ice algae under various stress conditions**  
Maria A. van Leeuwe, Jacqueline Stefels

Sea-ice thickness and distribution is possibly the most sensitive indicator of global climate change. Current sea-ice-climate coupled models are still limited in covering all possible factors involved in climate regulation and sea-ice dynamics. One of these factors is the contribution of sea ice to the production of the climate active gas dimethyl sulphide (DMS). DMS acts as a climate-cooling gas through its effect on cloud formation. DMS originates from dimethylsulphoniopropionate (DMSP), which is produced in large amounts by sea-ice algae. DMSP has a range of functions (a.o. as osmoregulator and cryoprotectant) and may play a crucial role in the physiology of cells to survive the extreme sea-ice conditions.

Within this NWO-Polar Program funded project, we collaborate with colleagues from the USA to develop biogeochemistry modules in polar ocean-ice models. A first model incorporating DMS in an Arctic regional model was developed based on our experimental data from Antarctic sea ice core studies (Elliott et al. 2012). The model consisted of a relatively crude module of a biogeochemically active bottom-ice layer that acts as a source and sink for ice diatoms, which produce the sulfur compounds. Nutrients transfer from the ocean mixed layer to drive algal growth, while sulfur metabolites are reinjected in the surface ocean from the ice interface. The model calculates DMS concentrations that rise to well over 10 nM in remote, un-sampled locations (Figure 11).

![Figure 11. Baseline simulation of ice algal source organisms, (left) log10 mg/m² chlorophyll, along with (right) log10 nM dimethyl sulfide injected into the surface ocean, in May and June of 1992. Ice edges are defined by the 15% ice concentration contour (white), and thicknesses are superimposed in meters (black). (Elliott et al. 2012)](image-url)
This study indicated that bottom-ice can add substantially to reduced sulfur chemistry that may be dominant across the marginal Arctic environment. This first model exercise also concluded that real in-situ data are currently far too sparse to distinguish sea ice specific sulfur production levels.

In order to improve the DMS modules in climate models, algorithms for DMSP production in ice algae under a variety of environmental conditions are required. To develop such algorithms, several experiments were performed with ice algae. Two ice algae were selected based on their ecological relevance: the diatom *Fragillariopsis cylindrus* and the Haptophyte *Phaeocystis antarctica*. Diatoms are generally more abundant, especially in bottom-ice communities, whereas *Phaeocystis* is less abundant but known to produce more DMSP and often occupies the surface community of sea ice. The diatom *Fragillariopsis cylindrus* was cultured at a range of salinities, mimicking conditions that occur in sea ice. Basic parameters such as growth rate and DMSP content were recorded to feed models. In addition, photosynthetic parameters were recorded to study the photophysiological response of the algae (fluorescence analysis by PAM, pigments by HPLC). Growth rate based on cell counts showed that growth was suppressed at lower and elevated salinity. At the same time, the DMSP content increased. Pilot experiments with *Phaeocystis antarctica* showed that the DMSP-content is an order of magnitude higher in the Haptophyte.

In sea ice, algae often experience multiple stress factors. When sea ice forms, not only salinity increases inside brine pockets but also the carbon availability changes. This can have important implications on photosynthesis and the ability to handle light stress. Experiments under combined stress conditions were performed with both species to determine which algae would grow best and to determine how they would respond in terms of DMSP production. Specific DMSP-production was in almost all cases found to be higher in *Phaeocystis antarctica* than in *Fragillariopsis cylindrus* (Figure 12).

![Phaeocystis in vivo DMSP production](image1)

![Fragillariopsis in vivo DMSP production](image2)

**Figure 12.** Specific DMSP-production during a 6-hour period in *P. antarctica* and *F. cylindrus* at low and high pH and at two different salinities. Shaded bars represent light-stressed cultures.

Production was highest at low pH and high salinity in *P. antarctica*, but not for *F. cylindrus*. The species also responded differently to light stress. These results argue for the use of two key-species in sea-ice models.
Plant Biology is an international journal of broad scope bringing together different subdisciplines, such as physiology, molecular biology, cell biology, development, genetics, systematics, ecology, evolution, ecophysiology, plant-microbe interactions, and mycology. Plant Biology publishes original problem-oriented full-length research papers, short research papers, and review articles. Discussion of hot topics and provocative opinion articles are published under the heading Acute Views. From a multidisciplinary perspective, Plant Biology will provide a platform for publication, information and debate, encompassing all areas, which fall within the scope of plant science. Plant Biology is the official journal of the German Botanical Society and the Royal Botanical Society of the Netherlands. In 2012, six regular issues were published and one Special Issue: “Plant-Pathogen Interactions”.


This Special Issue on the Wadden Sea Region is inspired by the Trilateral Scientific Symposium on the Wadden Sea Region, organized by the Wadden Academy, on December 8–10, 2010, in Leeuwarden, The Netherlands.

Organisation of international meetings

Within the European network “Biochemical and Genetic Dissection of Control of Plant Mineral Nutrition (BIONUT-ITN)” a meeting was organised: Complementary Skills Course 2 “Scientific Publishing and Presentation Skills” & Mid-Term Review, Hampshire Hotel - Plaza Groningen, The Netherlands, October 28-31, 2012.
Publications 2012

Publications in journals:


Publications in edited books:


Evolutionary Genetics

http://www.rug.nl/fmns-research/evolutionary-genetics/index

**Group leader**
Prof. dr. L.W. Beukeboom

Composition of the group in 2012:

**Tenured Staff**

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**PhD-students**

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**Technical and Administrative Staff**

**Secretary**
E.E.J. Plender-Hartman RUG 0.4

**Analyst/Technician**

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**Adjunct chair**

Prof. dr. M. Schilthuizen NCB Naturalis 01-12-2007 – 01-12-2012

**Guest**

dr. M. Trienens Volkswagen Stiftung 1.0 01-08-2012 – 01-08-2014
At Evolutionary Genetics we investigate genetic mechanisms and life-history traits of organisms from an evolutionary perspective. We ask how population structure and genetic variation affect and, in turn, are shaped by, evolutionary and ecological processes. Research focus is on the genetics and evolution of reproductive systems and life histories, genetics of speciation, genetics and genomics of evolutionary adaptations, and the genetics and evolutionary ecology of biodiversity. We focus on the link between genotype and phenotype, combining molecular and genomic approaches with genetic crosses and measurements of individual life-history traits. Our main research themes are: (1) Genetics and evolution of sex determining mechanisms; (2) Genetics and evolution of reproductive isolation; (3) Genetics, ecology and evolution of life-history traits and reproductive strategies; and (4) Genomics of host-parasitoid interactions. Our main study organisms are parasitoid wasps (a.o. Nasonia, Asobara, Leptopilina) and flies (Drosophila, Musca). Below we present our highlights, as well as several projects that showcase the diversity of our research.

Highlights of Evolutionary Genetics

In June 2012 we organised a mini-symposium on "Adaptive and non-adaptive processes in life-history evolution and speciation" in conjunction with the promotion of Tosca Koevoets. She successfully defended her thesis entitled “Deadly combinations. Hybrid incompatibilities in the parasitic wasp genus Nasonia” and was awarded the cum laude degree. The abstract of her thesis was published as an article in Entomologische Berichten. We were very successful in obtaining grants for five new Phd students. Three new positions are within the Marie Curie ITN network “INsecTIME” (figure 1) for research on photoperiodism and diapause in Nasonia. The other two positions are funded by an Open Competition grant of the Netherlands Organisation for Scientific Research (NWO) in collaboration with colleagues at the University of Zurich, and an Ubbo Emmius sandwich position with the University of Göttingen, both for investigating sex determination evolution in houseflies. A new postdoc joined our lab with funding from the German Volkswagen Stiftung, working on the genomics of adaptations in fly-fungal competitive interactions. We were invited for writing a review article entitled “Polyploidy in animals: effects of gene expression on sex determination, evolution and ecology” for a special issue of Cytogenetics and Genome Research on “Trends in Polyploidy Research in Animals and in Plants”. As a wrap up of our ITN network SPECIATION we co-authored an invited article in Trends in Ecology and Evolution entitled “What do we need to know about speciation?”

Figure 1. The Marie Curie ITN Network INsecTIME logo
Overview of academic results

Speciation genetics
Leo Beukeboom, Louis van de Zande, Tosca Koevoets, Maartje Giesbers, Wenwen Diao, Silvia Paolucci

Hybrid incompatibility is an important factor during speciation and studying its genetic basis will provide better understanding of the emergence of new species. Haldane’s rule states that early during speciation, hybridization affects the heterogametic sex more than the homogametic sex and the question was raised whether this is due to phenotype (sex) or genotype (heterogamety). We showed previously that haplodiploid reproduction obeys Haldane’s rule, but were not able to resolve the genetic basis of the observed pattern. Using advanced genetic tools in Nasonia, we explored the separate effects of heterozygosity and maleness. To this end we generated sex-reversed diploid Nasonia males and compared incompatibilities across sexes and ploidy levels. We found that hybrid mortality and sterility were significant for haploid males, confirming previous results, but minimal for both diploid males and females, which indicate the absence of sex-specific incompatibilities. The genomes of hybrid diploid females and hybrid diploid and haploid males were analyzed with microsatellite markers to identify genomic regions associated with hybrid mortality (figure 2). Similar genomic regions were identified in both diploid males and females, but fewer as compared to haploid males. Most incompatibilities identified under hemizygosity were rescued under diploidy in hybrid males and females. Our results indicate dominance effects at some genomic regions, but more often dosage effects seem to rescue hybrid mortality. These dosage effects revive a neglected theory in explaining Haldane’s rule, which has implications for X-linked incompatibilities in diploid systems.

Figure 2. Quering the Nasonia genome for hybrid incompatibilities (cover of PhD thesis T. Koevoets 2012)

Genetic variation for mate choice is believed to be abundant in natural populations, but we have little knowledge about how this variation is maintained and how fast it can respond to selection. Likewise, few studies have investigated how selection on mate discrimination affects other aspects of reproductive behaviour. Nasonia giraulti and N. vitripennis are two parasitoid wasp species that occur in sympathy in eastern North-America. Infection with species-specific strains of the endosymbiont Wolbachia prevents production of hybrid offspring, making interspecific mating very costly. We investigated the response of N. vitripennis females to artificial selection for lower mate discrimination against N. giraulti males. Mate discrimination changed from 80% to 50% within 8 generations of selection with a heritability of 0.29 (figure 3). Relaxation of selection over five generations did not change the degree of mate discrimination. Correlated responses to selection were observed in within-species mate discrimination and in interspecific mate discrimination against males of a third species (N. longicornis), indicating a partially similar genetic basis of intra- and
interspecific female mate discrimination. Female re-mating rate and male mating behaviour were not affected by selection for reduced female mate discrimination, revealing an independent genetic architecture for those aspects of reproductive behaviour.

Figure 3. Results of artificial selection for reduced interspecific mate discrimination. Mate discrimination of *N. vitripennis* females against *N. giraulti* males in percentage, measured during eight generations of selection followed by within-line random mating. Error bars display calculated standard error.

One current focus in speciation research is on the underlying genetics of *reproductive isolation*, with the aim to identify the chromosome regions and genes that prevent hybridization. We used interspecific crosses within the parasitoid wasp genus *Nasonia* (Hymenoptera, Pteromalidae), to investigate the genetic architecture of two main pre-zygotic isolation traits: behavioural mate preferences and male sex pheromones. Among *Nasonia* species, *N. oneida* females have a high mate discrimination against *N. giraulti*, but *N. giraulti* females are less choosy. Both species differ in male sex pheromones that are used to attract females. We analysed mating behaviour and the production of sex pheromones in around 500 recombinant hybrid males and mate choice in almost 3000 reciprocally backcrossed females. Several courtship components were measured in both male and female hybrids, including “latency time”, “duration of series” and “copulation occurrence”. Hybrid males showed intermediate courtship and production with ample variation for quantitative analysis. Hybrid females showed intermediate mate discrimination. Quantitative Trait Locus (QTL) analysis using a genetic linkage map with 52 SNPs markers that were equally spaced at 10cM intervals covering all 5 chromosomes, plus 40 candidate mating behaviour genes identified two QTLs for amount of male sex pheromones. No QTL were found for female mate discrimination, suggesting a complex genetic basis with large environmental effects.
Evolutionary genomics of ecological interactions

Bregje Wertheim, Kirsten Jalvingh, Laura Salazar-Jaramillo, Sylvia Gerritsma, Ammerins de Haan, Monika Trienens

One of the key challenges in biology is to unravel how organisms can evolve and adapt to their complex, changeable environments. Until only 5 years ago, it was virtually impossible to study how whole genomes are affected by ecology and evolution. Although genomic technology provided us with the whole genome sequences of multiple species, the high costs and time required for sequencing a single genome typically prevented the sequencing of more than one individual or species per taxon. Yet, to understand evolutionary processes and dynamics, the important step is to study the genetic variation. With the latest advances in DNA technology and genome sequencing, it has now become possible to sequence many species and individuals per species. As a result, we can now associate complex traits and phenotypes with variation across the whole genome, or re-sequence individuals after an evolutionary adaptation. This allows us, for the first time, to quantify how much genetic variation exists between individuals or species, and which part of that variation is impacted by ecological interactions and the evolution of the genome.

We study the evolutionary genomics of ecological interactions in *Drosophila* fruitflies. In fruitflies, we can combine our extensive understanding of their ecology with the formidable molecular toolbox and knowledge on mechanistic processes for various life history traits. We aim to elucidate the genomic variation and genetic networks underlying the evolution of complex traits in ecological interactions, using a combination of experimentation, genomic approaches and bioinformatics. In our group, we investigate the changes in the genome that occur during host-parasite co-evolution, competitive turf wars for food, sexual conflicts, and the composition of the microbiome.

Evolution of parasitoid resistance

The ability to resist parasitoid wasps varies hugely, both among and within species of the *Drosophila* genus, from completely absent in some species and natural populations to high resistance in others. Using a combination of phenotypic assays and genomics approaches, we identify genomic changes associated with gains, losses and increases of parasitoid resistance.

![Figure 4. We found large geographic variation in resistance to parasitoids, which was related to the proliferation of two differentiated types of blood cells in optimal ratios.](image-url)
We investigated the possible causes for the variation in parasitoid resistance among 24 natural *D. melanogaster* populations, and concluded that the mechanisms underlying between-species variation did not explain the within-species variation. Unlike between-species variation, the variation in resistance was not correlated to the load of total hemocytes (i.e., insect blood cells), but depended on the proliferation of two differentiated types of blood cells in optimal ratios. The geographic variation in parasitoid resistance among these natural populations is persistent, and indicates that they evolved along different trajectories in response to the local community structures (Gerritsma et al, 2013). To elucidate how the genetic architecture for fine-tuning the immune response has evolved so differently among natural populations of *D. melanogaster*, we are now investigating the underlying genomic variation for this ecologically important trait.

![Tree diagram showing parasitoid resistance evolutionary history](image)

**Figure 5.** Parasitoid resistance is restricted to a sub-lineage of *Drosophila*, and co-occurs with the acquiring of a special type of blood cells and duplications of 11 immunity genes.

In a comparative approach across 11 sequenced *Drosophila* species, we showed that the ability to resist parasitoids is not highly conserved as is commonly assumed, but evolved in a branch of the *Drosophila* lineage and was lost in one species of this group. We associated the between-species evolution of parasitoid resistance with the acquiring of a special type of differentiated blood cell and with the duplication of 11 genes that are up-regulated during the immune response against parasitoids. Furthermore, these genes have undergone large-scale sequence changes, likely associated with a loss-of-function in the species that secondarily lost immunity against parasitoids. Our approach allowed us to show that a combination of co-option of existing gene interaction networks, recent gene duplications and rapid divergence co-occurred with the gain of parasitoid resistance in *Drosophila* (Salazar-Jaramillo et al, submitted).
Figure 6. A selective sweep for parasitoid resistance caused pronounced shifts in allele frequencies in narrow genomic regions.

To assess the effect of a rapid and strong selective sweep across the genome and to infer candidate genes potentially conferring increased resistance against parasitoid attack, we used an experimental evolution approach. We generated **replicated selection lines** for increased resistance to parasitoids, and used whole-genome sequencing to identify regions that showed signatures of that positive selection. Several narrow genomic regions across all chromosomes carried a strong signature of selections, and we identified 42 genes within these regions that were potentially the targets of selection. By using a large, genetically diverse natural population as basis for our replicated selection lines, we were able to realistically map the genomic changes due to a sudden and strong selective pressure on a complex trait. We conclude that even under rapid evolutionary adaptation, not one or few loci are under strong selection, but the natural genetic variation across a suite of genes is exploited (Jalvingh et al, in preparation).
Competitive turf wars

Figure 7. The competition for food among Drosophila larvae and fungi resulted in co-evolutionary arms races, including toxins and behavioural adaptations.

Competition among species is a major organizing force in community ecology, and species can evolve complex strategies to survive competition. Two major players for ecosystem function are filamentous fungi and insects, and the turf wars between these taxa resulted in various adaptions and counter-adaptations. Fungi can engage in chemical warfare producing various insecticidal toxins, while insects can evolve detoxification mechanisms, immunity and behavioural adaptations (“social immunity”). In this project, we use Drosophila flies and Aspergillus fungi for the functional genomic analysis of this co-evolutionary arms race and to characterize the genomic changes associated with it. The detailed phenotypic characterization of the effect of the fungus on the fruit fly is now complemented by measuring the whole-genome transcriptional responses to the chemical shield produced by the fungus. We also started an experimental evolution experiment with fruit fly larvae for increased survival probabilities upon confrontation with the fungus and mycotoxin.

Genetics of sex determination
Louis van de Zande, Leo Beukeboom, Eveline Verhulst, Elzemiek Geuverink, Ammerins de Haan, Anna Rensink

In 2012 we continued our research on Nasonia sex determination, exploiting the Nasonia Genome Sequence and building upon our two previous Science publications on the subject. We focused on identification of the previously discovered imprinting factor. Although sex determination is a universal process in sexually reproducing organisms, sex determination pathways are among the most highly variable genetic systems found in nature. Nevertheless, general principles can be identified among the diversity, like the central role of transformer (tra) in insects. When a functional TRA protein is produced in early embryogenesis, the female sex determining route is activated, while prevention of TRA production leads to male development. In dipterans, male development is achieved by prevention of female-specific splicing of tra mRNA, either mediated by X-chromosome dose or masculinizing factors. In Hymenoptera, which have haplodiploid sex determination,
complementary sex determination and maternal imprinting have been identified to regulate timely TRA production. In the parasitoid Nasonia, zygotic transformer (Nvtra) expression and splicing is regulated by a combination of maternal provision of Nvtra mRNA and silencing of Nvtra expression in unfertilized eggs. It was unclear, however, if this silencing is directly on the tra locus or whether it is mediated through maternal silencing of a trans-acting factor. We showed that in Nasonia, female sex determination is dependent on zygotic activation of Nvtra expression by an as yet unknown factor (figure 8). This factor, which we propose to term womanizer (wom), is maternally silenced during oogenesis to ensure male development in unfertilized eggs. This finding implicates the upstream recruitment of a novel gene in the Nasonia sex determining cascade and supports the notion that sex determining cascades can rapidly change by adding new components on top of existing regulators.

Although the role of DNA methylation in insect development is still poorly understood, the number and role of DNA methyltransferases in insects vary strongly between species. DNA methylation appears to be widely present among the social hymenoptera and functional studies in Apis have suggested a crucial role for de novo methylation in a wide variety of developmental processes. The sequencing of the Nasonia genome revealed the presence of three Dnmt1 (Dnmt1a, Dnmt1b and Dnmt1c) genes and one Dnmt2 and Dnmt3 gene, suggesting a role of DNA methylation in Nasonia development. Here we show that in Nasonia all Dnmt1 mRNAs and Dnmt3 mRNA are maternally provided to the Nasonia embryo of which Dnmt1a is essential during early embryogenesis in Nasonia. Lowering of maternal Dnmt1a mRNA results in embryonic lethality during the onset of gastrulation. This dependence on maternal Dnmt1a during embryogenesis in an organismal group outside the vertebrates, suggests evolutionary conservation of the function of Dnmt1 during embryogenesis.

Figure 8. Nascent transcription of Nvtra compared to that of a control developmental gene Nvcad. Nuclear spots of Nvtra (green) and Nvcad (red) reveal that there is no parent dependent imprinting in either of the Nvcad or Nvtra locus in early (A) or late (B) blastoderm stage female embryos. In male embryos (C,D) there is moderately less Nvtra expression in early blastoderm stage (C) and significantly less in late blastoderm stage (D), which is in agreement with previous expression results.
Doi:10.1371/journal.pone.0063618.g003
Consequences of Complementary Sex Determination
Leo Beukeboom, Jetske de Boer, Wen-Juan Ma, Bram Kuijper (Theoretical Biology), Bart Pannebakker, Louis van de Zande

Many parasitoids in the insect order Hymenoptera have a sex determination mechanism based on allelic variation at a single locus, or in some cases multiple loci, referred to as Complementary Sex Determination. Reduced genetic variation is especially problematic in these parasitoids because of the production of diploid males. Males are normally haploid in sexually reproducing Hymenoptera as they develop from unfertilized eggs. Fertilized eggs usually develop as diploid females as long as they are heterozygous at (at least one) sex locus. Diploid males however result from homozygosity at the sex locus under and are thus produced instead of females. Because they are generally unviable or (effectively) sterile, they represent a severe form of inbreeding depression. Inbreeding may thus lead to male-biased sex ratios and can reduce population growth rates and rates of establishment. Natural enemies used for importation biological control are at risk of going through population bottlenecks during collection, culturing or establishment. These bottlenecks may be associated with reduced genetic variation and are expected to impair biological control efficacy. Diploid male production under CSD may even theoretically lead to extinction through a so-called diploid male vortex: decreased population size leads to a reduction in sex allele diversity, leading to an increase in the production of diploid males, which in turn leads to further decreases in population size and so on. However, surprisingly little information is available on the occurrence of diploid males in field populations of parasitoid wasps and on the consequences of CSD for biological control.

We investigated the sex determination mechanism of Cotesia rubecula Marshall in a field population in North America. Based on the presence of CSD in other Cotesia species and previous unpublished results, we expected C. rubecula to exhibit CSD as well. We observed that diploid males occur at a relatively high rate (8-13% of diploid adults) in a field population in Minnesota, USA, where this parasitoid was introduced for biological control of the cabbage white Pieris rapae. However, our laboratory crosses suggested two locus CSD in a native Dutch population, and moderately high diploid males survival (~70%); a scenario expected to produce low proportions of diploid males.

We also investigated the presence of CSD mechanisms in the braconid wasp genus Asobara, which comprises species with diverse life-histories. Multiple generations of inbreeding were monitored for offspring sex ratio patterns, mortality and the occurrence of diploid males as indicators for CSD. In addition, simulation models were developed to compare the observations to predicted patterns for increasing numbers of CSD loci. The inbreeding regime did not lead to altered offspring sex ratio patterns, increased wasp mortality, or increased diploid male frequencies in all four tested Asobara species. The simulation models allowed us to reject CSD with up to ten loci (Figure 9). This result is in contrast with most other tested braconid species. They further support the diversity and rapid evolution of sex-determining mechanisms in the Braconidae and in the Hymenoptera in general.
Figure 9. Simulation of secondary offspring sex ratios.
(a): Asobara tabida, (b): A. japonica, (c): A. citri and (d): A. pleuralis. 10 000 replicates of each experiment were simulated, assuming different numbers of unlinked csd loci, $n_{loci} = \{1, 2, 5 \text{ and } 10\}$. Blue shading with solid line represents predicted offspring sex ratio under CSD with one locus; green shading with dashed line represents the trend under CSD with two loci; red shading with dotted line for five loci, and pink shading with dot-dashed line for ten loci. Each color-shaded polygon represents the 95% confidence intervals of offspring sex ratio for a particular number of csd loci $n_{loci}$, which is listed on the left side of the polygons. Black dots represent observed mean offspring sex ratio per generation, and corresponding error bars represent 95% confidence intervals of the observed mean offspring sex ratio. Note that in A. pleuralis, the low initial brood size (on average five) in the 1st generation of the B-S cross makes the stochastic effects more pronounced, resulting in overlapping confidence intervals for model predictions during the first generations.

The signature of inbreeding depression in gene and protein expression
Corneel Vermeulen

In sexually reproducing species, increased homozygosity often causes a decline in fitness, called inbreeding depression. Inbreeding depression is thought to be mainly caused by the increased expression of recessive deleterious alleles (Figure 1). Although some of these alleles are known, we know little about the functional genomic causes of inbreeding depression. Recently, researchers started describing the functional genomic changes that occur during inbreeding, mainly in genetically accessible organisms like Drosophila melanogaster. Studies of genome-wide gene expression showed that, despite differences in the genetic background among inbred lines, there is a coordinated transcriptional response to inbreeding. This involves upregulation of genes involved in stress response, basic metabolism and immunity, suggesting that inbreeding is a source of cellular stress. I focus on Drosophila lines carrying conditional lethals. These flies display large and reproducible inbreeding effects at restrictive thermal conditions, but are otherwise easy to culture and handle. I have now finished analysis of genome-wide gene and protein expression during inbreeding.
depression in these lines. These studies were part of a collaboration with colleagues from Aarhus University (Denmark) and the Danish Technological Institute. The set of differentially expressed genes overlapped with that found in other studies of inbreeding depression in *D. melanogaster*, demonstrating that it is possible to detect a common signature across different genetic backgrounds. In addition, I also found differential expression that was lineage-specific, which may give clues about the causative mutations of the inbreeding effects. For example, a line that shows increased mortality after a mild cold shock had a transcriptional profile suggestive of an exaggerated cold stress response. Maybe the cold sensitivity in this line is caused by mutations in genes with a function in cold resistance.

![Figure 10. Quering the Drosophila genome for conditional lethals](image)

The interplay between native insects and non-native plants

*Kim Meijer, Menno Schilthuizen, Leo Beukeboom*

We continued our research on the interaction between native herbivorous insects and non-native plants. We ask whether non-native plants escape from their enemies (herbivorous insects) compared to native plants. We found differences between populations of two species of native insects which occur both on a native and a non-native plant. Larvae and pupae of *Rhagoletis alternata*, a true fruit fly, that lays eggs on rose hips are larger on introduced Japanese roses compared to native roses. Moreover, pupae of Japanese roses are parasitized five times less than those from native roses, which may be due to the fact that they can live deeper in the rose hips and are sheltered from wasp attack. The leaf beetle *Gonioctena quiquepunctata* feeds on the native rowan and the non-native black cherry (originally from North America). Beetles on black cherry were larger than those on native rowan. In a choice experiment, beetles collected on rowan showed a preference for rowan, but beetles from black cherry did not show a preference. These data indicate that, potentially adaptive, changes can occur rapidly in insect populations that colonize introduced host plants.
Publications 2012

Dissertations

Koevoets, T. Deadly combinations - hybrid incompatibilities in the parasitic wasp genus *Nasonia*. Promotor Prof.dr. L.W. Beukeboom, Copromotor Dr. L. van de Zande, University of Groningen.

Publications in journals


Books/chapter in books


Professional publications

Marine Benthic Ecology and Evolution (MarBEE)

Group leader
Prof. dr. J.L Olsen

Composition of the group in 2012:

Tenured staff

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Adjunct Chairs

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Overview of academic results

The theme of the Marine Benthic Ecology & Evolution group is “diversity in space and time”, which is spread over two broad work areas, each with several projects: 1) marine population genetics-genomics, hybrid zones, speciation and phylogeography (Olsen lead); and 2) experimental marine community ecology, focusing on factors that promote resilience in complex benthic communities (Eriksson lead). There is also a sub-programme related to invasive marine species (Olsen). The group utilizes field and laboratory methods with emphasis on links between ecological and evolutionary processes. There is a strong emphasis on the development and use of molecular ecological tools in combination with manipulative field experiments.

http://www.rug.nl/fmns-research/marbee/index

Research Line 1 - From the MarBEE portfolio of population genetics-genomics and phylogeography

ZEN and the art of seagrass ecosystem function

Beginning in 2011 the Zostera Experimental Network (ZEN) was established among 15 labs throughout the northern hemisphere (http://zenscience.org). The objectives of the project are to test: 1) mesograzers influence on structure and function; 2) the association of biodiversity (genetic and species levels) on structure and function along natural gradients; and 3) the context-specificity of bottom-up, top-down and diversity effects by assessing their variation along major global gradients in abiotic drivers.

The program is coordinated by Prof. Emmett Duffy, VIMS, USA with US-NSF funding, 2011-2015). Olsen and Eriksson are responsible for the northern Norway site near Bodø. In 2011-12 the first data and field experiments were conducted with all participants conducting the same experiment at their site (Figs 1 and 2).

Figure 1. Factorial design of the ZEN site in northern Norway.

Figure 2. Tripod design of treatments used in the ZEN experiments.
A preliminary analysis of this incredibly complex data set revealed the classical latitudinal gradients in diversity and strong bivariate correlations between biomass of mesograzers with nutrient status and genotypic richness. Model selection (n=26) based on biogeography, metabolic theory, biodiversity, resources, top-down control and interactions suggested that a saturated model (all main effects and interactions) was optimal. Preliminary results for the multi-level, mixed model analyses suggest that eelgrass genetic diversity (along with nutrient availability) is a key driver of global patterns in epifaunal biomass; that top-down control is pervasive; and that biodiversity may be of comparable importance to other fundamental drivers of major ecosystem processes and state variable on a sub-global scale.

Interestingly, genetic richness was the single strongest predictor of global variation in crustacean grazer biomass. The mechanism appears to be that genetically richer eelgrass plots tend to have higher biomass and/or shoot density. A second set of experiments involving an additional group of labs and measurement of additional parameters is under development for 2013. The ZEN programme has provided an excellent opportunity for Olsen and Eriksson to combine forces.

Seagrass ecosystems in the high north

Phylogeographic theory predicts that northern range edges will retain lower allelic diversity as a function of leading edge dynamics following recolonisation after the last glacial retreats 18,000 y ago. Many marine and terrestrial species follow this classic pattern. Although rear-edge populations of eelgrass have been studied extensively, leading edge populations have not. In cooperation with colleagues in Tromsø (69°N), Olsen surveyed three fjords, including 15 locations within the 60-km-long Balsfjord (Fig. 3).
Allelic richness in the regional pool was unexpectedly high suggesting that some fjord locations served as refugial pockets and that effective population sizes in many fjords are much larger than previously thought.

The three fjords showed strong population differentiation and isolation, whereas substantial gene flow was characteristic among locations within Balsfjord. Large clones were seldom encountered. However, strong linkage disequilibrium and high inbreeding coefficients suggested family structure within meadows. Thus, despite gene flow, meadows are probably demographically isolated as a consequence of sporadic recruitment.

Characterization of population structure (genetic and demographic) provides critical information about connectivity and ecological coherence that are relevant to marine spatial planning and protected area design. In Balsfjord eelgrass meadows provide spawning ground for herring and capelin, as well as grazing areas for numerous waterfowl. Thus, loss of eelgrass is of general concern. The widely perceived notion that populations found in high latitudes and/or at the extreme northern edge of a species’ distribution are genetically depauperate and ‘struggling’ is unsubstantiated, as our study showed that meadows of *Z. marina* along northern Norwegian fjords have a higher than expected level of regional diversity, turnover and were generally “healthy” in appearance.

**Comparative transcriptomics of a heat wave response in *Zostera marina* and *Zostera noltii***

To address the molecular correlates of the different thermal niches occupied by *Z. marina* (subtidal) and *Z. noltii* (intertidal), plants of both species (co-occurring at each location) were exposed to a realistic heat wave scenario in a common-stress-garden experiment. Large-scale RNA-seq was performed on four libraries per species from northern and southern Europe with 7 million reads per library. Both species were negatively affected by the heat wave but displayed major differences in their transcriptomic responses (Fig. 4).

![Figure 4. Heat map of expression profiles of 267 up-regulated genes during heat stress in *Z. marina* and *Z. noltii*. White, highest expression; red, lowest expression.](image)
For example, no up-regulation in heat shock proteins (HSPs) was found in *Z. noltii* indicating a higher temperature threshold of response. Further analyses by post-doc Susanne Franssen (U. Münster) are still in progress but differential regulation of expression variation clearly involves different gene sets and the higher induction thresholds for *Z. noltii* may reveal an advantage for this species in the face of climate change; particularly in southern latitudes.

*Olsen* along with senior research associate *Jim Coyer* and our Münster colleagues conducted another similar experiment utilizing the same conditions described above but involving populations of *Z. marina* individuals obtained from replicate northern and southern locations from both sides of the Atlantic (US and Europe). Here we test the hypothesis of independent parallel response diversity at the individual, population and geographic levels, i.e., comparative phylogeographic transcriptomics. In these experiments, more time points were sampled during the heat wave and recovery totaling 92 libraries for subsequent RNA-seq with 15-20 million reads/library. At present, we are struggling to analyze this massive amount of transcriptomic data.

**Devil rays roam the Pacific**

Mobulid rays are widely distributed in the world’s oceans. *Mobula japonica* is found throughout the Pacific. However, phylogeographic structure has not been investigated. PhD student *Marloes Poortvliet* has recently sequenced the mitochondrial genomes from 60 individuals of *M. japonica* throughout its range, as well as having genotyped >300 individuals with 12 nuclear microsatellite loci. Results so far suggest no significant population structure. All locations tested are dominated by a single haplotype with a small set of local, low frequency haplotypes and an historical demographic analysis suggests recent expansion within the past 300,000 years. Further analyses are in progress.

As with other elasmobranchs of the open ocean, rays are subject to high mortality from bycatch and targeted fisheries. Marloes’ results provide a compelling argument for the development of broad international approaches for management and conservation of this charismatic species as well as a re-evaluation of the recent species evolution of this ancient lineage. Her presentation at the 16th Annual Meeting of the European Elasmobrach Association Congress in Milan was awarded best student paper (See CEES Highlights).

**Newly minted PhDs**

*Andreas Zipperle* investigated reproductive dynamics of dwarf eelgrass, *Zostera noltii*, in the northern Wadden Sea. Populations were found to be predominantly outcrossing, genetically diverse, regularly recruiting and well connected by gene flow with the exception of barriers across the German Bight and Eems-Dollard Estuary (Fig. 5). These same barriers to gene flow also applied to *Z. marina*. 
Using genetic assignment tests over a four-year period of sampling, it was also determined that a seed bank is present and viable for at least 3 years in *Z. noltii*; an important discovery, as this species is often characterized as an annual with no seed bank. Year to year survival is most successful if winter survival is >10%. In a manipulative field experiment, grazing pits formed by geese were shown to act as seed traps and to facilitate recruitment (Fig. 6). This mechanism could provide a valuable tool of conservation of intertidal meadows. He defended his thesis in October 2012.
Silva Santos investigated dispersal dynamics, recruitment and population structure in the peppery furrow shell, Scrobicularia plana (Fig. 7) at different spatial and temporal scales, from the Western Wadden Sea to its entire range along European shores.

Figure 7. The peppery furrow shell Scrobicularia plana.

S. plana is a key species of intertidal communities and an important food source for birds, fish and humans. Phylogeographic patterns revealed a single, wide-spread mitochondrial haplotype with many local haplotypes (Fig. 8) consistent with a recent expansion (0.3-1.1 Ma), large effective population size and very low migration rates.

Figure 8. Distribution of CO1 haplotypes for Scrobicularia plana.
Relatively strong isolation by distance and low migration at the basin scale further indicate that populations are locally distinct, probably as a consequence of oceanographic entrainment.

At the smaller scale (Western Wadden Sea), weak population differentiation reflects connectivity as settlement patterns and observed aggregations were not correlated. At the scales of tens to hundreds of meters, mapping and spatial autocorrelation of aggregated patterns (Fig. 9) suggested that variation is mediated by density dependent ecological processes such as inter- and intraspecific competition for food and space. No discernible relation was found between spatial aggregation patterns in relation to densities of other bivalve species belonging to similar niches. Sediment type also affected patch formation above 1 km scale. Silvia defended her thesis in July 2012, a co-production with colleagues Dr. Pieternella Luttikhuizen and Dr. Henk van de Veer (NIOZ).

![Fig. 9. Changing density of *Scrobicularia plana* in the Dutch Wadden Sea.](image)

**Moving On**

Long-term post-doc/research associate, Dr. **Jim Coyer**, retired in 2012. He has returned to the US, where he has taken up a new position as Assistant Director for Appledore Programs at Shoals Marine Laboratory on Appledore Island (Portland, ME).

**Research Line 2 – From the MarBEE portfolio on marine, experimental community ecology**

The overall aim of this research line is to understand how biodiversity loss (in the broad sense) affects the resilience of communities. Focusing on experiments in natural conditions, group leader Britas Klemens **Eriksson** and students tests how trophic levels, ecosystem engineers and species diversity determine the capacity of marine benthic communities to adapt to changes in the environment and tolerate disturbances or stress. The research line addresses fundamental ecological questions in three specific systems: 1) what are the consequences of losing higher trophic levels in
the Baltic Sea; 2) what is the importance of ecosystem engineering for the function of intertidal flats in the Wadden Sea; and 3) how does species trait in periphyton metacommunities translate to the capacity to adapt to climate change.

**Biodiversity and resilience in the Baltic Sea system**

Large-scale exploitation of higher trophic levels by humans, together with global-scale nutrient enrichment, highlights the need to explore interactions between predator loss and resource availability. Research by PhD student Katrin Reiss (formerly Sieben) and colleagues have shown that declines in off shore predators have led to trophic cascades along the Swedish coast. In the Baltic Sea, declines in the dominating coastal predators, perch (Perca fluviatilis) and pike (Esox lucius), as well as declines in offshore populations of cod (Gadhus morhua) have led to massive increases in the medium-sized-predator stickleback (Gasterosteus aculeatus L.). Sticklebacks live off shore during the winter and migrate in late spring to the coast to spawn in sheltered bays. Today, the number of sticklebacks migrating to the coast can add up to a million individuals per hectare in early summer. Eriksson and Reiss have shown that, together with nutrient enrichment, invasions of sticklebacks promote blooms of filamentous algae by trophic cascades. Recent compilations of fisheries data suggest that the observed effects of coastal mesopredators on lower trophic levels may, in fact, be triggered by fishery induced changes in offshore food webs.

**Eriksson** and colleagues demonstrated the general importance of trophic level diversity for ecosystem structure in a benthic food web in the southern Baltic Sea (Eriksson et al. 2012). Oksanen’s hypothesis of ecosystem exploitation suggests that, in food webs with three trophic levels, primary producers and predators should increase with primary production; whereas in food webs with two trophic levels, only herbivores should increase. Eriksson and colleagues provided experimental support for these model predictions in a natural benthic community with three effective trophic levels, where the number of algal recruits (but not the biomass of gastropod grazers) increased with algal production. In contrast, when the food web was reduced to two trophic levels (by removing larger predators such as crabs), the number of algal recruits was unchanged whereas gastropod grazer biomass increased with algal production (Figs 10 and 11).

**Fig. 10.** The relation between the net production of algal biomass and the biomass of gastropod grazers (above) and the number of algal recruits (below), in the absence of top predators (crabs) (2 trophic levels, graphs to the left) and in the presence of top predators (crabs) (3 trophic levels, graphs to the right).
Biodiversity and resilience in the Wadden Sea system

Eriksson along with former post-doc Johan Eklöv, PhD student Serena Donadi and colleagues are studying interactions between benthic soft-bottom communities dominated by ecosystem engineers and local resource conditions. In 2012, Johan Eklöv, together with former COCON post-doc, Tjisse van der Heide, finalized a series of papers showing that landscape properties on the Groninger Wad are determined by a three-way interaction among lugworms (Arenicola marina), seagrass (Zostera noltii) and waterfowl (Eklöv et al. 2011, Heide et al. 2012). In Spring, the intertidal of the Groninger Wad is covered by a patchy seagrass landscape (Fig. 12). Elevated patches of seagrass are interspersed by hollows dominated by lugworms. In summer, seagrasses outcompete lugworms creating a homogenous seagrass cover. However, in autumn brent geese (Branta bernicla) and widgeon (Anas penelope) migrate through the area. They preferentially graze in the former hollows and thereby recreate the heterogeneous hollow-hummock landscape. Johan and Tjisse showed in a combination of field experiments and modeling, that while seagrass promotes the elevated hummocks by accumulating sediment, the lugworms promote the hollows through bioturbation, making them more attractive for the geese in the autumn.
In 2012, Serena Donadi showed that ecosystem engineers strongly affect the biomass of benthic algae, which are the main primary producers on the intertidal flats. In a large-scale experiment she demonstrated that sediment stabilizing species such as cockles (Cerastoderma edule) and blue mussels (Mytilus edulis), promoted algal biomass through biodeposition and hydrodynamic stress alleviation; whereas lugworms (Arenicola marina), which are well-known bioturbators, had negative effects on algal growth (Fig 13; Fig. 14A).

By modifying sediment properties, cockles and mussels were also found to promote the recruitment of cockle spat (Fig. 14B), indicating that positive feedbacks between bivalves and sediment stability might guarantee the persistence and survival of cockle populations in the Wadden Sea. Therefore, these results demonstrate the importance of ecosystem engineering for the productivity of intertidal communities and suggest that thoughtless exploitation of ecosystem engineers can seriously affect
the functioning of the Wadden Sea ecosystem and lead to deteriorated habitat conditions that are hard to reverse.

Fig. 14B

Biodiversity and resilience in the laboratory

In the coming decades, climate change will force biological systems to adapt to new ecological equilibria affecting the basic conditions for provisioning of ecosystem services and goods. Post-doc Karin de Boer studies the ecology of climate change and has shown that the capacity of periphyton metacommunities to adapt to different climate change scenarios depends on the diversity of diatom species. Final analyses are currently in progress.

For many benthic ecosystems (including the Wadden Sea), the most immediate effects of climate change are an increase in extreme temperatures and other weather related disturbances as opposed to gradual changes in overall conditions. In 2012, PhD student Lena Eggers, Prof. Birte Matthiesen (GEOMAR) and Eriksson published a paper demonstrating the importance of dispersal for recovery of biomass in diatom metacommunities exposed to a short term heat wave (Eggers et al. 2012). However, the results also demonstrated a limited capacity for recovery after heat stress. The metacommunities did not recover fully even after a prolonged period, indicating the possibility for short-term heat stress to cause long-term shifts in community structure.
Publications 2012

Doctoral Dissertations


Publications in journals


Books & Book Chapters

Marine Evolution and Conservation (MarECon)
http://www.rug.nl/research/marine-evolution-and-conservation/

**Group leader**
Prof. dr. P.J. Palsbøll

Composition of the group in 2012:

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**Technical and Administrative Staff**

*secretary*
H.G. Tjoelker
RUG-CEES
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*technicians*
M. Bérubé
RUG
1.0

Y.I. Verkuil
RUG/H&K (Sweden)
0.8
Marine Evolution and Conservation

The research unit Marine Evolution and Conservation was established in September 2011 and conducts research basic and applied questions in conservation, ecology, and evolution using population genetic/omic technology and inference methods. Stripped down to its bare essentials our research interests lie with those processes that structure natural populations. The structuring of individuals within species and consequently the partitioning of their intra-specific genetic variation are fundamental to evolution, ecology and conservation as the basis upon which selection act leading to local adaptation and ultimately speciation. The structuring of individuals and intra-specific genetic variation is also central to conservation by elevating extinction rates through increased demographic and genetic stochasticity, human-induced evolution and loss of adaptive potential. Despite their general appeal and wide-applications, molecular-based inference methods represents only one of many complementary research approaches from which we may gain insight into key basic ecological and evolutionary processes and how we humans affect those processes. Accordingly our research is not conducted in a “molecular” vacuum but we are part of an extensive network of collaborators for field data and samples as well as by our complementary knowledge and skill sets, which is necessary to tackle the increasingly complex conservation issues and research questions.

Most of our published work has been aimed at marine mammals, more specifically cetaceans (whales, dolphins and porpoises). The research has been conducted across a broad range of organizational levels from mutational processes at the level of the DNA itself (e.g., Nielsen & Palsbøll 1999; Palsbøll et al. 1999), individual identification and paternity (e.g., Nielsen et al. 2001; Palsbøll et al. 1997a) as well as behavior (e.g., Best et al. 2003; Clapham et al. 1992; Ramp et al. 2010b), migratory patterns (e.g., Palsbøll et al. 1995; Stevick et al. 2003a) and site fidelity (e.g., Berube et al. 2002; Palsbøll et al. 1997b) as well as the effects of geological events and speciation (e.g., Berube et al. 1998; Pastene et al. 2007). Research questions have ranged from basic, such as the effects of past global warming on speciation or glaciation on current population structure (e.g., Berube et al. 1998; Pastene et al. 2007), to more applied issues such as estimation of abundance (e.g., Bertorelle et al. 2010; Palsbøll et al. 1997a; Smith et al. 1999) and the effects of anthropogenic habitat fragmentation on dispersal (e.g., Epps et al. 2005; Epps et al. 2010; Peery et al. 2010a). In some cases we have presented more conceptual developments, such as a general approach to the delineation of units of conservation (e.g., Palsbøll et al. 2007), the establishment of wildlife DNA registers to detect illegal trade of protected species (e.g., Palsbøll et al. 2006) or kinship-based estimation of dispersal (e.g., Palsbøll et al. 2010; Peery et al. 2008). In other cases, the research questions have been highly specific such as determining the degree of isolation of specific presumed endangered populations (e.g., Berube et al. 2002; Hall et al. 2009) or estimation of population trajectories in abundance (e.g., Stevick et al. 2003b) and mortality rates (e.g., Ramp et al. 2010a).

While some studies may appear somewhat out of context, such as estimating rate of mutation at microsatellite loci, such insights are necessary to improve the understanding and hence interpretations we derive from our population genetic data. We have also published reviews/assessments of population genetic inference methods commonly used in conservation and ecology (Jombart et al. 2010; Palsbøll et al. 2010; Peterson et al. 2012a).

Although our main focus is marine staff in the Marine Evolution and Conservation group are also involved in research in terrestrial species (e.g., aphids, Panda bears, bighorn sheep as well as marbled murrelets and shorebirds).
Overview of academic results

Current and future research directions

As is the case for most ecology/conservation genetic labs, we are in the transition to genome-enabled methods, which entails optimizing and adopting new genome-enabled methods and technologies, acquiring bioinformatics skills as well as facilitating the analysis to capitalize on the information contained in the massive amounts of data we now are able to generate from each specimen. Although there are so-called next generation sequencing (NGS) methods not requiring a reference genome sequence (e.g., ddRAD sequencing or GBS), the potential of NGS data increases dramatically if a reference genome is available. To this end, Dr. Carlo Maley (an evolutionary cancer biologist at UC San Francisco) and I are sponsoring the sequencing of the humpback whale and, hopefully, the harbor porpoise genome. The actual genome sequencing is performed by Dr. Nader Pourman at the genome facility at UC Santa Cruz and is almost as complete as one can expect for a non-model species (https://gtc.soe.ucsc.edu/content/humpback-whale-sequencing). Armed with these new tools we are able to estimate key evolutionary, population, ecological and genetic parameters with a significantly higher degree of precision. However, perhaps equally, if not more, important we will now be able to detect past and current footprints of adaptation across the genome in non-model species, which in many ways is the Holy Grail in evolution, ecology and conservation.

Our current research is heading down three different but complementary/interacting main trajectories; (i) past and current adaptation, (ii) multi-species interactions and (iii) development of more realistic “population” genetic inference methods in ecology and conservation. These represent the kind of basic and conceptual understanding and developments which it is expected that academic research provides. In addition, MarECon continue to be involved and contribute to solving specific conservation issues in collaboration with conservation agencies and management bodies.

Examples of ongoing research

*Development of kinship-based methods to estimate population parameters in ecological time (with Prof. Dr. M.Z. Peery and Prof. Dr. Jon Pauli, Wisconsin University Madison, WI, USA).* We developing a spatially explicit individual-based statistical framework to estimate population parameters from the spatio-temporal distribution of closely related individuals (kin) (Palsbøll 1999; Palsboll *et al.* 2010). This approach complements traditional population genetic approaches, which use the average degree of relatedness within and among populations. In contrast to traditional population genetic inference methods, kinship-based methods yield estimates (e.g., migration rates, abundance and potentially individual fitness) that apply to the last one or two generation(s). In other words, this approach is complementary the current population genetic inference methods, which (with a few exceptions) yield parameter estimates that are evolutionary averages. The proposal, which is funded by the US National Science Foundation, will apply the developed methods to study the landscape use of two- and three-toed sloths across a landscape gradient (from native jungle to banana plantation mono-culture) in Costa Rica.

*Are Baltic harbor porpoises demographically isolated? (Prof. Dr. P. J. Palsbøll and Dr. Martine Bèrubè, MarECon)* With finding rom the Swedish Environmental Agency we are applying genome-wide ddRAD sequencing (Peterson *et al.* 2012b) of Baltic Sea porpoises to estimate current immigration rates from the distribution of genetically identified close kin. Large resources are allocated every year to reduce fisheries related mortalities of harbor porpoises in the Baltic Sea. However, the value of such an effort depends upon whether porpoises in the Baltic Sea constitute a small isolated population. Current, more traditional, population genetic analyses have revealed very low levels of genetic divergence between the Baltic Sea and adjacent regions where porpoises are common (Andersen *et al.* 1997; Fontaine *et al.* 2007; Wang & Berggren 1997). This result has two possible
explanations; either (i) high current migration rates or (ii) low current migration rates coupled with high historical connectivity and subsequent isolation. In order to discern between these two contrasting hypotheses which have radically different conservation and management implications we are applying a kinship-based approach we previously employed to marbled murrelet to estimate current immigration rates into a small isolated and endangered population outside Santa Cruz (CA, USA) (Peery et al. 2008; Peery et al. 2010b).

*Effects of past environmental change on Arctic marine mammals (PhD student A. A. Cabrera and MSc student J van der Zee, in collaboration with staff at the Norwegian Polar Institute (Tromsø, Norway), Centre for GeoGenetics (Copenhagen University, Denmark), Mingan Island Cetacean Study (Quebec, Canada), Natural History Museum (Oslo University, Norway), Greenland Institute of Natural Resources (Nuuk, Greenland), and Marine Research Institute (Bergen, Norway). The Arctic is home to marine mammals (e.g., polar bear, ringed and bearded seal as well as bowhead whale) that are uniquely adapted to an environment dominated by sea ice. During the summer large whales, which winter in Temperate and Tropical waters, migrate to the Arctic and sub-Arctic waters where they take advantage of the very high primary production, which ultimately is driven by the annual dynamics of sea ice. Genetic data are employed to estimate past changes in population sizes in representative species from these two groups of mammals, in order to assess the effect of global changing temperatures. Previous, similar, analyses by staff at MarECon revealed a strong effect of past global warming events (3-5 million years ago) upon the distribution and abundance of minke whales (Pastene et al. 2007), suggesting that marine mammals are highly sensitive to global warming (e.g., Fontaine et al. 2007; Fontaine et al. 2010).

*MHC selection in humpback whales (L. Wilkens (former MSc student, now a PhD student at Lausanne University, Switzerland) and Dr. J. Robbins (Provincetown Center for Coastal Studies, MA, USA)). The current paradigm in evolutionary and conservation genetics stipulate that outbreeding elevates individual and consequently overall population fitness. The universal example of this “good genes” theory is the high levels of variation universally detected at the Major Histo Compatibility (MHC) locus across a wide range of animal species. The MHC genes drive immune-competence and so-called “dissortive” mating (a behavioral preference for mates with different MHC genotypes) is thought to drive selection for mates with “good genes”. This argument, however, could lead to what is termed “outbreeding depression”, which in turn would prevent local adaptation. Our analyses in humpback whales show the opposite trend; that cows and their calves are more similar than expected by chance at the MHC locus, suggesting selection for (rather than against) the maternal MHC type. Individual humpback whales migrate to the maternal summer feeding area throughout their life time and hence acquiring the (successful) maternal MHC genotype could increase individual fitness.

Cited literature


Publications 2012

Publications in peer-reviewed journals


Interviews

Microbial Ecology

http://www.rug.nl/research/microbial-ecology/

Group leader
Prof. dr. J.D. van Elsas

Composition of the group in 2012:

Tenured Staff

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Introduction

The research programme in the Microbial Ecology Department focuses on the adaptation of prokaryotic microorganisms to a variety of environmental niches, and the ways in which the genomes involved allow plasticity to enhance fitness. The heterogeneous and fluctuating conditions in most current ecosystems, microbial competition and adaptation to biotic and abiotic factors, and consequent evolution, have resulted in the fascinating microbial diversity that is currently found in natural habitats. Microorganisms inhabit almost every thinkable ecological niche on the Planet, and the diversity in the microbial world is daunting by its sheer magnitude. Only a minor fraction of this diversity has been accessed to date.

Understanding the ecology of the diverse microbial communities and their interaction with each other and the environment thus represents a major challenge to current microbial ecology research. At present, most of the studies of microbial diversity and function focus on organisms at the community level, using, for instance, the 16S ribosomal RNA (rRNA) gene sequence as a marker of microbial diversity. Thus, an inventory is made of the microbial diversity extant in target ecosystems (soils, aquatic systems), and the putative ecophysiological role of members of that community is inferred from the data. However, finding similar populations of species in similar or different environments does not necessarily mean that these are also functionally identical. This is caused by the fact that microbial populations are genetically much more diverse than can be concluded from the diversity of 16S rRNA genes, which belong to the core set of bacterial genes. In fact, genetic diversification within microbial populations is the key step in microbial adaptation and evolution. Several mutational processes, ranging from horizontal gene transfer through genomic rearrangements to small mutations, may yield variants with traits that enhance fitness. The genetic diversity within a community or population of cells may thus often be missed by 16S rRNA gene-based culture-independent methods, although we do not fully understand the limits to using 16S rRNA genes to infer community function. This lack of sound information provides the rationale for current innovative work that aims to address key questions such as “how well does community structural diversity reflect functional diversity” and “how does horizontal gene transfer interfere with the putative relationship between structural and functional diversity”.

Hence, questions in respect of how bacteria adapt genomically to the environmental challenges they encounter, on what occasions they show adaptive responses, how horizontal gene transfer impacts on the adaptational process and at which speed adaptations occur, represent main topics addressed in the Microbial Ecology research program. In terms of ecosystems, the focus is on soil and soil-related habitats, as (1) in the light of the multitude of niches and gradients present in this habitat, there are challenging and largely unstudied processes of niche adaptation and niche differentiation to be found in this habitat, and (2) a number of key and major drivers of microbial activity, including adaptational activity, such as plants and soil fungi, are available. Several other projects deal with non-soil systems, such as freshwater and surface marine waters (sponges).

Overview of academic results

Adaptation of *Escherichia coli* to heterogeneous conditions

The purpose of this work is to assess the direction and level of divergence of *Escherichia coli* under conditions of fermentation in simulated industrial systems. For this, we evaluated the long-term adaptive response of *Escherichia coli* MC1000 in Luria-Bertani (LB) broth under three main selective forces (aerobic, fluctuating and anaerobic conditions). The emergence of forms with enhanced fitness was evident in competition experiments of all evolved forms versus the ancestral strain. The evolved forms were then subjected to genomic (next generation sequencing - NGS) analyses and phenotypic / transcriptome (metabolic profiling and gene expression) microarrays relative to the
ancestor. Profound changes were observed in the whole-genome sequences as well as in the phenotypes. Consistently occurring parallel genomic and phenotypic responses were found across all populations, suggesting a response likely driven by the LB medium. The evolved forms all contained a mutation in galR, a repressor of the galactose utilization operon. Concomitantly, they revealed enhanced growth on galactose as well as galactose-containing disaccharides. We concluded that parallel responses in independent populations can occur from similar ecological settings which can be an indication of predictability in the evolution of these systems. However, because of differential phenotypic outcomes, we found that the effect of parallelism is affected by differing genomic backgrounds. Interestingly, considerable heterogeneity was found even at the intra-populational level. These non-parallel genomic and phenotypic responses propose the establishment of different ecological roles adopted by forms in the populations, which is suggestive of diversification. At the current final stage of this work, specific phenotypic traits and distinct ecological roles in one of the evolved populations are being examined, this, with the purpose of unraveling the positioning of niche differentiation within the population as a result of adaptation.

The role of plasmids in bacterial adaptation to the mycosphere

In previous work on mycosphere-responsive bacterial communities, we found a plasmid in a Variovorax paradoxus-like responder. The plasmid was classed into the IncP1 group of broad host range (BHR) plasmids and was found to contain, next to its backbone, about 12 Kb of accessory sequence. We are on the track of determining the accessory load. Moreover, recent evidence has suggested that plasmids belonging to the prom2 group of BHR plasmids, exemplified by our rhizosphere-isolated plasmid pIPO2, also perform a role in mycosphere-associated bacteria. This study initiated in December 2010, aims to assess the dynamics and role of such mycosphere bacteria-associated plasmids. One hypothesis is that key adaptive yet dispensable genes are carried by the plasmid, whereas other thinking departs from the principle of plasmids behaving as the ultimate parasitic genetic agents. We did find evidence for the prevalence of IncP1 as well as PromA plasmids in the mycospheres of diverse fungi (fruiting bodies). We are in the process of sorting out both the nature of the plasmid backbones and that of the accessory fgenes present in the insertion hot spots.

Bacterial-fungal interactions and the eco-molecular mechanisms involved: getting to the limits using the “Omics” toolbox.

The project is part of the Marie Curie Trainbiodiverse consortium which aims at highlighting possible bioindicators for soil health, taking bacterial-fungal interactions in soil as a system. The ecology of bacterial-fungal interactions has been studied already, and it appears that Burkholderia terrae BS001 and its fungal counterpart Lyophyllum strain Karsten share a microhabitat in soil, based on their mutual demands and benefits. The interactions between these two organisms has already been reported to be beneficial in nature both for bacteria and fungi, where the former has been privileged with easy availability of carbonaceous compounds while the later in return of its generosity gets protection against hostile entities and environments. Understanding the bacterial-fungal interactions in general and between Burkholderia terrae BS001 and Lyophyllum strain Karsten in particular, requires a deep insight into the genetic systems that are potentially driving these interactions. Thus questions need to be answered applying different approaches by unleashing the Omics toolbox. Currently we are investigating the interactions between the bacterial and fungal partner on the basis of transcriptome analysis of Burkholderia terrae, with the hope that we would enable us to report on genetics systems or genes that are switched on and off during the course of their interactions. Another aspect of the project deals with comparative genome analysis of fungal- and plant-interactive Burkholderia species, with the intentions to find the common core genome contents of these species and further emphasize on their potential fungal interactive systems. We
also are focusing on those genetic systems which are believed to make these species successful in the complex soil environments where nutrient acquisition and survival is tough task for them. The preliminary results show that all seven selected *Burkholderia* strains have genome sizes ranging from 8-11.5 Mb. Such enormous genome sizes carry many genetic systems which are supposed to make these species so successful in soil environments. We are also planning to investigate the conversation between *Burkholderia* and *Lyophyllum* during their physical interaction. For that, fine-scale metabolomics will be applied to explore and if possible characterize new metabolites that are exchanged during this chemical cross talk. Studying these systems in detail will greatly contribute to our understanding of the ecology of these interkingdom relationships.

The dynamics of key microbiota in soils in the face of stress

Soil is a living entity with global significance. The need to maintain its functions, to sustain biological productivity and to serve as an environmental buffer against disturbances, is large. It is commonly known that the local environment in which soil organisms dwell is never constant. Temperature, water and nutrient availability often fluctuate over time, affecting process rates and the associate microbial players. One important question is what can thus be considered to be “normal” in a soil system? By studying the fluctuations in population dynamics of key sensitive microbial groups (e.g. ammonia oxidizers or nitrogen fixers; Pereira e Silva et al., 2012) we propose that it is possible to establish a baseline of soil functioning and to create a proto-type monitoring tool for educated judgment of soil normality (Pereira e Silva et al., 2013). Following this line, microbial communities involved in nutrient cycling, such as nitrogen, are essential for the ecosystem functioning and maintenance. We also investigated the patterns of microbial nitrogen cycling communities (ammonia oxidizers, nitrogen fixers and denitrifiers) along a natural salt marsh chronosequence, which spans more than one hundred years of succession. Preliminary data showed functional differences in nitrogen cycling dynamics along the salt marsh chronosequence, revealing an interplay of changes in community structure interpretable in terms of adaptation to the local environment (Salles et al. in preparation). Furthermore, by combining a spatial-temporal sediment sampling with 454-pyrosequencing of the bacterial 16S rRNA gene, we observed that, possibly, high rates of immigration might be involved in bacterial assemblages at initial succession, while at later stages, the increase in nutrient partitioning (i.e. niche establishment) might act as a selective effect (i.e. determinism), driving to a more constant assemblages. Putting all together, physicochemical factors as well as biological input are likely the major determinants of the microbial dynamics and succession in soil systems.

Metagenomics guided exploration of ecosystems – mining for novel bacterial chitin degrading enzymes

Chitin is the second most abundant biopolymer on Earth. Although chitin is not produced by bacteria, it is used by them as a source of nitrogen, carbon and energy. In most ecosystems, the degradation of chitin is, thus, mainly microbial, and comes about as a result of excreted chitinases. The majority of bacterial chitinases is assigned to family 18 of the glycosyl hydrolases, in particular to type A encoded by chiA genes. The main objective of the present study was to obtain information about possible new chitin degraders and chitinases with improved features. We prepared large insert (fosmid) libraries using metagenomic DNA from 5 different habitats: chitin amended and native soil, bog soil, rhizosphere of arctic plant *Oxyria digyna*, and bacterial symbionts of the freshwater sponge *Ephydatia fluviatilis*. In total 450000 clones were generated and stored. The largest library was one based on metagenomic DNA from chitin amended and it contained 145000 clone. The library was screened (genetic screening based on PCR and Southern hybridization) for the presence of chitinase-like genes. Six individual fosmids were identified as bearing inserts with chitinase-like gens. The positive inserts were completely sequenced using Illumina paired-end
technology. The annotation of sequences and protein predictive model revealed the presence of putative four novel chitinases and one chitin deacetylase. Gene synteny analysis offered a clue about the donor organism and the other genes involved in the regulation of the carbohydrate small oligomers transport. Chitinases were assigned to bacterial groups like Chloroflexi, Acidobacteria, Bacillus and Streptomyces. One gene was further processed for heterologous expression and protein characterization.

Fig. 1. Fosmid isolated from soil chitin amended library - Gene synteny and transcription orientation. Chitinase gene in progress for heterologous expression

Additionally, the chitin amended soil was tested, by our partners from Plant Research (dr. G. Korthals) for suppressiveness against plant pathogens. The diversity and abundance of microbial community were measured as well as the effect on plant pathogens (Verticillium dahliae and nematodes of the genus Pratylenchus). The data revealed that chitin amendment had raised the suppressiveness of soil, in particular towards Verticillium dahliae, 9 months after the (second) treatment, extending to two years following treatment. Moreover, major effects of the added chitin on the soil microbial communities were detected. The abundances and structures of soil actinobacteria and Oxalobacteriaceae were affected by chitin. At the functional gene level, the abundance of specific (family-18 glycoside hydrolase) chitinase genes carried by the soil bacteriota also revealed upshifts as a result of the added chitin.

Metagenomics for bioexploration – lignocellulose breakdown

The purpose is of this project is to search for novel consortia of microorganisms, as well as their genes/enzymes, involved in the bioconversion of lignocellulosic substrates and toxic compounds, such as furfural or 5- hydroxymethylfurfural (SHMF). We constructed two replicated sequential-batch aerobic enrichment cultures with (un)pretreated wheat straw as carbon sources. The communities evolved along different paths in accordance with the pretreatment applied. In each batch, the final bacterial abundances were between 8.7-9.5 log 16S rRNA gene copy numbers/ml, and PCR-DGGE analyses showed that the bacterial-fungal consortia reached approximate structural stability after six transfers. A total of 124 bacterial strains was isolated from the two types of enrichment cultures. The most abundant strains were closely related to the genera Raoultella-Klebsiella, Kluyvera, Acinetobacter and Pseudomonas. Totals of 54 and 47 strains showed (hemi)cellulolytic and 5-HMF oxidoreductase activity, respectively. Also, fungal strains related to Coniochaeta, Plectosphaerella, Penicillium and Trichosporon were isolated. We revealed the succession and bacterial-fungal composition in the enriched cultures by 16S and ITS region pyrosequencing. Most of bacterial taxa that could be made the lignocellulosic degradation or involved in bioconversion of furanic compounds belong to Enterobacteriales, Pseudomonadales, Xanthomonadales, Flavobacteriales and Sphingomonadales orders. Overall, two novel stable microbial consortia were obtained that could be the starting points for lignocellulose degradation applications, construction of metagenomic libraries, enzymatic evaluation of degrading microbial players and plasmidome analysis. Two papers has been writing to publish. In the next steps, and in order to evaluate the influence of microbial source and substrates in the enriched cultures, we will
take more samples that received input of lignocellulosic material (as agricultural soils) or furanic compounds (as bioethanol industrial hydrolysates). We will apply stable isotope probing - SIP using $^{13}\text{C}$-labelled wheat straw as a strategy for recover lignocellulolytic microbial communities.

**Microbial community ecology – diversity and community niche**

Research on biodiversity and ecosystem functioning takes many angles, from the diversity and stability of communities to the preservation of ecosystems and ecosystem services. In spite of the overwhelming microbial diversity, the concern about how environmental changes affect life in our planet has also taken microscopic scales. It is therefore crucial to understand how ecosystem processes are affected by microbial diversity, but also the mechanisms through which microbial diversity affects community functioning. These are key questions in the research carried out by Joana Falcão Salles involved in this line of research.

Predicting biodiversity effects on ecosystem functioning requires adequate evaluation of the mechanisms explaining why more diverse systems could perform better than less diverse ones. In this context, tackling functional diversity has become an important issue. In microbial ecology, genetic diversity of phylogenetic or functional markers is widely used as a proxy of microbial diversity. However, the extent to which functional diversity is linked to gene sequence diversity remains unclear, and which diversity proxy is the most useful to predict community functioning is still debated. We have recently analysed (Salles et al 2012), for a range of denitrifying bacteria, the relationships between (i) the similarity of functional traits evaluated from metabolic profiles (BIOLOG plates) or from N2O accumulation patterns on different C sources, and (ii) the similarity of phylogenetic (16S rRNA gene) or functional (nir gene) markers. We also calculated different proxies for the diversity of denitrifier community based on taxa richness, phylogenetic or functional similarities, and evaluated their performance in inferring the functioning of assembled denitrifying communities. For individual strains, the variation in the 16S rRNA gene sequence explained only 10% of the variation in metabolic patterns and were not related to N2O accumulation patterns. This indicates that this phylogenetic marker should be used with caution when inferring ecological similarities between taxa. N2O accumulation patterns were correlated with the similarity of nitrite reductase residues. When nir genes were analysed separately, the similarity in amino acid sequences coded by the nirS genes accounted for 48% of the variance of the observed pattern of N2O accumulation, whereas coded to the nirK gene was unrelated to N2O accumulation pattern. The distinct patterns between nirS- and nirK-harbouring bacterial strains can be explained by different rates of horizontal gene transfer observed for these genes. Furthermore, it indicates that using the diversity of nir gene sequences might be more meaningful for nirS- than nirK-harbouring bacterial species when inferring ecological similarities. For bacterial assemblages, phylogenetic diversity (PD) was calculated using similarity distances based on 16S rRNA gene sequences. The 16S rRNA gene was also used to calculate the mean community dissimilarity (Diss). These diversity metrics predicted poorly the variation in the functioning between assembled communities (<15%). In contrast, the proxies of functional diversity based on N2O accumulation patterns and determined either using Functional Attribute Distance (FAD), Functional Diversity measure (FD) or Community niche (CN; Salles et al 2009), performed better and explained from 23 to 42% of the variation in community functioning. CN was the best metric, indicating the importance of complementarity for carbon resource use among taxa in the context of denitrifier community functioning (Salles et al 2009). Overall, our results indicate that in order to understand the functioning of bacterial communities, focus should be placed on functional diversity. In this respect, the choice of both adequate functional traits and diversity metrics is a key issue when quantifying bacterial community diversity.
Microbial diversity and invasiveness

Biodiversity is implicated in the likelihood of a community to be invaded by alien species. Theoretical and experimental studies have indicated that biologically-diverse plant communities are often less prone to being invaded than simpler ones, but effects of microbial diversity on invading organisms have remained unexplored. Previous experiments in our laboratory have supported an inverse relationship between an invading species, E. coli O157:H7, and the diversity of the microbial community it encounters. These experiments have also hinted to the idea that at least one of the mechanisms behind this inverse relationship is resource complementarity. This year we were able to show that in experimental single niche systems composed of one carbon source more diverse bacterial communities are able to use more resources, and at a faster rate, than less diverse communities. In the context of invasion, this high rate and consumption of resources limits the dominance of E. coli in a microbial community.

Expanding on this idea that resource use limits an invader’s access to resources, we plan to test this hypothesis with experimental soil microcosms. We used two approaches to test this hypothesis: an assemblage and dilution-to-extinction approach. In the assemblage approach, we followed E. coli’s survival in soil communities composed of 1 to 80 strains. In the dilution-to-extinction approach, we diluted soil microbial communities in a stepwise fashion to obtain communities of different diversities and then followed E. coli’s survival after inoculation. This enabled us to examine resource use and invasion at much higher levels of species richness than the assemblage counterpart. In both these approaches, we measured community resource utilization and found that higher diverse communities will extract more resources than less diverse communities, thus aiding to E.coli’s decline. The data were laid down in a publication in PNAS and form the basis for the thesis of Cyrus Mallon.

The microbiology of bird eggs

A collaborative project with animal ecology (Prof. Dr. Irene Tieleman) aims to explore how environmental conditions shape variation in microbial communities and to determine the evolutionary consequences of these changes for the protective systems of birds against microorganisms. Because of the complexity of the avian immunity, an ‘egg-nest’ dyad represents a simple model system to study the association between microbial communities on egg shells and antimicrobial defence proteins into albumen. Until now, the project has been focused on both microbial and immune methodology. Further experimental approaches will aim (1) to experimentally manipulate the microbial community in nests and to look at the effects on antimicrobial proteins of eggs, in a captive bird (zebra finches, Taeniopygia guttata), and (2) to perform a comparative study on eggs of two tropical bird species across a range of environmental conditions in Kenya.

Bacterial endophyte communities of arctic plants are cold-adapted and host plant specific

Arctic and subarctic climates are highly demanding environments for plants, due to low temperature average and extreme cold, short growth season (1-4 mo) and low solar radiation, with high annual fluctuations. Water stress, either in the form of drought or flooding, is common. As recycling of nutrients, especially nitrogen and phosphorus, in cold climates is very slow, the soil is often very nutrient-poor. To survive in this highly demanding environment, arctic plants employ diverse anatomical and physiological adaptations like low-growth morphology, dominantly vegetative propagation, low optimal temperature for photosynthesis and physiological hardening to prevent frost damage. This study characterized the taxonomic and functional diversity of endobacterial flora of three arcto-alpine plant species (Oxyria digyna, Diapensia lapponica and Juncus trifidus) in the low Arctic in Kilpisjärvi area, northwestern Scandinavia (69°03’N). The endobacterial flora was isolated from plant samples collected from three distinct sites, and endophyte community diversity was
analyzed by cultivation and DNA-based methods. Functional diversity of culturable endobacteria was analyzed by activity assays and by PCR amplification of functional genes.

Analysis of bacterial endophyte library of over 350 isolates as well as 6 clone libraries each encompassing 90- sequences revealed a high diversity of bacteria living in association with arctic plant species, representing Actinobacteria, Bacteroides, Firmicutes, Acidobacteria and α-, β- and γ-proteobacteria. The most common taxonomic groups were α- and β- proteobacteria and Actinobacteria (30.6%, 26% and 27.4%, respectively). Taxonomic distribution of the culturable isolates as well as the clone libraries were dependent mainly on host plant species, but also on (properties of) sampling sites (Figure 1).

Several bacterial groups associated tightly with specific plant species: *Burkholderia* spp. dominated *D. lapponica* and *J. trifidus* samples, but were nearly absent from *O. digyna*. *Sphingomonas* spp. were common in *D. lapponica* as well as in *O. digyna*, and sequence alignment of sphingobacterial isolates revealed their plant host specificity. Moreover, *Sphingomonas* spp. from different plant hosts showed divergent metabolic profiles.

The isolated bacteria were well adapted to low temperatures; this is reflected in both taxonomy, with the closest relatives often representing psychrophilic isolates, as well as in their physiology: most isolates grow well at +4 °C. Phosphate solubilization is a very common trait in isolates analyzed, as well as production of ACC deaminase. In contrast, cellulase or amylase activity were detected in less than 10 % of the analyzed isolates. Many of the isolates retained full enzymatic activity at +2–+5 °C.
Publications 2012

Doctorate granted by the institution, prepared within the institution


Publications in journals


Garbeva, P., van Elsas, J.D., de Boer, W., Draft Genome Sequence of the Antagonistic Rhizosphere Bacterium *Serratia plymuthica* Strain PRI-2C. *Journal Bacteriology* 194: 4119-20


Nazir, R., Zhang, M., de Boer, W., van Elsas, J.D., 2012. The capacity to comigrate with *Lyophyllum* sp. strain Karsten through different soils is spread among several phylogenetic groups within the genus *Burkholderia*. *Soil Biology & Biochemistry* 50: 221-233


Composition of the group in 2012:

Tenured staff

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Postdocs

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<td>1.0</td>
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PhD students

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PhD students – thesis in preparation

Drs. A. Duarte

drs. A. Hinsch

drs. A.B.F. Ivens

drs. A.L.W. Kuijper

drs. E. van der Vaart

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Introduction

Most of the models developed in the Theoretical Biology group address research questions in ecology, evolution, and behavioural biology. We see it as one of our main tasks to bridge the gap between theoretical and empirical research. The group intends to contribute to this task in three ways. First, our models are framed in terms of ‘concrete’ variables and parameters with a clear biological meaning. In other words, the models are more ‘mechanistic’ and less phenomenological than is standard in theoretical research. Second, we strive to include slightly more complexity in the models than is typical for the ‘toy models’ that are often used to illustrate conceptual ideas. Overly complex models do not inspire much insight, while overly simplistic models may be misleading when applied to particular real-world situations. With models of intermediate complexity, we hope to achieve a good balance between conceptual simplicity and analytical tractability on the one hand, and realism and testability on the other. Third, the model predictions are systematically confronted with real-world situations. In close collaboration with colleagues from various biological disciplines, the group tries to test its model predictions both in the lab and in the field. With the modelling approach indicated above, we address questions in three interrelated research areas:

Adaptive variation

Variation and diversity are key concepts in the biological sciences. Much of the variation found in nature is the result of stochastic processes like mutation, recombination, genetic drift, and environmental or demographic stochasticity. However, at all levels of biological organisation part of the variation is structured in a systematic way. Most probably, much of this patterned variation results from directional processes like competition or selection. It is one of our core aims to investigate how such ‘adaptive variation’ does arise and how it is maintained on an ecological or evolutionary time scale. At the behavioural level, we are interested in the evolution of ‘animal personalities’, the phenomenon that individuals differ systematically in whole suites of behavioural tendencies, and that, moreover, these tendencies are stable in time and correlated across contexts. How can various behavioural types coexist; why isn’t there a single type of highest fitness that outcompetes all other types? And why are behavioural tendencies stable in time and consistent across contexts; shouldn’t one expect a more flexible structure of behaviour? At the life-history level, we are interested in the evolution of alternative phenotypes, like the differentiation of a primordially homogeneous population into distinct morphs, distinct mating types or distinct castes of workers. Under which circumstances should one expect the evolution of coexisting specialist phenotypes, rather than a single generalist phenotype? Why, for example, is the evolutionary transition from a state where all individuals are simultaneous hermaphrodites to a population with two separate sexes common in animals, but rare in plants? What are the evolutionary and ecological consequences of morph differentiation? What are, for example, the evolutionary and ecological implications of the interplay between natural and sexual selection? At the population level, we are interested in the emergence of new species. To what extent can speciation be viewed as an adaptive process? Can we predict speciation on the basis of intraspecific and environmental factors? How can our understanding of speciation be used to explain global biodiversity patterns? At the community level, we are interested in species coexistence and the interaction of ecology and evolution in shaping community patterns. Why can hundreds of algal species coexist in a droplet of water while the ‘principle of competitive exclusion’ predicts that the number of coexisting species will not exceed the number of limiting resources? To what extent are the structure of a food web and the energy flow pattern within a food web the result of adaptive evolution?

Interestingly, the answer to one type of question at one level of organisation is often of a similar structure as the answer to a quite different question at a different level of organisation. Indeed, many explanations of adaptive variation can be traced back to two basic principles: evolutionary branching resulting from architectural constraints and non-equilibrium dynamics caused by non-
transitive interactions as in the Rock-Scissors-Paper game. Based on this general insight, there is hope that the detailed study of seemingly diverse examples of adaptive variation may in the end result in a general theory for the emergence and stability of biodiversity patterns.

**Cooperation and conflict**

The history of life on earth can be seen as a sequence of major transitions in which lower-level entities evolve to cooperate with each other and form ever higher levels of biological organisation. Genes cooperate to form genomes and cells, cells cooperate to form multicellular individuals, individual organisms cooperate to form social communities and species cooperate in mutualistic interactions. Yet the stability of higher-level cooperative units is constantly threatened by potential conflict between lower-level entities over their share in the genetic contribution to future generations. We study how this balance between conflict and cooperation shapes individual development, the social structure of populations and the dynamics of mutualistic interactions.

A fundamental developmental process is sex determination. Will an individual develop into a female, a male or both and become a hermaphrodite? What cues are used to trigger sexual development? We build models to investigate how the evolution of sex determination is affected by parent-offspring conflict, sexual conflict and environmental variability. A great advantage of studying sex determination is that model predictions are relatively easy to test by observing sex ratios in the lab or in the field. We collaborate with various national and international partners to test our models in a wide range of taxa, from tiny short-lived hermaphroditic worms to long-lived reptiles. More generally, we develop models to study the role of maternal effects and epigenetics on the evolution of individual developmental plasticity. To what extent should an individual ‘listen’ to cues transmitted by its mother or by the environment? What are the evolutionary implications of epigenetically mediated transgenerational inheritance?

Highly social species, such as humans and eusocial insects, are characterized by strong division of labour. We study under what conditions division of labour can ‘self-organize’ in groups of phenotypically plastic individuals. How can development of discrete casts evolve from an initially homogeneous workforce? How important is cultural evolution as opposed to genetic evolution? Mutualistic interactions between species are common and ecologically important. Yet it is still far from clear how mutualisms remain stable in view of the temptation to exploit partners. What mechanisms are expected to evolve to ensure stable cooperation? How important are partner choice and sanctions against non-cooperative partners? What is the role of horizontal versus vertical transmission of partnership? By applying evolutionary principles to these lower-level processes, those patterns and processes will be singled out that are evolutionarily stable. The integration of an evolutionary and a self-organization approach is a major challenge and one of the central goals of our research programme.

**Overview of Academic Results**

Ana Duarte and Aniek Ivens successfully defended their thesis and are now continuing their scientific careers as post-docs at the University of Cambridge (UK) and Rockefeller University of New York City (USA), respectively. Sander van Doorn joined the Theoretical Biology Group after obtaining an ERC Starting Grant and VIDI grant, and will start a new research line on evolutionary systems biology (more on that below).

The following contributions give an impression of the work being done in the Theoretical Biology group. Sander van Doorn gives an introduction to his new research project; Piet van den Berg gives an overview of our recent work on social learning and the evolution of cooperation; Andrés Quiñones shows how evolutionary transitions to eusociality emerge from coevolving sex allocation.
and life history strategies. Finally, Aniek Ivens summarizes some results from her thesis on “Evolutionary Ecology of Mutualism”.

Sander van Doorn

With the recent establishment of a junior research group in evolutionary systems biology, the Theoretical Biology group has strengthened its research activities at the interface of evolutionary biology and the molecular life sciences. Funded by an ERC Starting Grant and a VIDI grant of the Netherlands Organization for Scientific Research, the new research team, headed by Dr. Sander van Doorn, will investigate the evolution of gene- and protein-interaction networks. Understanding how such networks function is prerequisite to unlocking the wealth of molecular data that has become available.

The work in our group concentrates on bacteria, which are currently most amenable to an evolutionary systems biology approach. Using bacterial chemotaxis, natural competence, biofilm formation and metabolic switching between carbon sources as model systems, we ask how the complexity of biomolecular interactions influences the dynamics of evolutionary adaptation, and how it constrains or facilitates phenotypic diversification through either plasticity, bet-hedging or the establishment of genetic polymorphism. To answer these questions, we rely on theoretical modeling techniques from systems biology and traditional evolutionary theory, as well as on evolution experiments (in collaboration with the Molecular Genetics group).

Figure 1 – Molecular, phenotypic and evolutionary similarity between solutions to a complex optimization task.

In a model for the evolution of phenotypic plasticity, we studied how a signal-transduction network of interacting proteins could process information from the environment in order to realize an optimal pattern of phenotypic adjustment over the lifetime of the organism. This is a complex optimization task with many (locally) optimal solutions, each of which is represented by a node in A–C. Some of the optimal solutions can be implemented by a simple network of three interacting proteins (red circles), others require four (blue circles) or up to five proteins (green circles). We also generated solutions by the trial-and-error process of evolution (grey squares). These were clustered together with the optimal solutions based on molecular similarity (i.e., similar network structure; A), phenotypic similarity (i.e., similar developmental trajectories of the phenotype; B) and evolutionary similarity (i.e., reachability by a series of nearly neutral small-scale network modifications; C). The resulting graphs show that the evolved solutions are nearly-neutral equivalents of the simplest possible network architectures capable of implementing age-dependent plasticity (C). This equivalence is also apparent at the phenotypic level (B), but not at the level of network structure (A), because neutral and redundant interactions obscure which interactions in the network are important for its function. Both (A) and (C) recover the same clusters of structurally similar optimal solutions, but only evolutionary similarity (C) predicts which ones of these can be reached by evolution.

A second research objective is to utilize evolutionary insights to develop new model-reduction strategies for systems biology. Preliminary results suggests that complex interaction networks can often be reduced to functionally equivalent simpler networks that are reachable by a series of nearly neutral evolutionary steps each involving only a local rewiring of the network (Figure 1). This observation is consistent with the idea that evolution typically first converges on a simple solution for a problem, which is subsequently refined by a process of tinkering.
Joint evolution between conditional dispersal and cooperativeness can promote both within – and between species cooperation in unexpected ways
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Cooperation between individuals of the same species (intraspecific cooperation) as well as cooperation between different species (interspecific cooperation, mutualism) are ubiquitous in nature. Although cooperation provides benefits to all parties involved, it does not evolve easily, because cooperators can be exploited by cheaters. This problem may be overcome if cooperative individuals predominantly interact with other cooperators. A conditional behavioural mechanism leading to such positive assortment is partner fidelity (staying in cooperative and leaving non-cooperative neighbourhoods).

We used individual-based simulations to investigate whether partner fidelity indeed favours the evolutionary emergence and subsequent stability of both within-species and between-species cooperation in patch-structured populations. We organized our simulations on within-species cooperation as a public goods game, where investment in cooperativeness could jointly evolve with the tendency to leave a patch to join another patch. Dispersal decisions were allowed to be conditional on average cooperativeness in source and target patches. The simulations on between-species cooperation were modelled analogously, with cooperative behaviour modelled as substance exchange between two species co-inhabiting the patches.

In both scenarios (intra- and interspecific cooperation), conditional dispersal had a strong effect on the evolution of cooperation. In case of within-species cooperation, if the cost-benefit ratio of public good investment was very low (making any investment profitable for the investors themselves), conditional dispersal destabilized the cooperation that readily evolved when dispersal was kept constant. If the cost-benefit ratio of investing in public goods was high (making the public goods game a social dilemma where investors lose while their group gains) conditional dispersal strongly promoted the evolution of cooperation, but only in simulations where individuals evolved the tendency to leave cooperative patches (figure 1). In simulations where individuals tended to stay in cooperative patches (partner fidelity), cooperation quickly went extinct. This unexpected finding is explained by the fact that a high level of cooperation is associated with a high intensity of local competition in our model, making it profitable to leave cooperative environments and cash the benefits of cooperation elsewhere. Our results imply that conditional dispersal can both promote and destabilize intraspecific cooperation, and that the evolutionary consequences of conditional strategies may thus be difficult to predict.

In the model on between-species cooperation, cooperation also evolved, but only under extremely low cost-benefit ratios. When these conditions were met, conditional dispersal indeed promoted cooperation following the same dynamics as observed in our model on intraspecific cooperation. Although the two species considered in our model were identical, evolved patterns of cooperation were typically unilateral, with one species contributing a lot and the other very little. This finding matches the observation that many natural mutualisms exhibit strong asymmetry between partners. Moreover, our findings on the importance of low cost-benefit ratios show that this kind of interactions can readily evolve when initially based on by-products, for example the honeydew of aphids.
Figure 1. Joint evolution of cooperativeness and conditional dispersal in a representative simulation run leading to the evolution of cooperation. The three panels to the left are heat plots illustrating the evolution of the frequency distribution of the alleles for cooperativeness $x$ (A) and the parameters $\beta^x$ (B) and $\alpha^x$ (C) characterizing the evolving conditional dispersal strategy. For generation 5,000, panel D shows the representative dispersal strategy and the average cooperativeness in the population (vertical line). A single dispersal strategy is predominant with individuals tending to leave their patch when its cooperativeness is higher than the population average.

An evolutionary transition in social behaviour and life-history
Andrés E. Quiñones & Ido Pen

Eusociality is the most advanced form of social life found in the animal kingdom. The hallmark of this social life is reproductive division of labour, where some individuals refrain their own reproduction (workers) to increase the reproduction of their parents (queens). The first hypothesis that tried to explain the phylogenetic distribution of eusocial life “the haplodiploidy hypothesis” came from the theoretical framework developed by W. D. Hamilton. Haplodiploidy is a sex determination system under which haploid males are derived from the female genome, and diploid females have an even genomic contribution from parents. This sex determination system implies that females are more related to their sisters than to their own daughters. The haplodiploidy hypothesis states that the relatedness caused by haplodiploidy increases the inclusive benefits to females that help to produce sisters. This increment in inclusive fitness implies that eusociality evolves more often in haplodiploid groups. However, this hypothesis ignored the fact that the relatedness between a female and her brothers is lower than to her sons. Thus on a nest that has even sex ratio, haplodiploid inclusive fitness benefits are cancelled when both sexes are accounted. Therefore, female workers can only obtain the inclusive fitness benefit of haplodiploidy when there is a female bias sex ratio.

The theoretical problem of the haplodiploidy hypothesis is maintenance of female bias sex ratios. Seger showed that taking into account realistic life-history conditions in a model, sex ratios biases can be stable along evolutionary time. He considered two bivoltine life-histories common in the hymenoptera. First, “female hibernation” is when the reproductive season in a year starts with overwintering mated females (Fig. 1a). Second, “larval diapause” is when both females and males overwinter, and the reproductive season starts with mating (Fig. 1b). In both cases two broods are produced. The second brood is composed of both offspring and grand offspring of the founders. This two types of life-history change the reproductive value of males and females born at different stages of the life cycle. Which leads to bias sex ratios in both progenies. Even though, Seger’s findings, in light of Hamilton’s hypothesis, are very relevant for the evolution of eusociality, there has been no models of the consequences of the biased sex ratios maintained by these life-history set up. In order to assess the effect of these two life-histories and the biased sex ratios derived from them, we used a dual approach combining individual-based simulations and inclusive fitness methods.

Our model assumes a population that is structured in different classes. The classes are defined by the moment of the season in which an individual is born, and the sex. We assume a partially bivoltine life-history, a condition that is present in groups with wide variation in social behaviour. Under these life histories two broods are produced in one season, the first one at the end of the spring, and a second one at the beginning of autumn. In species that have social behaviour, some of the females born in the first brood, do not leave the nest and instead stay to help their mother to
produce the second brood. We assess the evolutionary change in traits that determine the sex ratio of the first and second brood, and the tendency of females from the first brood to stay in the nest and help.

The inclusive fitness approach of our model shows that in accordance to Seger’s findings, “female hibernation” is a life history that selects for sex ratios that are male biased in the first brood, and female biased in the second brood. This type of bias facilitates the evolution of eusociality (tenency of females to stay and help) only under haplodiploidy, due to the fact that female offspring are higher related to their sisters than to their own offspring (Figure 7). Moreover, the dynamic picture coming from the individual-based simulations show that the sex ratio that is selected at the beginning of the evolutionary process changes later on. Once helping behaviour evolves in the population, the selection pressure on the sex ratios is reversed. In the new condition, where females are no longer reproducing but instead stay to boost their mother’s fertility, it is more profitable from a fitness perspective to produce these female workers. In other words, helping behaviour is facilitated by sex ratio evolution. And later, helping behaviour feeds back to sex ratio evolution (Figure 8).

It is noticeable that due to the evolution of only three traits, the population we model goes from a solitary partially bivoltine life-history to a eusocial bivoltine life-history. Thus, the evolution of these three traits causes an evolutionary transition in social behaviour and life-history.

**Figure 7.** Required fitness benefits for helping to evolve. The inclusive fitness model predicts the benefit (b), from one worker to the foundress, necessary for a positive selection for helping. means that the foundress gets as many extra offspring as what the worker would have gotten by herself. In the y axis is the selection differential for helping behaviour, positive values mean that selection favours helping. Blue lines assume female hibernation, red lines larval diapause life-history. Filled lines assume haplodiploidy and dashed lines diploidy. a) Assumes even sex ratios in both broods. Dashed lines cross the limit of neutrality exactly at 1. Thus, without regarding of the life-history, under diploidy helping is required for helping to evolve. Filled blue line crosses the limit of neutrality before 1. Filled red line crosses the limit above 1. Under female hibernation, haplodiploidy favours helping; under larval diapause it hinders it. b) assumes the level of sex ratio biases that are selected for in each case. Bias in sex ratio increases the differences between the two haplodiploid cases. Once again, diploid examples show no change in the intercept with the neutral limit. Under female hibernation, haplodiploidy favours helping; under larval diapause it hinders it. To obtain these lines we assume full overlap between the generations, these means the best survival probabilities for males and females.
Figure 8. Evolutionary dynamics of helping behaviour and sex ratios. This is an example of a simulation run matched by the dynamics predicted by the inclusive fitness model assuming female hibernation and haplodiploidy. The x axis shows the generations, and y the average genotypic value of the population. Green lines represent the sex ratio of the summer brood, brown lines the sex ratio of the autumn brood, and blue lines represent values for helping behaviour. Thin lines are results of the individual based simulations. Thick lines are predictions from the inclusive fitness model. The evolution of helping behaviour is favoured by the initial sex ratio biases, male in the summer, female in the autumn. Then helping behaviour feeds back to the sex ratios, reversing the selective pressures on them. Summer sex ratio turns completely female biased. The colony now only produces female workers. Autumn sex ratio becomes even again. At the end of evolutionary process the population is eusocial and presents a univoltine life history.

Social learning and the evolution of cooperation
Piet van den Berg & Lucas Molleman

Especially in humans, social learning has been conjectured to drive an evolutionary process of its own: cultural evolution. Together with our capability for cooperation on large scales, culture is a main factor that has contributed to our success as a species in colonizing almost every habitat on earth. In our group, we have run a number of experiments with the aim of investigating social information use in different social interaction contexts. We do these experiments in laboratory facilities in which subjects have anonymous interactions with each other using software that was specifically developed to this end. We have been able to show that there is strong individual variation between people in both the amount and the kind of social information they use in social interactions. Moreover, a large group of subjects is very consistent in the amount and kind of social information they use, across the different contexts (contexts included games of cooperation, coordination and evasion). Some subjects always based their own decisions on information about the decisions of their group members, but others consistently requested information about both the payoffs and the decisions of their group members. In addition, we have shown that this variation in social learning strategies has significant implications for behaviour in a context of cooperation, where individuals are faced with making a choice between their individual interest and the interest of their group. Individuals that choose to view only information about decisions act more cooperatively than individuals that choose to view information about both decisions and payoffs of their group members. Indeed, when interacting in groups that were sorted for social learning strategy, groups of individuals that learn by decisions of others only reach significantly higher cooperation levels than groups of individuals that learn by decisions and payoffs.
Publications 2012

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