CEES

progress report 2004

Colofon

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Ecophysiology of Plants

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Theoretical Ecology

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CEES Highlights in 2004

Recent developments

In 2004, the Evolutionary Genetics group split into two research groups. As of 1 September 2004 an independent research group Theoretical Biology was founded. The reason was that the focus of Theoretical Biology had become much broader than population and evolutionary genetics and now includes ecological interactions, behavioural ecology and artifical intelligence. Hence, in the future there will be two independent programmes, Evolutionary Genetics (comprising the subprogrammes Evolutionary Genetics and Population and Conservation Genetics) and Theoretical Biology that will remain scientifically integrated.

Prizes

The **Royal Dutch Zoological Society** (KNDV) awarded **dr. Irene Tieleman** the **Dutch Zoology Prize 2004** on the occasion of the annual meeting of the KNDV on 3rd June 2004 at the Biological Center, Haren

Irene Tieleman won the Dutch Zoology Prize 2004. Photo Joris M. Koene

Die **Deutsche Ornithologen Gesellschaft** awarded **Prof.dr. Theunis Piersma** the **Ornithologen Preis 2004** on the occasion of the 137th annual meeting in Kiel on 30th September 2004. The president of the DOG, Prof.dr. Franz Bairlein, presented the prize to Theunis Piersma "für seine integrativen Untersuchungen zu Ökophysiologie, Wanderverhalten und zur weltweiten Verbreitung des Knutts *Calidris canutus*".

WOTRO presented **Prof.dr.ir. Jan Komdeur** one of the four **WOTRO 40 Year Anniversary Awards** at the WOTRO anniversary meeting at Amsterdam on 10th November 2004. The prize was awarded to Komdeur for his publication "Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs" (Nature, 1997, 385: 522-525) and its relevance in tropical and developing countries (co-authors Serge Daan, Joost Tinbergen & Christa Mateman).

The **"Prins Bernhard Cultuurfonds Prijs voor Natuurbehoud"** was awarded to **Theunis Piersma** for his affectionate, science-based, anxiety for the Wadden Sea and his fight on first principles for this valuable landscape and its natural inhabitants. The award ceremony took place in de Nieuwe Kerk in Amsterdam on 11th November 2004.

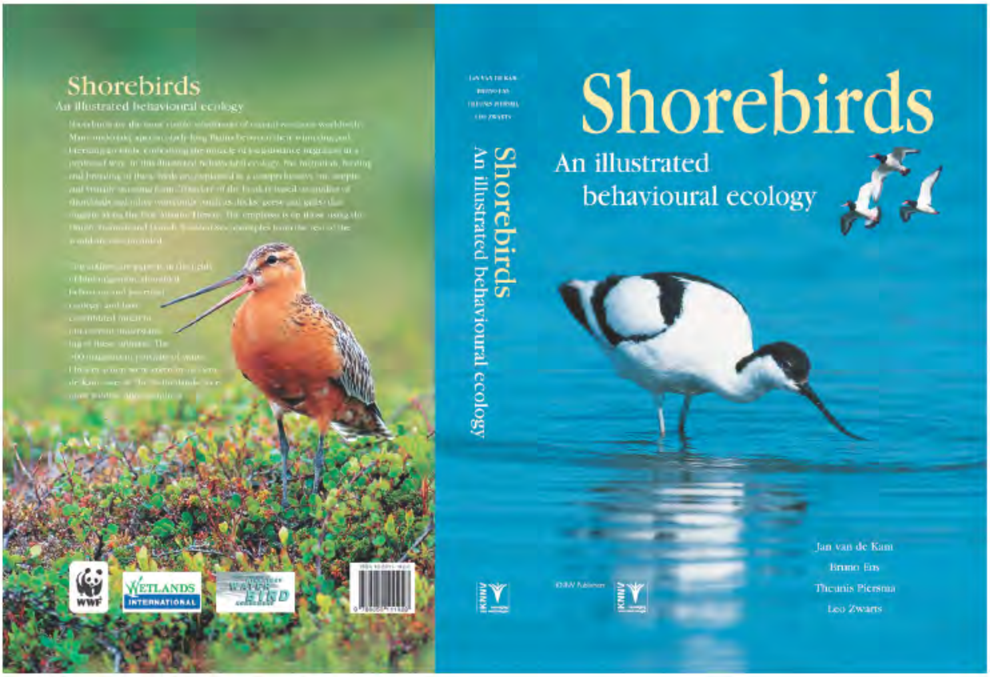
On the occasion of the 50th Anniversary of **Wetlands International** the first triennial **Luc Hoffmann Medal** for excellence in Wetlands Science and Conservation was awarded to **Prof.dr. Theunis Piersma** at Bangkok on 25th November 2004.

Awards

The NWO "Innovational Research Incentive Scheme" awarded dr. Irene Tieleman a VENI grant for her project "Solutions to seasonal challenges: the interplay of energetics, corticosteroid response, and immune function in Avian Life History"

Books

British Birds and the British Trust for Ornithology announce the winner of the Award for BEST BIRD BOOK OF THE YEAR. 2nd BEST: **Shorebirds: an illustrated behavioural ecology** by Jan van de Kam, Bruno Ens, Theunis Piersma and Leo Zwarts. KNNV Publishers, Utrecht, 2004.



Symposia

CEES Symposium "Wetenschappelijke evaluatie van de ecologische effecten van schelpdiervisserij in de Waddenzee" 29 January 2004, Biological Center.

Dr. Anneke Breeman retired in 2004 after a long career in seaweed ecophysiology and coral reef ecology. To commemorate this event a symposium was held on April 16 during which colleagues, both foreign and Dutch, as well as former students addressed

Anneke's contributions to science and teaching of marine biology. Anneke started her career at Groningen University as a PhD student in 1971; she retired as an associate professor.

On the occasion of the presentation of the Dutch Zoology Prize 2004 to dr. Irene Tieleman the Royal Dutch Zoological Society, Animal Ecology Group and Animal Behaviour Group organised the symposium "Coping with Environmental Challenges: adaptations of physiology, behavior and life history" 3rd June 2004, Biological Center.

CEES Jubilee symposium: 10 years Centre for Ecological and Evolutionary Studies "Biodiversity: adaptation versus chance", 1-3 September 2004, Biological Center.

Lectures/Oration

On the occasion of his retirement Prof.dr. Rudolf H. Drent held his public lecture "De onvrijheid van het vrije veld: hoe het energiebudget het dierenleven regeert" on 14th September 2004 at the Academic Hall of the University.

On the occasion of his retirement Prof.dr. Jelte van Andel held his public lecture "Tussen theorie en werkelijkheid: oneindige wetenschap" on 19th October 2004 at the Academic Hall of the University.

On 21st December 2004 Prof.dr. J. Dick van Elsas held his oration "Wie het kleine niet eert", to mark his acceptance of the chair in Microbial Ecology.

Appointments

Dr.ir. J. Komdeur has been nominated to the chair Avian Evolutionary Ecology.

From June 1, **Dr. R. Bijlsma** was appointed to the new personal Chair in "Population and Conservation Genetics", indicating that the field of conservation genetics has become quite mature and an integrated part of CEES. In addition to general evolutionary and population genetic topics, his chair is particularly targeted at understanding the dynamics of genetic processes in small populations subject to stochastic processes.

Prof.dr. J.J. Videler has been nominated to the chair Marine Zoology on behalf of the Foundation 'Leonardo da Vinci'.

Prof.dr. J.P. Bakker has been nominated to the chair Coastal Conservation Ecology.

The G.P. Baerends Visiting Professorship has been awarded to **Prof. Mark D. Bertness** (Department of Ecology and Evolutionary Biology, Brown University, Providence, USA), who will be paying a sequence of visits to Haren over the academic year 2004/2005.

Dissertations granted by the institution in 2003

E. de Deckere	Faunal influence on sediment stability in intertidal mudflats. Promotores prof.dr. C.H.R. Heip and prof.dr. W.J. Wolff, University of Groningen
I. Ferro	Sedimentary cycling of iron and manganese in freshwater, estuarine and deep-sea sediments. Promotor prof.dr. C.H.R. Heip and co-promotor dr. J.J. Middelburg, University of Groningen
P.C.Luttikhuizen	Spatial arrangement of genetic variation in the marine bivalve *Macoma balthica* (L.). Promotor prof.dr. W. van Delden and co-promotor dr. T. Piersma, University of Groningen
W.H. van de Poll	Patterns in ultraviolet radiation sensitivity of tropical, temperate and Arctic marine macroalgae. Promotor prof.dr. W.J.Wolff, co-promotores dr. A.M. Breeman and dr. A.G. Buma, University of Groningen
E. Stam-Bolink	Causes of leaf area reduction and implications of acclimation to UV-B radiation in *Pisum sativum* L. Promotor prof.dr.ir. P.J.C. Kuiper, co-promotor dr. P.R. van Hasselt.
H.J. van der Strate	As tides go by (spatial genetic structure of the seaweed *Cladophoropsis membranacea*). Promotores prof.dr. J.L. Olsen and prof.dr. W. van Delden, co-promotores dr. W.T. Stam and dr. L. van de Zande, University of Groningen.
P. Wiersma	Working for a living: Physiological and behavioural trade-offs in birds facing hard work. Promotor prof.dr. R.H. Drent, co-promotores dr. J.M. Tinbergen and dr. S. Verhulst, University of Groningen
Z.A. Zekeria	Butterfly fishes of the Southern Red Sea: Ecology and Population Dynamics. Promotor prof.dr. J.J. Videler, University of Groningen
H.J.Zemmelink	Dimethyl sulphide: measuring emissions from the ocean to the atmosphere. Promotor prof.dr. H.J.W. de Baar, co-promotores dr. W. Klaassen and dr. W.W.C. Gieskes, University of Groningen

Dissertations granted by the institution in 2004

M. Ateweberhan	Seasonal dynamics of coral reef algae in the Southern Red Sea : functional group and population ecology. Promotores: Prof.dr. W.J. Wolff and Prof.dr. J.J. Videler
L.W. Bruinzeel	Search, settle, reside and resign: territory acquisition in the Oystercatcher. Promotor Prof.dr. R.H. Drent, co-promotor Dr. J.M.Tinbergen, referents Dr. B.J. Ens and Dr. S. Verhulst
G.S. van Doorn	Sexual selection and sympatric speciation. Promotor Prof.dr. F.J. Weissing
J. Drent	Life history variation of a marine bivalve (*Macoma balthica*) in a changing world. Promotores Prof.dr. W.J. Wolff and Prof.dr. T. Piersma
A.H. Engelen	Flexibility without compromose. Population biology of the brown seaweed *Sargassum polyceratium* around the island of Curaçao. Promotor Prof.dr. J.L. Olsen, co-promotores Dr. A.M. Breeman, Dr. P. Åberg and Dr. W.T. Stam
J. van Gils	Foraging decisions in a digestively constrained long-distance migrant, the Red Knot (*Calidris canutus*). Promotores Prof.dr. R.H. Drent and Prof.dr. T. Piersma
I. Hendriks	Flow dependent processes in settlement of intertidal bivalve larvae. Promotores Prof.dr. C.H.R. Heip and Prof. P.M.J. Herman
M. van Himbergen-Gondwe	Quantifying the role of marine phytoplankton (DMS) in the present day climate system. Promotor Prof.dr.ir. H.J.W. de Baar, co-promotores Dr. W.W.C. Gieskes and Dr. W. Klaassen
G. Hoarau	Population genetics of plaice (*Pleuronectes platessa* L.) in Northern Europe. Promotores Prof.dr. J.L. Olsen, Prof.dr. A.D. Rijnsdorp and Prof.dr. W.T. Stam
D.P.J. Kuijper	Small herbivores losing control: plant animal interactions along a natural productivity gradient. Promotores Prof.dr. J.P. Bakker and Prof.dr. H.H.T. Prins (Wageningen University)

A.M. Mouissie	Seed dispersal by large herbivores -implications for the restoration of plant biodiversity. Promotor Prof.dr. J.P. Bakker, co-promotor Dr. R. van Diggelen
T. van Oijen	Iron and light limitation of carbohydrate production by phytoplankton in the Southern Ocean. Promotor Prof.dr.ir. H.J.W. de Baar, co-promotores Dr. W.W.C. Gieskes and Dr. M.A. van Leeuwe
J. Prop	Food finding: the trail to successful reproduction in migratory geese. Promotor Prof.dr. R.H. Drent
C.J. Vermeulen	Genetics of lifespan determination in *Drosophila melanogaster*. Promotores Prof.dr. R. Bijlsma and Prof.dr. W. van Delden, co-promotor Dr. L. van de Zande
V.E. Villafane	Ultraviolet radiation and primary productivity in temperate aquatic environments of Patagonia (Argentina). Promotor Prof.dr.ir. H.W. de Baar
C. Winter	Ecological role of planktonic bacteriophages in the North Sea and the tropical Atlantic Ocean. Promotor Prof.dr. G.J. Herndl

CEES PhD Students in 2004

Y. Aferworki	*WOTRO* - *Marine Biology* 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
A.C. Alderkamp	*Marine Biology* The role of laminaran within the microbial loop following a *Phaeocystis* bloom
A.H. Ambrosio de Castro	*Plant Physiology* Signal metabolites involved in the coordination between nitrate and sulphate assimilatory pathways
A.G.J.M. Ayrinhac	*IOP Genomics* - *Evolutionary Genetics* Evolution of ageing
J. Bakker	*NWO-WOTRO* - *Theoretical Biology/Evolutionary Genetics* Dynamics and significance of genetic variation in a metapopulation context: an integrated theoretical and experimental approach
M. te Beest	*NWO/Pionier-Community and Conservation Ecology* The invasion of *Chromolaena odorata* in South African savannas
T.W. Berngruber	*UE bursary* - *Theoretical Biology* Constraints on viral evolution
F.G.H. Boersma	*Microbial Ecology* The role of a plasmid with high mobilizing and retromobilizing capacity on the ecological behaviour of plant-associated bacteria
K.M. Bouwman	*ALW-Animal Ecology* Partner choice and sex ratio adjustment as exponents of sexual selection the the Reed Bunting
V.S. Brauer	*UE bursary* - *Marine Biology / Theoretical Biology* The effect of temperature on diversity, dynamics and abundance of phytoplankton: towards a synthesis of matter and energy-based approaches

L. Brouwer	*NWO-WOTRO-Animal Ecology* Population regulation and persistence of small isolated populations
D.M. Bühler	*UE bursary-Animal Ecology* The effect of genetic and environmental variation in immunocompetence on long distance migrations in Red Knots
J.F.M.F. Cardoso	*FCT-Portugal - Koninklijk NIOZ / Marine Biology* The importance of predation by epibenthos as forcing function for the structure of the benthos of the North Sea
E.R. Chang	*Community and Conservation Ecology/Dept. of Botany University, Toronto, RUG Bursary/Nat. Sci. and Engin. Res. Council of Canada* Seed bank dynamics in a natural salt-marsh system
M.S.C.O.M. Chevolot	*NWO-ALW (PRIORITEIT) - Marine Biology* Population structure and population dynamics in species with low dispersal capacity (rays) based on high resolution genetic markers and metapopulation modelling
T. Compton	*UE bursary-Marine Biology/Animal Ecology/NIOZ* Biodiversity of tellinid bivalves on a tropical mudflat: bird and fish predation as a driving force
J.P.G.M. Cromsigt	*WOTRO/Community and Conservation Ecology* Resource partitioning among grazers of different actual and functional body weight in spatially structured savannas of South Africa
M. Durenkamp	Plant Physiology Whole plant regulation of sulfate uptake and assimilation
G. Eichhorn	*UE bursary-Animal Ecology/Community and Conservation Ecology* Nutritional ecology and migratory strategy of a herbivore migrant bird - the barnacle goose *Branta leucopsis*

C. Eikenaar	*ALW VICI/Animal Ecology* Long-term inclusive fitness consequences of sex allocation in the Seychelles Warbler
B.V. Feldmeyer	*Robert Bosch Stiftung bursary - Theoretical Biology/Evolutionary Genetics* Temperature-dependent sex determination
S. Ferber	*Marine Biology* Mechanisms underlying the genetic structure of seagrass meadows: historical stochastic events versus adaptation
H. Flores	*NWO-ALW (NAAP) - Marine Biology / Alterra* Seabird foodchains in the Antarctic seasonal sea-ice zone: challenging the dominant role of krill
E.O. Folmer	*Animal Ecology/Community and Conservation Ecology* Patchiness of food and the structure of shorebird communities
B.P. Freymann	*Robert Bosch Stiftung/Community and Conservation Ecology* Interactions between invertebrates and vertebrates in savanna ecosystems: consequences of elevated temperature
V. Fuld	*NWO-ALW Pionier project/Community and Conservation Ecology* Spatial ecology of tropical savannas
R. Geerts	*Evolutionary Genetics* Genetic dissection of long-term evolved insecticide resistance in *Drosophila*: In search of compensatory evolution
R. Gesser	*Marine Biology* Flatfish flight in a salinity gradient
A.J. van der Graaf	*Animal Ecology/Community and Conservation Ecology* Herbivore facilitation in salt-marsh systems: from temperate to arctic
N. Hagenah	*PE&RC/Community and Conservation Ecology* The interplay of small mammals with large herbivores

D. Haydar	*NWO-ALW (PRIORITEIT) - Marine Biology* Introduction of exotic species: a process contributing to coastal marine biodiversity
S.A. Hazelaar	*STW - Marine Biology* The role of proteins in silica biomineralization in diatoms
M.E. de Heij	*ALW-Animal Ecology* Defining limitations to egg care in avian life histories
S.K. Heijs	*Microbial Ecology* Microbial communities at deep-sea mud volcanoes in the Eastern Mediterranean Sea
M. Hinsch	*RuG bursary/ALW VICI/Theoretical Biology/Animal Ecology* Sex specific competition, co-operation and dispersal in Seychelles warblers
F. Hoffmann	*NWO-ALW/Community and Conservation Ecology* Pollinators and flowering plants: metacommunity dynamics and biodiversity in the agricultural landscape
H.J.T. Hoving	*Marine Biology / University of Cape Town* Age, growth and reproduction of South-African mesopelagic squid
P.J. Janknegt	*Marine Biology* Oxidative stress in marine Antarctic phytoplankton resulting from exposure to solar ultraviolet radiation
J. Jansen	*NIOO-CEME - Marine Biology* Exploring ecotypic variation in European shellfish populations
D. Joubert	Evolutionary Genetics Fluctuating asymmetry as a monitor for genetic and environmental stress in *Drosophila*: Fundamental and applied aspects
R.K.H. Kats ALTERRA	*NWO-Alterra Texel/Animal Ecology* Large-scale population of Eiders and their long-term impact on commercial shellfish

A. Klimkowska	*IMUZ/OECD/PIN-MATRA (Min. Agr.)/Prins Bernhard Cultuur Fonds* The ecological feasibility of fen restoration - Opportunities and constraints of top-soil removal?
N. Klomp	*NIOO-CEME / Marine Biology* Food quality in the Wadden Sea
A. Koralewska	*Plant Physiology* Significance of expression level versus activity of sulfate transporters in *Brassica* - on a whole plant level - in the regulation of uptake, transport and subcellular distribution of sulfate in relation to sulfur requirement for growth
M.A. Kozielska	*Evolutionary Genetics/Theoretical Biology* Evolution of sex determining mechanisms in the housefly
P. Korsten	*ALW-Animal Ecology* Sex-biased investment in blue tits (*Parus caeruleus*): primary sex ratio and paternal quality
G.D. Kramer	*NWO-ALW* - *Koninklijk NIOZ / Marine Biology* Bacterioplankton dissolved organic carbon production and the distribution of bacterioplankton derived DOC in the oceanic water column
I.V. Mateo Leach	*UE bursary* - *Evolutionary Genetics* Genetics of sexual and parthenogenetic reproduction in *Venturia canescens* (Hymenoptera)
S.P.M. Michler	*ALW VICI/Animal Ecology* Offspring fitness and sex-specific dispersal in great tits
J. Mieog	*WOTRO* - *Marine Biology / Australian Institute of Marine Science* Understanding the flexibility of the coral-algal symbiosis on the Great Barrier Reef (Australia) as a mechanism to respond to environmental change
H. Najafi Zarrini	*Republic Iran* - *Plant Physiology* The role of receptor-like kinases in salt, drought and osmotic stress

M. Nicolaus	*UE bursary/Animal Ecology* Offspring fitness and density dependent dispersal: an experimental study in the great tit
D. den Os	*NWO-AWL* - *Plant Physiology* Integration of light- and ABA-signalling processes in leaf development
V.S. Parada	*NWO-ALW* - *Koninklijk NIOZ / Marine Biology* Virioplankton dynamics and diversity in relation to bacterioplankton diversity in open ocean systems
A. Peire Morais	*Evolutionary Genetics* Genetics of reproductive isolation in *Nasonia*
H.P. Perez de Vladar	*RUG bursary* - *Theoretical Biology* Molecular evolution under population constraints
A.M.T. Piquet	*Marine Biology* Adaptations of marine bacteria to (increased) solar ultraviolet - B radiation and consequences for the decay of diatom blooms
M. van de Pol	*Animal Ecology/Theoretical Biology* Dispersal and settlement decision in a long-lived shorebird
J.J. Poos RIVO	*NWO-PRIORITEIT-RIVO/Animal Ecology* The dynamics of effort allocation of fishing fleets with special emphasis on spatial scale of exploitation
A. Purwoko	*Asian Development Bank* - *Marine Biology* Factors determining the benthic fauna of Sembilang Peninsula, Sumatera, Indonesia
T. Reinthaler	*NWO-ALW / EU* - *Koninklijk NIOZ / Marine Biology* Bacterioplankton growth yield dynamics in the oceanic water column
T.A. Revilla-Rimbach	*NWO Computational Life Sciences* - *Theoretical Biology* Emergence of biocomplexity

M. Roodbergen ALTERRA	*NWO-ALW-ALTERRA/Animal Ecology* Pollutant induced changes in life support functions of soils and its impact on meadowbird populations
A.L. Rutten ALTERRA	*STW-Alterra/Animal Ecology* Long-term effects of human disturbance on waders feeding on intertidal flats: the use of telemetry and cage experiments
M.J.A. Rijkenberg	*NWO-ALW (NAAP) - Koninklijk NIOZ / Marine Biology* Positive feedback of enhanced UV-B via iron chemistry of sea water on phytoplankton growth and CO_2 fixation in the Southern Ocean
M.E.C. van Rijswijk	*Evolutionary Genetics* Dispersal and local adaptation in experimental metapopulations of *Drosophila melanogaster*
C. Schmitz	*Robert Bosch Stiftung* - *Plant Physiology* Universal temperature dependece of plant growth: physiological and biochemical principles
M. Schotten	*Marine Biology / University of Hawaii* Use of echo location and communication signals of wild dolphins
J. Schröder	*Animal Ecology* Individual fitness correlates in Black-tailed Godwits (*Limosa l. limosa*)
M.W. Smith-Kleefsman	*Evolutionary Genetics* Determinants of metapopulation dynamics: stochastic processes versus adaptation
J. Steinhauser	*RUG bursary* - *Theoretical Biology* Self-organization of social structure
P. Stevens	*Microbial Ecology* Adaptation of *Ralstonia solanacearum* to temperate climates

R. Trifonova	*Microbial Ecology* Microbial maturation of novel substrates
K. Troost	*NWO-ALW* - *Marine Biology / RIVO* Will an introduced species, the Japanese cupped oyster *Crassostrea gigas*, force Dutch estuarine ecosystems in a new stable state?
W.K. Vahl	*Breedtestrategie 1 -NIOZ/Theoretical Biology/Animal Ecology* Interference and carrying capacity
T. Veen	*NWO-ALW* - *Theoretical Biology* Evolution of mating decisions in hybrid zones
R.M. Veeneklaas	*Community and Conservation Ecology/Evolutionary Genetics* Dispersal and adaptation in two salt-marsh plant species
H. Verkiel	*Breedtestrategie 2* - *Theoretical Biology* Allometric scaling laws
Y. Verkuil	*Animal Ecology* Population structure in Ruffs
J.A. Warmink	*Microbial Ecology* Interactie tussen bodembacteriën en *Mycorrhiza* schimmels
H.E. Wolters	*Community and Conservation Ecology* Restoration of salt marshes
L. Yang	*WOTRO* - *Plant Physiology* Impact of atmospheric sulfur and nitrogen deposition on crop cultivars in relation to fertilizer practice in rapidly developing regions in China
A. Zipperle	*UE-bursary* - *Marine Biology* Inter-relationships between reproductive ecology, genetic structure and fitness in populations of the seagrass *Zostera noltii* in the Wadden Sea - North Sea

PhD Projects - theses in preparation

M. Berg	*UE bursary-Animal Ecology* Mutual sexual selection in the European wren
H. Beukema	*WOTRO/Community and Conservation Ecology/ICRAF-Indonesia* Pteridophyta in rubber agroforests at a range of management intensities and the choice between segregation and integration of biodiversity conservation and agricultural productivity
G. Boedeltje	*RWS/Community and Conservation Ecology/Radnoud University* Establishment of species-rich canal verges
M.K. de Boer	*NWO-ALW - Marine Biology* Raphidophyceae, microalgae potentially hazardous to marine life in Dutch coastal waters: environmental control of neurotoxicity
O.G. Bos	*NWO-ALW (PRIORITEIT) - Koninklijk NIOZ / Marine Biology* Settlement chance and post-settlement mortality in marine soft-sediment communities in relation to the amount of larval supply, the size of the local adult stock, and feeding conditons
J.C.A. Creuwels	*NWO-GOA (NAAP) - Marine Biology /Animal Ecology /Alterra* Individual quality of Antarctic petrels
G.J. Geertjes	*WOTRO - Marine Biology / Evolutionary Genetics* Relations between reproductive strategies of reef fishes, hydrographic conditions and the genetic differentiation between parrotfish populations on the reefs of Caribbean islands
R. Geerts	*Evolutionary Genetics* Genetic dissection of long-term evolved insecticide resistance in *Drosophila*: In search of compensatory evolution
A.V. de Groot	*NWO-ALW/KVI/Community and Conservation Ecology* Rejuvenation of salt marshes
G.N.J. ter Heerdt	*Community and Conservation Ecology/RIZA* Development of helophytic vegetation as related to hydrological management in inland polders

I.E. Hendriks	*NWO-ALW (PRIORITEIT) - NIOO-CEME / Marine Biology* Flow-dependent processes in recruitment of intertidal bivalves: research into direct effect of shellfisheries
P.J. van den Hout NIOZ	*NIOZ-Animal Ecology* Adaptive responses of non-breeding shorebirds to avian predators
F. de Jong	*CWSS* - *Marine Biology* Marine eutrophication: ecology and policy
J. de Jong	*Animal Ecology* Ecology of the barn-owl
D. Joubert	*Evolutionary Genetics* Fluctuating asymmetry as a monitor for genetic and environmental stress in *Drosophila*: Fundamental and applied aspects
R.H.G. Klaassen NIOO	*Netherlands Institute of Ecology, Centre for Limnology/ Animal Ecology* The scale of foraging decisions in waterfowl
C. Kraan NIOZ	*NIOZ-Animal Ecology* Spatial dynamics of macrobenthos in the western Wadden Sea
Y. Malherbe	*UE bursary* - *Evolutionary Genetics* Molecular genetic analysis of quantitative traits involved in stress resistance and fitness
M.P. McDonnell Alexander	*EU bursary* - *Community and Conservation Ecology/Plant Physiology* Plant competition and coexistence in successional grasslands: effects of heterogeneous soil conditions
L. Mendes	*Universidade de Lisboa, Portugal/NIOZ/Animal Ecology* The role of parasites and immuno competence in habitat selection of waders

D. Micu	*National Institute for Marine Research 'Grigore Antipa' - Marine Biology* Diversity and ecology of the malacofauna of the Romanian Black Sea
E.P. Morris	*EU - NIOO-CEME / Marine Biology* Quantifying primary production of microphytobenthos: application of optical methods
J. Reneerkens NIOZ	*NIOZ-Animal Ecology* Preen waxes of marine birds: is sexual selection involved in the evolution of complex chemical compounds?
H. Schekkerman ALTERRA	*Animal Ecology/Alterra* Growth and survival of the blue-tailed godwit *Limosa limosa* in relation to habitat use in an agricultural landscape
E.W.M. Stienen ALTERRA	*Animal Ecology/Alterra* Population persistence of the sandwich tern *Sterna sandvicensis* in relation to the local food stocks
H.M.C. Verhagen	*Community and Conservation Ecology/Fryske Gea* Nature restoration on mineral soils
M. Vorenhout	*WWF/Community and Conservation Ecolog/VU/Radboud University* Estuarine and coastal ecosystems affected by pollutants
B.K. Wesenbeeck	*NIOO-CEMO/Community and Conservation Ecology* Rejuvenation of salt marshes

Animal Ecology

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Group leader Prof.Dr T. Piersma

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prof.dr. T. Piersma	RUG	0.7	
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drs. M. Nicolaus	RUG-Ubbo Emmius	1.0	041101-081101
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Introduction

A fundamental understanding of the distribution and abundance of animals naturally leads to an examination of the factors constraining distribution and abundance. The traditional focus of our research group concerned the quantification of the relationships between animal populations and their food supplies, trying to trace the causal links at the individual level. Whilst maintaining a focus on the individual, i.e. the ‘decision making’ unit of selection that integrates the complex array of social and ecological pressures, the spectrum of factors that mould evolutionary responses, has been expanded to also include energy expenditure, disease factors and the social environment. Our work is done primarily in natural settings, especially those available in the northern Netherlands, and hinges upon detailed behavioural and physiological measurements, as well as fitness measurements to judge the consequences of behavioural variation. When possible, an experimental approach is chosen to determine the causality of relationships between behavioural variation and fitness. We keep an open mind to what new findings and developments in other fields of biological enquiry (such as theoretical biology, ethology, biophysics, genetics, endocrinology, immunology and molecular genetics) have to offer. Indeed, we particularly encourage the use of modern molecular techniques, both to determine genetic relationships between competing and cooperating individuals in a population and to decipher the long-term demographic and genetic histories of populations with a suite of consequences for today’s functioning. Most work is on higher vertebrates, especially birds, but in several cases studies of predator-prey interactions have lead to detailed work on (invertebrate) food organisms as well. This year we present reports on a selection of topics that have received detailed scrutiny by our group.

Overview of academic results

Tracking Russian barnacle geese by means of light-level geolocation

Götz Eichhorn, Rudi H. Drent, Henk van der Jeugd

In more than one way migration is the most demanding period in the annual life cycle of migratory birds. They may spend half or more of their lives on the way between breeding and wintering sites. The study of individual travel schedules, however, still represents a challenge to the researcher. Recently, several tracking devices became available, of which satellite transmitters (PTTs) have been the most commonly used ones so far. They are able to convey very accurate positions. Nevertheless, their application is limited by low sample sizes due to high costs, a working duration rarely exceeding 1-2 years due to limited battery power, and a possible negative impact on the bird’s behaviour and/or condition, which may even prevent it from breeding. To noninvasively study migratory strategies of barnacle geese, the application of GLS (Global Location

Sensing) techniques based on the theory of geolocation by light levels appeared promising. In GLS, archival tags equipped with a 'clock memory chip' record light-level data, from which dusk and dawn events are estimated, and these form the basis to calculate geographical positions (two fixes daily); day (night) length determines the latitude and time of local midday (midnight) the longitude. The working duration of loggers of the type used in this study can reach eight or more years. GLS units of same type and manufacturer were evaluated by other researchers during a study on seabirds. Positions of free ranging albatrosses could be determined with a mean error ± SD of 186 ± 114 km, an accuracy much worse than PTT systems, but still sufficient to monitor the spatial-temporal pattern of barnacle goose migration over a distance of 3,500 km. A drawback inherent to the method is an increased latitudinal error close to the times of equinox, especially at the winter side of the equinox. However, the longitude estimate is not affected by the equinox, and is also in general more accurate (SD c. 110 km and 185 km for longitude and latitude, respectively). Fortunately, the largely east-west movement within a narrow corridor along coastal lines of the barnacle geese studied by us, allows the reconstruction

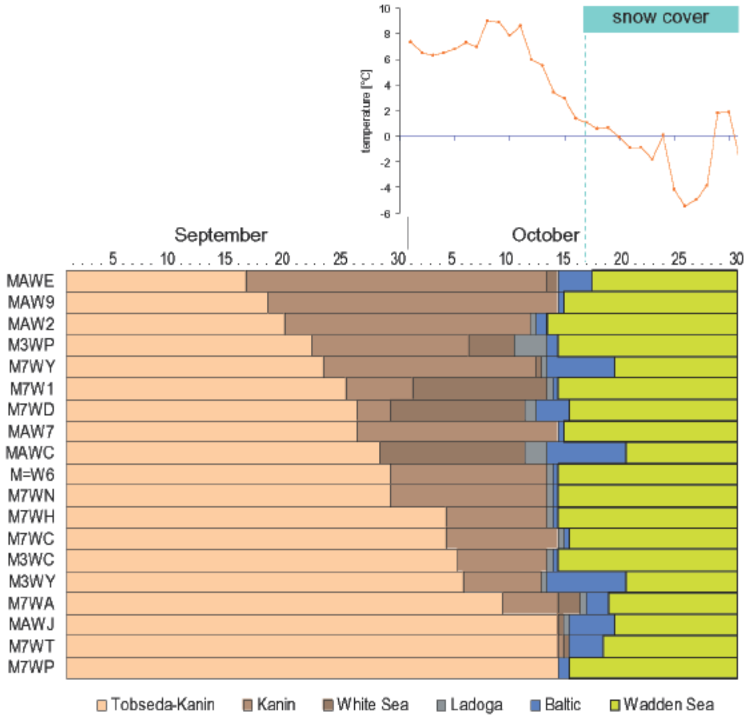

Figure 1. Spatiotemporal pattern of 19 females (codes given at the left side) in the period September to October 2003. The individual cases are sorted by the departure date from the breeding grounds or adjacent sites within 200 km. The graph above shows ambient temperatures and start of snow cover (17 Oct.) measured at the weather station in Shoina, Kanin.

of shifts between stopover sites even by means of solely the longitude estimates. A bonus of the technique is that the incubation rhythm can be detected in these arctic breeders.

In 2003 during the breeding or moulting periods of barnacle geese, GLS loggers were fitted on 54 females at the colonies at Tobseda in northern Russia (for location see Fig. 2). The nine gram device was attached to one of the plastic rings around the legs (see photo in Fig. 2). Total mass of logger and all rings was 21 g, corresponding to 1.5% of females' body mass at the end of incubation. To retrieve the information it is essential

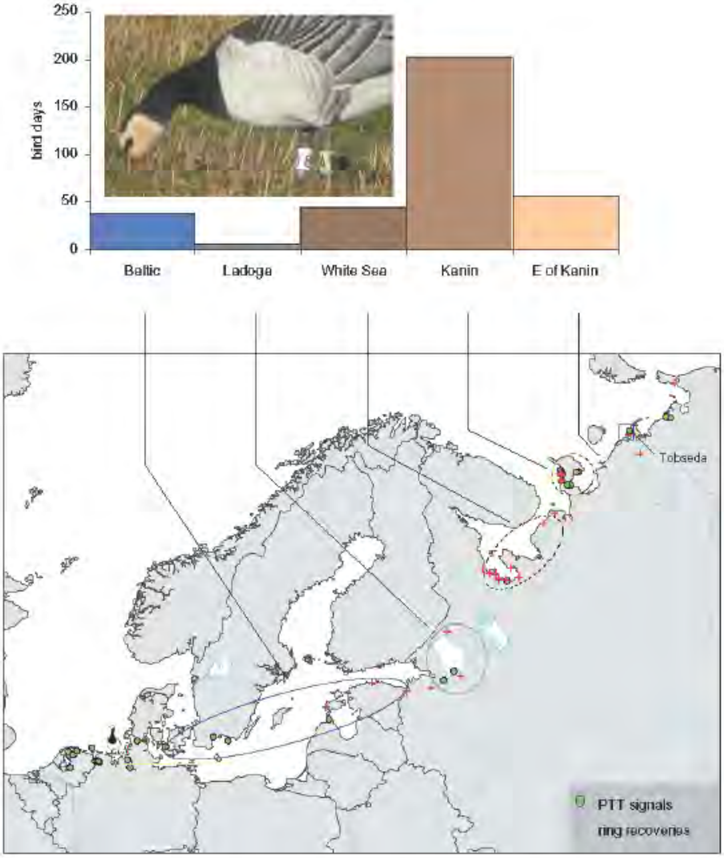

Figure 2. Positions from satellite tracked birds (2004) and ring recoveries (1965-2003). The yellow shaded area shows the migratory corridor reconstructed from these accurate positions and direct observations published in literature. Stopover sites for geese from the Tobseda colonies on fall migration are encircled and the relative use by the 'logger birds' in 2003 is expressed in 'bird days' in the figure above. The photo (by H. van der Slot) shows the logger attachment to the legring.

to recapture the bird, and the data treated here derive from nineteen female ‘logger birds’ out of 24 recaptured in 2004; five units failed working. Valuable information can thus be obtained, even when birds were not under direct observation. The spatial-temporal pattern for the 19 ‘logger females’ displayed considerable variation in individual travel schedules (Fig. 1). On their long-distance travel the geese used 0-3 stopover sites where they staged longer than 1 day. Most birds (63%) travelled within 1-3 days directly from arctic staging sites (White Sea and east of it) to the wintering grounds in the Wadden Sea. Arctic sites were used as long as possible, such that the geese left the Arctic during 11-16 October (mean 13 October) shortly before the onset of frost and snowfall (17 October). Many geese staged extensively in or near the breeding colonies before commencing migration. The Kanin Peninsula was the most important stopover site used by 80 % (15/19) of the birds for on average 2 weeks (3-27 days, Fig. 2). Little use was made of staging sites in the Baltic, where only 8 birds spent more than one day.

Currently we investigate whether individual travel schedules during spring migration is related to subsequent breeding performance. Arctic geese with a supposedly mainly capital breeding strategy (i.e. bringing along stores from wintering and spring staging sites to the breeding destination) and a typical incubation rhythm with very restricted food uptake, need to be prepared before they even reach the breeding site. Therefore, spring migration has not only to account for the nutrient budget to cover the travel costs - it represents to a large extent also the process of preparation for reproduction.

Northward migration of ruffs and reeves

Yvonne Verkuil, Petra de Goeij, Jan Wijmenga, Jos Hooijmeijer, Christiaan Both, Theunis Piersma

In 2004 we started a project on the migratory flyways, population dynamics and genetic population structuring of a shorebird species that has almost disappeared as a breeding bird in The Netherlands, but is still commonly found in the boreal zone of Eurasia, the ruff *Philomachus pugnax*. We have focussed on three topics:
(1) The colour-ringing program. In March, April and May, migrating Ruffs staging in southwest Fryslân were caught in cooperation with ‘wilsterflappers’ (traditional Frysian bird catchers, Fig. 3). We banded 1134 birds with an individually unique combination of colour rings, of which 26% were resighted within the study area before they continued their northward migrating. We also obtained quite a few resightings further north (see Fig. 4). The local resightings suggest a stopover duration of 3-4 weeks, with possibly as many as 70,000 birds using the study area. Interestingly, the population consisted predominantly of males; only after medio April the proportion of females increased to about 50%. But while on first sight males seem to dominate the numbers, females (also called ‘reeves’) may stopover for shorter periods and thus have an higher turnover rate, meaning that the numbers of males and females migrating through Southwest Fryslân could even be equal.

Figure 3. 'Fryske wilster-netters' catch various wader species with the aid of a clapping net, whistling sounds and handmade decoys. This ages-old catching method was in the past predominantly applied by farm workers to earn an (extra) living, but currently the method is only allowed when supporting scientific research. Next to 'wilsters' (the Frisian word for golden plover) many ruffs are caught. All birds are ringed, weighed and measured by the wilsternetter, after which he gives us a cellphone call. When we arrive at the catching site we apply the birds with an unique colourring combination. In this way we are able to ring much more birds than if we would had to catch them all by ourselves.

Figure 4a and 4b. Resighted Ruffs in Norway. Most resightings were made in the Fryslân and within days or weeks after catching. But soon birds were reported from other localities as the Wadden Sea islands and Lauwersmeer. Ruff were reported from several countries along the migration route, Germany and southern Sweden, and from the breeding areas in northern Scandinavia. Birds were also reported on southward migration. Again mostly in Southwest Fryslân and Lauwersmeer, but also in Belgium, France and Italy. Even in winter, observations have been mailed in, lately mostly from Belgium.

(2) Molecular evidence for temporal and spatial population structuring. We started using molecular markers to track possible genetic population subdivision or admixture in ruffs. DNA was isolated from blood samples taken from each colour-ringed individual and we received samples from international co-workers in Belarus, Sweden and Siberia. Using nine microsatellite markers, the genotypes of 213 individuals were obtained. Our first analyses showed a weak but significant population differentiation within ruffs migrating through The Netherlands, i.e. allele frequencies varied significantly with the timing of migrating of migration. Moreover, 15 genotyped individuals from the two breeding areas (Siberia and Sweden) showed a distinctive segregation in allele frequencies for all nine loci.

(3) Habitat requirements. In last decades Fryslân has changed dramatically. It lost many extensively managed, wet grasslands, with herb rich vegetations on peat soils. Open landscapes have become more scarce and many roosting sites are in decline, while agricultural activities keep changing and intensifying. To assess how further changes could affect staging conditions for the migrating Ruffs (who predominantly feed in meadows), we studied habitat use in spring 2003 and 2004. In 125 meadows on a transect from Lake IJsselmeer to 25 km inland we monitored ruffs and 13 meadow characteristics. It turned out that ruffs mostly foraged nearby roosts and in meadows with short vegetation. Also meadows in an open landscape were more often visited than meadows fringed by grove/trees or buildings. The expected preference for meadows on peat soils was not found for male ruff, but in reeves. Reeves also stayed even closer to the roost and chose ‘wetter’ grasslands.

Individual fitness correlates in the black-tailed godwit

Julia Schröder, Jos Hooijmeijer, Christiaan Both, Theunis Piersma

Black-tailed godwits *Limosa limosa limosa* are a true symbol of the Dutch lowland meadow landscapes which harbour more than 80% of the West-European population Despite concerns and serious conservation activities, black-tailed godwits continue to suffer a strong population decline. Numbers of Dutch breeding pairs dropped dramatically from 120,000 in 1983 to 42,000 in 2003 and the decline has not yet stopped. The most common explanation for this decrease is the low breeding success, caused by habitat loss, increased agricultural practise and strong predation pressure. But even in high quality habitats, with low or even no agricultural activities and extremely low predation pressure during the breeding season, reproductive success usually does not reach values high enough to sustain the population, while at the same time there are concerns about annual survival.

In spring 2004 we embarked on what hopefully will be a long-term population study of black-tailed godwits on the Workumerwaard, an area of low-intensity farmed grasslands

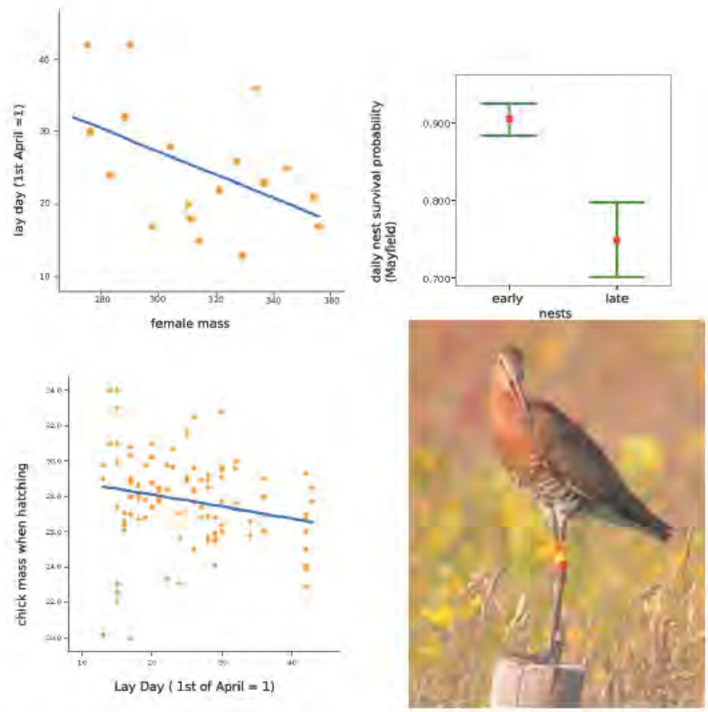

Figure 5. Date-dependence of parameters related to the breeding success of black-tailed godwits in the Workumerwaard, Friesland. Top left: Female mass in relation to lay day (r= -0.424, p<0.05, N = 28). Top right: Mayfield nest survival of early (<24th April) and late nests (>24th of April). Bottom left: Lay day in relation to chick mass measured shorly after hatching, pearson correlation (2-tailed): -0.22, N = 107, p<0.05), bottom right: one of our color ringed birds.

in western Friesland, with the aim of determining correlates between individual traits and reproductive success. Consistent with many studies on birds in temeparte climatic zones, we found a strong effect of individual timing of breeding on reproductive success. Early egg-laying black-tailed godwits were heavier, had heavier eggs with a higher probability to hatch (Fig. 5), and they produced heavier chicks with higher growth rates. For a godwit it is obviously important to breed early, which raises the question why not all black-tailed godwits do so?

A 11 000-km-long nonstop flight from Alaska to New Zealand by bar-tailed godwits?

Theunis Piersma, Robert E. Gill, Jr

Populations of the bar-tailed godwit *Limosa lapponica* embark on some of the longest migrations known among birds (Fig. 6). The *baueri* race breeds in western Alaska and spends the nonbreeding season a hemisphere away in New Zealand and eastern

Figure 6. A group of very fat bar-tailed godwits in southwestern Alaska, close to embarkation on a transPacific, 11,000 km long migratory journey (photo by Robert E. Gil, Jr).

Figure 7. Distribution of records of bar-tailed godwits throughout Oceania during the southward migration period (September-November). Solid circles = sites reporting godwits; clear circles = sites at which no godwits were noted. Map projection = Orthographic (central meridian = 180; reference latitude = -10). Lateral bounds of stippled region = plotted great circle routes.

Australia; the *menzbieri* race breeds in Siberia and migrates to western and northern Australia. Although the Siberian birds are known to follow the coast of Asia during both migrations, the southern pathway followed by the Alaska breeders has remained unknown. Two questions have particular ecological importance: (1) do Alaska godwits migrate directly across the Pacific, a distance of 11 000 km? and (2) are they capable of doing this in a single flight without stopping to rest or refuel? We explored six lines of evidence to answer these questions. The distribution of resightings of marked birds of the *baueri* and *menzbieri* races was significantly different between northward and southward flights with virtually no marked *baueri* resighted along the Asian mainland during southward migration. The timing of southward migration of the two races further indicates the absence of a coastal Asia route by *baueri* with peak passage of godwits in general occurring there a month prior to the departure of most birds from Alaska. The use of a direct route across the Pacific is also supported by significantly more records of godwits reported from within a direct migration corridor than elsewhere in Oceania (Fig. 7) and during the September to November period than at other times of the year. Flight simulation models, extreme fat loads, and the apparent evolution of a wind-selected migration from Alaska further support a direct, nonstop flight.

Functional ecology of intraspecific pectoral muscle size variation

Maurine W. Dietz, Theunis Piersma

Birds should be as light as possible, because flying requires high power and energy and the more so when you are heavy. Birds are magnificent in saving weight by adapting their organs and structures to the prevailing requirements during each life-stage, the bar-tailed godwits of the previous account being no exception. The flight machinery of a bird is the pectoral (breast) muscle which is also the largest avian organ (20-25% of

Figure 8. Life cycle stages of the red knot. The abbreviations stand for the following life phases: F*i*, fuelling period *i*, PD*i*, pre-departure period *i*, FL*i*, migration flight *i*, and A*i*, just arrived after migration period *i*.

body mass). For non-stop long-distance migration birds must store large amounts of nutrients, but at the same time they should minimize their weight, including their pectoral muscle. But this muscle is also needed for locomotion and for carrying the weight, and hence birds have to compromise between being as light as possible and performing their task. Using power models and available data, we calculated the theoretical rate of change in pectoral muscle mass with changing body mass: on a double logarithmic plot of pectoral muscle mass against body mass the slope of the regression is 1.25.

Red knots *Calidris canutus* are extremely long-distance migrants and are champions in adjusting their organ sizes in response to these peak performances. In the annual cycle of red knots, the birds cover long-distance flights of over 5000 km between breeding and wintering areas. These migration periods consist of a fuelling, a pre-departure, a flight and an arrival phase (Fig. 8). Using a large set of dissection data of red knots (n=185) we investigated the relationship between pectoral muscle mass and body mass in detail. Pectoral muscle mass changes with body mass but there were clearly two phases: light, mainly starving, birds in winter had a steeper rate of change in their pectoral muscle with body mass than heavy birds in healthy condition (Fig. 9). The breakpoint between the two phases lies around 125 g, the normal winter weight of a red knot. The theoretical slope of 1.25 applies to birds in healthy state only. The breakpoint of the biphasic regression

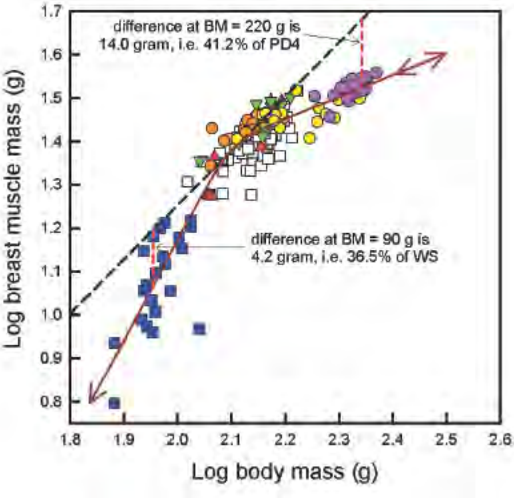

Figure 9. The allometric relationship between pectoral muscle mass and body mass for adult red knots. The red solid line represents the biphasic allometric regression through the data (intercept: -3.481; left slope: 2.327; right slope: 0.501; and breakpoint 2.098, i.e. at 125.3 g), the black dotted line is the theoretical allometric regression (see text). The life phases are indicated as follows: breeding, green triangles down; F2, orange circles, A3, red triangles up; F4, yellow circles; PD4, pink circles; wintering, white squares; winter starved (WS), blue squares. For an explanation of the abbreviations see previous figure.

should thus be part of the theoretical allometric relationship which enables placement of the theoretical line in the graph. Now we see that the pectoral muscle of the average winter starved bird is much smaller than expected: in 90 g red knot are 36.5% too small. During starvation birds use their protein stores and reserves. Inevitably the pectoral muscles are also used as protein source which explains why they are so small as compared to the theoretical expectation. This part of the relationship describes thus the rate of *decrease* in pectoral muscle mass with decreasing body mass.

However, also heavy predeparture red knots in a healthy state have much smaller pectoral muscles than expected (Fig. 9). The rate of change in pectoral muscle mass with body mass after the breakpoint is not even half of the theoretical rate of change (0.501 vs. 1.25, respectively), and pectoral muscles are more than 40% smaller than expected at a body mass of 220 g. We can come up with three explanations for this phenomenon: (1) There is a constraint in cell size that limits maximal pectoral muscle mass (in general organs do not increase in mass via new cell growth). (2) Red knots increase the energy conversion efficiency of their pectoral muscle with increasing body mass and thus increasing power demand. (3) There is a trade-off between pectoral muscle mass (weight saving to increase migration flight range) and power margin (flight apparently abilities to e.g. decrease predation risk), and for a predeparture knot weight saving is more important than maintaining optimal power margin.

The effect of density on extra-pair paternity in great tits

Marco van der Velde, Stephanie Michler, Richard Ubels, Joost Tinbergen, Christiaan Both, Jan Komdeur

Since the introduction of molecular techniques in bird ecology, it has become clear that extra-pair paternity (EPP) is a widespread phenomenon among socially monogamous birds. For example, extra-pair young (EPY) have been observed in approximately 86% of all passerine species. This finding has urged ecologists to study the evolutionary/adaptive significance of EPP (e.g. ‘good genes’ hypothesis), and to pinpoint (ecological) factors that explain variation in EPP among populations and species. Breeding pair density has been proposed as an important factor that might significantly affect the level of EPP within a population. Aim of our study was, therefore, to examine the effect of experimentally manipulated breeding pair density on the level of EPP.

In 2004, we studied EPP in a population of great tits *Parus major* in the Lauwersmeer (The Netherlands). In this population nest-boxes were distributed over 8 different plots. These plots had different nest-box densities and, consequently, different breeding pair densities. For all occupied nest-boxes both parents and chicks (Fig. 10) were blood sampled and genotyped for 6 different microsatellite markers (Fig. 11). In total, paternity was determined for 897 chicks of 106 first broods and for 382 chicks of 57 second broods.

Figure 10. Nest with great tit chicks.

We found that 41% of the first broods and 58% of the second broods had at least one EPY, and that within broods on average 9.5% and 16.0% of the chicks were EPY for first and second broods, respectively. These observed levels of EPP for great tits in the Lauwersmeer were comparable to those reported by other studies on *Parus* species. The higher level of EPP in second compared to first broods in the Lauwersmeer population was also consistent with a study on a great tit population in Bahrdorf (Germany). 47% of the breeding males fathered chicks in other nests, the number of EPY of these males ranged from 1-10 EPY in 1-4 different nests per male. In contrast to a similar study in blue tits, we observed no significant correlation between the breeding pair density in a plot and the percentage of extra-pair chicks produced in that plot (Fig. 12). Differences

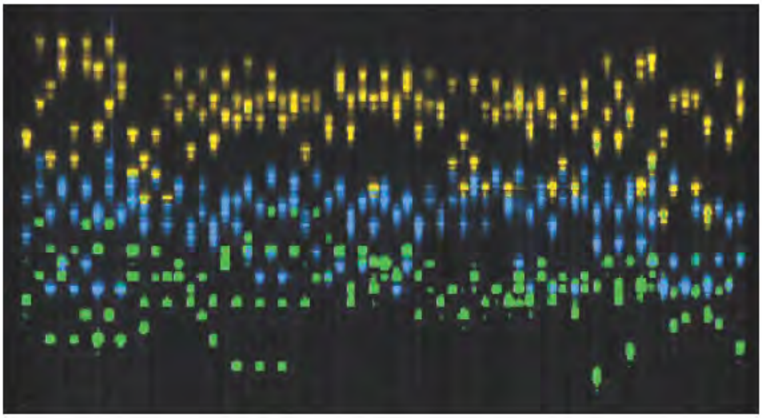

Figure 11. Microsatellite analysis of 64 great tit samples for 3 loci.

in density, therefore, can not explain the observed variation in EPY among plots. Possibly, other differences among plots, e.g. breeding synchrony or habitat quality, might be more important in explaining variation in EPP in the Lauwersmeer population. Future research should tell us to what extent these factors may be important determinants of EPP levels in great tits.

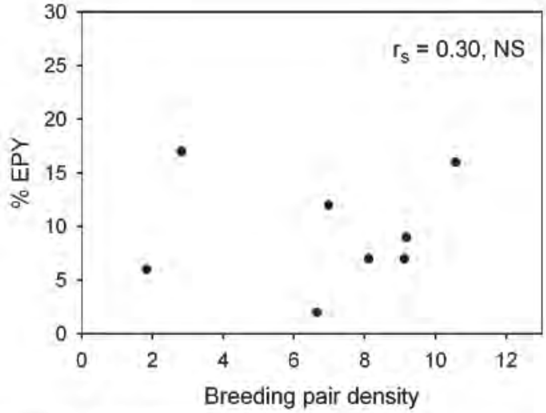

Figure 12. Spearman rank correlation (r_S) between plot breeding pair density (# breeding pairs/10 ha) and percentage extra-pair young (EPY) produced in that plot (N= 8 plots).

Sexual conflict over care in penduline tits

Istvan Szentirmai, Sjouke Kingma, Maarten Bleeker, Tamas Székely, Jan Komdeur

Although parents often cooperate in raising their offspring, their evolutionary interests regarding the amount of care are rarely similar. Potentially both males and females can benefit from shifting the care to their partners and then obtaining additional mates. One extreme outcome of this conflict is that one of the parents deserts the offspring while the other stays with them. Whether the male or the female stays depends on the outcome of the sexual conflict over care and on the attractiveness of the partner.

The penduline tit, *Remiz pendulinus*, is a small passerine bird, which has a uniquely diverse breeding system, in which both males and females may desert the clutch and mate polygamously. Thus parental care is provided strictly by a single parent (5-20% male-only care and 50-70% female-only care in European populations) and both sequential polygamy and polyandry exist in the same population. Due to accelerated sexual conflict over care about one third of the clutches is deserted by both parents. Males and females are dimorphic in a number of characteristics: males have larger and brighter black eye-stripe and they build a sophisticated hanging nest (Figure 13) and attract females to the nest by their song.

Figure 13. Male and female penduline tit with their nest.

We work in Fehértó, a 3,000 ha fishpond system in South Hungary. Our research showed that males with a larger eye-stripe were more attractive to females because these males found a mate earlier and obtained a higher rate of polygyny over the breeding season (Figure 14). Parental decisions of males and females, however, were not influenced by male eye-stripe size. Females also seemed to prefer those males that had large song repertoire size, because these males found a female earlier. Interestingly, instead of caring for the offspring of their preferred males, females deserted the clutches of males with large repertoire size more often than the clutches of males with small repertoire size. Finally, males that built a large nest had a better chance to get their females to care for the clutch, whereas if they built a small nest they cared themselves (Figure 15). Therefore males with bigger nest had higher reproductive success than males with smaller nests.

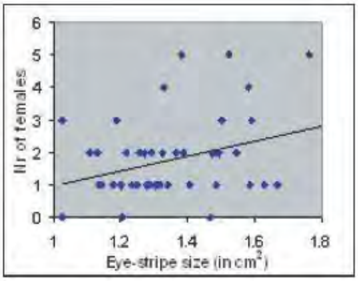

Figure 14. Male penduline tits with large eye-stripe have more mates over a breeding season.

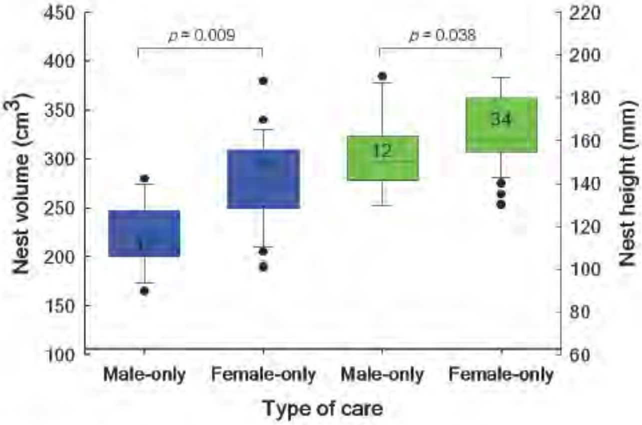

Figure 15. Female penduline tits care more often for the clutches of those males that have a large nest.

Our results indicate that although large eye-stripe and large song repertoire size make males attractive to females during mate choice, they do not make females more willing to raise the offspring. Large repertoire size even made females more likely to desert, thus it shifts the outcome of sexual conflict over care toward the interest of females. One potential explanation for this controversy between female mating and parental decision may be that males with large repertoire size attract good quality females, but these females are also more likely to desert them, because they have better chances to become polyandrous. In contrast to repertoire size, nest size of males shifted the outcome of sexual conflict towards the male's interest and thus increased male reproductive success.

Gosling adoption as mutually beneficial strategy?

Ellen Kalmbach, Maarten Loonen and Jan Komdeur

Adoptions of unrelated young by successful breeders are a form of alloparental care which has been observed in many species of geese. Depending on costs and benefits to the parents, adoptions might represent an inter-generational conflict or a mutually beneficial strategy. Although most studies of wild populations suggest benefits of large brood sizes, incidental observations mostly report aggressive behaviour of parents towards lone goslings. No studies have investigated mechanisms and behaviour during adoptions in order to test whether adoptions are driven by parents or goslings. The captive population of greylag geese (*Anser anser*) near the Biological Centre was used to test whether goslings might use adoption as a strategy to obtain better parental care. An experiment was carried out where lone two-day old greylag goslings, which were hatched in an incubator, could choose between a dominant and a subordinate foster family. Except for one case, all lone goslings ($N = 16$) chose the dominant family (Figure 16). A second

Figure 16. Greylag goose family. Female in the front, male in the back with seven goslings from their own and three adopted goslings.

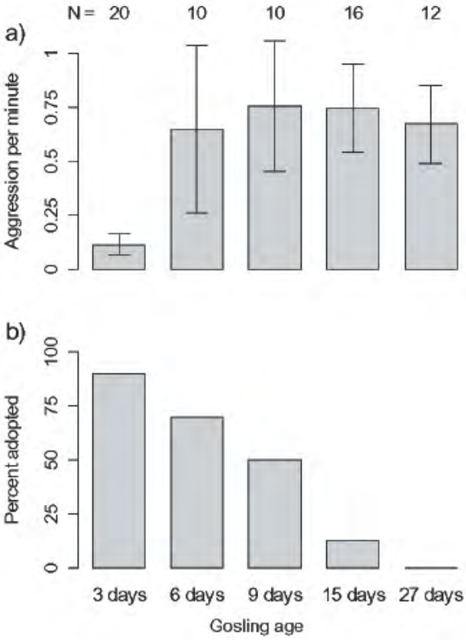

Figure 17. Aggression from parents towards lone goslings (a) and rate of successful adoption (b) during 30 minute experiments in relation to gosling age. Error bars represent ± 1 SE, and N denotes sample size.

experiment was conducted to test whether adoption was age-dependent. Parents showed very little aggression towards lone goslings at three days after hatch, but aggression increased until 9 days and remained high thereafter (Figure 17). At the same time as aggression increased, the chance of successful adoption decreased. These results show that goslings might choose foster families according to dominance. The fact that with increasing gosling age parents are less willing to adopt could be due to improved individual recognition and reflect decreasing benefits of gaining an additional family member. An alternative explanation could be that in order to gain the (later) benefits of large broods, families have to work as a well coordinated unit. This might be much easier achieved if family members join at a young age, as the early phase is likely to be important for imprinting, social learning, establishing group coherence and group coordination.

Publications 2004

Dissertations

Doctorate granted by the institution, prepared within the institution

Bruinzeel, L.W. 2004. Search, settle, reside & resign. Territory acquisition in the oystercatcher. Promotor prof.dr. R.H. Drent; co-promotor dr. J.M. Tinbergen; referents dr. B.J. Ens and dr. S. Verhulst, Rijksuniversiteit Groningen

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Drent, J. 2004. Life history variation of a marine bivalve (*Macoma balthica*) in a changing world. Promotores Prof.dr. W.J. Wolff and Prof.dr. T. Piersma, Rijksuniversiteit Groningen

Van Gils, J. 2004. Foraging decisions in a digestively constrained long-distance migrant, the red knot (*Calidris canutus*). Promotores Prof.dr. R.H. Drent and Prof.dr. T. Piersma, Rijksuniversiteit Groningen (cum laude)

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Community and Conservation Ecology

Group leaders

prof. dr. H. Olff	NWO/ALW (v.d. Leeuw)	1.0	020901-040901
	RUG	1.0	since 040901

Composition of the group in 2004:

Tenured Staff	**source**	**fte**	**period**
prof. dr. J. van Andel	RUG	1.0	until 040901
prof. dr. J.P. Bakker	RUG	1.0	
dr. R. van Diggelen	RUG	0.8	
dr. A.P. Grootjans	RUG	0.8	
dr. M.M. Kwak	RUG	0.2	

Post-docs

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dr. M.E.F. Apol	NWO/ALW+EW (CLS)	1.0	031201-061201
dr. R.M. Bekker	NWO/ALW (Biodiv.)	0.5	021101-041101
	EU/LEDA	0.5	021101-041101
dr. R.S. Etienne	NWO/WOTRO	1.0	020901-040301
dr. R.S. Etienne	RUG/Breedtestrategie	1.0	040301-070301
dr. P.A. Jansen	NWO/WOTRO	1.0	040401-060801
dr. A. Jolles	NWO/Pionier	1.0	031001-051001
dr. I.C. Knevel	EU/LEDA	0.5	021101-041104
	EU/LEDA	1.0	041101-051101
dr. R.C. Looijen	NWO/WOTRO	0.5	990101-041105

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ir. J. Cromsigt	NWO/WOTRO	1.0	020901-050901
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ir. V. Fuld	NWO/ALW (Pionier)	0.9	040101-080701
N. Hagenah, MSc	Robert Bosch Stiftung bursaal	1.0	020901-040801
drs. F. Hoffmann	NWO/ALW (Biodiv.)	0.8	010601-041216
drs. A.M. Mouissie	NOW (Biodiv.)	1.0	000401-040401
drs. R.M. Veeneklaas	RUG/Breedtestrategie	0.8	010901-060901
ing. H.E. Wolters	RUG	1.0	010901-050901

Technical and Administrative staff

secretary			
M.J. Boertje-van den Bergh	RUG	0.5	
J.G. Rietveld	NWO/Pionier	0.5	030101-080101
chemical analists			
N.D. Eck	RUG	1.0	
W. Kanter	RUG	0.8	030901-050901
B.J. Venema	RUG	0.5	
botanical analist			
J.J. Hogendorf	RUG	1.0	
Y. de Vries	RUG	0.5	
IT-assistent			
ing. G.J. van den Burg	RUG	0.8	

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N. Mbata	UND/RUG	1.0	040101-050101
S. Mhlongo	UND/RUG	1.0	040101-050101
K. Mpanza	UND/RUG	1.0	040101-050101
J. Ngobese	UND/RUG	1.0	040101-040701
C. Khumalo	UND/RUG	1.0	040701-050101
T. Shelembe	UND/RUG	1.0	040101-040401
E. Buthelezi	UND/RUG	1.0	041101-041201

Guests

Prof. dr. J. van Andel	Groningen, NL
drs. G.J. Baaijens	Ministry of Agriculture
prof. dr. M. Bertness	Brown University, USA
prof. dr. R.L. Jefferies	Univ. Toronto, Canada
prof. dr. E. van der Maarel	Groningen, NL
prof. dr. K. Prach	Univ. South Bohemia, Czech Republic

Overview of academic results

Hydraulic Conductivity a discriminating factor in buoyancy driven water flow

Buoyancy driven water flow may act as an important nutrient transport mechanism in *Sphagnum* bogs. It may mechanistically explain the nutrient recycling that is necessary to account for the observed bioaccumulation of peat.
The research in our group on this theme focussed on the hydraulic conductivity of different peat systems with respect to buoyancy driven water flow. Besides a sufficient temperature difference between day and night that on a large global scale determines the possibility of water flow, hydraulic conductivity seems the discriminating factor on a small spatial scale.
We measured hydraulic conductivity in different peat types on several sites to reveal information over the variability of hydraulic conductivity. For this purpose we developed an instrument to measure the vertical hydraulic conductivity of an intact peat monolith.
The results show a decrease of the hydraulic conductivity with depth for most monoliths. (Fig. 1) This decrease is related to the increase of biomass with depth.

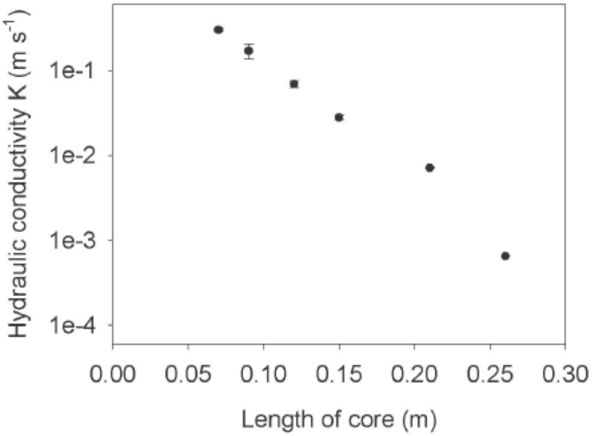


Figure 1. Change in hydraulic conductivity with depth in peat monoliths.

The hydraulic conductivity showed differences between the peat types. The pool and hollow vegetation showed high hydraulic conductivity whether the lawn and hummock vegetation showed more variation between sites. Some lawn and hummock sites buoyancy driven water flow may occur but in others the hydraulic conductivity is too low to support water flow.

These results suggest that buoyancy driven water flow occurs frequently in pools and hollows. *Sphagnum fimbriatum* and *Sphagnum recurvum* vegetation both show a sufficient hydraulic conductivity to support buoyancy driven water flow at a temperature difference of ~10°C. On *Sphagnum* lawns and hummocks the situation appears to be more complex. Peat monoliths of *Sphagnum magellanicum* collected from the same areas (Rancho Hambre, Argentina, 54° 44.7', S 98° 49.5' W for lawns and Konotop, Poland, 53° 7.7' N, 15° 48.9' E for hummocks) showed a large variation in hydraulic conductivity (Fig. 2). Most of this variation could be explained by the density of the peat (Fig. 3).

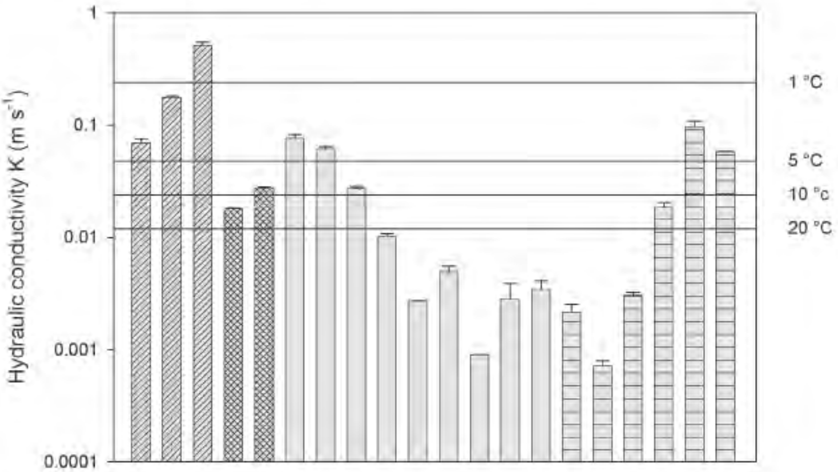


Figure 2. Spatial variation between peat cores in hydraulic conductivity.

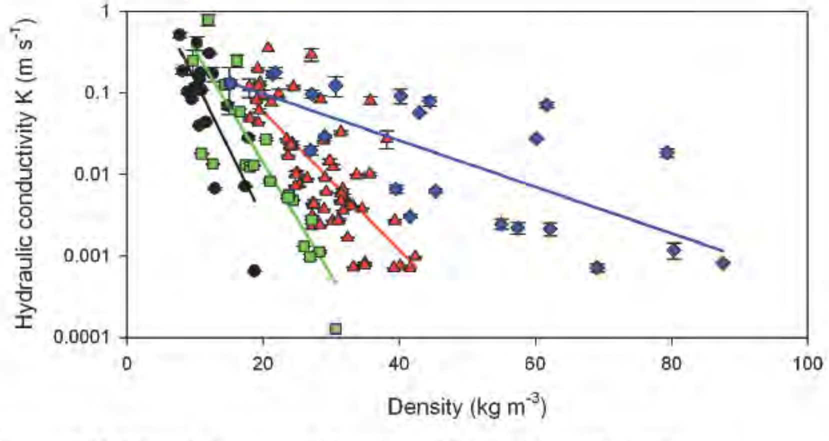


Figure 3. Relation between peat density and hydraulic conductivity.

Besides the hydraulic conductivity the position of the water table is also an important factor for the occurrence of buoyancy driven water flow. In the hollow vegetation the water table is always almost at the peat surface but in the lawn and hummock vegetation the water table may drop notably below the peat surface. Although, under these circumstances buoyancy driven water flow together with capillary rise may still transport nutrients upwards, the peat above the water table will act as insulation and the chance of buoyancy driven water flow will be reduced due to lower temperature differences.

Concluding: buoyancy driven water flow frequently occurs in hollows. The occurrence in lawn and hummock vegetation depends on the peat density and water table, which makes it likely that in these systems buoyancy driven water flow occurs most frequently in spring and autumn when the water tables are high and the temperature differences are still sufficient.

Life history traits as predictors of plant species rarity

Red list plant species in the Netherlands tend to become rarer whereas common species tend to become more common. It is often hypothesized that each of those two groups of plants combines different plant characteristics, resulting in different response to environmental changes. If we could relate plant rarity with plant traits this would also mean that we would be able to predict which plant species presently still common might become rare in the near future.

This analysis requires extensive knowledge on traits representative for all parts of the life cycle of plants. Building upon our large experience on flower biology and pollination ecology of plants we were able to create a dataset which includes reproductive traits of plant. We also made use of the increasing data availability on plants life history traits built up in e.g. the LEDA-Traitbase project (www.leda-traitbase.org). Thus, we could analyse whether two groups of Dutch plants, rare (30) and common (105) species, all dicotyledonous and forbs with insect-visited flowers, differ in their life history and reproductive characteristics.

The traits life span, clonality, breeding system, seed production, seed dispersal, and soil seed bank longevity were considered. Some traits include several characteristics. All trait values have been ranked according to their possible effect on the vulnerability of a

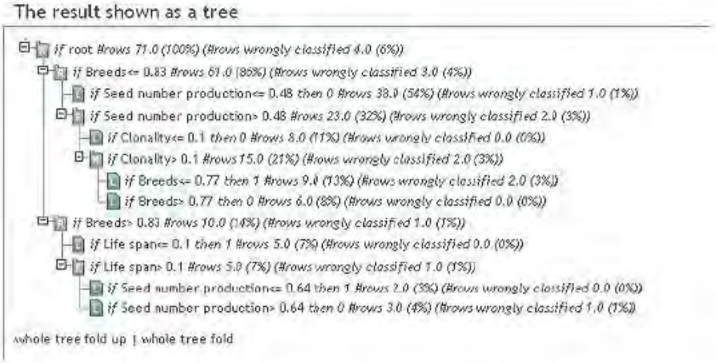
Table 1. Results of the discriminant analysis of rare and common plant species.

	Wilks Lambda	*F* statistic	d.f.	Sig. (=*P*)	*P* < 0.05
Life span	1.00	0.37	1, 133	0.545	n.s.
Clonality	0.95	6.95	1, 133	0.009	**
Breeding system	0.92	12.36	1, 133	0.001	***
Seed production	0.87	20.27	1, 133	0.000	***
Seed dispersal	0.97	3.81	1, 133	0.053	n.s.
Seed bank longevity	0.94	10.75	1, 133	0.001	***

species for extinction, where low values indicate a low risk and high values a high risk for a species.

Rare and common species differed significantly in four traits: seed production, breeding system, seed bank longevity and clonality (Table 1). Discriminant analysis showed that 79% of the 135 species was correctly classified in the group they presently belong to. Interestingly, species that are rare now but were much more common 50-100 years ago were classified as being common species, pointing at the detrimental effect of habitat loss these species encountered at which they have not yet been able to adapt to.

Using the recently developed data mining tool DIONE we were able to construct a predictive model for plant rarity. DIONE was developed at the Computer Science department of the Carl von Ossietzky University of Oldenburg in close co-operation with the LEDA-Traitbase project (University of Groningen) for the analyses of large datasets. Clustering techniques, decision trees and predictive models are all available within this web based free software package. Even with the relative small dataset we could define in much detail a set of decision rules for the prediction of plant rarity. An example is shown in Figure 4.



The result shown as a tree

```
if root #rows 71.0 (100%) (#rows wrongly classified 4.0 (6%))
  if Breeds<= 0.83 #rows 61.0 (86%) (#rows wrongly classified 3.0 (4%))
    if Seed number production<= 0.48 then 0 #rows 38.0 (54%) (#rows wrongly classified 1.0 (1%))
    if Seed number production> 0.48 #rows 23.0 (32%) (#rows wrongly classified 2.0 (3%))
      if Clonality<= 0.1 then 0 #rows 8.0 (11%) (#rows wrongly classified 0.0 (0%))
      if Clonality> 0.1 #rows 15.0 (21%) (#rows wrongly classified 2.0 (3%))
        if Breeds<= 0.77 then 1 #rows 9.0 (13%) (#rows wrongly classified 2.0 (3%))
        if Breeds> 0.77 then 0 #rows 6.0 (8%) (#rows wrongly classified 0.0 (0%))
  if Breeds> 0.83 #rows 10.0 (14%) (#rows wrongly classified 1.0 (1%))
    if Life span<= 0.1 then 1 #rows 5.0 (7%) (#rows wrongly classified 0.0 (0%))
    if Life span> 0.1 #rows 5.0 (7%) (#rows wrongly classified 1.0 (1%))
      if Seed number production<= 0.64 then 1 #rows 2.0 (3%) (#rows wrongly classified 0.0 (0%))
      if Seed number production> 0.64 then 0 #rows 3.0 (4%) (#rows wrongly classified 1.0 (1%))
```

Figure 4. Results of data mining tool DIONE. This tree gives respectively the parameter values, the outcome of the targetted parameter Rarity=1 when plant species are rare and Rarity =0 when species are common, the number and percentage of data rows correctly and wrongly clasified. In this example half of our data set was randomly selected (n=71) so that the other half could be used for verification.

Adaptive and neutral theory

There are two contrasting scientific views on the mechanisms underlying biodiversity: the adaptive view and the neutral view. The adaptive view is the traditional view that is inspired by the Darwinian theory of evolution by natural selection (only species that are best adapted (best fit) to their environment survive). In this view, species are functionally different. Evolution has led to a situation in which each species has a unique combination of traits that enable it to occupy a niche. Whenever two species are so similar that they occupy the same niche, one will always be (slightly) better in utilizing or consuming the essential resources (light, space, food, water) and will eventually outcompete the other species, thus reinstating the one niche - one species situation. The neutral view is a radical departure from the traditional view, because it assumes that species are functionally equivalent (hence the name ‘neutral’). They may have different traits, but in the end they all utilize and consume the available resources in the same way. Interestingly, an evolutionary argument is used to support this view: species have evolved in different ways to reach the same fitness, that is, they are equivalently adapted to their environment (if not, they would not coexist). In a system with such functionally equivalent species, chance processes will eventually lead to extinction of all but one species (which one is inherently unpredictable because all species are equivalent) unless this extinction is balanced by immigration and ultimately speciation. There is evidence for both adaptive and neutral mechanisms, but it is unclear what their relative contribution is in maintaining biodiversity. This is, however, of crucial importance for conservation, because the adaptive view stresses the maintenance of different habitats to optimize biodiversity, whereas the neutral view stresses the connectedness of habitat.

How can we determine what these relative contributions are? Although controlled experiments are the most reliable tools to judge which mechanism is active, they are only possible on relatively small space and time scales, whereas it is generally believed that the mechanisms may be most important on intermediate to large scales. Therefore, we need to look for other sources of information about these mechanisms. Large-scale observational data, which have been collected for many years, provide such a source. Two such patterns are the species-body size patterns and the species-abundance pattern. The former is visualized as a bar graph in which each bar represents the number of species in a body size class, the latter is also a bar graph where each bar represents the number of species in an abundance class. A technical detail: the body sizes and abundances are usually considered on a logarithmic scale, that is, 0.1, 1, 10, 100 rather than 1, 2, 3, 4.

The species-body size pattern is generally hump-shaped with a thick tail on the right: the number of species is highest for intermediate body sizes and falls off more slowly to the larger body size classes than to the smaller body size classes. Many explanations have been offered for this pattern, but none is totally satisfactory. We have developed a theory which combines both adaptive and neutral elements that can explain this pattern. The key role is played by body size: species of similar body size are functionally similar (and hence interact more neutrally), whereas species of different body size are functionally different (and hence interact more according to niche theory). To study the effect of this theory on the body size-diversity pattern, we divided all species in body size classes. In each class species have similar body size and obey the rules of neutral competition, whereas all body size classes are linked by so-called allometric scaling laws that underlie niche theory. The most important scaling law is the one for active dispersal distance: larger species can travel larger distances. Thus, we predicted a species-body size pattern that resembles qualitatively the observed pattern; it can even explain the observed variation in the species-body size pattern.

The species-abundance pattern is also hump-shaped, with a sometimes thicker tail on the left side. A currently popular model based on the neutral view has been claimed to describe this shape particularly well, but there are many other models, including ones based on the adaptive view, that do so. To be able to judge which model describes observed patterns best, a careful statistical analysis is needed. To this end, we developed a Bayesian approach. Bayesian statistics is a field in statistics that assumes that we always have some idea of the likelihood of events, values of quantities, etc. (called prior knowledge) and that data are used to update this (intuitive) idea (which becomes the posterior knowledge). Thus, expert knowledge and data can be treated together in a single framework that provides full uncertainty distributions of its predictions. Our Bayesian approach can be used to determine which model describes observed data better than others. However, to apply it for a comparison of the neutral model with other models, a more mathematical treatment of the neutral model is required. We have provided such a treatment. This is not just a mathematically handy tool; it also provides us with more insight in the neutral model. The basic idea is that the species-abundance pattern of a community of animals or plants can be fully traced back to the species-abundance pattern of

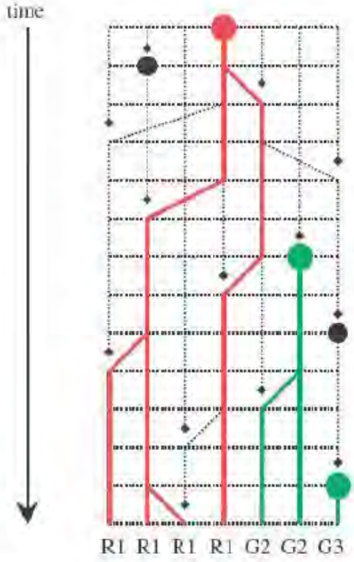


Figure 5. Ancestry tree of a local community with seven individuals of two different species R and G (red and green). Sites become vacant due to death of individuals (small diamonds). Either off-spring of a local individual colonizes the vacant site (branching points), or an immigrant from the regional species pool colonizes it (large dots). The seven individuals can be traced back to three immigrating ancestors by following the solid lines backwards in time; the number next to the species code denotes the ancestor. The dotted vertical lines represent individuals that have no importance for the composition of the local community at the current time; branching and immigration events along these dotted lines do not contribute to local diversity. The dotted horizontal lines represent the time units. The zero-sum assumption requires all seven sites to be occupied at each time unit.

their ancestors that once immigrated into the community (Fig. 5). Thus, the genealogy of the community largely determines the current species-abundance pattern, pointing at a link between genetic diversity and biodiversity. In fact, our mathematical treatment has parallels in population genetics.

Community ecology of large herbivores in savannas

The vegetation of many savannas is highly heterogeneous, and our study site in Hluhluwe-Umfolozi is a good example of this (Fig. 6). Heterogeneity is displayed at different scales: large-scale heterogeneity originates from topography (hills, river valleys, flood plains) with associated differences in parent material, soil type and hydrology. On smaller scales (kilometres), more dynamic woodland and grassland patches alternate, with less clear underlying differences in soils. On even smaller scales, very short (2-5 cm) grazed grassland patches ('grazing lawns') alternate with tall grassland (bunch grassland), each with a characteristic plant species composition. We found that the patches are maintained through the interplay between plant species, fire and different herbivores, with a key role for white rhino (Fig. 7). The 'grazing lawns' contain a suite of grasses with a particular prostrate growth form, that propagate through stolons that stay very close to the ground. They have a high species richness in forbs as well, mostly geophytes

Figure 6. A diverse assemblage of large herbivores in the tropical savanna of the Hluhluwe-Umfolozi park, South Africa. Photograph by H. Olff

Figure 7. Spatial heterogeneity created by white rhino in Hluhluwe-Umfolozi park, South Africa. Photograph by H. Olff

(species with taproots and bulbs). The grazing lawns spatially alternate with bunch grasses, such as *Sporobolis pyramidalis* and *Themeda triandra*. The grazing lawns seem to provide the highest food quality for herbivores: they have a high leaf/stem ratio, and high nutrient contents, and are frequently visited by several species. Within the bunch grasses, large differences exist in palatability to herbivores. In plant tissue nutrient analyses we found that species as *S. pyramidalis* and *B. insculpta* have low palatability, while species as *Panicum maximum*, and *Themeda triandra* have high palatability. These groups of species will be referred to as high and low quality bunch grasses, respectively, later on.

Experimentally manipulated feeding patch sizes in the savanna by mowing and fertilizing revealed that large herbivores separate out spatially in resource utilisation according to patch size and patch quality. Although the number of animal species in the experiment was too low to draw clear conclusions on allometric rules, it did provide first experimental evidence of the idea of 'spatial exclusive niches' that we theoretically predicted. In addition, an exciting mechanism of 'context-dependent vegetation change' was discovered, that is now further being investigated. It was found that above a critical size of 4 m x 4 m, a positive feedback develops in which once created patches attract herbivores, which cause nutrient input and keeps the vegetation short, which contributes to the further maintenance of these patches. This contributes to invasion of so-called grazing lawn grasses (short, stoloniferous, high quality plants) that further attract grazers. In addition, nutrient cycling seems to be speeded up by increased activity of termites.

A nested herbivore exclusion experiment provided interesting insights on the interplay between the different-sized herbivores, the dominant plant species in the vegetation, and heterogeneity in vegetation height. In the full-grazing guild, allometric analysis revealed that white rhino were responsible for half of the biomass removal. They are thus a very important species in the park. We however found that exclusion of white rhino did have much effect on the visitation by the next smaller herbivore group (buffalo, wildebeest, zebra), the exclusion of white rhino thus resulted in a decline of the overall visitation by herbivores. Height mosaic mapping revealed that excluding white rhino changed the scale of the vegetation, causing the patterns to become more 'fine grained' after removal of the largest herbivore. Also, the vegetation became on average taller, because the grazing lawns grew out taller. This provided evidence that the very short (2-5 cm) grazing lawns are maintained exclusively by white rhino. Despite the changes in vegetation height mosaics upon exclusion of white rhino, the relative abundances of the dominant grasses remained the same, leading to the conclusion that white rhino did mostly affect the height structure of the vegetation, not so much its plant species composition (at least in the first years of the experiment). White rhino mostly maintained the mosaics of

Figure 8. Termite mounds, migrating herbivores and smoke from a fire in the Serengeti, Tanzania. Photograph by H. Olff

grazing lawns in between patches of tall bunch grasses. A small herbivore group - nyala and impala - on the other hand was found to have an important effect of the species composition of the vegetation by effectively removing most palatable bunch grasses. This is expected to have effects on fire intensity, decomposition rates and primary productivity and biodiversity. This finding is particularly important as nyala and impala were introduced in the park in the 1930s. There are no reported occurrences of both species in the park in the last part of the 19th century, when the park was first visited by European hunters. The introduction of both species (that now rank in 3rd and 4th place with respect to 'metabolic' densities in the park (numbers corrected for size)) must have been a major alteration to the ecology of the park.

We conclude from the experimental work that not a single herbivore species or size group can be identified as keystone species. Different-sized herbivores partition resources and each have unique, very different effects on different aspects of community structure and ecosystem functioning, possibly in interplay with termites and fire (Fig. 8).

Award

Esther R. Chang was winner of the prize for best oral presentation given by a student 'Relationship between the vegetation and seed bank along a community state sequence'. The prize was awarded during the Seed Ecology Meeting, Rhodos (Greece).

Publications 2004

Dissertations

Doctorate granted by another institution, prepared at another institution

Kuijper, D.P.J. 2004.Small herbivores losing control - Plants-herbivore interactions along a natural productivity gradient. Promotores: Prof.dr.J.P.Bakker and Prof. dr. H.H.T. Prins. University of Groningen

Mouissie, A.M. 2004. Seed dispersal by large herbivores. Implications for the restoration of plant biodiversity. Promotor: Prof. dr. J.P.Bakker. Copromotor: Dr. R. van Diggelen. University of Groningen

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Ecophysiology of Plants

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

Composition of the group in 2004:

Tenured Staff	**source**	**fte**	**period**
prof.dr. J.T.M. Elzenga	RUG	1.0	
dr. L.J. de Kok	RUG	1.0	
dr. G. Stulen	RUG	1.0	
Post docs			
dr. F.C. Lanfermeijer	RUG	1.0	till 050815
dr. J.H. Venema	NOVEM / Wageningen	0.8	000501-040501
dr. J. Stefels	NWO	0.8	040101-071031
PhD Students			
drs. A.H. Ambrosio de Castro	RUG	1.0	011010-051001
drs. M. Durenkamp	RUG	1.0	010301-050430
drs. D. den Os	NWO-AWL	0.8	010901-050901
drs. L. Yang	NWO-WOTRO	1.0	011001-051001
drs. C. Schmitz	Robert Bosch Stiftung	1.0	040906-081001
drs. A. Koralewska	RUG	1.0	041001-080930
drs. H. Najafi Zarrini	Republiek Iran	1.0	041101-081101
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secretary			
J. Nuijten-Tjalkes	RUG	0.4	
administrator			
G.P. Telkamp	RUG	1.0	
research analist			
M. Staal	RUG	1.0	
C.E.E. Stuiver	RUG	0.7	
F.S. Posthumus	RUG	1.0	

Overview of academic results

Transport processes and plant acclimation

Plants carefully ‘monitor’ their environment. Many developmental changes in plants depend on the perception of environmental cues. One of the principal signals is light. Plants possess pigments that can perceive quality, quantity, duration and polarity of light and that are used to measure the length of the light period. These properties of light are used to time flowering, to perceive the presence of nearby neighbours competing for light and to sense whether the plant is receiving enough light for photosynthesis. Leaves of plants that grow in the light unfold and expand, while in the dark they stay folded and small. The epidermal cell layer is the tissue that controls this response to a large extent. The increase in leaf area is completely due to expansion of individual epidermal cells as cell division is not affected by the light conditions. For a cell to expand the turgor of the cell must exceed the yield threshold of the cell: in other words the force exerted by the cell on the cell wall must be greater than the strength of the cell wall itself. To achieve this the cell acidifies the cell wall by activating membrane bound H^+-pumping ATPases. The lowering of the pH results in lowering of the yield threshold and an increase in the extensibility of the cell wall, while simultaneously increasing the possibility to take up potassium into the cell and thereby increasing the turgor pressure. We found that both red and blue light induce extracellular acidification. In blue light the response is both fluence rate and fluence dependent, while in red light the response depends on the fluence rate. As the response to blue light is fluence dependent (short pulses of intense light have the same effect as long pulses of weak light, as long as the number of photons in the pulse are the same) we could work out a kinetic model for the blue light dependent H^+-pumping ATPases activation. By doing this for wild type plants and for mutant plants that lack the red light photoreceptor phytochrome, we discovered that the activation in blue light depends on the interaction between phytochrome and a blue light receptor, possibly cryptochrome. Electrophysiological measurements (patch clamp and microelectrode studies) on these plants and studies with specific inhibitors indicated that this activation involves cytoplasmic calcium changes and involves calmodulin as a so-called second messenger. When the calcium fluxes across the membrane are measured with a technique called Multiple Ion Flux Estimation (MIFE), a modification of the vibrating probe technique, they proved to be below the measuring threshold of the technique. However, direct measurements of cytoplasmic calcium concentration changes (ratio imaging of calcium-sensitive fluorescent probes) confirmed the hypothesis that a pulse of blue light leads to the transient [Ca^{2+}] changes that are associated with signal transduction cascades. In the recent year our lab has invested in developing the MIFE technique further, such that it can be adopted as a powerful tool to study membrane transport processes in intact tissue, single celled organisms and protoplasts.

Now that we have a model for the effect of light on leaf expansion and are able to measure the kinetics of signal transduction processes, we are in a position to address the question how plants are able to integrate changes in the environment that are imposing often opposite constraints. As light signals the plant to increase the leaf area, drought requires the plant to reduce the leaf area to minimize evaporation. Drought is perceived initially by the roots, which respond by synthesizing the stress hormone abscisic acid (ABA) that is transported to the shoot with the transpiration stream. In the leaf ABA accumulation leads to inhibition of leaf expansion. The effect of ABA on stomatal closure, which is another functional response to limit water loss, is mediated by $[Ca^{2+}]$ changes, we hypothesize that the reduction of epidermal cell expansion also involved interference in calcium signalling by ABA. Currently this hypothesis is being tested, measuring the kinetics of the blue light-induced calcium changes in the absence and presence of ABA. For the measurements of the expected changes in cytoplasmic calcium concentration, several techniques are being used. In protoplasts derived from leaf epidermal cells it is possible to introduce a Ca-sensitive fluorescent probe, Fura, that displays a shift in excitation spectrum when is binds calcium. After callibration a shift in excitation wavelengths can thus be used as a measure of the calcium concentration. In figure 1 false colour image of a protoplast is shown after 20 minutes in the dark (A), 5 minutes after a 10 second blue light pulse (B) and 25 minutes after the blue light pulse (C). The intensity of the colour is representative of the cytoplasmic calcium concentration. (The crescent shape of the coloured area results from the fact that the fluorescent probe is excluded from the vacuole.

The MIFE technique has also been succesfully used in a study (in cooperation with the ecophysiology of plants department of the Utrecht University) on the hyponastic response of *Rumex palustris*, a plant that occurs in the floodplains of rivers and that swivels its leaves to an upright position when it experiences flooding. This reaction involves a differential expansion on the abaxial (outer) side of the petiole. The outer epidermal cells

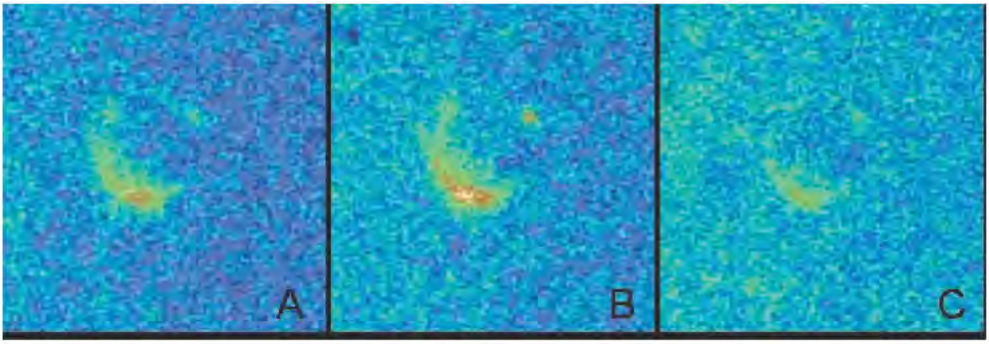

Figure 1. Protoplast of Pisum sativum leaf epidermal cell loaded with Fura-2-AM. The protoplasts were kept in the dark for at least 20 minutes (panel 1A) before they were illuminated for 10 seconds with blue light with an intensity of 100 microM.$m^{-2}.s^{-1}$. This results in a transient increase in the cytoplasmic calcium concentration which reaches a maximum after about 5 minutes (panel 1B). After 20 minutes the calcium levels have dropped to the calcium level level before the light pulse (panel 1C).

start to elongate faster than the cells on the inner side. We tested the hypothesis that the faster elongation rate is the result of an acidification of the cell walls of the epidermal cells, which would lead to an increase in the extensibility of these cell walls. In figure 2 the result is shown of a typical experiment in which in the control plant the submergence induced a long lasting increase in the H^+ efflux while in the plant that was treated with 1-MCP, an inhibitor of the ethylene signalling pathway, this response is much reduced. It can be seen that when the H^+ efflux is enhanced the response becomes eratic. This eratic behaviour is the result of the increase in growth rate, the tissue that is being measured moves to a more vertical position and starts to expand. As a result the electrode 'moves' along the tissue and records the response of different cells, with slightly different responses (Vreeburg *et al*. Plant Journal (in press)).

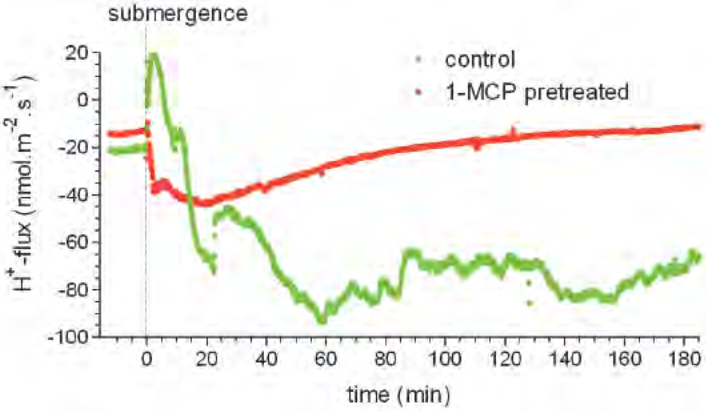

Figure 2. MIFE measurement of proton fluxes at the adaxial (outer) surface of a leaf petiole of *Rumex palustris* before and after submergence. Before submerging the whole leaf, the base of the petiole has been kept just emerged for at least 1 hour until a steady state proton efflux level has been reached. At the indicated time the whole plant is submerged. In the plant labelled 1-MCP was treated with a blocker of the ethylene signal transduction pathway before the plant was submerged.

Negative Versus Positive Functional Plant Responses to Air Pollution: A study with SO_2 and H_2S in establishing cause-effect relationships

The atmosphere in rural areas generally contains only trace concentrations of SO_2 and H_2S. However, high concentrations may occur in areas with volcanic activity and in the vicinity of industry and/or bioindustry and may be matter of environmental concern. In developing countries, the rapid economic growth, industrialization and urbanization are generally associated with a strong increase in energy demand and emissions of various gaseous pollutants and here the vegetation is still at risk from SO_2 and other air pollutants and their negative side effects. For instance, in China 22.4% of the cities had in 2002 a higher annual average SO_2 concentration than 0.024 µl l^{-1} and in one-third of the monitored cities the annual average precipitation pH value was lower than 5.6. In contrast, the atmospheric concentrations of sulfur gases in the USA and Europe have strongly decreased during the last two decades as the consequence of strict regulations on sulfur gas emissions for the combustion of fossil fuels in order to diminish the negative effects of acid rain deposition. This has resulted in a diminished atmospheric sulfur deposition; for instance in Western Europe it has decreased from 70 kg ha^{-1} yr^{-1} in 1970s till less than 10 kg ha^{-1} yr^{-1} currently. However, in the direct vicinity of emission sources sulfur gas concentrations may still incidentally exceed the threshold concentrations for potential phytotoxic effects via both dry and wet deposition. The paradoxical effects of atmospheric sulfur gases complicate the establishment of cause-effect relationships of these air pollutants and their acceptable atmospheric concentrations in natural and agro-ecosystems. For instance, it is still unclear to what extent metabolism contributes to the detoxification of the absorbed sulfur gases. Furthermore the physiological basis for the wide variation in susceptibility between plants species and cultivars for atmospheric sulfur gases is still largely obscure.

Plants are able to transfer from sulfate to foliarly absorbed SO_2 or H_2S as sulfur source and concentrations of $\geq$ 0.06 µl l-1 appear to be sufficient (or nearly sufficient) to cover the sulfur requirement of e.g. *Brassica* species (Fig. 1). Sulfate deprivation of the root environment generally results in a shift in shoot to root biomass partitioning, in favor to that of the root resulting in a decreased shoot/root ratio. However, in Brassica the decreased shoot/root ratio upon sulfate deprivation is generally not rapidly alleviated when the pedospheric sulfate is replaced by atmospheric SO_2 or H_2S as the sulfur source for growth. Root growth is even stimulated upon exposure to atmospheric sulfur gases, when compared to that of plants grown under sulfate-sufficient conditions (Figure 3). In case these laboratory experiments could be extrapolated to outside field conditions, foliar sulfur absorption by plants growing on sulfur-deficient soils might benefit from a relative higher investment in root development especially when the plants are grown under stress conditions such as limited available nutrient resources and/or drought. The latter may have practical significance, especially in agro-ecosystems, where during recent years sulfur deficiency of soils has become a major problem in various areas in the world due to an imbalance of S in relation to N, P and K in the fertilizers.

Exposure of plants to atmospheric sulfur gases may result in an enhancement of total sulfur content of the shoot, which upon SO_2 exposure is predominantly due to an accumulation of sulfate (Figure 4). Upon H_2S exposure the increase in total sulfur content may also be due to an accumulation of organic sulfur compounds, *e.g.* in spruce *Allium* species, where accumulation is due to secondary sulfur compounds γ-glutamyl peptides and alliins; Durenkamp, M. and De Kok, L.J., in preparation).

There is evidence for interaction between atmospheric and pedospheric sulfur utilization, since exposure of plants to sulfurous air pollutants may repress the uptake of sulfate by the roots and its transport to the shoot. SO_2 or H_2S exposure may affect the sulfate

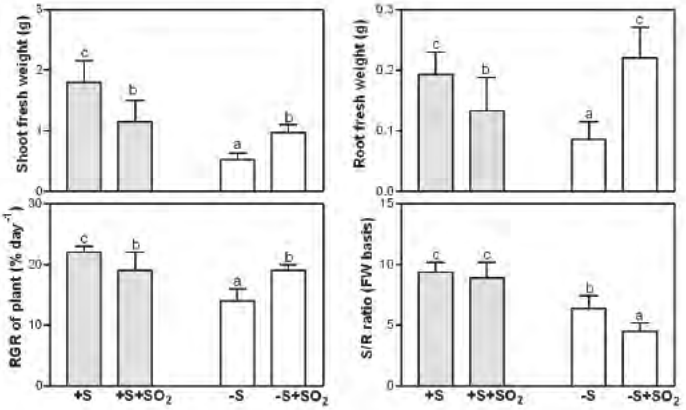

Figure 3. The effect of SO_2 exposure on growth of Chinese cabbage (*Brassica pekinensis*, cv. Beijing 3). Ten-day-old seedlings were grown in a 25 % Hoagland nutrient solution with or without sulfate and simultaneously exposed to of 0.06 $\mu l\, l^{-1}$ SO_2 for two weeks. For experimental conditions see Buchner *et al.* (2004). Data on shoot and root fresh weight represent the mean of 2 experiments with 10 measurements in each (± SD). Relative growth rate (RGR) of plant was calculated on a fresh weight basis and was determined over the exposure period. S/R ratio, shoot to root ratio. Different letters indicate significant differences at $p \leq 0.01$ between different treatments (Yang, L., De Kok, L.J. and Stulen I., in preparation).

reduction pathway by a decrease of the activity of ATP-sulfurylase and adenosine 5'-phosphosulfate (APS) reductase with sulfide, *O*-acetylserine or cysteine being the most likely regulators. Especially in curly kale (*Brassica oleracea* L.) there is a strong interaction between atmospheric H_2S and pedospheric sulfate utilization. The shoot to root signal transduction pathway and the signal compounds therein involved are still yet unsolved. The de-repressed sulfate uptake capacity and expression of genes encoding the different sulfate transporters, which are characteristic upon transfer of plants to sulfate deprived conditions are not rapidly alleviated in *Brassica* upon exposure to H_2S. There is apparently (at least in *Brassica*) no strict and direct shoot to root signaling (e.g. via reduced S compounds) in the tuning of the factors involved in the regulation of the sulfate uptake capacity, expression of the sulfate transporters, root development in sulfate-deprived roots.

Laboratory studies support the idea that the effect of sulfurous air pollutants on plant growth strongly depends on the pedopheric sulfur supply. For instance, growth of *Brassica oleracea* (Chinese cabbage) was reduced upon a two weeks exposure to concentrations as low as 0.06 $\mu l\, l^{-1}$ SO_2 (Figure 4). Sulfate deprivation does not only result in a strong decrease in sulfate and thiol content of both shoot and root, but also in strongly reduced plant growth, whereas at this stage organic sulfur content was hardly affected. However, when sulfate-deprived plants are simultaneously exposed to 0.06 $\mu l\, l^{-1}$ SO_2 then growth is largely maintained. On a plant basis, growth is still less than that of sulfate-sufficient plants and comparable to that of sulfate-sufficient SO_2-exposed plants.

Still it remains unclear to what extent these effects can be explained by an interfering toxicity of SO_2 or a limitation in sulfur supply.

It is evident that on basis of the current knowledge on the impact of atmospheric sulfur gases on plant functioning it is hard to establish cause-effects relations. First, the physiological basis for the variation in susceptibility of plant species to SO_2 and H_2S is still largely obscure. Second, there is no clear-cut transition in the level/rate of metabolism of the absorbed sulfur gases and their phytotoxicity. Third, the impact of sulfurous air pollutants on plants may be strongly dependent on the soil sulfur status.

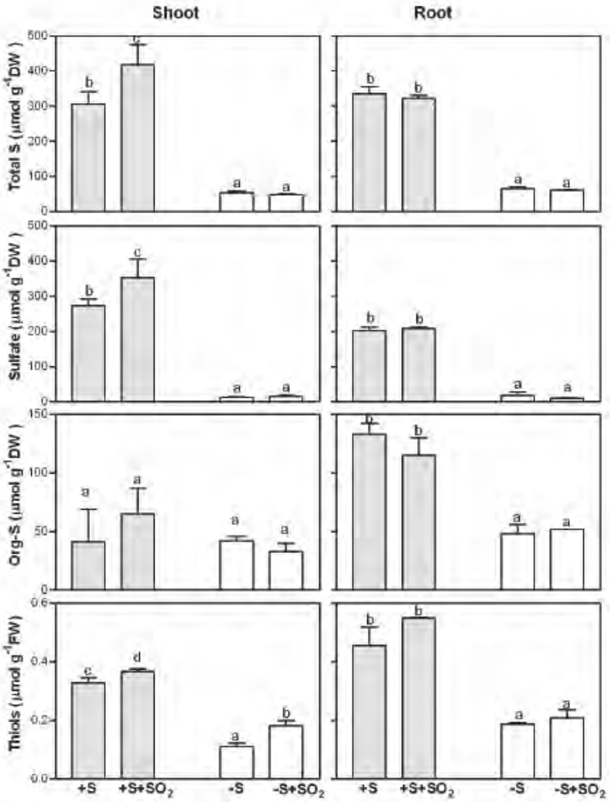

Figure 4. The effect of SO_2 exposure on sulfur metabolites. Ten-day-old seedlings of Chinese cabbage (*Brassica pekinensis*, cv. Beijing 3) were grown in a 25 % Hoagland nutrient solution with or without sulfate and simultaneously exposed to of 0.06 $\mu l\ l^{-1}$ SO_2 for two weeks. Data on total S and sulfate content of shoot (open bars) and root (gray bars; measured in freeze-dried material) represent the mean of 5 measurements with 9-12 plants in each (± SD). For analytical methods see Buchner *et al.* (2004). Data on total water-soluble non-protein thiol content (μmol g^{-1} FW) in shoot (open bars) and root (gray bars) represent the mean of 3 measurements with 3 plants in each (± SD). Different letters indicate significant differences at $p \leq 0.01$ between different treatments (Yang, L., De Kok, L.J. and Stulen I., in preparation).

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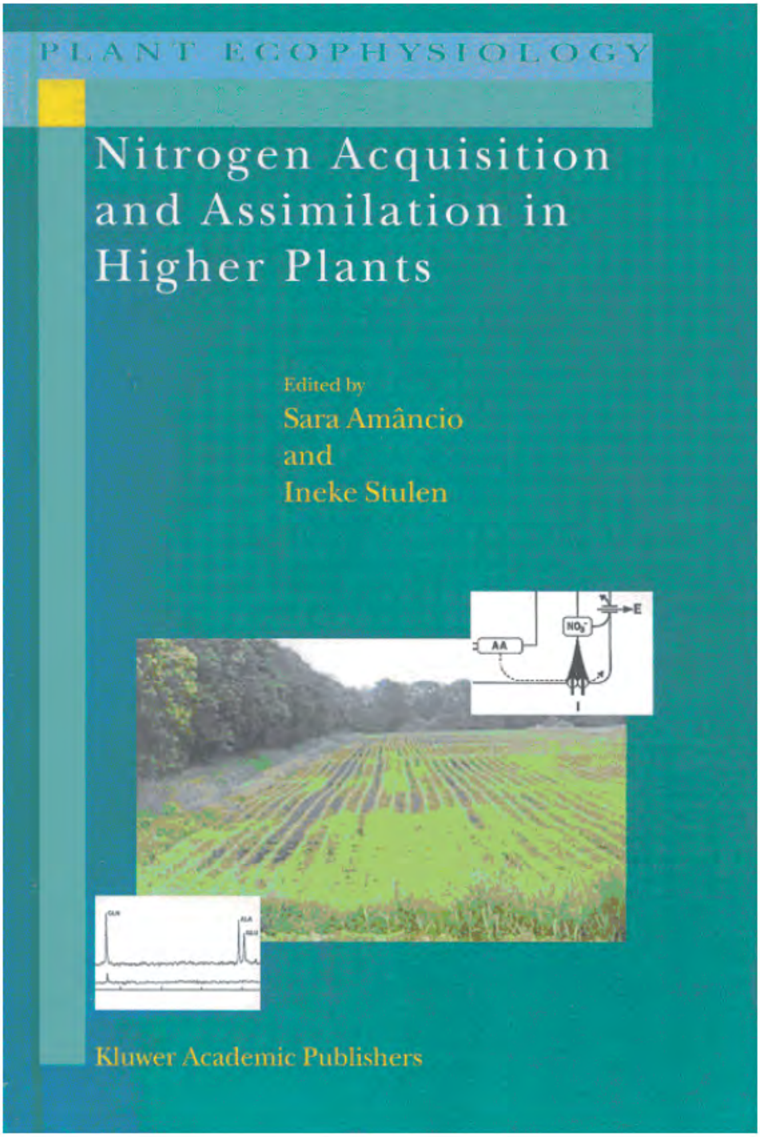

PLANT ECOPHYSIOLOGY

Nitrogen Acquisition and Assimilation in Higher Plants

Edited by
Sara Amâncio
and
Ineke Stulen

Kluwer Academic Publishers

Evolutionary Genetics

Group leader Prof.dr. L.W. Beukeboom

Composition of the group in 2004:

Tenured Staff	**source**	**fte**	**period**
prof. dr. L.W. Beukeboom	RUG	1.0	
prof. dr. R. Bijlsma	RUG	1.0	
dr. L.P.W.G.M. van de Zande	RUG	1.0	
Post-docs			
dr. N. Noorman	NWO-PIONIER	1.0	020701-040701
dr. W.F. van Putten	NWO-WOTRO	0.8	011216-040616
dr. R. Bertossa	Swiss Science Foundation	1.0	040401-051001
PhD-students			
drs. A. Ayrinhac	SENTER-IOP Genomics	1.0	020915-060915
drs. J. Bakker	NWO-WOTRO	1.0	990901-030901
drs. R. Geerts	NWO-ALW	1.0	000201-040201
drs. B. Grillenberger	NWO-PIONIER	1.0	040901-080831
drs. D. Joubert	NWO-ALW	1.0	971001-020401
drs. M.W. Smith-Kleefsman	RUG	1.0	040116-080115
drs. M.A. Kozielska	RUG	1.0	030923-070922
drs. I.V. Mateo Leach	RUG Ubbo Emmius	1.0	020201-060201
drs. A. Peire Morais	RUG	1.0	020301-060301
ir. M.E.C. van Rijswijk	RUG	1.0	020201-060201
drs. C.J. Vermeulen	RUG	1.0	990416-030916
Technical and Administrative Staff			
secretary			
E.E.J. Plender-Hartman	RUG	0.5	
analyst/technician			
A.C. Boerema	RUG	1.0	
M.H. Edelenbos	NWO-WOTRO	1.0	001115-030515
P.J. Hes	RUG	0.4	
R.S.S. Houwerzijl	RUG	1.0	
A. Kamping	RUG	1.0	
M.W. Kleefsman	RUG	1.0	031015-040115
J. Prijs	NWO-PIONIER	0.7	020601-070601
A. Rumahloine	DSW	1.0	
R. Veenstra	NWO-PIONIER	0.6	030315-070601

Overview of academic results

Our group aims at unraveling the interplay between genetic and evolutionary processes. On the one hand we study how the course of evolution is affected by genetic constraints, the mode of reproduction, and the availability of genetic variation. On the other hand, we aim at understanding how evolutionary forces and processes shape (i) the structure of genetic variation within and among populations, (ii) life histories and reproductive systems, (iii) adaptations of organisms and populations to non-genetic (environmental stress) and genetic (genetic load, inbreeding depression) challenges, and (iv) affect speciation and extinction probabilities.

In the broad field of evolutionary genetics the group addresses a variety of research questions that for simplicity are grouped in two research programmes (administratively structured in two research units, here referred to as subprogrammes). For the remainder of this report, these subprogrammes are described separately. Although each subprogramme has a clear research focus, it is important to realize that the two units do not work in isolation, and that many research problems are jointly, e.g. integrated and bridged by Ph.D student projects that are co-supervised.

- The *Evolutionary Genetics* subprogramme seeks to understand the genetic regulation of reproductive systems and how reproductive mode has shaped mating systems and life-history characteristics. The main focus is on elucidating the underlying genetics and evolution of sex determining mechanisms, sexual versus asexual reproduction and the process of speciation. In addition, we study the consequences of these reproductive modes and processes for the structure of genetic variation in natural populations. We use parasitoid wasps (Hymenoptera) and houseflies as model systems.
- The *Population and Conservation Genetics* subprogramme is for an important part targeted on investigating the genetic processes in small, fragmented and isolated populations, where genetic drift and inbreeding are predominant and result in genetic erosion (loss of genetic variation and fitness). We aim (*i*) to elucidate the consequences of genetic erosion for population persistence, particularly, in changing and deteriorating environments, (*ii*) to evaluate the nature of genes involved in inbreeding depression, and (*iii*) to asses the role and importance of gene flow and local adaptation in fragmented populations. We use *Drosophila* as a model organism to get fundamental insights into these problems, but part of the research has also more applied goals and studies these processes in natural populations of endangered species. Other research sublines aim at understanding the consequences of different reproductive strategies for the structure of genetic variation in plant and animal populations and at elucidating the evolution of ageing and assessing the genes involved in this process.

Evolutionary Genetics

Evolutionary genetics of species differences and speciation

The genetic architecture of species differences in adaptive traits has again become a major issue in evolutionary biology. The *Nasonia* species complex has developed into a model system to study these questions in detail due to conspicuous trait differences between the three species and the possibility to generate viable and fertile hybrids. The research project on behavioural and speciation genetics initiated at Leiden, was continued in Groningen. We investigated courtship and mating behaviour of interspecific hybrids. Characteristics of the displays were intermediate between those of the parental species, but at the same time were biased towards the paternal side. Due to the haplodiploid sex determination system of the Hymenoptera, the nearest male parent of the haploid (hence, fatherless) *Nasonia* males are their grandfather. Therefore, we have called this bias the 'grandfather effect'. We investigated one of the possible causes of the 'grandfather effect': nucleo-cytoplasmic interaction. In interspecific hybrids, the paternally donated nuclear genes must operate in an 'alien' environment: the maternal (heterospecific) cytoplasm. Nucleo-cytoplasmic incompatibility effects may prevail in this situation and result in a biased gene transmission (although to the maternal side). With introgression techniques and subsequent hybridisation of introgressed lines, we constructed male progeny in which paternally contributed nuclear genes are conspecific with the maternal cytoplasm. Courtship of these males provide a test of the interaction hypothesis. Because our results showed a persistence of the 'grandfather effect', a simple nucleo-cytoplasmic interaction seems an unlikely explanation of the phenomenon.

Our results indicate that the basis for the grandfather effect is a stronger expression of paternally donated genes than maternally donated genes in hybrid males, hence, some epigenetic phenomenon. It would require that males activate the genetic substrate underlying courtship behaviour, and that this imprint 'survives' transmission through the hybrid daughters. This is not completely surprising since genomic imprinting effects have previously been invoked from studies on sex determination in *Nasonia*. Homologous genes of paternal and maternal origin seem to act differently in inducing maleness or femaleness. We have confirmed the grandfather effect in hybrid crosses of different species and have found some evidence for it in intraspecific crosses. Presently we do not know whether the grandfather effect has any adaptive siginificance or is merely an outcome of hybridisation. We currently explore the biological significance of this phenomenon theoretically and experimentally (screening natural populations for variants, using selection and female choice experiments) in a Ph.D project. The possibility that the maternal and paternal genomes of hymenopteran females are differently expressed could prompt a rethink of three decades of theoretical models on the evolution of social behaviour in ants, wasps and bees.

As part of an international collaboration collections of recombinant inbred lines (RIL) containing different degrees and combinations of the genome of two species have been generated and are maintained in Rochester (USA) and Groningen. We currently have 30 *giraulti-vitripennis* (GV) RILs and are in the process of generating 100 *longicornis-vitripennis* (LV) RILs. All RIL's will be behaviourally typed and genetically characterised with molecular markers. These RILs prove particularly useful for genetic dissection of species specific traits. We have constructed an intraspecific *vitripennis* and an interspecific *vitripennis-longicornis* genetic linkage map containing >200 AFLP and microsatellite markers with a resolution of approximately 5 cM. Maps for other species pairs are being assembled. Attempts to microclone genes with the aid of closely linked markers (positional cloning) are currently underway in our laboratory using introgressed wing size lines.

The *Nasonia* courtship behavior was split up in multiple quantifiable components to perform QTL analyses and screen for epistasis. Five-hundred hybrid males derived from an interspecific cross between *N. vitripennis* and *N. longicornis* were phenotyped for 12 quantitative and two qualitative components of the courtship behavior. Almost all traits showed an independent genetic architecture with at least one major QTL, based on a genotypic analysis of a subset of 100 males. Genome wide two-way epistatic analyses for the traits revealed multiple unique interactions between QTL for several courtship components. Epistatic interactions were involved in transgression and explained reversed allelic effects for some QTL. The biased recovery of maternal alleles in our cross together with nuclear-nuclear epistatic interactions explained the observed grandpaternal bias in two courtship components. Both analyses supported the notion of an independent genetic architecture of the observed courtship components contradicting a pleiotropic model explaining the generally more agitated male courtship behavior in *N. vitripennis* compared to *N. longicornis*. We are now refining our QTL analyses and improving the statistical support of some of our QTL by selectively genotyping the remaining males.

Evolution and genetics of sex determining mechanisms

All Hymenoptera (ants, bees and wasps) have haplodiploid sex determination. The most common mode of reproduction is arrhenotoky, i.e. males develop from unfertilized eggs and are haploid, whereas females develop from fertilized eggs and are diploid. As a consequence, males inherit genes from their mother only and have no genetic father. In haplodiploids there are no heteromorphic sex chromosomes; the only genetic difference between males and females is the number of chromosome sets. The question of how this difference in copy number of the genome can lead to the development of either males or females can still not be answered satisfactorily. Available data indicate that at least two different sex determining mechanisms exist in the Hymenoptera.

We established that *Venturia* has the common mode of single locus Complementary Sex Determination using inbreeding crosses that lead to the production of diploid males. We also showed that the sex locus in thelytokous females must be located near to a centromere given the cytological mechanism of diploidy restoration that we established cytologically to resemble central fusion automixis. In contrast, we firmly established that *Nasonia* does not have single locus Complementary Sex Determination, in which sex is determined through the allelic composition of a single locus. We characterised two mutant strains for sex determination of *N. vitripennis*. A polyploid mutant strain carries two recessive eye-color mutations; *oyster* (*oy*) and *scarlet* (*st*) which makes it possible to distinguish between haploid and diploid offspring. We confirmed preliminary data that suggested that unmated triploid females sometimes produced daughters and found significant line, temperature and age effects on parthenogenetic daughter production. Another naturally collected mutant produces gynandromorphs (male-female mosaics) and females spontaneously from haploid unfertilized eggs at high frequency (Figure 1). These gynandromorphs appear to be true intersexes rather than haplodiploid mosaics as established with flow cytometry and confirmed by cytological counts of ploidy levels in embryos of different ages. With genetic crosses we have established that the gynandromorphism comes about through an interaction between a nuclear gene and a maternal effect product. We mapped the gynandromorphism locus to chromosome 3 using visible eye-colour mutations. Using crosses between both mutants strains, we are now establishing whether the aberrant sex determination shares common features in both lines, e.g. act synergistically. Taken together, our breeding data indicate that the sex determining mechanism in *Nasonia* is most likely more complicated than the existing models predict. Our current working hypothesis is that the female produces a cytoplasmic product that 'counts' the number of chromosome sets in the zygote, since we found evidence for both a maternal effect and an effect of the ploidy level of the egg on sex determination.

We recently started a research programme on the evolution of sex determining mechanisms in houseflies. Houseflies are ideally suited for this because different natural populations carry different sex determining mechanisms (XY and autosomal male heterogamety, autosomal female heterogamety) and some additional variants are available from laboratory populations (A. Dübendorfer, Zürich). In addition, houseflies are easy to culture and some mutant lines and molecular markers are available. We have developed

Figure 1. A gynandromorphic parasitoid wasp (*Nasonia vitripennis*). The wasp developed from a haploid egg and has a male abdomen, but female antennae, legs and wings (indicated by arrows).

the infrastructure for culturing houseflies and started to characterise the sex determining mechanism of a number of field collected lines from different latitudes in Europe. The first results already indicate a large variety of different sex determining mechanisms. This material will be very useful for future experimental work on identifying the selective forces behind this diversity.

Genetics of reproductive mode

Underlying genetics and consequences of reproductive modes are studied in a number of organisms in our group including plants, endangered vertebrates and insects (see also subprogramme *Population and Conservation Genetics*). Sexual versus parthenogenetic reproduction is studied in the parasitoid wasp *Venturia canescens* in which both modes co-occur. We showed with nuclear and mitochondrial markers that considerable gene flow occurs between both modes in field populations in the Mediterranean. The underlying genetics of parthenogenesis has not been elucidated in any organism, although such information is crucial for understanding the evolutionary dynamics of sexual versus asexual reproduction. How fast can parthenogens invade a sexual population? Unfortunately, we were unable to repeat previous crosses between sexual males and parthenogenetic females, which would have allowed us to introgress sexual genes into parthenogens, and hence analyse the genetics of parthenogenesis. We now take a molecular approach using differential display to search for genes that are differentially expressed in eggs of sexual and parthenogenetic females. The first putative bands are being characterised. We are constructing a linkage map with approximately 100 microsatellite markers to determine the speed at which the parthenogenetic genome becomes homozygous, as predicted by the mode of diploidy restoration.

Population fragmentation may have drastic effects on the genetic load of populations. However, the effects of inbreeding may be smaller for haplodiploid organisms where recessive genes are exposed to selection in haploid males. The haplodiploid wasp *Nasonia vitripennis* is a parasite of several fly species that occur at bird nests and carcasses. Due to the patchy distribution of its host *Nasonia* is believed to have strong inbreeding in nature, because males mate with emerging females within a patch that may be founded by one or few females. The population genetic structure of *N. vitripennis* at the Hoge Veluwe Park revealed large differences in the size of locally founded populations. About 50% of all nestboxes infested with blowflies were parasitised by *Nasonia* and estimated foundress numbers from multilocus microsatellite genotypes ranged from 1-10. A first analysis of the data appeared to confirm expectations of a strong substructuring of the population with much inbreeding.

Conservation and Population Genetics

Highlights of the programme

From June 1, Dr Kuke (R.) Bijlsma was appointed to the new personal Chair in 'Population and Conservation Genetics', indicating that the field of conservation genetics has become quite mature and an integrated part of CEES. In addition to general evolutionary and population genetic topics, his research is particularly targeted at understanding the dynamics of genetic processes in small populations subject to stochastic processes.

The importance of conservation genetics is also acknowledged by the fact that on the initiative of Prof. Kuke Bijlsma and Prof. Volker Loeschcke (Aarhus, DK), and in collaboration with colleagues from other European countries, the European Science Foundation (ESF) did launch the Scientific Programme "Integrating population genetics and conservation biology: merging theoretical, experimental and applied approaches" (*ConGen* = Conservation Genetics) in 2004. This programme was started in November for the duration of five years and is chaired by Kuke Bijlsma and co-chaired by Volker Loeschke. The programme aims to stimulate further advancement of the field of conservation genetics by organising and funding international workshops, funding short visits (1-2 weeks) and long-term exchanges (3-5 months) of European scientists.

In co-operation with Prof. Volker Loeschcke, Kuke Bijlsma organised a workshop 'Progress in environmental Stress, Adaptation and Evolution' August 22-26 in Sandbjerg (DK). This workshop with 15 invited and 25 contributed speakers was sponsored by the European Society of Evolutionary Biology and the University of Aarhus.

At 8 October Corneel Vermeulen successfully defended his Ph.D. thesis 'Genetics of lifespan determination in *Drosophila melanogaster*' and was awarded the doctor's degree by his promotores Prof. Kuke Bijlsma and Prof. Wilke van Delden with Dr L. van de Zande as co-promotor.

January 16, Willemien Smith-Kleefsman started the Ph.D. project "Determinants of metapopulation dynamics: stochastic processes versus adaptation" in the framework of the Breedtestrategie programme "Understanding biodiversity: neutral versus adaptive explanations". In the framework of this project she conducted a literature study aimed at quantifying outcrossing probabilities of genetically modified plants including the development of a predictive computer model. This project that was done at the request of the COGEM (Commission on Genetic Modification) in collaboration with Theoretical Biology (Prof. F.J. Weissing) resulted in a report of which the summary can be found below (the complete report can be obtained from the COGEM office).

Academic results

The research of the Conservation & Population Genetics section is focused on various population genetic topics. Considerable emphasis is directed towards understanding the dynamics of genetic variation in small, endangered populations (**conservation genetics**), and focuses in particular on studying the occurrence, extent, and consequences of genetic erosion in relation to the persistence of such populations. We approach these questions both theoretically (in collaboration with the Theoretical Biology section) and for a major part experimentally. The latter approach includes both fundamental research to gain an insight into the genetic processes that underlie genetic erosion, inbreeding depression, and population extinction by using *Drosophila melanogaster* as a model organism, and practical research to analyse the extent and organisation of genetic diversity in endangered species, such as the pine marten (see below), using powerful molecular tools. Other topics that are addressed can be broadly summarised as **population genetics** and, among others, does focus on reproductive strategies in plants and animals, the evolution of ageing (see below), and stress adaptation in *Drosophila*.

Fundamental conservation genetics. Over the past years we have studied in *D. melanogaster* the consequences of inbreeding for the persistence of small populations. Our results did show that inbreeding significantly increases the extinction probability of these experimental populations. Moreover, we showed that the impact of inbreeding on the probability of population extinction became intensified under stressful environmental conditions. Much more important, however, was the observation that the impact of stressors, such as high temperature, becomes significantly enhanced for higher inbreeding coefficients. This showed that inbreeding and environmental stress were not independent but did act synergistically, and indisputably demonstrates that more inbred populations will be more prone to environmental stochasticity and hence have higher extinction probabilities. This finding is the more important as many endangered species have to cope with changing and deteriorating environmental conditions.

We further investigated the causes of inbreeding depression and how the level of inbreeding depression changed under environmental stress. We assessed the amount of inbreeding depression for a number of different fitness components by comparing inbred and non-inbred lines. Although lines differed considerably in reaction for the individual fitness components, nearly all inbred lines were clearly affected for one component or the other. Consequently, when the different fitness components were combined in an overall fitness measure, all inbred lines showed a lower fitness than all non-inbred lines. This indicates that it is very doubtful to assess the possible fitness consequences of inbreeding by studying only one or a few fitness components. We also compared the level of inbreeding depression expressed under benign temperature (25˚C) and stress temperature (29˚C). In this case we found the amount of inbreeding depression for some fitness components to be greatly elevated by the thermal stress while others seemed not to be affected at all. For instance, for survival in the larval stage the level of inbreeding depression observed was almost the same under both temperature conditions, whereas for the pupal stage inbreeding depression was almost exclusively expressed under thermal stress and not at 25˚C. This shows that inbreeding depression for a significant part is only conditionally expressed, in this case due to temperature sensitive mutations. Thus to value the consequences of inbreeding correctly, one should not only study many fitness components, but it is also necessary to assess inbreeding depression under a suite of relevant environmental conditions.

The underlying causes of inbreeding depression and the synergistic genotype-by-environment interaction have been further investigated by pre-adapting the flies to different environmental stresses either as large population prior to inbreeding or during inbreeding before assessing the extinction probability of small populations. Only when the flies had been adapted to stress during inbreeding, increased persistence was observed at the specific stress the population was adapted to. This suggests that many of the genes responsible for the genotype-by-environment interaction are recessive with a low frequency in the founder population, which become highly deleterious under stress and in fact can be regarded as conditional deleterious alleles.

Importantly, conditionally expressed *deleterious* alleles can be regarded as being (nearly) neutral under permissive conditions and as such easily become fixed by genetic drift as they will not be purged by natural selection from an inbreeding population. The other side of the coin is, that alleles that are conditionally beneficial under specific conditions, such as alleles that confer pesticide or disease resistance during pesticide application of disease outbreak, will be easily lost from a population under ‘permissive’ conditions. Given that an appreciable number of mutations may show conditional expression (in *Drosophila* the estimates are higher than 10% of all mutations), we predict that conditionally expressed inbreeding depression will pose a more important long-term threat for the persistence of small endangered populations than inbreeding depression expressed under all conditions. Experiments are now in progress studying the affectivity of purging in relation to the rate of inbreeding and the dynamics of genetic rescue, and seem to confirm this idea.

Applied conservation genetics. Over the years, we have studied the genetic structure of several endangered species and developed microsatellite markers for a number of these species. Most relevant questions that we addressed concerned whether populations were genetically eroded and whether populations were isolated and genetically diverged from each other. Most of the time this work has been done in collaboration with the research institute Alterra or with the University of Aarhus. The most significant findings for each of the species are listed below (for more detailed information see CEES progress reports 2001 and 2003).

Root vole (*Microtus oeconomus*). The extent and consequences of genetic erosion in fragmented populations of the root vole was investigated using 8 polymorphic microsatellite loci. We found relatively high levels of variation, H_e ranging from 0.6-0.8. Regional Dutch root vole populations had become genetically differentiated to a significant level (mean $F_{\mathrm{ST}} = 0.158$), that was of the same order of magnitude as among samples from different countries including Germany, Norway and Finland, indicating that the Dutch regions are really isolated. A much lower level of genetic differentiation was observed within each of the regions, i.e. among local populations (mean $F_{\mathrm{ST}} = 0.038$). Although low, the level of differentiation was significant for most pairs of local populations examined, possibly indicating recent but progressive isolation of local populations.

Badger (*Meles meles*). The genetic structure of three Dutch badger populations (Veluwe, Noord-Limburg and Zuid-Limburg) was assessed using 8 polymorphic microsatellite loci. Compared to other Mustilidae, average expected heterozygosity was low, ranging from 0.33-0.42 indicating possibly loss of genetic diversity. No significant genetic differentiation was observed between the populations. This may indicate the sufficient migration between the badger populations either naturally through corridors or artificial migration by humans. Incorporating data from three populations from Denmark mean overall F_{ST} was observed to be 0.322 indicating strong genetic differentiation between countries.

Pine marten (*Martes martes*). Three regional Dutch populations (Veluwe and Utrechtse Heuvelrug) and a Spanish population were analysed with microsatellites. The level of variation observed for this species, mean H_e=0.63, did not differ significantly between populations, and was comparable to the level found for non-endangered mustelids. Genetic differentiation was substantial between the Spanish and the Dutch population, but among the Dutch populations there was little divergence. Further research using mtDNA confirmed these results.

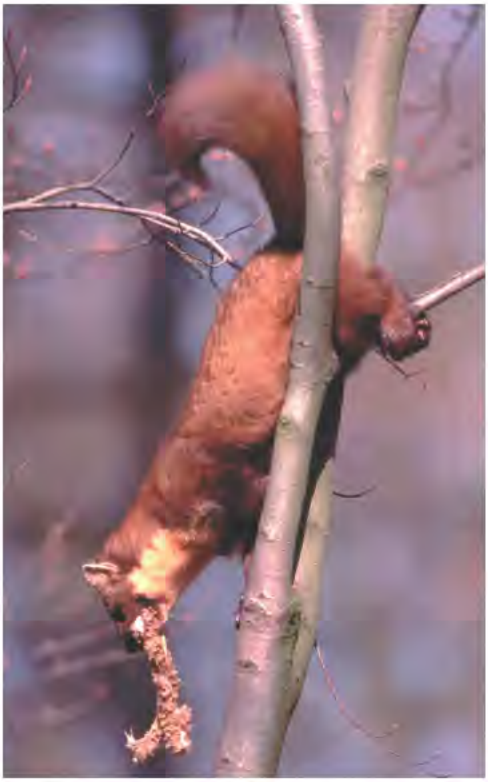

Figure 2. Pine marten (*Martes martes*). Picture kindly supplied by Hugh Jansman (Alterra)

Harbour seal (*Phoca vitulina*): The genetic variation at the Major Histocompatibility Complex (MHC) of harbour seals from the Dutch Wadden Sea has been analysed by DNA sequencing. A comparison of PCR fragments from at least two members of the multigene MHC class I family revealed variation both within and between these fragments. Evidently, the low genetic variation as observed for minisatellite and microsatellite loci does not extend to the MHC, possibly through strong balancing selection acting on this complex.

Quantifying Outcrossing Probabilities of Genetically Modified Plants: Development of a Predictive Model (Willemien Smith-Kleefsman)

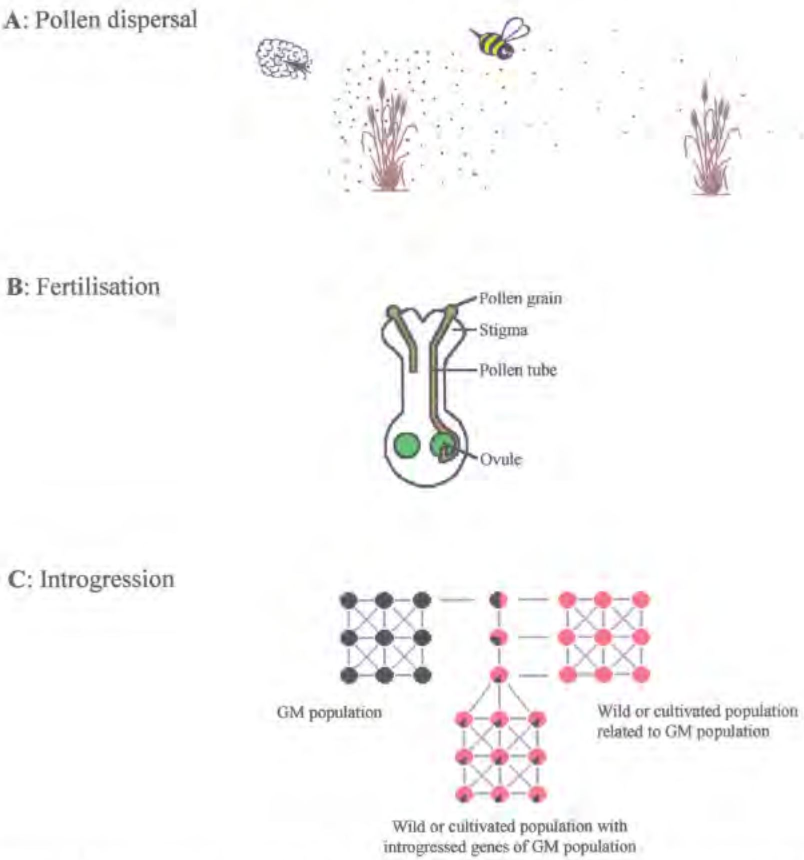

Figure 3. Schematic depiction of the outcrossing process, which can be divided into three distinct parts (A, B, C). A. Viable pollen grains must disperse over the distance between the GM population and related wild or cultivated populations. B. After reaching such population, the pollen must fertilise an ovule, resulting in hybrid seed. C. The modified DNA present in the hybrid must establish itself and persist (i.e. introgress) in the genome of the receiving population.

Literature survey - The process that leads to outcrossing can be divided into three steps (see figure 3): *(A)* First, viable pollen must reach the stigma of a compatible specimen, which is usually, but not necessarily of the same species as the GM crop. The main processes and mechanisms that affect pollen dispersal are considered. (*i*) Self-fertilising species can be expected to lack specific (long-distance) dispersal mechanisms. Most self-fertilising species, however, show appreciable levels of outcrossing, but they are expected to have lower chances of outcrossing than typically outcrossing species, since pollen of the former species, being at low concentration in target populations, has to compete with much selfing pollen. Therefore, differences between outcrossers and selfers should be a component of the model. (*ii*) Pollen grains are not able to disperse themselves actively, but need dispersal vectors. In our biogeographic region, insects and wind seem to be the predominant dispersal vectors. Pollen flow by insects as well as by wind can be highly variable among species, among plant populations and over time, depending on the weather, on population characteristics and on the environment. Distances travelled by pollen of wind-pollinated species are generally larger than the distances travelled by insect-dispersed pollen, but the shape of wind and insect pollination curves relating pollination probability (or relative pollen density) to dispersal distance is generally highly similar (see figure 4).

A large fraction of the pollen lands close to the donor plant and only a small fraction disperses further, some of which may travel large distances. From a modelling perspective, this similarity between the dispersal curves for wind-and insect-dispersed pollen has the

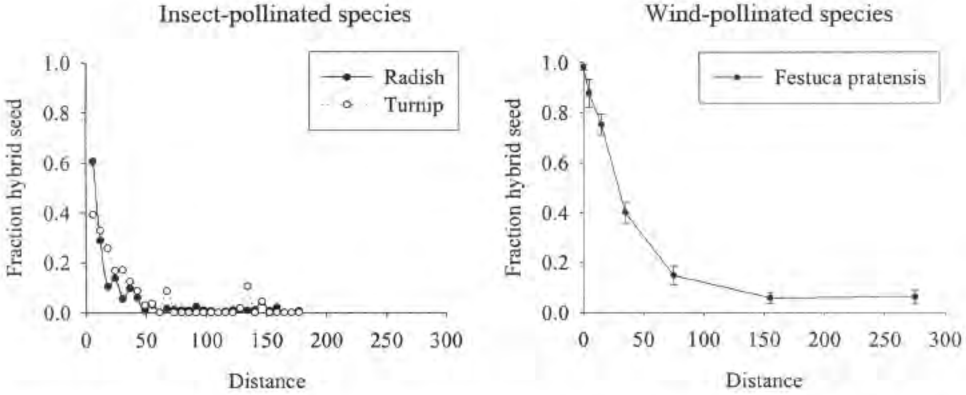

Figure 4. Examples of pollen dispersal curves for two insect-pollinated species (radish and turnip) and one wind-pollinated species (*Festuca pratensis*). In all cases, two different morphological types were used, one as a source, the other as a target. Pollen dispersal was measured as the fraction of hybrid seed in the target. More pollen grains would certainly have travelled over these distances than those that resulted in hybrids. Data from Bateman 1947ab and Rognli *et al.* 2000.

great advantage that the same mathematical approach can be used for both. (*iii*) During the dispersal process, a certain percentage of the dispersing pollen will have lost viability. These pollen do not contribute to the fertilisation process. It is questionable whether, on the basis of current knowledge, pollen viability rates of crops growing in the field can be predicted.

(B) After landing on a suitable stigma, the pollen has to succeed in fertilising an ovule. Therefore, it has to compete with other pollen that has landed on the same stigma. The competitive ability of GM pollen may be different from that of pollen from the target population. Some important mechanisms that influence fertilisation success are reduced viability, time of arrival, the exact place where the pollen lands and the presence of self-incompatibility mechanisms. Since the fertilisation process is very complicated that is affected by many mechanisms, the process needs to be incorporated the model in a simplified mode to be workable.

(C) When hybrid seed is developed successfully, the modified DNA of the GM species must be incorporated into the gene pool of the receiving species (introgression). Whether this will happen, is in the beginning mainly dependent on stochastic processes that determine whether the modified DNA will establish in the receiving population. Later on, when the modified DNA is established in sufficient numbers of plants to render stochastic effects negligible, deterministic processes will determine the persistence of the modified DNA. Introgression is a very complicated process on which little information is available; therefore, modelling of this part is not considered at present.

Modelling approach. An overview of the proposed mathematical that estimates probabilities of pollen to land in populations of compatible species and to achieve fertilisation in such population is given in Figure 4. This proposed model consists of three modules, but only the first two are programmed at present.

(A) The first module addresses the question: how does pollen, originating from a GM source population, disperse over the landscape? In this module, pollen dispersal of a source population is simulated. Different equations are pre-programmed that can be used to describe the dispersal pattern of a species. These are a negative exponential equation, an inverse power law, and a uniform distribution. The user can also choose to estimate the dispersal process using two equations, one describing the first part of the curve (i.e. describing the dispersal pattern of the pollen that lands close to the dispersing individual), the other one describing the tail of the curve (i.e. describing the dispersal pattern of the pollen that travels farther away) (see figure 5).

If the dispersal pattern is described by another known curve than the ones that are pre-programmed, the user can enter this equation using the custom function. In this module, data on pollen viability can be added; pollen survival can be described by a negative cumulative normal distribution, or by an equation added by the user.

(B) The second module addresses the question: what is the expected frequency of seeds in a target population that is fertilised by pollen originating from a given GM source population? In this module, the percentage of seeds that is the result of a cross between

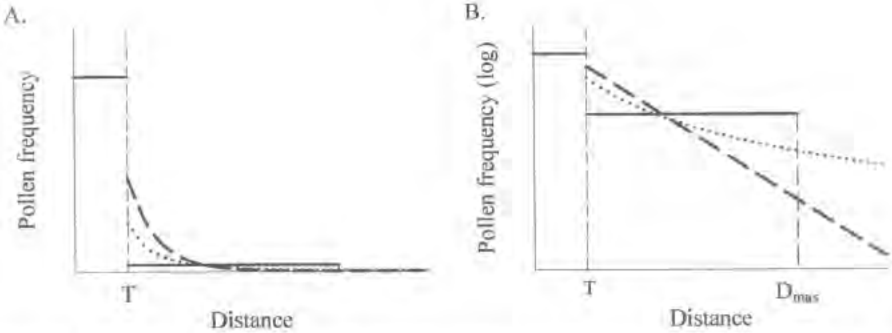

Figure 5. Schematic representation of how the pollen distribution pattern is estimated with three different equations. Here, the first part of the pollen dispersal curve is estimated with a uniform distribution (solid line), the second part of the curve (the tail) is estimated by a negative exponential curve (dashed line) an inverse power law (dotted line) and a uniform distribution (solid line). To be able to estimate the pollen distribution pattern in this way, there should be a clear 'cutting point', i.e. it should be clear where to end the first equation and to start the second one (T in this figure). Furthermore, it should be known what fraction of the pollen is described by the first equation and what fraction by the second equation.

pollen from the source population and ovules from the target populations is calculated, thus giving an estimation of the contamination level of the target population with DNA from the GM source population. Relative competitive ability of the pollen is included in this module.

Conclusions. The model will be helpful for estimating the separation distances required to reduce contamination levels with modified DNA to acceptably low levels. The outcome of the model will largely depend on the parameter values entered by the user. The user should be aware of two types of uncertainty associated with this. One type of uncertainty is whether the parameter values used have been estimated correctly. The other uncertainty is caused by variation in parameter values due to stochastic processes. The model, however, only handles 'standard' situations: it does not reckon with fluctuations in time and/or space. The process of pollen dispersal, however, appeared to be highly variable.

We aim at extending and refining of the present model, by (*i*) including more complex situations, like estimation of gene flow at landscape level, and/or (*ii*) including stochasticity, allowing to estimate possible deviation from the contamination levels found. By adding modules and functions like these, the procedure to estimate outcrossing probabilities of populations will be continually improved. The data presented in the report and the accompanying computer model can be easily adapted to model gene flow between natural populations and as such are of great value for the Ph.D. project.

Publications 2004

Doctorate granted by the institution, prepared within the institution

Vermeulen, C.J. 2004 Genetics of lifespan determination in *Drosophila melanogaster*. Prof. dr. R. Bijlsma, Prof. dr. W. van Delden, Dr. L. van de Zande, University of Groningen.

Doctorate granted by another institution, prepared at another institution

Pannebakker, B.A. 2004 Evolutionary consequences of *Wolbachia*-induced parthenogenesis in the parasitoid *Leptopilina clavipes*. Prof. dr. J.J.M. van Alphen, Prof. dr. P.M. Brakefield, Dr. B.J. Zwaan, Prof. dr. L.W. Beukeboom, University of Leiden.

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Chauvet S, Velde M van der, Imbert E, Guillemin ML, Mayol M, Riba M, Smulders MJM, Vosman B, Ericson L, Bijlsma R, Giles BE, 2004. Past and current gene flow in the selfing, wind-dispersed species *Mycelis muralis* in western Europe. *Mol Ecol* **13**: 1391-1407

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Rütten KB, Pietsch C, Olek K, Neusser M, Beukeboom LW, Gadau J, 2004. Chromosomal anchoring of linkage groups and identification wing size QTL using markers and FISH probes derived from microdissected chromosomes in *Nasonia* (Pteromalidae: Hymenoptera). *Cytogenet Genome Res* **105**: 126-133

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Marine Biology

Group leader W.J. Wolff

Composition of the group in 2004:

Tenured Staff	**source**	**fte**	**period**
dr. A.M. Breeman	RUG	0.8	till 040901
prof.dr. H.J.W. de Baar	RUG	0.3	
dr. W.W.C. Gieskes	RUG	1.0	
dr. W. Klaassen	RUG	1.0	
prof.dr. J.L. Olsen	RUG	1.0	
prof.dr. W.T. Stam	RUG	0.6	
dr. E.J. Stamhuis	RUG	1.0	
prof.dr. J.J. Videler	RUG	1.0	
prof.dr. W.J. Wolff	RUG	1.0	
Post-docs			
dr. A.G.J. Buma	NWO-MEERVOUD	0.8	020201-060201
dr. J.A. Coyer	NWO-ALW	1.0	030501-060501
dr. G.G. Hoarau	RIVO	1.0	040315-040615
	EU-Marine Genomics	1.0	041001-051001
dr. M.A. van Leeuwe	EU-Marcassa	0.3	030501-040301
	EU	0.5	020901-050301
dr. W.H. van de Poll	NWO-NAAP	1.0	030501-040501
	NWO-NAAP	0.9	040501-060801
dr. H.J. van der Strate	EU-Silibiotec	0.8	030101-051001
PhD-students			
drs. Y. Afeworki	WOTRO	1.0	041101-081101
drs. A.C. Alderkamp	RUG	0.8	000701-050401
dipl.ing. V. Brauer	RUG - Ubbo Emmius	1.0	040801-080801
J.F. Cardoso, lic.	Kon. NIOZ - FCT	1.0	000701-040701
drs. M.S.C.O.M. Chevolot	NWO-ALW	1.0	020901-060901
T.J. Compton M.Sc.	RUG - Ubbo Emmius	1.0	020415-060415
drs. S. Ferber	RUG-Breedtestrategie	1.0	030901-070901
dipl. biol. H. Flores	NWO-ALW	1.0	031115-071115
dipl. biol. R. Gesser	RUG	1.0	990901-050301
drs. D. Haydar	NWO-ALW	0.8	031101-061231

drs. S.A. Hazelaar	NWO-STW	1.0	000801-040801
P.M. van Himbergen-Gondwe, M.Sc.	NWO-SRON	1.0	990901-040901
drs. G. Hoarau	NWO-ALW	1.0	000115-040115
drs. H.J.T. Hoving	Various grants	1.0	031101-071101
drs. P.J. Janknegt	RUG	0.8	020615-070615
drs. J. Jansen	NIOO-CEME - EU	1.0	
drs. N. Klomp	RUG	1.0	040815-080815
drs. G.D. Kramer	Kon. NIOZ - ALW	1.0	000915-040915
drs. J. Mieog	WOTRO	1.0	040401-080401
drs. V.S. Parada	Kon. NIOZ - ALW	1.0	
drs. A.M.T. Piquet	RUG Breedtestrategie	1.0	020301-060228
ir. A. Purwoko	Asian Developm. Bank	1.0	030701-070701
drs. Th. Reinthaler	Kon. NIOZ - ALW/EU	1.0	001005-041005
drs. M.J.A.Rijkenberg	NWO-ALW	1.0	000301-040701
drs. M. Schotten	Various grants	1.0	031201-071201
drs. K. Troost	NWO-ALW	0.8	031201-070401
dipl. biol. A.M. Zipperle	RUG - Ubbo Emmius	1.0	030901-070901

Technical and Administrative Staff

secretaries			
G. van Roon-ter Horst	RUG	0.5	
J. de Vries-Veldkamp	RUG	0.5	
technicians			
S.A. Boele-Bos	RUG	0.5	
H.W. de Groot	RUG	1.0	
H. Peletier	RUG	0.5	
J. Roggeveld	EU	1.0	020901-050301
M.R. Tyl	NWO-ALW	1.0	010915-040701
J.H.Veldsink	various	1.0	
L.H. Venekamp	RUG	0.5	
R.J.W.Visser	RUG	0.5	
J.J. de Wiljes	RUG	1.0	

Guests/detachment

Prof.dr. V.N. de Jonge D.Sc.	RIKZ	1.0	010701-050701

Adjunct Chairs

Prof.dr. C.H.R. Heip	NIOO-CEME
Prof.dr. G.J. Herndl	NIOZ
Prof.dr. A.D. Rijnsdorp	RIVO

Overview of academic results

Workgroup 'Evolutionary ecology of benthic plants and animals'

The distribution of populations of a species over a range of temporal and spatial scales - from deep historical timeframes to contemporary timeframes; and from meters to thousands of kilometers spanning ocean basins - is the result of both historical events and ecological processes. The interlocking roles of life histories, short- and long-dispersal capacities, and adaptive tolerances to temperature and light constraints are key variables of interest in our research program.

Seaweeds and seagrasses, as well as corals (including their symbionts), flatfish and rays are being investigated in one or more of the following ways: 1) Analysis of population genetic structure and phylogeography; 2) The coupling of molecular phylogenetic and ecological data sets to test hypotheses in 'historical ecology'; and 3) Development of new tools for ecological genomics to include subtractive and EST libraries (mini- and microarrays) in fucoids and seagrasses in order to begin to explore gene expression in nature. Other lines are 4) Studies on invasive benthic seaweeds and invertebrates, and 5) The application of our results in Integrated Coastal Zone Management.

The year 2004 was one of continuing transition. Dr. Anneke Breeman retired in April and Prof. Wytze Stam accepted the additional post of Adjunct Director for the *Opleiding Levenswetenschappen*. In anticipation of Prof. Wim Wolff's retirement in November 2005, Prof. Jeanine Olsen took over from him in October. Preparations for hiring of new permanent staff were initiated. Three PhDs were completed. One new PhD project and one new Post-doc project were started.

Field work took place on the Great Barrier Reef but also closer to home in the Wadden Sea, Denmark, Norway and Iceland (Fig. 1).

Figure 1. Galice Hoarau and Jim Coyer working on *Fucus* hybrid zones in Iceland.

2004 Also saw the beginning of our participation in two new EU Networks of Excellence - *Marine-Genomics-Europe* and *Marine Biodiversity and Ecosystem Function (MarBEF)*. Both of these networks facilitate our ongoing studies in population genetics (especially adaptive population divergence, which involves the new post-doc position) and phylogeography (especially historical demography) of benthic algae, seagrasses, fish and selected invertebrates. Our overall goal is to link biotic and abiotic processes that affect distribution and, especially, adaptation.

Population genetic structure, dispersal and phylogeography. Galice Hoarau defended his thesis on the population structure and genetic stock assessment in the flatfish *Pleuronectes platessa* along the European continental shelf in October (See earlier CEES reports for other aspects of his project). As noted previously, high inbreeding coefficients have been repeatedly found in plaice. Further analyses revealed that the frequency of lower individual multilocus heterozygosity (MLH) classes was higher than expected under random mating confirming the presence of inbred individuals. In a companion analysis, effective population size (N_e) was also estimated from archived otolith DNA isolated between 1924 and 2002. Results showed that plaice N_e is five orders of magnitude smaller than the census size with $N_e = 2000$ in Iceland and $N_e = 20{,}000$ in the North Sea. Although no reduction in genetic diversity could be detected over the last century, a dramatic increase in inbreeding was found in samples after 1950, which coincides with industrial-scale fishing and fishing on the spawning grounds (Fig. 2). Results of these two studies were among the first to document genetic changes due to fishing pressure and have, therefore, required extra documentation in anticipation of exceptionally critical peer-review. This manuscript was accepted in 2004 but will appear in the *Proceedings of the Royal Society B* in 2005.

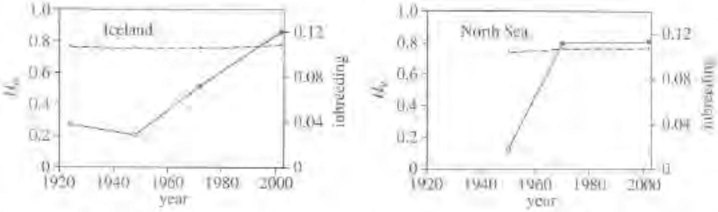

Figure 2. Temporal changes in genetic diversity of plaice based on otolith data. H_e (dashed line) and inbreeding (solid line). Open circles: n.s., filled circles: significant at $p<0.001$. Inbreeding is defined as the excess of homozygotes $(MLH_{obs}-MLH_{exp})/MLH_{exp}$.

In a companion project on the common thornback ray, *Raja clavata*, PhD student Malia Chevolot has just completed her first survey of population structure using newly developed microsatellite loci (Chevolot *et al.* submitted). First results suggest that some populations remain coastal while others migrate around large swaths of the British coast (Fig. 3).

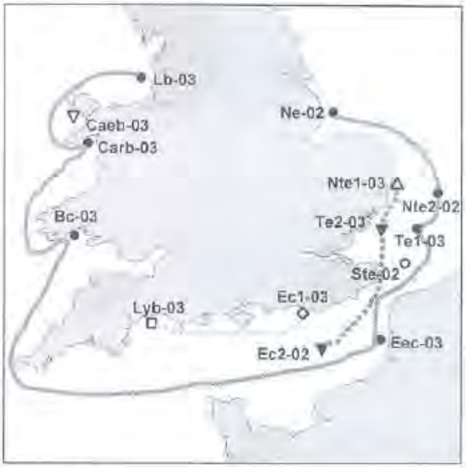

Figure 3. Suggested population structure of *Raja clavata* with wide-ranging (solid line) and more restricted (dotted) groupings, including singletons. Identical symbols represent locations groups following the FCA analysis.

Aschwin Engelen defended his thesis in January on the population biology of *Sargassum polyceratium* around the island of Curaçao (see earlier CEES reports).

Our phylogeographic and population-level studies on seagrasses continue to focus on *Zostera marina* and and *Zostera noltii*. Eelgrass, *Z. marina*, is the dominant seagrass throughout the northern hemisphere. Over the past several years the lab has embarked on an in-depth study of the population genetics and ecology of *Z. marina* throughout Europe. In 2004 we completed the first large-scale analysis of recolonization of the North Atlantic since the Last Glacial Maximum (Olsen *et al.* 2004). Two key findings were: 1) that gene flow is probably contemporary between the Pacific and North Atlantic based on shallow topologies of the gene trees; and 2) that genetic diversity for the species is highest in the Skagerrak-Kattegat area, as opposed to the Brittany peninsula which is typical for a number of other marine macrophytes (Fig. 4). The next phase of the study involves historical demography which is being pursued by PhD student Steven Ferber, who is currently developing suitable genetic markers (not microsatellites) for coalescence-based modeling.

In a parallel study involving *Z. noltii*, the Skagerrak area was also found to be the area of highest diversity (Coyer *et al.* 2004). Unlike *Z. marina*, *Z. noltii* has a more restricted North Atlantic distribution and a broader southern distribution.

PhD student Andreas Zipperle is studying the reproductive ecology and mating system of *Z. noltii*. An additional focus of the study is patch connectivity via gene flow and the

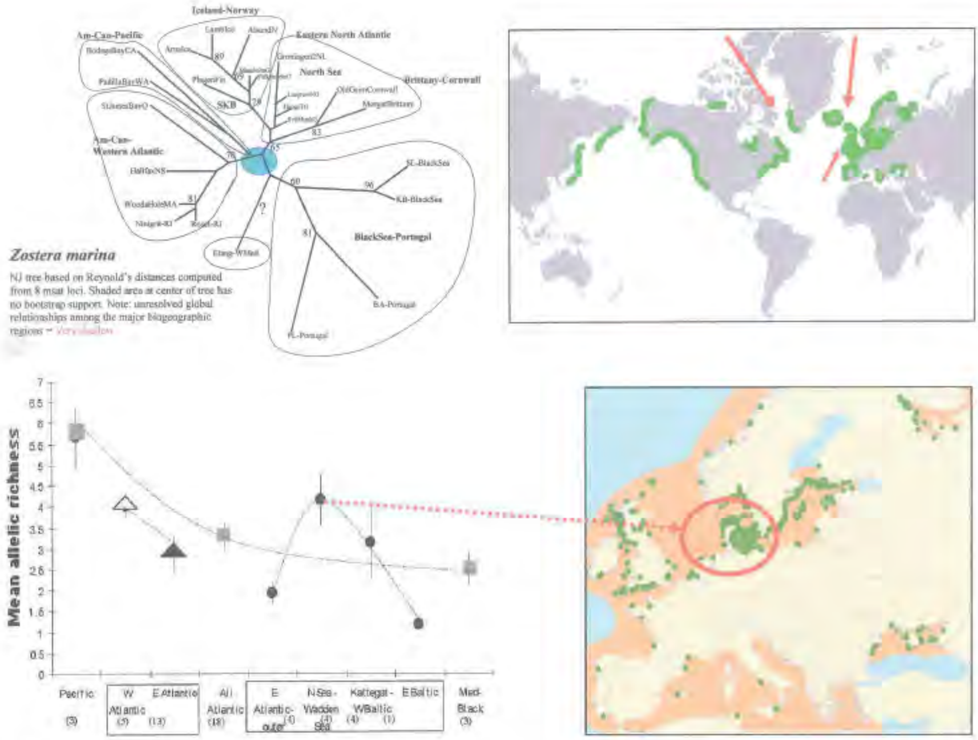

Figure 4. *Zostera marina*. Upper panel left: Shallow topology between Pacific and Atlantic; Upper panel right: Distribution of eelgrass in which there is evidence for strong Pacific-Atlantic gene flow. Lower panel: Allelic richness relationships in which each point on the graph represents the 9-locus mean and standard error. Squares show the trend among pooled Pacific, Atlantic and Mediterranean populations; triangles compare Western and Eastern Atlantic populations in which the filled triangle represents the mean of the circle populations for comparison. The circles compare sub-regions within the Eastern Atlantic. The highest point of the unimodal distribution corresponds to the area shown. Ends of the distribution reflect edge populations.

degree to which new recruitment is achieved from a seed bank vs. dispersal. Every year, huge piles of seagrass wrack form on the beaches of the German island of Sylt (Fig. 5). Classical genetic tests of isolation by distance using microsatellite loci (Coyer *et al.* 2004) suggest that populations of *Z. noltii* are in contact at scales of up to 150 km but that significant differentiation can be detected at scales as small as 2 km. Data of this kind are highly relevant for seagrass restoration and management.

Hybrid Zones in *Fucus*. Interspecific hybridization is a major mechanism for generating evolutionary novelty by offering prime opportunities to test hypotheses of gamete choice, differential fertilization and fitness that expand or reduce reproductive isolation.

Figure 5. *Zostera noltii* wrack on the beaches of Sylt.

In 2003 we began field and laboratory studies of these hybrid zones in the Kattegat area of Denmark (*Fucus evanescens* intrusive on resident *F. serratus* from ca. 1900) and Western Iceland (*F. serratus* intrusive on *F. evanescens* from between 1000 and 1900). A control area in Norway (where both species are found only in allopatry) was added in 2004. Preliminary results suggest lower fitness of the hybrids but also introgression with parents (Fig. 6). Data are not yet sufficient to test for geographic reinforcement.

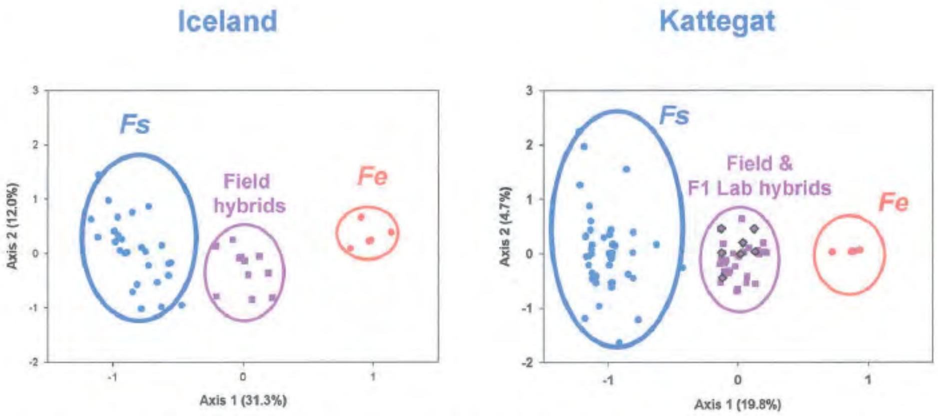

Figure 6. Hybrid zones between *Fucus serratus* and *Fucus evanescens*.

Moving towards genomics in ecology. Beginning in October 2004, Dr. Galice Hoarau began a post-doc position with partners in the Marine-Genomice-Europe Network to develop EST collections for species of *F. serratus* (full-length cDNA libraries for high temperature and light stress) and *Fucus vesiculosus* (full-length cDNA libraries for desiccation response. Sequencing will begin in mid-2005 with 5,000 reads per library (total of 20,000 reads). These EST collections will be used to identify stress-associated genes as well as to identify SNPs.

Phylogenomics. Phylogenetics at the genome level is quite a challenge. Marie-Curie-Post-Doc-Fellow Marie-Pierre Oudot-LeSecq sequenced six complete mitochondrial genomes from the Phaeophyceae (Fig. 7) totaling >240Kb of sequence. A concatenated phylogenetic analysis of 28 heterokonts using full sequences from 10 regions revealed multiple primary and secondary acquisitions of plastids. This project also produced a set of much-needed new mt loci suitable for population genetic analysis in heterokonts.

Figure 7. Coarse-scale alignment of heterokontic mitochondrial genomes (Oudot-LeSecq *et al.*, Mol. Biol. Evol. (submitted))

Symbiotic Associations and Coral Bleaching. Recently, it has been shown using qPCR that a single coral species or even a single coral colony harbours one dominant strain and 2-3 background strains (Fabricius *et al.* 2004). Corals can thus adjust their *Symbiodinium* strain to enhance both survival and fitness under different physical regimes. Predictably, however, there is a trade-off between strains that do well at depth or in shade (light adaptation) but are slow growers (poorer competitors) vs. those that do well in higher temperatures (global warming and the phenomenon of *coral bleaching*). As part of our ongoing studies in corals, a new WOTRO-financed PhD project (Jos Mieog) started in April 2004 in collaboration with the Australian Institute of Marine Sciences in

Townville. His project concerns stress-adaptation and switching of the algal endosymbiont (the dinoflagellate *Symbiodinium*) within common scleractinian species such as *Acropora* and *Favites*. During the first year, the goal has been to quantifiy background strains from different species and light-temperature gradients; and to begin coral-acquisition experiments in which coral larva are offered different symbionts (so-called infection experiments), grown in mesocosms and subsequently outplanted (Fig. 8).

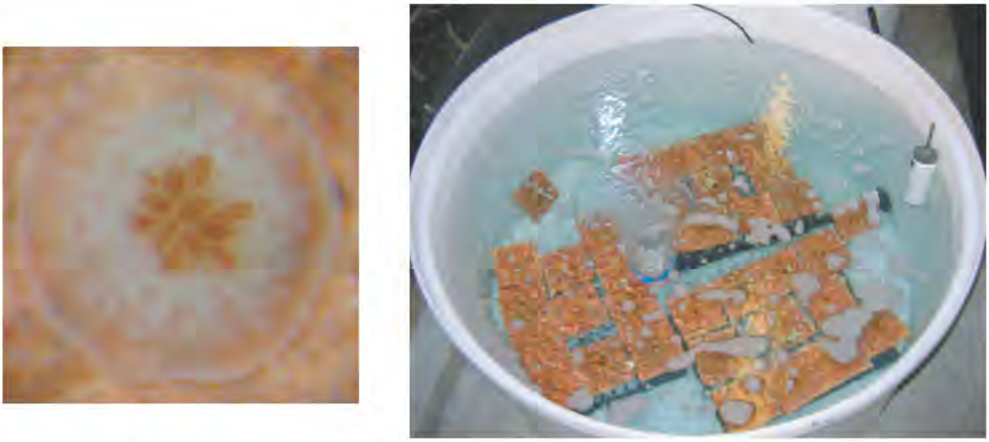

Figure 8. *Symbiodinium* acquisition is shown in this recently infected juvenile of *Acropora tenuis* (left). Different endosymbiont genotypes as well as combinations of genotypes are offered to the corals. Once the holobiont corals are established in the tanks, the tiles can be transplanted to the field to test physiological performance, fitness and so forth. Because corals are clonal and endosymbiont genotypes can be controlled, genotype x environment interactions can be separated.

Invasive species and phycological forensics. The impact of the introduced and highly invasive seaweed *Caulerpa taxifolia* in the Mediterranean and worldwide has been a point of continuing research since 1997. Work in 2003-2004 showed that hybridization among normally isolated geographic populations of *C. taxifolia* has led to the success of the invasive strain which is well-adapted to colder, turbid waters as compared with more tropical, clear-water strains (Meusnier *et al*. 2004, Fig. 9).

We have also joined forces with Dr. Linda Walters (Florida Central University and Florida SeaGrant) and Prof. Steven Murray (California State University) to survey *Caulerpa* species from around the coast of Florida (Fig. 10); and to assess the impact of internet sales of *Caulerpa* species on the aquarium trade. Results of the 2004 survey revealed that the source populations of internet sales of *Caulerpa* species originate from Caribbean/Florida.

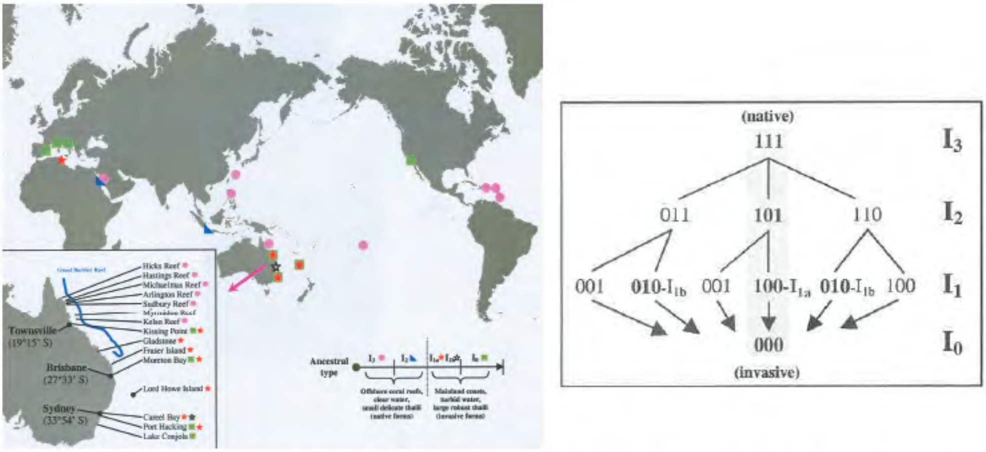

Figure 9. Biogeographic distribution of indelotypes in *Caulerpa taxifolia*. Right panel shows deletion pathways.

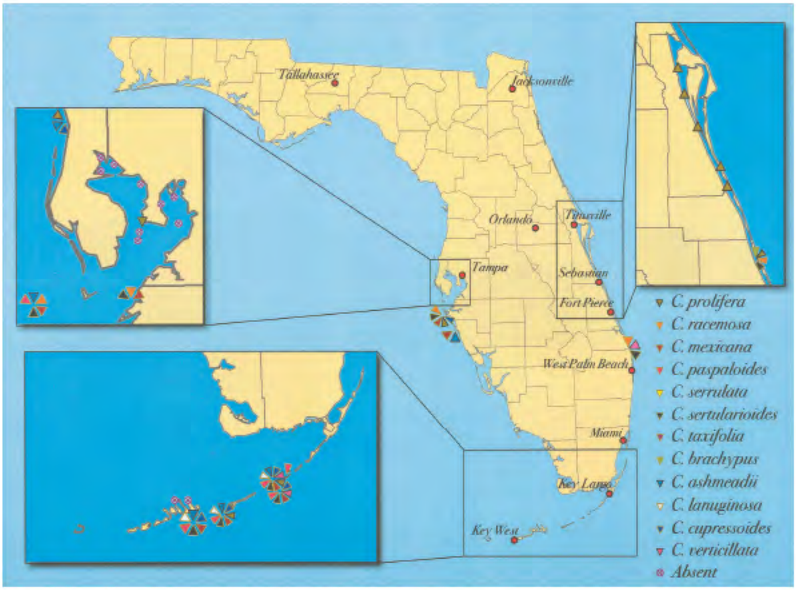

Figure 10. Sampling locations along the Florida coast including distribution of species.

Invasive species in northwest European coastal waters. A review of all non-indigenous marine and estuarine species in Dutch coastal waters was accepted for publication and published early 2005. In this paper an overview is presented of non-indigenous marine and estuarine plant and animal species recorded from The Netherlands. In this list both exotic species from outside NW Europe and non-indigenous species from elsewhere in NW Europe are enumerated. At least 99 plant and animal species appear to have been introduced from elsewhere in the world. Another 13 species have been introduced from other parts of NW Europe. The third category of dubious non-indigenous species enumerates 37 species.

In Fig. 11 the years of first observation of non-indigenous species in the Netherlands are shown. There is an increase in first observations of non-indigenous species, especially since the 1970s. This increased introduction rate is consistent with global patterns. Several reasons can be given for this increase, e.g. climate change, increased observer effort, invasional meltdown and/or increased rates of transport.

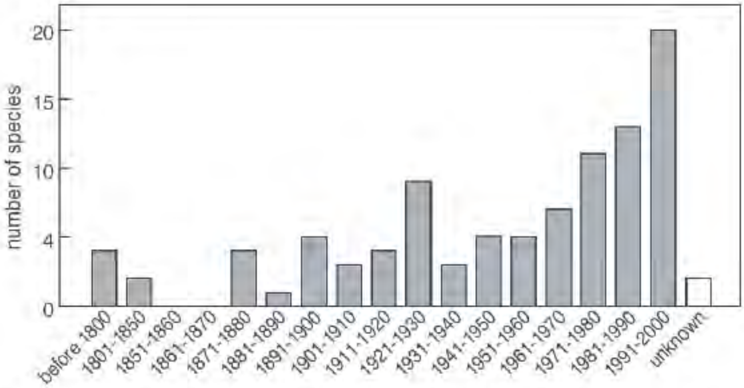

Figure 11. Years of first observation of non-indigenous species in the Netherlands (Wolff, 2005).

In the third year of the PhD study by Deniz Haydar a conceptual model was created in order to study this increased introduction rate. This model considers one donor region, in which species are taken up by one transport vector, which transports the species to one recipient region. In this process species have to overcome several steps before establishing a naturally reproducing population in the recipient region: uptake by the vector, survival of transport, and introduction in the recipient region. The introduction rate will be high at first and will *diminish* over time, assuming that there are no changes in both regions, and that vector intensity remains constant.

The different factors that determine invasion rate were investigated for the Pacific oyster (*Crassostrea gigas*) as a transport vector, focusing on algae that are transported with oysters. In order to estimate survival rates for the conceptual model, epiflora of oysters from the Oosterschelde estuary was studied during a period of two years. 41 species of algae were recorded. Rhodophytes were most abundant, this is also the group that has most frequently been introduced with oyster transports. All model parameters were established for this specific group, using the total number of Rhodophyte species in Japan and number these that have been introduced in the Netherlands. This analysis showed that the invasion rate of Rhodophytes transported with Pacific oysters is still high, which supports the observed increase of non-indigenous species introductions.

Oyster imports from Japan no longer take place. However, relayings of oysters within Europe are still common practice. Oysters from the Thau lagoon in the Mediterranean are relaid on the French Atlantic coast, from there oysters are transported to the Oosterschelde estuary. The Thau Lagoon has the highest number of exotic algal species in Europe, we can expect those to arrive in our waters in the foreseeable future.

Ecological impact of invasive oysters. The ecological impact of Pacific oysters (*Crassostrea gigas*, introduced in The Netherlands in 1964) on the coastal waters of the Netherlands, especially on native bivalve filter feeders, is studied in the PhD project of Karin Troost. One way in which the Pacific oyster might affect native bivalves is by filtering their pelagic larvae from the water column, thereby interfering with their recruitment. Collection of bivalve veliger larvae above oyster beds and above ‘bare’ reference sites revealed in 2003 that numbers of mussel (*Mytilus edulis*) larvae were significantly reduced above oyster beds in comparison with reference sites. On the other hand, significantly more oyster larvae were counted above the oyster beds. More observations on oyster larvae were made in 2004, showing the same relative distribution. Differences in larval numbers can not be accounted to differences in tidal current velocities. No significant differences could be found after measurements with an acoustic doppler current profiler.

The Pacific oyster may also affect indigenous bivalve filter feeders negatively by competing for space with them. Larvae of Pacific oysters were found to settle gregariously: the number of newly settled spat showed a positive relationship with the number of previously (1 - 7 days earlier) settled spat. Lab experiments were supported by field observations. This behaviour gives the Pacific oyster a significant advantage in the competition for space.

The Pacific oyster’s ability to compete for food with blue mussels was also studied this year. In a flow-through filtration set-up in the laboratory, the efficiency of both species in selecting organic matter for ingestion and in digesting ingested organic matter in the gut was studied, among other physiological parameters related to feeding. Although oysters clearly filter more water per hour dan mussels, they also reject much more, resulting in a similar ingestion rate. Between both species, no significant differences between selection and digestion efficiencies were detected.

Workgroup 'Ecophysiology of marine micro-algae'

Introduction. The programme 'Ecophysiology of marine micro-algae' went on with pursuing the understanding, quantification, and prediction of the interactions of marine micro-algae with the chemistry, physics and biology within the plankton ecosystem. The focus was on the Southern Ocean and Dutch coastal waters including the Wadden Sea, paying attention to the key role that marine micro-algae play in the geochemical cycling of C and S, elements that are relevant to climate: fluxes of the carbon-containing greenhouse gas CO_2 and of the cloud-inducing volatile sulphur component DMS are profoundly mediated through interactions in the plankton of seas and oceans. Our focus remained on the eukaryotic unicellular Haptophycaeans *Phaeocystis* sp. and the Coccolithophorid *Emiliania*, but also the diatoms (Bacillariophyceae), the other major class of micro-algae that is of importance for C and S element cycling. They constitute about 30 to 40 percent of all primary productivity in the oceans. Their heavy, silicified cells serve as carriers of organic carbon fixed near the ocean's surface that subsequently sinks to the deep sea, where this material is then 'sequestered' for many years, the so-called 'biological pump' for uptake and storage of CO_2 by the oceans.

Marine productivity, and factors that govern marine productivity, in the Southern Ocean around the Antarctic continent are also of vital importance to the global climate system. For these reasons much focus of our group lies in Antarctic ecological questions of the plankton food web, where diatoms and *Phaeocystis* also are key actors. Here we combine a strong record of scientific leadership in the role of Fe in photosynthesis and CO_2 fixation of algae, with cutting edge research on the impact of the UV part of the solar spectrum on plankton ecosystems. Diatoms also grow at the surface of tidal flat sediments and therefore play an important role in the Northwest European Wadden Seas. In the Dutch Wadden Sea our studies focus on benthic diatoms that by virtue of their primary products influence the sediment and the ecosystem, and moreover: diatoms of coastal seas regulate much of the carbon dioxide (CO_2) fluxes from sea to air and *vice versa*. In Dutch coastal waters we also investigated other nuisance algae than *Phaeocystis*: the class Raphidophyceae, with a focus on the environmental conditions that govern the level of their toxicity.

The following projects were run in 2004:

Ecophysiology of microalgae: bottom-up control by nutrients and light
Nutrient availability (P, N, Si, Fe) and the light climate (including harmful effects of ultraviolet radiation) are the major abiotic factors that control the growth of microalgae in marine environments ('bottom-up' control). It was our goal to investigate the impact of these growth limiting factors on the physiology and growth. Since growth limiting or stress factors often co-exist in the marine environment, our focus lies on the interactive effects of growth limiting factors, such as the interaction between Fe and light stress.

Biotic interactions: interactions between algae, bacteria, viruses and higher organisms
The interaction between microalgae and marine prokaryotes (bacteria, Archaea,

associated viruses) largely determines the transfer of the major greenhouse gas CO_2, from air to sea and between ocean compartments. The role of functional diversity in prokaryotic communities were investigated in the coastal North Sea and the Southern Ocean, as a function of micro-algal abundance and species composition, and as a function of the light climate. Phylogenetic diversity is assessed by molecular fingerprinting methods (DGGE, T-RFLP) combined with cloning and sequencing.

Plankton production and consumption of climate-driving trace gases
Fluxes of CO_2 and DMS were studied within the sea and from sea to atmosphere, both in open ocean regions and in coastal environments, through direct measurements and by means of modelling.

Antarctic ecosystems
Important phytoplankton growth controlling factors in Antarctic waters are iron (availability) and light (availability and stress). In iron enrichment experiments of Southern Ocean waters the CO_2 sequestration potential was studied. Harmful effects of high light, including ultraviolet radiation were also core business. Our Antarctic ecological field studies are the result of joint activities between members of our group and Royal NIOZ, in the context of international research teams, including German, Belgian, Dutch, American and Australian researchers.

Biological and physical Dynamics of intertidal Sediment Ecosystems
We processed all samples taken by our programme partners in shallow environments along northwestern Europe, from Portugal to Denmark by advanced HPLC methodology in order to obtain information on the distribution of the major classes of microalgae and the seasonal variation of biomass.

Biosilicification by diatoms
The molecular background of bio-silicification in diatom cells and frustule architecture continue to be unraveled. An understanding of this bio-inorganic synthesis not only is pivotal for understanding the functionality of the wide variety of frustule shapes in nature (up to 80,000 different ones !), their sinking rate, their preservation/dissolution, but also opens the way for industrial scale bioengineering of ‘artificial’ silicas. The search for proteins that play a role in bio-silicification continued with isolation, purification and sequencing of relevant proteins.

Harmful algal blooms
Life cycle, survival strategies and environmental triggers (temperature, light, nutrients etc.) that induce bloom formation and toxicity were the focal issues here. The final goal was to predict risks, occurrence and abundance of this microalgae in areas like Dutch coastal waters.

Carbon dioxide fluxes in coastal regions
A tower of 65 m height is now located on the edge of the Wadden Sea near Lutjewad. There is continuous observation of CO_2 and a suite of ancilliary atmospheric tracers serving as constraints to resolve the source/sink terms of the CO_2 (marine versus land,

natural versus anthropogenic). A pilot study of direct CO_2 gas flux detection by the eddy correlation technique has shown fascinating reproducibility of the diurnal cycle of CO_2 fluxes of the tidal flats due to daytime photosynthesis and net nocturnal respiration of the bentic diatom community.

Methods implemented.

1 PTR-MS for detection of traces of gases (such as DMS, halocarbons).

2 Flow Injection systems for trace analyses of iron and zinc, algal culture rooms with options of low temperatures (such as those of the Southern Ocean around Antarctica) as well as for ultra-clean trace metal-biota experiments and special gastight incubation systems as necessary for assessing biogenic gases (DMS, halocarbons).

3 Light systems have been designed for studies at high light intensities (>1000 µmol. m $^{-2}$.s $^{-1}$) as well as the simulation of a range of vertical mixing regimes.

4 Assays for stress-related enzymes and an array of biomolecular approaches (rep-PCR, t-RLFP, RNA expression patterns, micro-FISH).

Impact of phytoplankton on global climate. Sulphate aerosols cool climate by reflecting sunlight. The cooling effect of sulphate aerosols is comparable to the heating by greenhouse gases. Yet, the impact of sulphate aerosols of marine biogenic origin is difficult to assess because of a non-linearity: the first aerosols have a great impact and additional aerosols have a small impact. We may assume that volcanic aerosols were first, next came marine biogenic aerosols and finally anthropogenic aerosols. In the model, we could only distinghuish between marine aerosols and others. Assuming that marine aerosols are the additional ones, the radiative forcing of marine biogenic aerosols could be calculated. It was only -0.03 W m $^{-2}$. Sulphate aerosols also contribute to the cloud droplet number concentration (CDNC), the indirect radiative forcing by aerosols. Here again we noticed the non-linearity that first aerosols have largest impact, but now it was assumed that marine biogenic aerosols were first and anthropogenic and vulcanic aerosols additional. Then sulphate of marine biogenic origin contributes 49% of global CDNC and 77% of CDNC in the Southern Hemisphere. The largest impact of marine biogenic sulphate was found over the tropical southeastern Pacific Ocean where by contrast the lowest chlorophyll a concentrations (a measure of algal abundance) occur. This seemingly contradiction can be understood by advection from the Southern Ocean and by the relatively long residence time of aerosols over the area of interest. It is concluded that a global analysis is needed to understand the climatic relevance of algal derived DMS. This conclusion is particular relevant in the context of the original SRON proposal where a focus on the North Atlantic Ocean was suggested.

Photo-induced oxidative stress in marine diatoms. Although phytoplankton is typically characterized as low-irradiance adapted, the dynamic nature of irradiance in water (due to diurnal cycles, cloud cover, vertical mixing) holds the risk that algae experience excessive irradiance, i.e., irradiance well above photosynthetic requirements, for example when the algae reside near the surface. High irradiance increases the probability that electrons generated during photosynthesis combine with oxygen and form reactive oxygen species. These are harmful for the algae and may cause photo-induced oxidative stress. However, algae possess numerous mechanisms to divert the danger of photo-induced oxidative stress, such as heat dissipation of excessive energy by xanthophylls and the detoxification of reactive oxygen by enzymatic and non-enzymatic systems. Abiotic conditions that potentially influence oxidative stress are the focus of our research, such as the irradiance that the algae experience, UV radiation, nutrient availability and temperature. Effects of photo acclimation and nutrient limitation on sensitivity to excessive irradiance were investigated for the marine diatoms *Chaetoceros brevis*, *Thalassiosira antarctica* and *Thalassiosira weissflogii*. We used photo induced viability loss, pigment composition and PSII efficiency as measures for high irradiance sensitivity. Photoacclimation to high irradiance and nutrient limitation enhanced protection against high irradiance, whereas effects of UVB radiation were not observed. The experiments point to dramatic differences in high irradiance sensitivity of low and high irradiance acclimated algae due to a larger xanthophylls pool in the latter.

The genus *Phaeocystis*, a colony-forming, planktonic micro-alga. During and after the spring bloom, we determined the number of bacteria that can use laminarin as a substrate, using the Most Probable Number dilution technique and comparing those numbers with direct fluorescent counts of the bacteria (Fig 12).

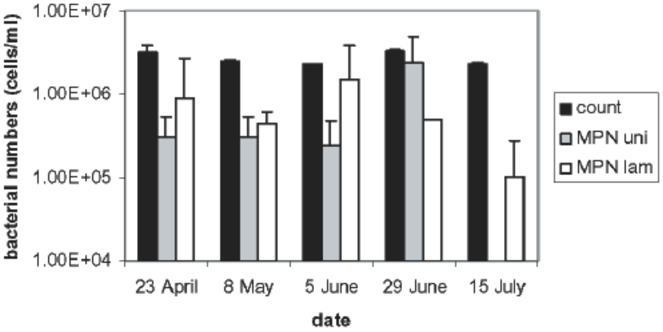

Figure 12. Bacterial abundance in the Marsdiep, The Netherlands, during the spring 2002. Total bacterial numbers counted by microscopy (black column) and Most Probable Number technique on universal medium (light grey column) and laminarin as a sole carbon source (white column). Error bars indicate standard deviation of the average of at least 10 counted fields (microscopy), or 95% confidence interval of eight replicates (MPN).

Generally 10-80% of the bacteria counted by microscopy where culturable. During and directly after the spring bloom the fraction of bacteria growing on laminarin was higher then on the universal medium. Later in the season this was inversed. Bacterial strains that could use laminarin as a sole carbon source were isolated both from the lowest and highest serial dilutions on laminarin. From the lowest dilution the fastest growing strains were isolated, whereas the most abundant strains were isolated from the highest dilutions. Sequencing of the 16S-rDNA revealed that strains belonging to the genus *Vibrio* were present in both groups. Therefore the degradation of laminarin was further examined in 3 different *Vibrio* strains. They can grow on laminarin with growth rates of approximately half of those on glucose. Enzymes from the *Vibrio* strains degrade laminarin to glucose. The enzymes are specific for laminarin, and are not active on other types of glucan. They are expressed in the presence of laminarin and low activities are expressed when *Vibrio* cultures are starved for carbon. Probably, induction of low activity is part of the general starvation response of *Vibrio*, whereas full induction of activity requires the presence of laminarin.

Silica biomineralization in diatoms. Other micro-algal groups in the plankton are less liable to sediment downwards and act in the ‘biological carbon pump’ because they lack the typical feature characterizing diatoms: the silicified part of the cell wall (the ‘frustule’) is relatively heavy (nowadays called ‘ballast’ in ocean models of element cycling in the water column). At the same time, silicification of the cell wall can safely be considered as the cause of the evolutionary success of diatoms, the class of micro-algae that stands out for its huge species diversity. Every species has its own characteristic architecture which is accurately reproduced from generation to generation, implying a strict genetic control of the silica formation process. Knowledge of the metabolic pathway of silicon and of the integrative molecular and physico-chemical processes in silica exoskeleton formation may eventually explain biodiversity in general, beyond the one of the diatoms proper. So far, there are only a few genes that have been identified to be related to silicification and silicic acid transport. The silica biomineralization process in diatoms is supposed to take place in a specific vesicle, the so called silica deposition vesicle (SDV). In a synchronous *Navicula salinarum* culture we have shown that the formation of the new silica cell wall is an amazingly fast process (Fig. 13), but an SDV could not yet be detected, let alone isolated.

Given these synchronisation experiments, we are using now targeted differential display strategies to follow the expression pattern of mRNA (i.e. the active gene transcripts) during the formation of the new cell wall. This resulted in about 60 (parts of) genes, of which most are unknown. Ongoing spatio-temporal characterization of these found genes and corresponding proteins will help us to the reach the ultimate goal to unravel the processes underlying the ecological advantage of silicification and to allow prediction of diatom species diversity in an environmentally changing world.

Stress in micro-algae. Substantial progress was made with the research on oxidative stress indicators. The usefulness of MDA (malon dialdehyde), which is in many scientific disciplines considered as a ‘general oxidative stress’ indicator was tested for marine micro-algae. Experimental work with a range of marine diatoms showed that MDA

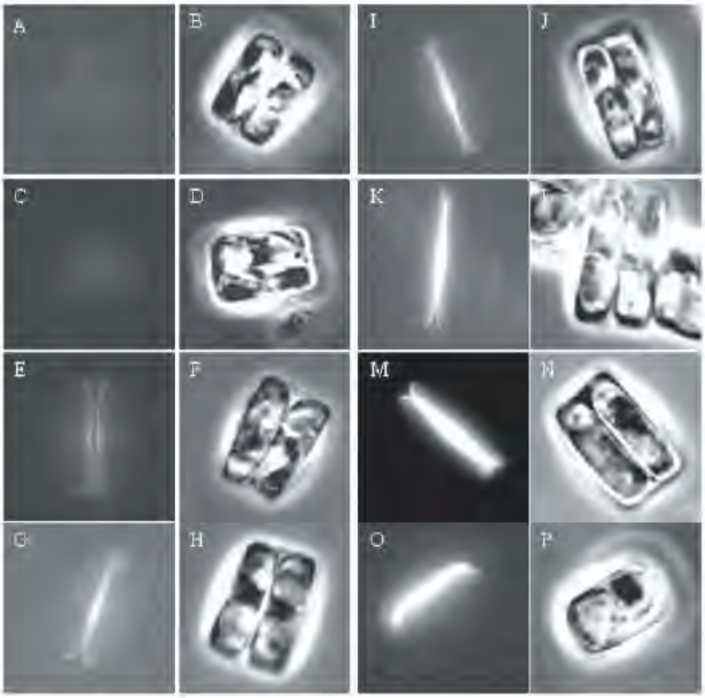

Figure 13. Valve formation in *Navicula salinarum* visualized by the PDMPO probe and observed in girdle view. Fluorescence images (A, C, E, G, I, K, M, and O) are aligned by their bright field ones (B, D, F, H, J, L, N, and P). The sequence of valve formation is as following: t = 0, before addition of silicic acid (A, B) and subsequently after addition of silicic acid and PDMPO at t = 10 min (C, D), t = 15 min (E, F), 25 min (G, H), 40 min (I, J), 60 min (K, L), 90 min (M, N), and 240 min (O, P).

content is highly variable in marine micro-algae, depending on the species under consideration and the physiological state of the algae. Cultures growing at higher growth rates as a result of higher irradiance levels show elevated MDA levels, without suffering from the negative consequences of high irradiance, for example reflected in a negative growth response. Also high light stress induced mortality (see elsewhere in this report) was not affected in many experiments where MDA levels were elevated. Simultaneously another stain was tested, for assessing intracellular oxiradical levels. First test runs, using flow cytometric end point detection were found to be promising, but more tests are required, i.e. in order to exclude possible negative effects of the (photo-oxidised) stain on the microalgae themselves. A PhD student from Argentina (V.E. Villafane, Estacion Fotobiologia, Chubut, Argentina, funded by CONICET) successfully finished and defended her thesis entitled *Ultraviolet radiation and primary productivity in temperate aquatic environments of Patagonia (Argentina).* This work was focussed on deleterious effects of ultraviolet radiation on natural plankton communities, both in marine and high altitude

(Andes) lakes (see Fig. 14). The work of Villafane has demonstrated that plankton populations from a range of Patagonian habitats are susceptible to UV stress and that an increase in UVR as a result of ozone depletion in the Southern hemisphere will affect plankton production and species composition depending on the location. Work done at an Australian Antarctic station shows that microbial diversity, as measured by DDGE and DNA sequencing, is affected by natural light regimes. Furthermore, the data clearly show distinct microbial communities in one and the same water sample: i.e. a free living community and a group of 'satellite bacteria', attached to phytoplankton cells. A next field campaign, in the new Koldewey lab, Ny Aalesund, Spitsbergen, is now prepared.

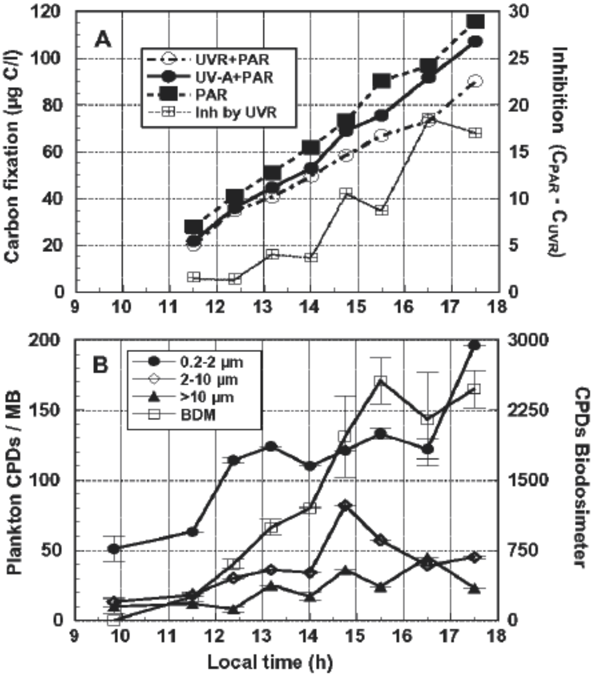

Figure 14. Daily course of UVR photosynthetic inhibition and CPD (UVB-related DNA damage) accumulation for phytoplankton from Lake Moreno. A) Carbon fixation as a function of different radiation treatments and inhibition due to UVR. B) CPD accumulation in three different phytoplankton size fractions and DNA effective dose, as measured with the biodosimeter (BDM).

Wadden Sea micro-algae. Despite large efforts that have been made in phytoplankton ecological research, important questions still remain open. E.g. it is unclear whether natural phytoplankton communities are predictable or whether it is possible to scale up from properties of single individuals to the properties and functioning of whole communities and ecosystems. These two central issues, however, are stimulated by two very different approaches. While the former question originates from a dynamic, nutrient-based approach, the latter is a result of a static, energy-based view. In this project we want to link classical nutrient-based competition theory with the energy-based 'metabolic theory of ecology' by exploring the effect of temperature on abundance, dynamics and diversity of phytoplankton.This project is a combined theoretical and experimental approach. First, computer simulations have been done where 2 species compete for 1 resource. One species was made a temperature specialist that grows very fast at a narrow and intermediate temperature range, the other being a generalist and having a slower but more constant growth rate over a wide temperature range. Under constant temperature and steady-state conditions a constant temperature level can decide over the winner, while under temperature fluctuations, amplitude and frequency of temperature variation can also determine the outcome. Besides this, preliminary observations in experiments also show a significant effect of temperature on the dominating species. For these studies, water has been sampled in the Ems Estuary near Borkum once a month from February 2005 onwards to be incubated in the lab under 5 different temperatures ranging from 3 to 26˚C. After one week, the dominating species largely depends on temperature. Interestingly, species composition differs between the replicates. This may give first indications about the stability and predictability of the system. The experiment will continue until summer 2005. Further result will show later, how temperature influences nutrient requirements, metabolism, and biomass but also whether these features are dependent of the species composition. Following steps will be the modeling of multispecies competition under fluctuation temperature and the setting-up of a continuous culture in order to create multispecies competition under temperature fluctuation and chemostat conditions.

In 2004, the European sponsored program 'Hierarchical Monitoring Method' (HIMOM) entered its final stage. Part of our task was to analyze pigment samples that were send in high numbers by the various European partners (Uni. St. Andrews, UK; ERI, Ireland; IOFCUL, Portugal; GKSS, Germany; NIOO-CEME, the Netherlands). A compilation of the data indicates a high variance between the different sites. This variation can largely be ascribed to differences in environmental conditions, i.e. differences in anthropogenic influences as well as in hydrodynamical - and geophysical conditions. Seasonal patterns also were highly variable between the sites. Whereas in the Tagus estuary algal biomass was high throughout the year, strong patterns were observed in the Eden estuary (UK). In Scotland, low winter temperatures coincided with low biomass, whereas in spring large blooms were observed.

One of the other aims of the HIMOM project was to develop a 'book of protocols', i.e., a System of Hierarchical Monitoring Methods that can be used to determine changes within intertidal areas. The book of protocols constitutes a special toolbox that consists of a wide variety of protocols that are organised according to a hierarchical system. This hierarchy is meant to provide a structure along which specific protocols can be adopted.

Figure 15. A 'cryolander' has been designed to sample the sediment without disturbing the surface layer. The sediment is rapidly frozen by the addition of liquid nitrogen. In the laboratory, the top layer is sliced in small coupes of ~ 100 μm, which thereupon can be analysed for their pigment content.

For monitoring purposes basic protocols like chlorophyll *a* biomass analyses by spectrophotometry and nutrient analyses (nitrate, phosphate) would suffice. In case of more complex research questions, more complex and (more expensive) methods are required, e.g. pigment analyses by HPLC, photosynthetic capacity by fluorescence analyses. By assigning a low hierarchical ranking to the first and a high ranking to latter type of protocols, a structure could be developed that will help specific end users (managers of ngo's, civil servants, etc.), in selecting protocol(s) that fit their specific goals. A major effort was spend in the editorship of this book.

Workgroup 'Experimental Marine Zoology'

Two papers exemplify the research progress and diversity of the Experimental Marine Zoology group during 2004. In collaboration with Videler's evolutionary mechanics research line at Leiden University, a study on the flow around a highly swept-back bird wing was finalized and published in Science. The first paper of PhD student Henk-Jan Hoving on the reproductive biology of giant squid appeared in the Journal of Zoology London.

A leading edge vortex on sharp-edged swept back wings. Wings, fins and flippers of fast animals in air and water are very often curved backwards with respect to the direction of movement. Data showing what exactly happens during the interaction between these propellers and the surrounding fluid are not available. The common swift is extremely well adapted to prolonged flight because it hardly ever lands. The wings are used in strongly swept back position during fast gliding motion. The arm part of the wing has a conventional aircraft profile with a round leading edge on cross section; the hand has a sharp leading edge (Fig. 16).

Figure 16. The common swift (*Apus apus*) in flight. Cross-sections through the arm and hand wings are added.

Physical models of a common swift wing in gliding posture with a 60° sweep of the sharp hand-wing leading edge were tested in our water tunnel. Interactions with the flow were measured quantitatively with digital particle image velocimetry at Reynolds numbers realistic for the gliding flight of a swift between 3750 and 37,500. The results show that gliding swifts can generate stable leading-edge vortices at small (5° to 10°) angles

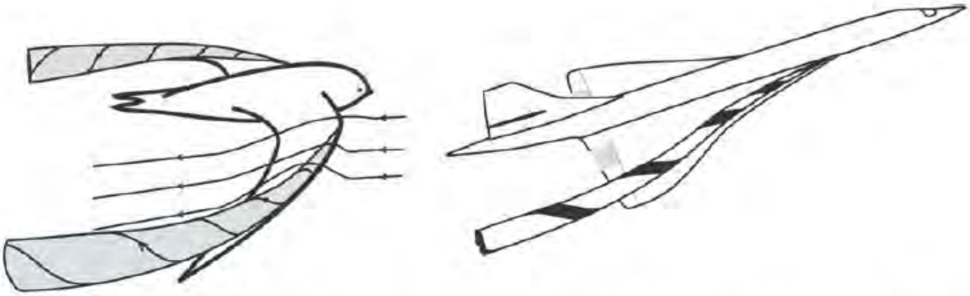

Figure 17. Left: Artist's impression of the conical LEV's on the wings of a swift in gliding flight. The oncoming flow is deflected downwards by the attached LEV-system, showing the lift generating downwash. Right: The conical spiral LEV on the wings of the Concorde.

of attack. We suggest that the flow around the arm-wings of most birds can remain conventionally attached, whereas the swept-back hand-wings generate lift with leading-edge vortices. The flow on the wing of a gliding swift closely resembles that on the wing of the Concorde (Fig. 17). That means that the current understanding of how birds fly must be revised, because birds use their hand-wings in an unconventional way to generate lift and drag.

The reproductive system of the giant squid *Architeuthis* in South African waters. The reproductive system is described from 15 giant squid *Architeuthis* sp., collected between 1972 and 2002 in South African waters. Distinctive features of the male reproductive system are the long muscular terminal organ, with elaboration of the anterior end, and modification of the tips of the ventral arms, probably hectocotilization. The spermatophoric organ has a long finishing gland that extends from the base of the gill. The terminal organ is differentiated internally into three distinct parts, involved in the guidance, storage, protection, expulsion and possibly the coating of spermatophores. Length of spermatophores in the terminal organ varied considerably. Several stages of spermatophores were found, from tentative to false to fully formed spermatophores, within a single animal. Distinctive features of the female reproductive system are a mesentery surrounding the main blood vessels of the ovary and attaching the ovary to the dorsal gladius chamber, multiple branching (at least three times) of the genital aorta that supplies the developing oocytes, high potential fecundity (3.5-6.2×106 oocytes), small eggs and short oviducts that suggest intermittent (extended) spawning. Large concentrations and single spermatangia were found in various places in females, indicating non-specific deposition. The transfer of spermatophores is probably rapid, perhaps because of considerable sexual size dimorphism (at maturity, males are much smaller than females). Implants in males are probably self-induced since the majority were found within reach of the terminal organ opening (primarily on the ventral arms in males).

Coral reef studies in Eritrea. The Eritrean PhD student Mebrahtu Ateweberhan successfully defended his thesis entitled '*Seasonal dynamics of coral reef algae in the southern Red Sea*.' The southern Red Sea is characterized by extreme seasonal variation in environmental conditions, related to the Indian Ocean monsoon system. The main theme of the study was to investigate the effects of this extreme seasonality on the reef algal community structure and on the population ecology of dominant species in shallow reef environments. The general characteristics, the factors controlling the seasonal dynamics and the life histories of these groups are described.

Yohannes Afeworki started his PhD study on: '*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*.', on the first of October.

Publications 2004

Doctorate granted by the institution, prepared within the institution

Ateweberhan, M., 2004. Seasonal dynamics of coral reef algae in the southern Red Sea: Functional group and population ecology. Promotores: prof.dr. W.J. Wolff, prof.dr. J.J. Videler, prof.dr. J.H. Bruggemann; co-promotor dr. A.M. Breeman, University of Groningen

Engelen, A.H., 2004. Flexibility without compromise: Population biology of the brown seaweed *Sargassum polyceratium* around the island of Curaçao. Promotor: prof.dr. J.L. Olsen; co-promotores dr. A.M. Breeman, dr. P. Åberg and dr. W.T. Stam, University of Groningen

Himbergen-Gondwe, M., 2004. Quantifying the role of marine phytoplankton (DMS) in the present day climate system. Promotor: prof.dr.ir. H.J.W. de Baar; co-promotores dr. W.W.C. Gieskes and dr. W. Klaassen, University of Groningen

Hoarau, G.G., 2004. Population genetics of plaice (*Pleuronectes platessa* L.) in Northern Europe. Promotores: prof.dr. J.L. Olsen, prof.dr A.D. Rijnsdorp, prof.dr. W.T. Stam, University of Groningen

Oijen, T. van, 2004. Iron and light limitation of carbohydrate production by phytoplankton in the Southern Ocean. Promotor: prof.dr.ir. H.J.W. de Baar; co-promotores dr. W.W.C. Gieskes and dr. A.M. van Leeuwe, University of Groningen

Doctorate granted by the institution, prepared at another institution

Drent, J., 2004. Life history variation of a marine bivalve (*Macoma balthica*) in a changing world. Promotores: prof.dr. W.J. Wolff, prof.dr. T. Piersma, University of Groningen

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Microbial Ecology

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

Composition of the group in 2004:

Tenured Staff	**source**	**fte**	**period**
Chair			
Prof.dr. J.D. van Elsas	RUG	1.0	0101-3112
Post-docs			
Dr. H. Bolhuis	RUG	1.0	0101-3112
Dr. F. Faber	EU	0.8	0107-3112
Dr. P.Garbeva	RUG	1.0	0911-3112
Project researcher			
Drs. P.W.J. van der Wielen	EU	1.0	0101-0108
PhD-students			
Drs. F.G.H.Boersma	RUG	1.0	0101-3112
Ing. P.Stevens	RUG	1.0	0103-3112
Drs. R.Trifonova	RUG	1.0	0105-3112
Drs. J.A.Warmink	RUG	1.0	0107-3112
Drs. S.K. Heijs	NWO-ALW	1.0	0101-1401
Guest workers			
Drs. P. Hill	Own	0.5	0101-3112
R.L.C.Cammaert	Ingeny	0.2	1506-3112
Technical and Administrative Staff			
secretaries			
M.T. Brink	RUG	0.6	0101-3112
B. Zand Scholten	RUG	0.8	0101-3112
M. Dusseljee	RUG	0.6	0101-3112
technicians			
Ing. J.K. Brons	RUG	1.0	0101-3112
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Ing. R.W. Dekter	RUG	1.0	0101-2107
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Overview of academic results

The research programme in Microbial Ecology has its focus in the bacterial niche adaptation - genome plasticity conundrum. For most of the time since its assumed origin, life has been exclusively microbial, that is, prokaryotic. Fluctuating environmental conditions, competition, adaptation to the niche and consequent evolution have resulted in the fascinating microbial diversity that is currently found in many ecosystems, with microorganisms inhabiting almost every thinkable ecological niche on the planet. This diversity is daunting by its sheer magnitude, and so far we understand just a minor fraction of it.

Microorganisms are known to play crucial roles in essentially all biochemical processes that drive ecosystems, such as the cycling of elements (carbon, nitrogen, sulfur, phosphorus, etc.); without microorganisms life, as we know it, would not exist in the same way. Understanding the ecology of the diverse microbial communities and their interaction with the environment represents one of the major challenges of current microbial ecology. At present, studies of microbial diversity and function focus mainly on organisms at the community level, using the 16S ribosomal RNA gene sequence as a marker of microbial diversity. Using culture-independent methods, an inventory is made of the microbial diversity extant in target ecosystems, 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 ribosomal RNA 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 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 rDNA-based culture-independent methods, although we do not fully understand the limits to using 16S rDNA to infer community function. This lack of sound information forms a basis for 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”.

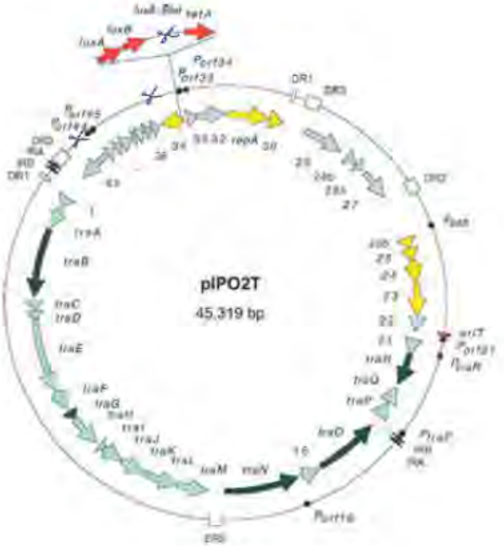
Hence, questions in respect of how bacteria adapt genomically to the environmental challenges they encounter, on what occasions they show adaptive responses, how horizontal gene transfer impacts on the adaptation process and at which speed adaptations occur, represent main topics addressed in the Microbial Ecology research programme. In terms of ecosystems, the focus will be 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. However, several other projects deal with non-soil systems, such as freshwater, deep-sea and surface marine waters, salterns and Antarctic systems.

Adaptational processes of bacteria in soil and the phytosphere

Like in any habitat, bacteria in soil and related systems can adapt to local conditions in the niche they occupy via horizontal gene transfer, genomic rearrangements and mutations. The former process allows for 'quantum leaps' in bacterial adaptation to the niche. There is emerging strong evidence for the dominant role that horizontal gene transfer (HGT) has played in the evolutionary shaping of bacterial communities in environmental habitats. Moreover, the impact of the putative capture of genetic material by bacteria from plants is becoming clear. Mobile genetic elements (MGEs) influence the behaviour of bacteria in their natural habitat, especially in the light of their occurrence in structured communities such as biofilms. Selection within the highly-structured soil/plant environment is likely to represent a dominant force shaping the genetic make-up of plant-associated bacterial communities, as it can act as an apparent accelerator of gene transfer. However, the current understanding of the triggering and impact of horizontal gene transfer remains limited by our lack of understanding of the nature of the selective forces that act on bacteria in situ. Therefore, a full understanding of the role of HGT in the adaptation of host bacteria to their environmental niche is still lacking.

The ecological role of plasmids with high gene-mobilizing capacity in plant-associated bacterial communities

In this Ph.D. project, the ecological impact of exogenously-isolated plasmids in bacterial communities that inhabit the phytosphere of crop plants is studied. The project makes use of the full sequence of a key gene mobilizer plasmid obtained via exogenous isolation from the wheat rhizosphere, named pIPO2. The plasmid was shown to confer gene-mobilizing and retromobilizing capacity to its host, and possesses the genetic machinery for establishment of a type IV secretion system in its host. The project focusses on the consequences of plasmid carriage for the ecological behaviour of the bacterial host, in particular taking into account the presence of a small region with open reading frames putatively encoding proteins of unknown function. Furthermore, the potential relevance



of carriage of this highly efficient gene-mobilizing element in respect of enhancing the adaptability of plasmid-carrying host strains in the phytosphere is addressed.

In an attempt to determine the original host species, a screening has been initiated on bacteria from the rhizosphere cultured on different media. Different primer sets, specific for two key regions of pIPO2 and pIPO2-like plasmids (reporting on the presence of *repA* and *virB10* producing genes), are unleashed on the bacterial populations from the rhizosphere of different plants. So far, at least eight candidate isolates looked promising as plasmid carriers. The strains mainly belonged to the γ-proteobacteria. Furthermore, to obtain a glimpse of the divergence and evolution of pIPO2-like plasmids, DNA was isolated from rhizosphere and bulk soil samples, and the same primer sets as mentioned above were unleashed on the total DNA. The amplified products are cloned and phylogenetic trees constructed. A key question here is whether there is a difference in diversity between rhizospheres of different plants and different soils, i.e. whether different rhizospheres tend to select different plasmid types.

To asses the function of the region of small ORFs, we started the construction of a strain containing pIPO2 lacking the contiguous region in which the small ORFs are localized. This strain will be compared to a strain containing the fully functional pIPO2 plasmid, with respect to, for instance, the colonisation of the rhizosphere.

Transfer of DNA from genetically modified plants to bacteria

The number of genetically modified plants being constructed worldwide is continually increasing, which has brought about a debate on the possibility of uncontrolled spread of transgenic DNA through the environment. A study of the available recent literature on Horizontal Gene Transfer (HGT) from transgenic plants to bacteria was performed, commissioned by the Netherlands Commission on Genetic Modification (COGEM). This literature study focussed on the (interkingdom) transfer of transgenic DNA from plants to bacteria, and in addition the topic was discussed in a two-day workshop (April 2004) with experts in the field.

HGT processes between bacteria cover conjugation, transduction and transformation. However, natural transformation is clearly the single key mechanism that can mediate the transfer of genetic material from plants to bacteria. The likelihood (frequency) of occurrence of gene transfer from plants to bacteria was found to depend on a range of ecological factors (mainly the occurrence of so-called hot spots and selection) as well as intrinsic genetic factors (e.g. sequence homology). Persistence of transgenic plant DNA in the environment is an important factor, which depends on the ecological factors reigning in the environment (e.g. soil, rhizosphere or gastrointestinal tract). In general, most released DNA is also degraded into smaller fragments (<200 and 400 bp), but protection against breakdown has also been observed. Furthermore, there is increasing evidence that larger numbers of bacteria than hitherto known are capable of capturing DNA in the environment. However, such transfer has so far been chiefly observed under rather artificial (laboratory) conditions in microcosms.

The presence of sequences in a transgenic plant with homology to sequences in bacterial genomes is a key factor for successful transfer of a transgene from plants to bacteria via natural transformation. In this respect, also the DNA sequences flanking the transgene in a transgenic plant are important. If these show homology to bacterial DNA, they can serve as 'anchor' sequences for incoming transgenic DNA and facilitate horizontal gene transfer. In the absence of any regions of homology of transgenic plant DNA with bacterial DNA, transfer to bacteria has not been reported so far (detection limit $<10^{-13}$). However, DNA that enters bacterial genomes via homologous recombination might simply not have been fixed due to the short time GM plants are present in our ecosystems.

The selectability (positive selection) of genes in case of a transfer out of transgenic plants should be a key criterion in the assessment of the potential impact of such a transfer (cf. antibiotic resistance genes). Transferred transgenic DNA will only have an impact in bacterial communities when it is 'correctly' integrated into the genome, which would allow its expression. Subsequently, fixation and spread of a transgenic gene in a bacterial population depends on its potential to raise the fitness of the recipient bacterium, leading to selection of the trait and proliferation of the organism.

Based on the available literature, but also concluded at the workshop on this topic, transfer of transgenes from plants to bacteria could not be excluded. However, taking into account all the prerequisites which should be fulfilled, the occurrence of such an event is highly unlikely. Furthermore, successful transfer of transgene from plants to bacteria in the environment has not yet been reported in all available literature.

Metagenomics - the quest for bacterial antibiotic biosynthetic operons from soil

Under the EU Metacontrol project, the Microbial Ecology group is involved in a metagenomics approach to the mining of the antibiotic biosynthetic machinery of soil bacterial communities, and to assess the role of antibiosis in soil microbial communities. We attempt to place the prevalence and diversity of such biosynthetic operons in the context of the extant soil bacterial diversities. The search for interesting biosynthetic operons is conducted in collaboration with several European partners. Evidence was obtained for the presence in soil of sequences presumably belonging to a novel class of antibiotic biosynthesis operons, which have not been detected before. Perhaps surprisingly, we also obtained evidence for the contention that the gene clusters involved might be localised on flexible DNA and thus make part of the flexible gene pool. We are currently investigating this link, and extend the findings to pose and test novel hypotheses on the role and evolution of such operons as molecular ticks in soil microbial communities.

Bacterial interactions with mycorrhizal fungi

Mycorrhizal fungi play an important role in the growth and development of plants and trees in ecosystems. The fungus delivers certain salts and water to the roots of the plants and also protects the plant against some pathogens. In return, the fungus receives sugars as energy source from the plant. Recent publications suggest that some, possibly specific, soil bacteria play an important role in these symbiosis systems. Until now, research in this area has been mainly focussed on the fungal partner in the interaction between bacteria and mycorrhizal fungi, and the exact role of bacteria in these systems has remained largely unknown. For instance, the genetic systems present in bacteria that are used to allow successful interaction with the fungal counterpart are unknown. The main goals of this project are to find answers on the following questions. What types of bacteria can successfully interact with fungal hosts? What genetic systems are involved, and what is the evolutionary or adaptational nature of these systems? Hence, in this Ph.D. project, the interaction between bacteria and mycorrhizal fungi is studied, with an emphasis on mechanisms that support such interactions. To find and identify bacterial strains that show an interaction with mycorrhizal fungi, we set out to use simple systems in which those bacteria that turn out to be fittest in the mycorrhizosphere are being selected. Finding mycorrhiza-adapted bacterial strains is the first goal of the project. After the identification of interacting bacteria, the project will continue with the study of genetic systems that are used by the bacteria to interact with their fungal partner. The bacterial strains are being isolated from naturally-existing symbiosis systems in the Netherlands. Also, *in-vitro* systems are being created to study the interaction between fungus and bacteria. Both ectomycorrhizal and arbuscular mycorrhizal fungi will be used in this project. The first data on bacteria that show putative interaction with mycorrhizal fungi revealed several promising candidate strains; these strains are currently being studied in respect of their interactive capabilities.

Adaptational processes that allow Ralstonia solanacearum *to survive under temperate climate conditions*

The Gram-negative bacterium *Ralstonia solanacearum* is a well known plant pathogen that causes wilting in a variety of plants and is responsible for great economic losses in tropical and subtropical areas. In the nineties, wilting disease was increasingly observed in temperate climate zones (biovar 2). Despite efforts to prevent re-introduction and spread, *R. solanacearum* was still detectable in the Netherlands in 2004. Two major causes for its persistence in Europe are the colonization of *Solanum dulcamara* (bittersweet), which is known to serve as a reservoir plant for *R. solanacearum*, and the potential of *R. solanacearum* to survive in canal sediment under local conditions.

Because survival of *R. solanacearum* in temperate climate zones (partially) depends on its capacity to cope with low temperatures, in this Ph.D. project we study the effect of cold stress on the genome. In 2004, *R. solanacearum* strains were isolated from canal sediment, bittersweet and surface water in a region where infected potato fields were reported in 1995. These strains were compared with other (sub)tropical biovar 2 strains using different molecular techniques such as fingerprinting and screening for IS elements.

It has previously been shown that *R. solanacearum* 1609 cells kept at 4˚C enter a VBNC state and loose virulence. To determine which genome changes occur during this process, several PCR systems were developed for relevant genomic regions. One of these was specifically designed to investigate a pathogenicity island involved in Type III secretion. In addition to using specific molecular techniques, the comparison of complete genomes (cold stressed vs WT) by using subtractive hybridization, DNA-chips and PFGE will hopefully lead to a better insight in how *R. solanacearum*, at a genetic level, responds to cold stress.

Microbial maturation of novel substrates

In this Ph.D. project, we aim to assess how micro-organisms are able to colonize a microbiological vacuum, i.e. thermally-treated grass fibers. The project is executed in collaboration with Plant Research International, Wageningen. What microbial players and what mechanisms play a role and what is the outcome? Can microbiological stability, i.e. the medium- to long-term persistence of a community of stable composition, be achieved? Can phytotoxic compounds be removed by the microbial colonizers of the substrate? So far, the work has focused on three major issues: the microbial colonisation of grass substrate, phytotoxicity diminishment in the grass fibers and a quest for the microbes which break down the phytotoxins.

PCR-DGGE bacterial fingerprintings demonstrated diverse bacterial communities developed in both grass fiber and grass extract. Community differences were found according to the inoculum and the nitrogen source used. Some populations were present during the total incubation period (7, 14, 21 and 28 days). Therefore, a stable community might have established on the grass substrate.

Evidence was found for the contention that bacteria from different phylogenetic groups (alfa-proteobacteria, gamma-proteobacteria, sphingobacteria) could colonise the grass fibers. Increases in the populations of bacterial spores, oligotrophic bacteria, filamentous actinomycetes and fungi were found upon inoculation of grass with a strongly diluted cell extract from soil.

Lettuce seed germination was strongly inhibited by extract from thermally-treated grass, which indicates that grass contains undesirable phytotoxic compounds. A ‘fireside’ soil microbial community was found to be a reliable source for isolation of strains in a comparison with two other soil communities (Wildekamp and forest soils) used as initial inocula for grass colonisation. After three subsequent enrichments, 44 strains were isolated. These were tested for their ability to diminish the phytotoxicity in the grass substrate. Five strains revealed enhanced seed germination.

Biological hydrogen production (EET-BWPII)

There are limited reserves of fossil fuels on Earth and the combustion of the fuels leads to serious problems such as global climate changes. It is widely acknowledged that hydrogen is an attractive clean energy source, which can be produced from biomass, as a product of biosynthesis. Energy crops or organic waste streams are thereby replacing the conventional fossil fuels. Biomass conversion can be realized by photosynthetic bacteria using sugars from lignocellulosic materials. The overall Biohydrogen project (EET-BWPII), in which the RUG participates, is a multi-step process, aiming at the complete conversion of biomass, energy crops or organic waste streams, into hydrogen and carbon dioxide. In short, it comprises three process units: (1) Biomass pretreatment; (2) dark fermentation; (3) photoheterotrophic fermentation.

The Microbial Ecology group is involved in the third process. Photosynthetic purple non-sulfur (PNS) bacteria are able to convert short-chain organic acids to hydrogen and carbon dioxide. PNS bacteria utilize sunlight to 'push' this reaction. Light energy is absorbed by their photosystems and used to liberate reducing equivalents from the organic acids and water. Supplied with reducing equivalents, the nitrogenase enzyme-complex is able to reduce protons to hydrogen. Although the main function of the nitrogenase enzyme-complex is to fix nitrogen during nitrogen-limitation, as a byfunction it can also catalyze the formation of hydrogen from protons, even in the absence of nitrogen. This process has been studied for many years. However, acetate was only rarely used as a potential substrate. Theoretically, 4 moles of hydrogen can be produced from 1 mole of acetate, but part of the acetate consumed will also be used for biomass production. The objective of the RUG is to characterize the metabolism of acetate, especially from various *Rhodopseudomonas* strains. Several important process parameters can/will be identified, which have an effect on the total hydrogen production.

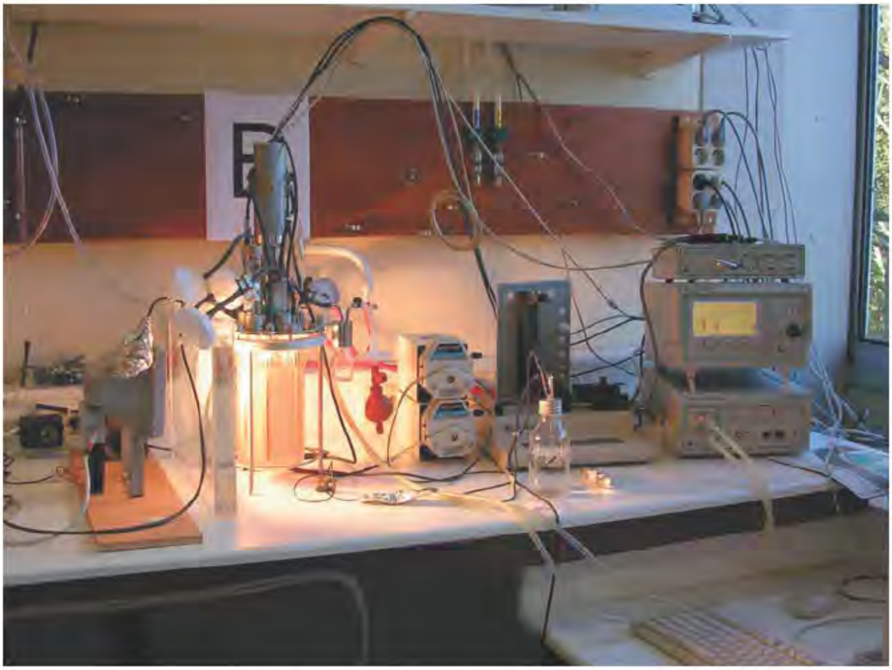


Figure 1. Continuous culture experiments with photoheterotrophic growth of *Rhodopseudomonas palustris* on acetate.

Currently, we perform long-term studies on the growth of *Rhodopseudomonas palustris* on acetate in chemostats (continuous cultures, Figure 1), using H_2-producing conditions (ammonium-limitation) as well as non-H_2-producing conditions (system flushed with N_2). Experiments clearly showed substrate inhibition above 20 mM acetate. Furthermore, with sufficient nitrogen present, acetate was fully degraded, showing only small amounts of intermediates (µM-range) from the citric acid cycle. During nitrogen (ammonium) limitation, acetate was not yet fully degraded and now are optimizing culture conditions. Moreover, nitrogen limitation resulted in the (unprofitable) production of polyhydroxybutyrate (PHB) and probably also the production of an extracellular polysaccharide (EPS), resulting in a clear formation of biofilms. We are currently studying the nature of this EPS.

From our vast collection of *Rhodopseudomonas* strains, isolated form the environment, several strains were selected for a preliminary genetic characterization of the differences in nitrogenases (*nifH* gene) and uptake hydrogenases (*hupSL* genes). Furthermore, using batch culture experiments, the acetate metabolism was also studied in these strains. Preliminary results clearly indicate major differences in the acetate metabolism. Future research will focus on the acetate metabolism under nitrogen limiting conditions in *R. palustris*, but similar experiments will be performed in *Rhodobacter capsulatus*. In addition, 'knock-out' strains will be constructed (e.g. for PHB-production) to study its effect on the efficiency of hydrogen production.

Haloarchaeal diversity along a natural salinity gradient

Hypersaline ecosystems form ideal model systems to study microbial evolution and niche adaptation. These coastal basins are easily sampled and have a relatively low microbial diversity. Due to the requirement of high salt concentrations, halophiles are easily cultivated without having to worry about contamination by the wide range of microorganisms and fungi that can frustrate microbial work. A large collection of halophilic archaea and some halophilic bacteria has been generated that are characterized by 16S rDNA analysis and molecular fingerprinting. The latter allows us to determine the natural genetic diversity within species. Samples were collected from salterns with different salinities and from different geographic locations. Combining genetic data with environmental parameters, we might get insight in the factors driving species diversity. The extant diversity is the result of mutation rate in the population versus the selection pressure. A low within-species diversity may result from low initial mutation rates or, alternatively, from high mutation rates in combination with a high selection pressure that diminishes the generated diversity.

Laboratory experiments are required to further investigate the role of mutation rates and selective pressure on halophilic archaea at different salinities.

Isolation, cultivation and characterisation of Walsby's square archaeon

In 1980, A.E. Walsby described a unique square halophilic archaeon. Its unique stamp-like shape raised many questions about how such a cell morphology and especially the straight corners can be formed. In addition to its weird morphology, the organism is abundant in different hypersaline ecosystems on earth. It can make up to approximately 40 to 60% of the total prokaryotic community at the highest, near saturating salinities, which suggests an important ecophysiological role. Undoubtedly, cultivation of the square archaeon is essential to understand their ecophysiological role and to unravel the nature of their unique morphologically features. However, ever since its discovery and despite their abundance and easy recognition by microscopy all cultivation attempts have failed up to now, marking the organism as one of the unculturables. In 2003, the square archaeon, *Haloquadratum walsbyi*, was isolated through long periods of enrichment and cultivation in low nutrient media. In 2004, a collaboration was started with D. Oesterhelt and F. Pfeiffer (Max Planck Institute for Biochemistry, Martinsried, Germany) and F. Rodriguez-Valera (Universidad Miguel Hernandez, Alicante, Spain) to sequence and analyze the genome of *H. walsbyi*. By this joint effort we were able to fully sequence the genome and start the annotation. Initial data revealed several unique genes that give insight in the nature and adaptive properties of *H. walsbyi* that allows it to survive under the harsh environmental conditions

Microbial ecology of brine lakes in the Mediterranean Deep Sea

The interdisciplinary research program entitled ‘BIOtechnologies from the DEEP (BIODEEP)’, started in 2001 and finished April 2004. It consisted of several scientific cruises in which four hyper-saline basins (L’Atalante, Bannock, Discovery and Urania) located at more than 3000 meters below sea level in the Mediterranean Sea were sampled. Combined analysis of the geochemical composition and 16S rDNA sequences amplified from DNA retrieved from these basins, revealed that each basin harboured its own unique microbial community and an equally unique geochemistry. From this, it is concluded that geochemical conditions drive the evolution of prokaryotic communities in these basins. 16S rRNA gene sequence analysis showed that high percentages of sequences belonged to γ-, δ-, and ε-Proteobacteria, Sphingobacteria, candidate division KB1, halobacteria and a completely new archaeal division termed MSBL1.

The Discovery brine is unique since in contrast to the other three NaCl rich brines, it is almost saturated with $MgCl_2$ (5 M) and represents one of the most saline environment known on earth. Due to the extremely low water activity, such an environment is expected to be sterile. Up to now only some haloarchaeal species are among the most $MgCl_2$ tolerant microorganisms, with growth occurring at 1 M $MgCl_2$. Nevertheless, Discovery basin appears to contain a unique, presently metabolically active microbial community. The brine contains $1.9 * 10^4$ microbial cells ml-1 and most of the 16S rRNA gene sequences are related to phylogenetic groups not found in normal seawater, indicating that a specific microbial community is present in the Discovery brine. Together with the detection of metabolic activities for different processes in the brine, these data suggests that life exists in such an extreme environment. In the final stage of the project, experiments were performed to study the diversity and distribution in functional genes, indicators for important geochemical processes such as methane oxidation, sulphate reduction and sulphide oxidation. Our manuscript describing the microbiology and geochemistry of the four basins and providing proof for life in the 5 M MgCl2 Discovery basin was accepted for publication in Science and published January 7th 2005.

Adaptation of bacteria to environmental change over the last 2,000 years - the fossil record in an Antarctic Firn Core (NAAP project)

Human activity has changed the chemical and physical composition of Earth's atmosphere at an increasing rate over the last two centuries; the accumulation of 'greenhouse gases' has had the most dramatic consequences (melting of the polar icecaps and glaciers worldwide; disruption of ecosystems). The degree of biological adaptation to these globe-wide environmental changes is unknown, but adaptational responses of ecosystem components in the past may be revealing in this respect, allowing predictions under future 'global change' scenarios. This is the reason why the fossil record receives much attention nowadays ('hindcasting'). The Antarctic forms an ideal fossil storeroom where even microorganisms are trapped, covered by snow and subsequently frozen into the permanent ice-cover. Ice cores still containing viable microorganisms have been dated back as far as 530,000 years before present.

In this project, we study how communities of prokaryotes, the key players in most biogeochemical cycles, have adapted to fluctuations in the climate over the last 2,000 years. A 160-meter deep firn core will be used which was drilled at site M (75˚S, 15˚E) in 2001 at the Antarctic polar plateau by a Norwegian/Dutch expedition as part of the European Project for Ice Coring in Antarctica (EPICA). The age and composition of the entrapped ancient atmosphere is known. In order to identify the genetic basis of evolutionary adaptation to the range of environmental conditions that succeeded one after the other over the period to be sampled, it is not sufficient to simply analyze microbial communities by culture-independent methods based on 16S-rDNA sequences. Indeed, random genetic changes may affect the relative fitness of a population without necessarily affecting the community composition. Samples will be taken from the cores (under conditions that prevent contamination with present day microorganism) to initiate cultivation experiments. Since complete sequencing of the genomes of multiple isolates is impossible, the best way to track down genetic changes is by using a combination of various molecular fingerprinting techniques (rep-PCR, PFGE). Multi-locus sequence typing will be applied to look for changes in the nucleotide composition of genes encoding proteins that are involved in carbon and nitrogen fixation and genes involved in mediating resistance to UV irradiation, that may have changed considerable over time also in the past. This project is being performed in collaboration with the institute for atmospheric research (IMAU) of the University of Utrecht and the Norwegian Polar Institute in Tromso, Norway.

So far, the project has been hampered by the inability to cultivate microorganisms from the firn-ice cores using standard cultivation techniques. Cultivation is intensified by changing medium composition and using micro-titerplates that allow us to generate an array of different nutrients, physical parameters (pH, salinity). Molecular tools (16S-rDNA gene amplification and sequence analysis) are applied to identify the microorganisms present and enabling us to design specific media. Pending the cultivation of Antarctic microorganism molecular tools and insight in evolutionary processes are developed using a spatial gradient of halophilic organisms growing at different salinities. Similar organisms are also present in Antarctic salt lakes and might be even better adapted to survive at low temperatures.

Bacterial diversity in deep sea mud volcanoes and fluid seeps

This Ph.D. work has been largely rounded off in 2004, and will lead to a Ph.D. thesis in June 2005. The text below is an excerpt of the findings reported in 2004.

In the past three decades mud volcanoes and fluid seeps have been discovered along the Eastern Mediterranean Ridge and were linked to tectonic activity in this area. Two of these areas (Olimpi Mud Volcano area (OMV) and Anaximander Mountains area (AM)) were studied in Dutch-French expeditions MEDINAUT and MEDINETH in 1998 and 1999. These studies revealed details on the occurrence of cold seeps and brine lakes on several mud volcanoes and showed the presence of communities of higher organisms including mollusks, tube-worms, urchins, shrimps and fish. A strong dependence of Eastern Mediterranean cold seep communities on methane was tentatively correlated with a higher abundance of macrofauna at sites of apparent greatest fluid emission. This was supported by lipid biomarker studies which showed that chemoautotrophy with methane as the main electron donor and carbon source supports the microbial communities in deep-sea sediments and carbonate crusts on mud volcanoes in the Eastern Mediterranean.

Knowledge on deep-sea microbial communities in the Eastern Mediterranean Sea is limited. Therefore we investigated microbial communities in carbonate crusts, a microbial mat and sediment samples collected from geographically distant mud volcanoes in the Eastern Mediterranean deep-sea with 16S rRNA gene sequence based techniques and compared these with chemical data.

Bacterial 16S rRNA gene sequences obtained were related to *Actinobacteria*, *Bacilli*, *Chloroflexi*, α-, β-, δ-, ε-, γ- *Proteobacteria* and unclassified novel bacteria, whereas archaeal sequences were affiliated with *Methanosarcinales*, *Thermoplasmales*, *Halobacteriales*, novel *Crenarchaea* and *Marine Group I* archaea. These bacterial and archaeal communities were diverse and unique at a species level for each of the sites studied, but similar microbial communities were found in comparable ecosystems and sediment depth layers at a phylogenetic group level. Linking sequence data to potential metabolic processes, our data suggests that aerobic heterotrophy and aerobic sulfide and methane dependant chemotrophy were predominant in the microbial mat and most of the mud volcano surface sediments studied. In addition, anaerobic processes such as the anaerobic oxidation of methane (AOM), sulfate reduction and anaerobic heterotrophy were predominant in deeper mud volcano sediment layers. The potential for these processes was supported by chemistry data.

We conclude that sulfide and methane dependent chemotrophy is important in Eastern Mediterranean deep-sea cold seeps. In addition, linking potential metabolic processes with a high species diversity and similarity among prokaryotic communities in the geographically distant sites studied, we conclude that our data support a conservation of function in the sediments of the Eastern Mediterranean mud volcanoes.

Effects of ultraviolet radiation on the diversity of marine microbial communities
This project is conducted in collaboration with the department of Marine Biology of the University of Groningen and the Australian Antarctic Division, Australia
Reporting over 2004 can be found in the report by Marine Biology.

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Workshop

'Transfer of genetically modified DNA from plants to bacteria', April 22-24, 2004, University of Groningen, Microbial Ecology, Biological Center, Haren

Report

Faber, F., van Elsas, J.D. (2005) Transfer of DNA from genetically modified plants to bacteria. COGEM Onderzoeksrapport: CGM 2005-02

Theoretical Biology

Group leader prof.dr. F.J. Weissing

Composition of the group in 2004:

Tenured Staff	**source**	**fte**	**period**
dr. C.K. Hemelrijk	RUG	1.0	
dr. I.R. Pen	RUG	1.0	
prof.dr. F.J. Weissing	RUG	1.0	
Postdocs			
dr. T.W. Fawcett	NWO-ALW	1.0	030301-041001
dr. G.S. van Doorn	RUG	0.8	041101-061201
dr. W.M.C. Edelaar	RUG/Honorary scholarship	0.5	031115-040115
PhD students			
drs. J. Bakker	NWO-WOTRO	0.8	020101-041101
drs. T.W. Berngruber	RUG Ubbo Emmius	1.0	020501-060501
drs. V.S. Brauer	RUG	1.0	040801-080801
drs. G.S. van Doorn	RUG	1.0	990801-040401
drs. B.V. Feldmeyer	Robert Bosch Stiftung	1.0	040901-080901
drs. M. Hinsch	VICI Jan Komdeur	1.0	040901-080901
drs. M.A. Kozielska	RUG VICI Beukeboom	1.0	030923-070923
drs. H.P. Perez de Vladar	RUG TT Pen	1.0	040901-080901
drs. T.A. Revilla-Rimbach	NWO	1.0	041101-081101
drs. J. Steinhauser	RUG Rosalind Franklin	1.0	040801-080801
drs. W.K. Vahl	RUG Breedtestr 1	0.8	000401-050801
drs. T. Veen	NWO-ALW	0.8	020401-070401
drs. H. Verkiel	RUG Breedtestr 2	1.0	030901-070901
Other PhD students			
drs. J. Wantia	University of Zürich		
drs. P. Girod	University of Zürich		
drs. H. Kunz	University of Zürich		
Administrative Staff			
secretary			
G. Ferber	RUG	0.5	

Guests

prof.dr. Isabelle Olivieri	University of Montpellier	January and April 04
dr. W.M.C. Edelaar	University of Leiden	April/May 04

Introducing the group

This is the first time that the Theoretical Biology group presents itself as a separate research unit. In the past, theoretical research on ecology and evolution was a research line within the Evolutionary Genetics group. Recently, the Theoretical Biology group started to grow rapidly (due to the appointments of Charlotte Hemelrijk and Ido Pen to tenure track positions), at the same time expanding its research area beyond questions related to evolutionary genetics. Therefore, it was decided to split it off from Evolutionary Genetics. Since 2004, the Theoretical Biology group forms a separate research unit with a separate research programme. Of course, there are still close links and close collaborations with the Evolutionary Genetics group.

Bridging the gap between theoretical and empirical research is a task of major importance for progress in evolutionary ecology. The Theoretical Biology 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 this field of 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:

Dynamics of ecological interactions. It is a major challenge, both from a fundamental and from an applied point of view, to obtain a better understanding of the dynamics of multi-species interactions. Even seemingly ‘simple’ interactions, like competition for nutrients, tend to be highly complex when more than three or four species are involved. However, systems that appear chaotic and unpredictable at the micro-level may look regular and predictable when viewed from a higher-level perspective. It is one of the goals of the programme to unravel whether, and in what sense, the ‘micro’ view of population ecology is consistent with the ‘macro’ view of community ecology. A second, independent goal is to find out whether, and to what extent, ecological insights are affected when approached from an evolutionary perspective. Ecologists tend to treat ecological parameters as fixed and externally given, even though these parameters are the result of an evolutionary process. Many parameter combinations considered by ecologists are not ‘reasonable’ from an evolutionary point of view, since they are not evolutionarily stable. Hence evolutionary arguments can be used to delimit the ‘relevant’ part of parameter space. By doing this we expect to obtain important new insights into the nature and dynamics of ecological interactions.

Evolutionary ecology of sex. As indicated above, ecology may learn a lot from evolutionary theory. The converse is true also. The fitness parameters of selection models are, for example, usually derived in an ad hoc way from plausibility considerations. The resulting model populations grow or decline exponentially and are obviously not ‘ecologically stable’. This is problematic, since ecological factors (like density-dependence) may strongly affect the course and the outcome of evolution. It is therefore one of our goals to follow an eco-evolutionary approach where evolutionary parameters, like fitness, are derived systematically from mechanistic and ecological considerations. In parallel, we develop new analytical tools that allow to cope with the increased complexity of the resulting models. This mechanistic approach is applied to a variety of research questions. Many of these questions are related to the causes and consequences of sexual reproduction. In close collaboration with empirical biologists, we try to contribute to the solution of questions like: Why are sex determining systems so diverse and rapidly evolving? What explains the enormous diversity of gamete recognition proteins? Why are sex ratios often close to unity, while theory predicts extreme sex ratios varying with local circumstances? How is sexual selection affected by sexual antagonism and the genetic basis of traits related to mating? Is sexual selection the key to understanding sympatric speciation?

Self-organization of social systems. In many biological systems, the understanding of patterns and processes is enhanced by the explicit consideration of different levels of organization. Seemingly well-understood phenomena at a higher level of organization can often in a surprising way be re-interpreted as emergent properties from the interactions among units at a lower level. For instance, in a model of groups of individuals that perform dominance interactions, a difference in a single parameter (intensity of aggression) affects the social structure in such a way that the society switches from the many characteristics of a despotic society to that of an egalitarian society. Thus, complex differences between societies may be caused by a difference in a single trait at a lower level, and the evolution of social systems may, correspondingly, reflect the evolution of a few individual traits. By applying principles of self-organization, we aim at a better understanding of the emergence and the evolution of complex structures in terms of lower-level processes. 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 the Theoretical Biology research programme.

Overview of academic results

In the research line ‘Dynamics of ecological interactions’ two PhD projects were started in 2004. In the project ‘Emergence of biocomplexity’, Tomás Revilla-Rimbach will investigate theoretically whether the interaction of many species, despite its inherent complexity, will lead to communities that are governed by relatively simple rules. In particular, we will address the question whether, and to what extent, multispecies systems can be characterized by allometric scaling laws, as is claimed by metabolic theories of ecology. This project will be performed in collaboration with the Community and Conservation Ecology group. In the project ‘The effect of temperature on diversity, abundance and dynamics of phytoplankton’, Verena Brauer will test these ideas with multispecies cultures of phytoplankton obtained from natural waters. In particular, we will investigate whether replicate cultures develop in parallel or whether they diverge from each other. By this, we hope to shed some light on the question whether and under what conditions the species dynamics in natural waters is inherently predictable. This project is performed in collaboration with the Marine Biology group.

Below, an overview is given of some of the results obtained in the other research lines. In particular, Sander van Doorn presents highlights of his thesis on ‘Sexual selection and sympatric speciation’, which was defended in October 2004. On the occasion of the thesis defense, we organized an international symposium on sympatric speciation, which attracted more than 100 participants. Speakers included Sergey Gavrilets (Knoxville), Ulf Dieckmann (Laxenburg), Trevor Price (Chicago), Olof Leimar (Stockholm) and Hans Metz (Leiden). The Dutch Zoological Society awarded Sander van Doorn with the Dutch Zoology Prize. This is remarkable, since it was for the first time that a theoretical thesis was awarded with this prize.

Sexual selection and sympatric speciation

Sander van Doorn

In the last couple of years there has been a revival of interest in the process of speciation. In particular, it has become clear that speciation might to a much larger extent be governed by adaptive (and, hence, predictable) processes than envisaged by classical speciation theories, where speciation is usually assumed to be initiated by external factors, such a geographical segregation. Two recent theoretical developments, from different lines of research, have alleviated two longstanding difficulties in the theory of sympatric speciation. First, sympatric speciation requires, almost by definition, the evolution of a specific mating structure enabling reproductive isolation. Classical models had problems to explain the evolution of assortative mating under general and plausible conditions. A suite of new models demonstrates that these problems can be overcome if sexual selection is the driving force behind the evolution of reproductive isolation. Second, reproductive isolation is not sufficient to ensure the sympatric coexistence of daughter species. These incipient species can only survive if reproductive isolation is associated with ecological differentiation. Only recently, evolutionary branching theory has provided a plausible mechanism for the evolution of ecological differentiation in the presence of disruptive selection. Based on these new insights, a new generation of 'ecological' and 'sexual selection' models of sympatric speciation has been developed. The two research lines focus on two different aspects (ecological differentiation and reproductive isolation), which are both crucial for a full understanding of speciation. Yet, both lines have largely been developed in isolation.

To provide a conceptual bridge between the two lines, we developed and analyzed a combined model, which, for the first time, integrates the ecological and sexual selection aspects

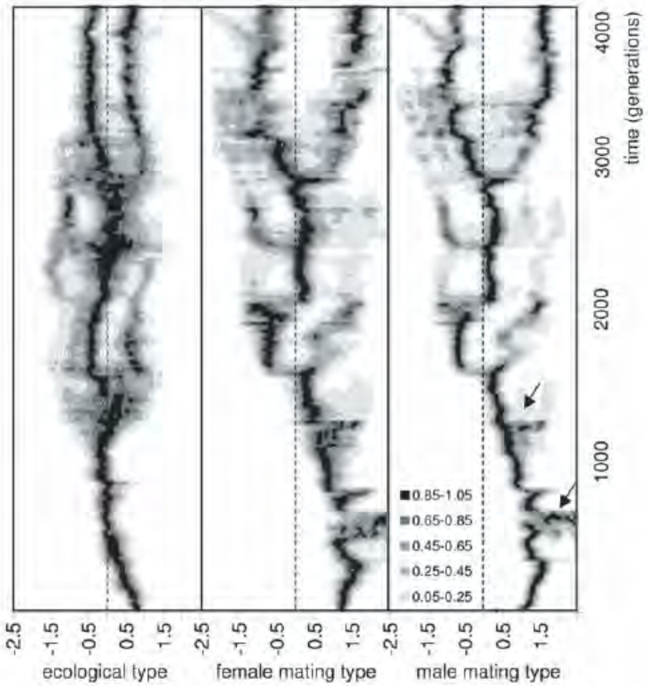

Figure 1. Sympatric speciation by the simultaneous evolution of ecological differentiation and assortative mating. The three panels show the distribution of ecological and mating types during 4000 generations of evolution. In the first phase of evolution, ecotype evolves towards the ecological optimum (dashed line). At the optimum, the population experiences disruptive selection. This does lead to an increase in the variation of ecotypes, but not to ecological differentiation, because assortative mating has not yet evolved. During this initial phase (the first 1500 generations), male and female mating type evolve jointly towards the optimum for male survival (dashed line). Several times (arrows), polymorphisms of mating type originate, but these turn out to be unstable due to competitive exclusion and viability selection against extreme mating types. After 1500 generations, simultaneous branching of ecotype and mating strategies repeatedly splits the population into groups that are ecologically differentiated and, at the same time, reproductively isolated.

of sympatric speciation (Van Doorn & Weissing, 2001; see Fig. 1). Using a combined analytical and individual-based simulation approach, we were able to show that sympatric speciation is feasible, and that it indeed requires the simultaneous development of ecological differentiation (‘ecological branching’) and assortative mating (‘mating type branching’). Both types of evolutionary branching can be understood as the outcome of a competition process in which individuals compete for a spectrum of either ecological resources or mating opportunities.

Our analysis highlights that an integrated ‘eco-evolutionary’ view is required to arrive at a really convincing theory of speciation. Keeping this basic insight in mind, we then specifically focused on the role that sexual selection plays in this context. In fact, several questions had not yet been answered satisfactorily by our speciation model. Like all other sexual selection models of sympatric speciation, our model required a high variation in female preferences to obtain divergence of male mating types. In our model, this variation was the result of mutation-selection balance, i.e. of a combination of high mutation rates and weak selection. Other models assumed a dramatic change in the environment, exposing previously hidden preferences to selection. These and similar assumptions are not very satisfactory in that they, again, require external or non-adaptive factors to get the process of sympatric speciation started.

To explore the possibility of a truly ‘adaptive’ road towards sympatric speciation, we investigated a model where the variation in female preferences could be caused by diversifying selection, rather than by mutation or by external events (Van Doorn, Dieckmann & Weissing, 2004). By means of a general argument, verified by individual-based computer simulations, we could show that selection on female preferences has to be frequency dependent in order to get divergence of male and female mating types. If the fitness of females only depends on their own preference, and not on the preferences of other females, mating type branching cannot occur, and the population will always end up in a monomorphic state with one type of preference and the corresponding male mating type (see Fig. 2).

Hence, selection on female preferences has to be disruptive and frequency dependent to initiate reproductive isolation and speciation. However, disruptive frequency dependent selection on female preferences is not sufficient to achieve the simultaneous divergence of female preferences and male mating types. On the contrary, we could show that for a broad class of models branching of female preferences and branching of male mating types exclude each other: whenever selection on female preferences is disruptive, selection on male mating types is stabilizing, and *vice versa*. From this, one may conclude that truly adaptive speciation by sexual selection is much less easy to achieve than many current models seem to suggest.

In principle, however, adaptive speciation by sexual selection *is* possible, provided that several disruptive forces are working in concert. We could demonstrate this by building the first truly adaptive model of sympatric speciation (Van Doorn, Dieckmann & Weissing, 2004; see Fig. 3). To this end, we added male-male competition as an additional disruptive factor into our model. The model structure was motivated by empirical findings

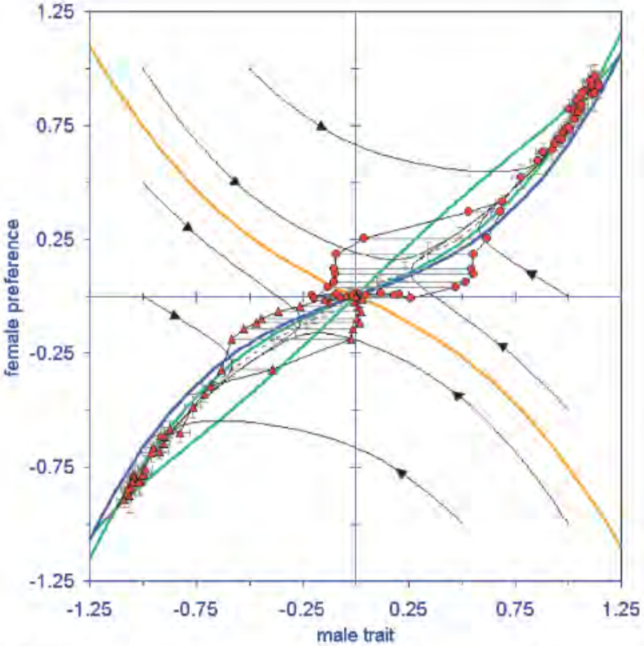

Figure 2. Disruptive selection on female preferences per se does not induce sympatric speciation. To formalize the idea that sympatric speciation by sexual selection is caused by the simultaneous occurrence of two Fisherian runaway processes, proceeding in opposite directions, we consider the example of a female choice function (thick blue line) that allows for such a bidirectional runaway. Two representative individual-based simulations (red circles and red triangles, respectively) are shown that both were started exactly at the point where sexual selection is disruptive. Nevertheless, the two simulations show no speciation. After an initial phase of dimorphism in male traits, each simulation run tends towards either one of two possible stable equilibria where female preference for extreme male traits has evolved. The simulation results are confirmed by an analytical 'adaptive dynamics' approach (grey lines with arrows: evolutionary trajectories; green and orange lines: null-isoclines). From this approach, the general conclusion can be drawn that population divergence is extremely unlikely if selection on female preferences is not frequency dependent.

on cichlids, where female choice and male-male competition are closely interrelated. Our model is, therefore, perhaps not too far removed from real-world situations. But it clearly indicates that sympatric speciation is not easy to achieve, and that much more research is required to fully understand the emergence of reproductive isolation in sympatry.

Sympatric speciation theory has, up till now, mainly considered Fisherian sexual selection as a potential source of divergence in sexually selected traits. In principle, however, there is a possibility that not only 'arbitrary' preferences diversify in the course of evolution, but also preferences for indicators of male quality. Here one might think of systems

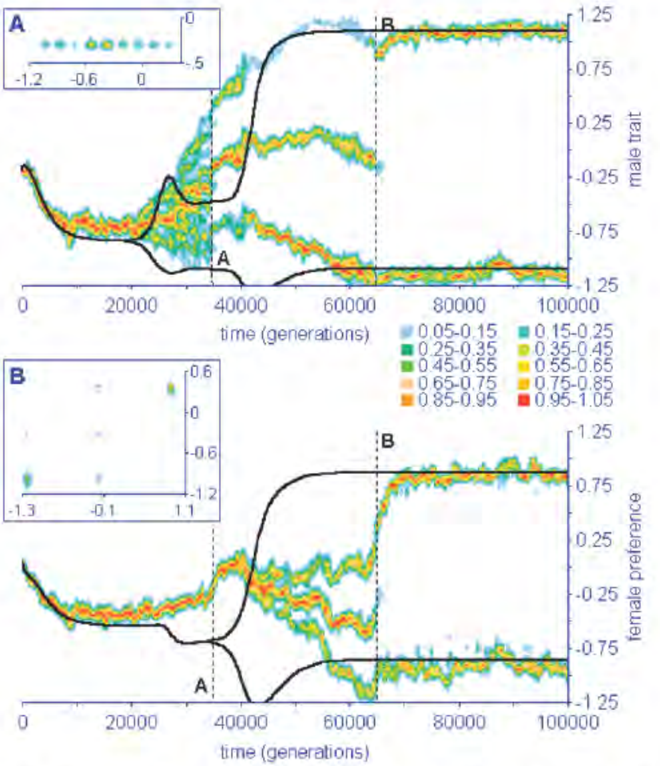

Figure 3. Sympatric speciation by sexual selection became possible when both competition between females (i.e., frequency dependent selection on female preferences) and additional disruptive selection in males (caused by male-male competition) were incorporated into our model. This is one of the first examples for truly 'adaptive' speciation by sexual selection. The two plots show the relative frequency distributions of male-trait and female-preference genotypes in an individual based simulation (indicated by a color scale) as well as the corresponding adaptive dynamics approximation (black lines). The good agreement between analytical prediction and simulation results indicate that speciation events are predictable and not purely the effect of chance events.

with *multiple* ornaments, where different preferences might give different weights to the various ornaments. To investigate this possibility, new theory had to be developed (Van Doorn & Weissing, 2004), since the existing models for the evolution of preferences for multiple ornaments are not very satisfactory. In particular, present models predict that only one of the ornaments can be an indicator of quality, while the other ornaments are 'arbitrary', i.e., maintained by Fisherian runaway selection. In contrast to earlier models, we considered the possibility that different ornaments provide information about different aspects of male quality (e.g., resistance to parasites vs. foraging ability). In other words, the ornaments in our model act as signals for distinct quality components. When the ornaments provide overlapping information about these quality components, we retrieve the results of earlier models. However, when the ornaments provide independent information, preferences for multiple ornaments may evolve by a 'good genes' process, even when exhibiting multiple preferences is costly (see Fig. 4). This is good news for behavioural ecologists, who in view of empirical counter-evidence tend to distrust the earlier theoretical results. We will take our new insights as a starting point for a re-evaluation of the role of sexual selection on quality indicators in the process of speciation. In the course of our work on multiple ornaments we made another observation that is potentially of great importance for the whole theory of sexual selection. Several general results of the theory rely on the assumption that female preference and male ornament expression eventually converge to a stable equilibrium. Examples of such results are the conclusion that no stable preference can evolve for ornaments that are purely epistatic indicators, or the conclusion that females should disregard all ornaments except the one

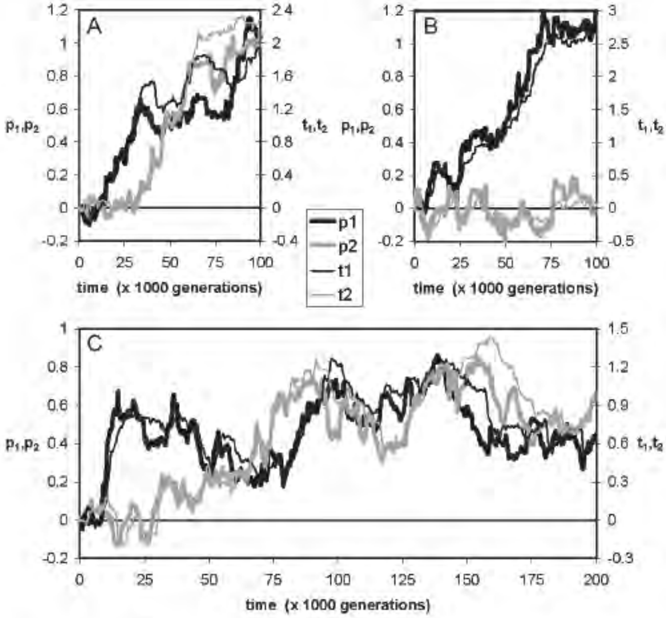

Figure 4. Sexual selection for multiple ornaments that act as signals for multiple components of male quality. In panels (A) and (B), the ornaments provide overlapping information about the quality components. This corresponds to earlier models viewing 'quality' as a one-dimensional trait. In line with earlier models, multiple preferences evolve in (A) but not in (B), due to the fact that the joint cost of choice is low in (A) but high in (B). In panel (C), the joint cost of choice is high, as in (B), but the ornaments now provide independent information about the quality components. Despite the high joint cost of choice, multiple preferences evolve. Analytical results confirm that the individual-based simulations shown in this figure are indeed representative.

that provides the most reliable information about genetic quality. We showed that the assumption of equilibrium dynamics is problematic and we characterized conditions under which female preference and male condition-dependent signaling continue to evolve without ever attaining stable equilibrium levels. Such limit cycles had been described before in the context of Fisherian runaway selection, but now we demonstrate that continual evolution can also be driven by the joint action of a 'good genes' process and a sexual conflict over the information content of signals used in mate choice. Although the existence of this conflict has long been acknowledged, its consequences had never been investigated. Our model illustrates that non-equilibrium dynamics may have major implications, since many of the standard results of sexual selection theory (including those mentioned above) do no longer apply away from equilibrium. The model also provides a mechanism for the apparent frequent loss of sexually selected traits, and it offers an alternative explanation the evolution of preferences for multiple ornaments. Moreover, the model illustrates the importance of genetic constraints on the quantitative *and* qualitative outcome of sexual selection (see Fig. 5). It is intriguing to see how strongly the new results parallel those in a very different research line, where we showed earlier (in the context of resource competition) that many seemingly well-established results in ecology and evolution only hold if the system does indeed settle on an equilibrium (Huisman & Weissing 1999, 2001ab, 2002, Scheffer *et al.* 2003).

In addition to female choice, we also addressed male-male competition, the second major aspect of sexual selection and an important ingredient in some of our speciation models (e.g. Van Doorn & Weissing, 2004). In two articles (Van Doorn, Hengeveld &

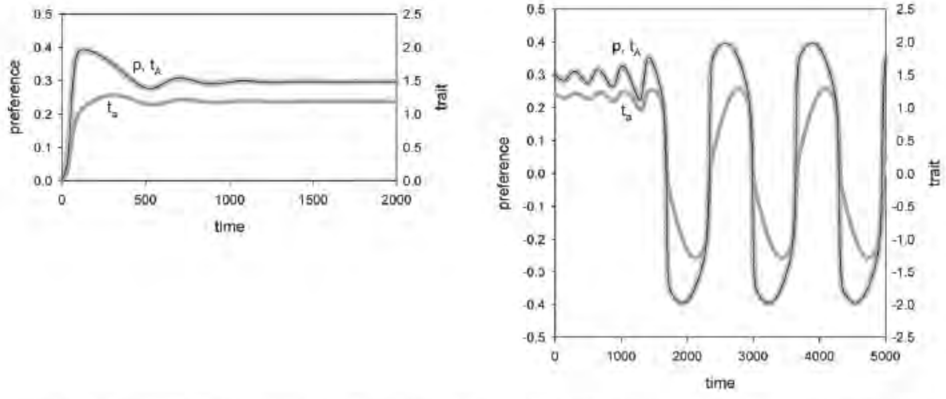

Figure 5. Sexual conflict may result in ongoing cycles in the strength and direction of female preference for a costly male ornament that is expressed conditional on male quality. The two panels illustrate that, for the same parameter combinations, genetic constraints can be decisive for whether sexual conflict results in an equilibrium (upper panel) or in ongoing oscillations (lower panel). In case of non-equilibrium dynamics, many of the standard results of sexual selection theory cease to hold.

Weissing, 2003ab), we addressed the question why dominance hierarchies do exist, why they are stable, and why one often observes ‘winner and loser effects’. In many species, difference in dominance rank is used as a cue to resolve conflicts between two animals without escalated fights. At the group level, adherence to a dominance convention efficiently reduces the costs associated with conflicts, but from an individual’s point of view, it is difficult to explain why a low ranking individual should accept its subordinate status. This is especially true if, as suggested by several authors, dominance not necessarily reflects differences in fighting ability but rather results from arbitrary historical asymmetries. According to this idea, rank differentiation emerges from winner and loser effects, in which winners of previous conflicts are more likely to win the current conflict, whereas the losers of previous conflicts are less likely to do so.

By means of game theoretical models, we investigated whether and under what conditions dominance, based on winner and loser effects, can indeed be evolutionarily stable. Even in highly simplified scenarios we found a multitude of alternative evolutionarily stable strategies (ESS). All these strategies utilize the asymmetries generated by the outcome of previous conflicts as cues for conflict resolution. One class of these strategies is based on winner and loser effects, thus generating evolutionarily stable dominance relations even in the absence of differences in strength or fighting ability. However, in view of the fact that alternative conventions are also evolutionarily stable, the question why winner and loser effects seem to occur so often in natural systems is by far not resolved. Still, our game theoretical results provide an interesting link to the research line of Hemelrijk (see below), who uses winner and loser effects as a starting point for the explanation of the various features of egalitarian and despotic societies.

The role of informational and genetic constraints on the evolution of sex allocation

Ido Pen

It was probably Hamilton (1967) who first drew attention to the effect that genetic asymmetries between males and females could have on the ‘unbeatable’ sex ratio. However, it wasn’t until nine years later that Trivers and Hare (1976) applied these ideas to hymenopteran eusocial insects in which both queen and workers might have a say in colony sex allocation decisions. In these decisions, the queen and the workers have different genetic interests, the queen being equally related to sons and daughters, but the workers being more closely related to the queen’s daughters than to her sons. This causes the workers to favor a higher proportion of females among the reproductives than the queen. Under the simplest of assumptions, the queen’s preference is a 1:1 male:female ratio, while the workers prefer 1:3 ratio. Trivers and Hare felt that workers are more likely to win the conflict, and until recently many studies of hymenopteran sex allocation supported this view. However, several new studies give examples of sex ratios close to the queen’s optimum. In order to understand these contradictory results, more sophisticated shared-control conflict models are needed to encompass the dynamic balance between queen and worker power over the colony sex ratio.

A potentially important aspect of the worker-queen interaction that has not been addressed in models so far concerns the flow of information between queen and workers. Indeed, Trivers and Hare (1976) argued that since workers in some sense might seem to have 'the last say', they should win the conflict and we might expect sex ratios to more closely match the workers' optimum than that of the queen. Similarly, Beekman *et al.* (2003) argue that "Because the workers act after the queen, this probably gives them greater power than the queen". If it is true that the workers have 'the last say', the workers might be able to observe the queen's actions and use this information to determine their own actions. We (Pen and Taylor, in press) studied a kin selection model of shared worker-queen control which explicitly allows workers to do just that and adjust their allocation to the queen's allocation.

In the model, we give control over the primary sex ratio (proportion haploid eggs) to the queen. Subsequently, the workers determine the proportion of diploid eggs that become workers (the rest becoming future queens), and they also control the distribution of resources among the developing larvae. In one version of the model, the workers cannot observe the queen's actions (i.e, the proportion of haploid eggs), whereas in a second version of the model the workers can observe her actions and act accordingly. The main result can be seen in Fig. 6. If the workers do not take the queen's behavior into account, the evolutionarily stable outcome of the conflict lies roughly intermediate between the queen's optimum and the workers' optimum. The unexpected outcome is that when the workers have the ability to act upon increased information, the queen appears to 'win' the conflict, in the sense that sex allocation is close to the queen's optimum. Apparently, the argument of Trivers and Hare that workers should win the conflict because they have the 'last say' does not always work. The key to understanding this result is to realize that natural selection acts on the queen's decision as if the queen knows that the workers will respond optimally to her actions, thus placing her in some sense in the driver's seat in being able to make the first move. Elsewhere (Taylor and Pen, in prep.), we study this 'paradox' more generally in the context of models of conflict and cooperation in biology and derive conditions under which the party that makes the first move gains a certain advantage over the party with the second move.

Is our model capable of explaining the cases where sex ratios close to the queen's optimum have been found? So far, very few species have been studied in sufficient detail to verify the assumptions of our models. However, there is one species of ant, *Lasius niger*, whose sex allocation and potential control mechanisms have received considerable study. This species seems to fit the assumptions of our model quite closely. There is clear evidence that the workers of *L. niger* can detect the sex of individual eggs: only either male or female larvae are placed in the uppermost chambers of the nest. It is therefore conceivable that workers could adjust their behavior to the fraction of haploid eggs. Indeed, spatial segregation of the sexes seems a very effective way for workers to adjust how much they feed to each sex. In agreement with our predictions, it has been found that *L. niger* has sex ratios very close to the 'queen's optimum', and that this relatively high proportion of males cannot be attributed to worker reproduction. What is unknown

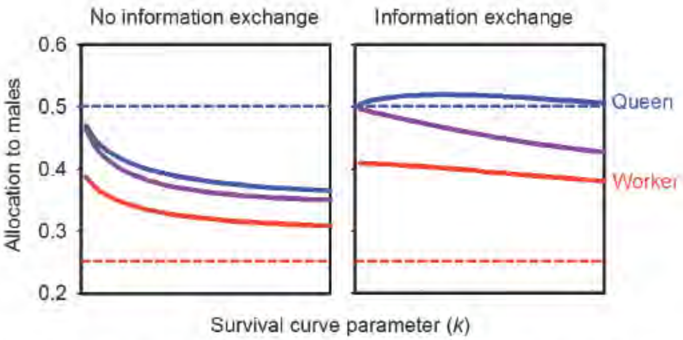

Figure 6. Effect of information exchange on the sex allocation conflict between queen and workers. The parameter k on the horizontal axis determines how resource investment translates into survival of reproductives. On the vertical axis the proportion of resources allocated by the queen (solid blue), by the workers (solid red) and the resulting sex ratio among reproductives (solid purple) are depicted. The dashed lines indicate the optimal allocation strategies if the queen is in full control (blue; 1:1 allocation) and if the workers are in full control (red; 1:3 allocation). In the left panel the workers cannot observe the queen's allocation decisions and act accordingly, in the right panel they can. Note that in the latter case sex allocation is closer to the queen's optimum despite of the fact that workers use information about the queen's strategy.

is whether *L. niger* workers have the crucial mechanism that is needed to make model 2 work: observing the queen's strategy and using this information to adjust their own decisions. It might be interesting to experimentally alter the number of haploid eggs in the nest and to observe whether this changes the workers' allocation decisions in the expected direction.

In another study we also investigated the role of information in sex ratio adjustment (Reece *et al*. 2004). In particular, we tested whether nonsocial haplodiploid wasps can discriminate between kin and nonkin. First we analyzed a model which predicted that if females can discriminate siblings from nonrelatives, then they are expected to produce a higher proportion of daughters if they mate with a sibling (see Fig. 7). This prediction arises because in haplodiploids, inbreeding (sib-mating) causes a mother to be relatively more related to her daughters than her sons. In the model, multiple females lay eggs in a patch, to mimic the biology of the parasitoid wasp *Nasonia vitripennis*. We investigated in the lab whether females of this species adjust their sex ratio dependent upon whether they mate with a sibling or nonrelative, in response to either direct genetic or a range of indirect environmental cues. It turned out that this was not the case, suggesting that females of *N. vitripennis* cannot discriminate between kin and nonkin.

Haplodiploid organisms, such as hymenopterans, have a simple mechanism to adjust the sex ratio of their offspring: fertilized eggs become diploid females and unfertilized eggs become haploid males. The situation is very different for diploid organisms. Sex determining (SD) mechanisms vary considerably between closely related taxonomic groups

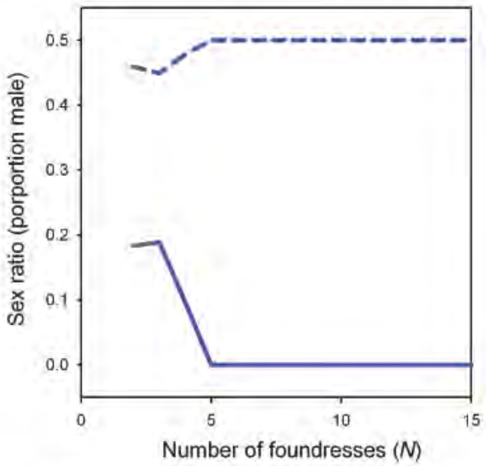

Figure 7. Inbreeding and facultative sex ratio adjustment. The predicted evolutionarily stable sex ratios for females who have mated with a sibling (solid line) or a nonrelative (dashed line) is plotted against the number of foundresses (*N*). Mating is at random in a patch, hence the probability of sibmating is 1/*N*.

and evolutionary transitions from one system to another seem to occur frequently. Common SD mechanisms are male heterogamety (males XY and females XX, such as in nearly all mammals and many insect groups), female heterogamety (females ZW and males ZZ, such as in birds, lepidopterans and snakes), and environmental sex determination (such as in some reptiles and fish), but there exist a variety of other mechanisms. Genetic SD mechanisms impose considerable constraints on sex ratio control. For example, an XY system that obeys the standard Mendelian laws of inheritance always produces equal numbers of sons and daughters. However, that is not to say that selection on the sex ratio has not played a role in the evolution of and transitions between different SD mechanisms. An interesting organism to study the potential role of sex ratio selection in the evolution of SD mechanisms is the housefly *Musca domestica*. In this species, several different SD mechanisms have been found to co-exist in field populations (see Fig. 8). In the so-called standard XY strains, a male-determining factor (M) is located on the Y chromosome and males are XY and females XX. The M factor blocks the action of an autosomal F which is necessary for female development. In addition to the standard XY system, field populations have been discovered in which an M factor is located on one or more of the five autosomes, or even on an X chromosome. These autosomal (more precisely, non-Y) M factors seem to have appeared relatively recently and may be spreading, replacing the standard XY system in many locations. Intriguingly, the frequency of autosomal M factors seems to decrease with latitude and altitude, northern and high altitude populations usually dominated by the standard XY system. Such geographical clines have been found in Europe, Japan and the USA. In most populations

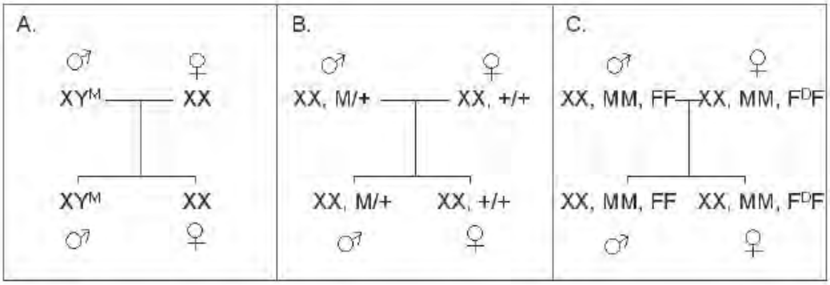

Figure 8. Common sex determining mechanisms in natural populations of *Musca domestica*. A. The standard XY system - male determining factor (M) present on the Y chromosome; B. Autosomal system with male heterogamety - M present on one of the autosomes, males and females homozygous for X; C. Autosomal system with female heterogamety - males and females are homozygous for X and autosomal M, sex is determined by presence (females) or absence (males) of the epistatic factor FD.

with autosomal M factors, an additional epistatic factor FD (FDominant) occurs, dictating female development, even in the presence of up to three M factors. It seems somewhat more likely that FD evolved after the invasion of autosomal M factors, instead of vice versa, since populations with FD always have autosomal M factors but not vice versa.

We (Kozielska *et al.*, submitted) studied a three-locus population genetic model that mimics the housefly system, with an XY 'locus', an autosomal M locus and an autosomal FD locus. We imposed sex ratio selection on the system by letting sons be more 'expensive' or 'cheaper' to produce than daughters. In the former case, selection favors male-biased sex ratios, in the latter case female-biased sex ratios. Our aim was to investigate how this would affect the dynamics of the frequencies of the various SD factors and the sex ratio. This might be useful for interpreting the patterns found in different field populations of the housefly, but also to help design and interpret future experiments that we will carry out with the housefly.

Some of the results are shown in Fig. 9, which shows the equilibrium frequencies of the various factors and sex ratio as a function of the parameter c, which measures the cost of a son relative to that of a daughter. For $c = 1$, sons and daughters are equally 'expensive', for $c < 1$ daughters are more costly, and for $c > 1$ sons are more costly. We were able to show analytically that for $c = 1$ the sex ratio is always even in equilibrium. For $c < 1$, FD disappears from the population and the equilibrium frequencies of Y and autosomal M lie on a curve of neutral equilibria. Surprisingly, the sex ratio at equilibrium is 1:1, even though a male-biased sex ratio would be more adaptive if it were under (unconstrained) control of either maternal genes or genes acting in the offspring. In case sons are more costly ($c > 1$), we find that, as expected, the sex ratio is female-biased, but the bias is much smaller than would be optimal under unconstrained control. We also

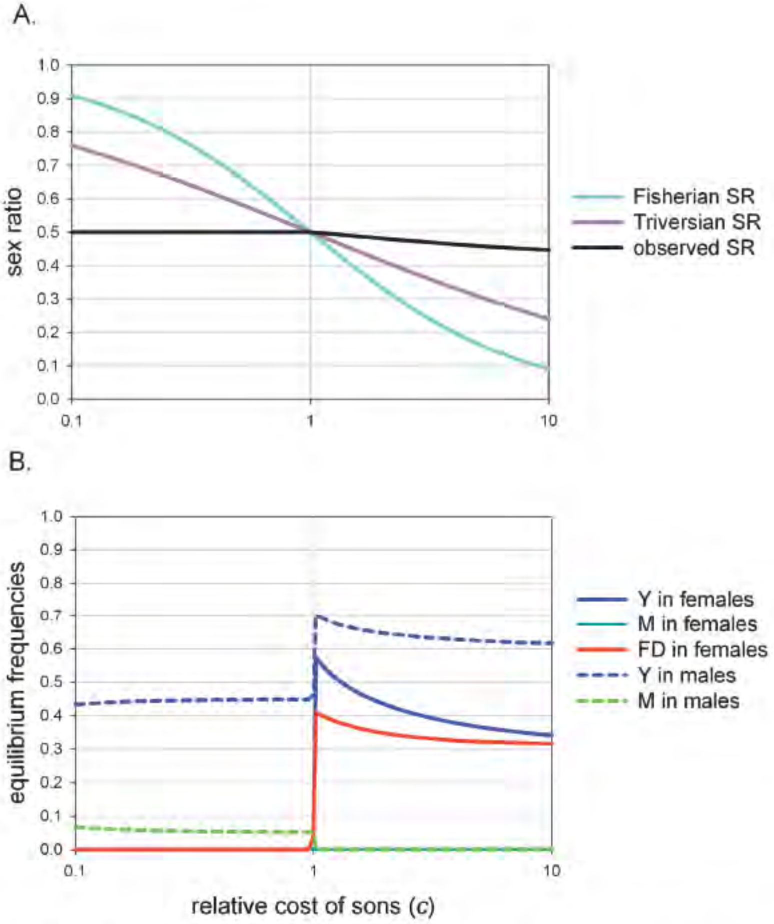

Figure 9. A: Equilibrium sex ratios compared to unconstrained optimal sex ratios from maternal point of view (Fisherian) and offspring point of view (Triversian). B: Equilibrium frequencies of sex determining factors as a function of the relative cost of sons (c).

observe that under this scenario all factors co-exist in equilibrium. FD reaches quite high frequencies in equilibrium, but a full switch to a female-heterogametic system does not occur.

To what extent can the observations on wild housefly populations be explained by our model? If we assume that in houseflies males are more costly, then the model predictions are not altogether inconsistent with the observations: co-existence of all factors and high frequencies of FD. However, there is no evidence that males are more costly in houseflies. If anything, females are more costly, since they are larger than males. On the other hand, there are other reasons (other than cost differences) why female-biased sex ratios might be selectively favored. For example, inbreeding and/or female-biased dispersal may select for female-biased sex ratios. There is some evidence that local housefly populations are sometimes small enough to expect some degree of inbreeding. But even if it turns out that there is no selection for biased sex ratios in wild populations, we can still impose such selection in the lab in order to mimic conditions that are thought to be important for the evolution of SD systems in general.

The main conclusions we draw from this analysis: (1) Even for a multi-factorial SD system that in principle allows a broad range of sex ratios, the genetic mechanism constrains the equilibrium sex ratios to deviate at most weakly from 50:50. (2) Sex ratio selection can cause substantial shifts in the frequencies of SD factors, but is probably not sufficient to explain the observed frequencies in the housefly.

Understanding social behaviour with the help of complexity science

Charlotte Hemelrijk

In many group-living animals competition leads to a dominance hierarchy in which some individuals outcompete others consistently. We study such dominance relations from the perspective of complexity science. For this we use computer models in which emergent phenomena at a higher level of organisation change the characteristics of the units at a lower level by self-organisation. As described below, this method leads to a number of new insights and hypotheses that could not be obtained otherwise.

In primate societies one distinguishes between two dominance styles, egalitarian and despotic. In a despotic society individuals differ greatly in fighting power, but in an egalitarian society power differences between individuals are small. The most extensive comparative studies on these differences have been made in the genus *Macaca*. It appears that both types of species differ not only in dominance style, but also in many other aspects of social behaviour. Compared to egalitarian societies, aggression in despotic societies is more intense, less frequent and less symmetric, groups are looser, grooming is more dominance-oriented, females are more choosy in their partner choice, and there is more migration by males. Usually, when species differ in several traits a separate adaptive explanation is sought for each difference. However, according to Thierry most of these traits co-vary and there are only two independent differences, namely in intensity of aggression and in the tendency to help kin. In a modelling study (Hemelrijk 2004), it could even be shown that all differences can be explained by differences in just one underlying variable, the intensity of aggression. The model, called DomWorld, consists of a world in which agents move around and group and compete. The effects of winning and losing competitive interactions are self-reinforcing. This implies that after winning the chance to win again increases and vice versa for losing. In the model, a change in the intensity of aggression (from mild aggression, such as slapping, to intense aggression, such as biting), results in a cascade of effects. Higher intensity of aggression accelerates the development of the hierarchy (Fig. 10A). Consequently, some individuals lose all the time. The losers flee from everyone else and, thus, the group as a whole spreads out, which in turn reduces the frequency of aggression (Fig. 10B). At the same time, a spatial structure develops with dominants in the centre and subordinates at the periphery (Fig. 10C). This spatial structure in turn strengthens the hierarchy, because individuals, when mainly interacting with those that are close by, interact mostly with agents of similar dominance. Thus if a dominance reversal takes place, it is only a minor one, because the opponents were similar before the fight. Consequently, the spatial structure stabilises the hierarchy and helps it to develop. In other words, the development of the hierarchy and the development of the spatial structure are mutually reinforcing processes. In short, at a high intensity, the gradient of the hierarchy is steeper, groups are looser, aggression is less frequent, spatial centrality is greater, etc. Many of these aspects strikingly resemble the differences found between the societies of despotic and egalitarian species, especially of the genus *Macaca* (Hemelrijk 2004).

An additional complexity was described recently by Preuschoft and co-authors. They found an unexpected sex difference in Barbary macaques: males behave in an egalitarian way whereas females are despotic. This seems difficult to reconcile with standard theory, since one would expect that, if there are sex-differences at all, the sex with the larger body and fiercer aggression should evolve a more despotic dominance style. Therefore, these authors raise doubt whether dominance style should be regarded as species-specific after all. To learn more about this problem, we extended the DomWorld model by including sex differences. The sexes differ in fighting capacity with females

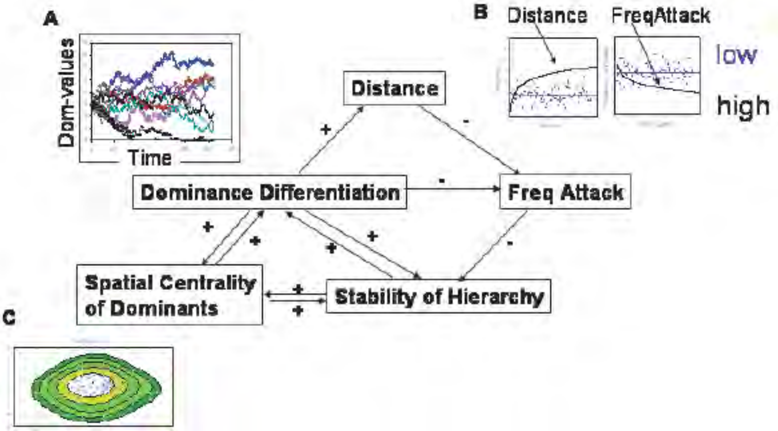

Figure 10. Feedbacks between the development of a hierarchy and the emergence of spatial structure. (A) A typical example of hierarchical differentiation over time. (B) The change in distance among agents (left graph) and the frequency of aggression (right graph) over time differs markedly between societies with high (black line) and low intensity of aggression (blue line). (C) The spatial-social structure with dominants in the centre and subordinates at the periphery (shades indicate rank classes).

having a lower (80% or 10%) intensity of aggression and a lower (50%) initial capacity of winning than males, assumptions that seem plausible for a species with a male-biased sexual dimorphism (Hemelrijk & Gygax 2004). Unexpectedly, and in contrasts to former findings on intensity of aggression, in the model the males' hierarchy turned out to be significantly weaker than that of females, leading to a less differentiated and more egalitarian society. The explanation is that, due to the higher initial dominance of males (in real animals caused by their larger body size), single events of victory and defeat have less impact on the dominance of males than they have on females. Thus such events lead to less hierarchical differentiation. The greater the sexual difference in initial dominance, the more egalitarian the males behave among themselves and the steeper the gradient of the hierarchy among females when compared to males (Fig. 11A). Further, at a greater degree of sexual dimorphism males approach other males more often non-aggressively to avoid the higher risks involved. As a consequence, the ratio of non-aggressive approaches to males compared to other group members increases (Fig. 11B). In short, different degrees of 'male-male tolerance' are a side-effect of sexual dimorphism.

Sexual dimorphism also influences the dominance relations between the sexes (Hemelrijk *et al*. 2003). Even though male macaques may be 1.5 times the size of females, females are sometimes dominant over one or more males. We quantified the degree of

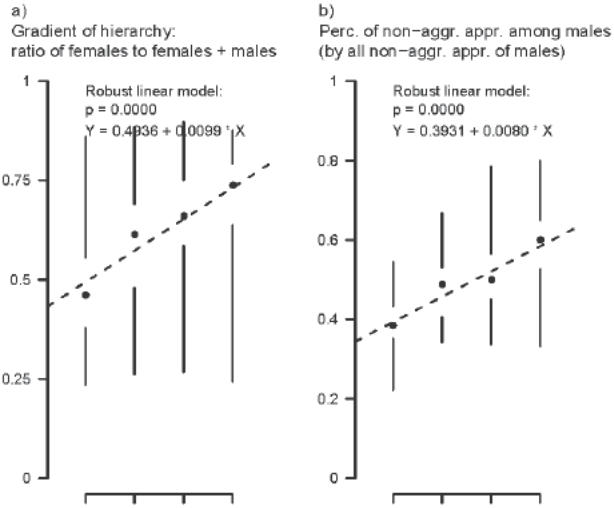

Figure 11. The effect of sexual dimorphism in initial dominance value on (A) the steepness of the hierarchy among females when compared to that among males, and (B) the relative frequency of non-aggressive approaches among males. Degrees of sexual differences in initial dominance values are: females-males of 24-24, 20-28, 16-32, 12-36.

female dominance over males and studied how female dominance is influenced by the intensity of aggression (Fig. 12ABC), the compactness of grouping (due to the distribution of food, Fig. 12D) and sexual attraction of males to females (Fig. 13).
Remarkably, intensity of aggression influences female dominance over males in an unexpected way: At a high intensity, female dominance appears to be greater than at a low intensity (Fig. 12C), a result corresponding to the greater female dominance described for fiercely aggressive despotic females over maturing subadult males. It has been argued in the literature that this effect reflects the fact that coalitions among females against (sub-adult) males are stronger in despotic than in egalitarian societies. In DomWorld, however, coalitions do not play a role, and the effect is due to the steeper hierarchy at a high intensity of aggression (because some females become very high in dominance and some males very low, see Figs 12A and 12B). In the literature, it is unusual to relate female dominance to the intensity of aggression. Typically, the focus is on other characteristics that directly relate to power - such as body size and coalitions against males. DomWorld shows that unexpected factors, such as intensity of aggression, group cohesion and male attraction to females (see below) may be key factors for a proper understanding of female dominance (Hemelrijk *et al*. 2003).

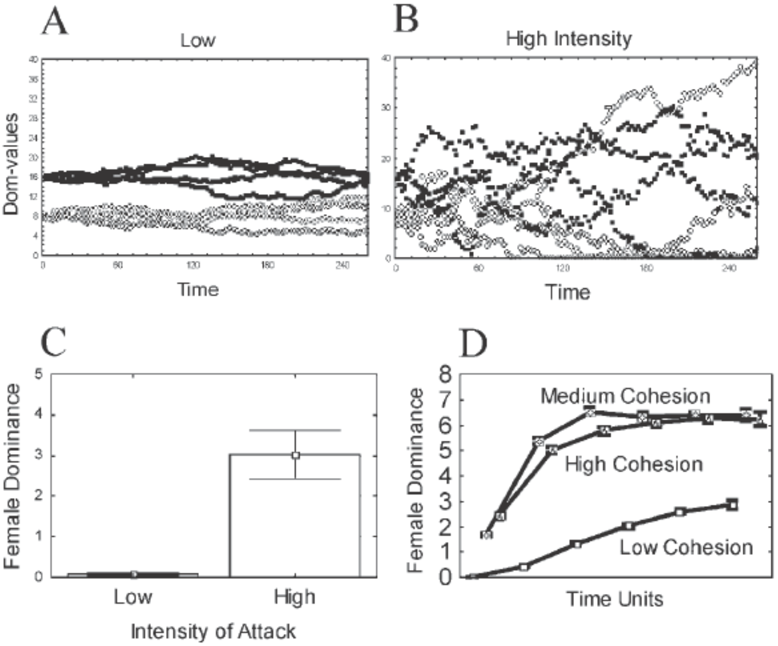

Figure 12. Effects of intensity of aggression and group cohesion on female dominance over males. Development of dominance values of 4 males (black dots) and 4 females (open circles) at a low (A) and a high (B) intensity of aggression. Female dominance over males occurs more often at high intensity of aggression (C) and in groups with a high degree of group cohesion (D).

Cohesion too promotes female dominance (Fig. 12D), because in denser groups the hierarchy develops more strongly due to the greater number of interactions and the marked spatial structure. This may in part explain the greater female dominance in pygmy chimpanzees compared to common chimpanzees: groups of pygmy chimpanzees are more compact. Once more, DomWorld offers a simpler explanation than suggestions in the literature, where it has been hypothesized that the larger degree of female dominance in pygmy chimpanzees is caused by their stronger tendency to form coalitions against single males.

Implementation of sexual attraction in DomWorld (as an increased tendency of a male to approach a female rather than a male) revealed that this effect automatically increases female dominance over males as a side effect (Fig. 13A). This happens if females are sexually attractive (tumescent) synchronously like in some species, or if they are tumescent asynchronously like in others. The process leading to increased female dominance differs, however, under both conditions as follows.

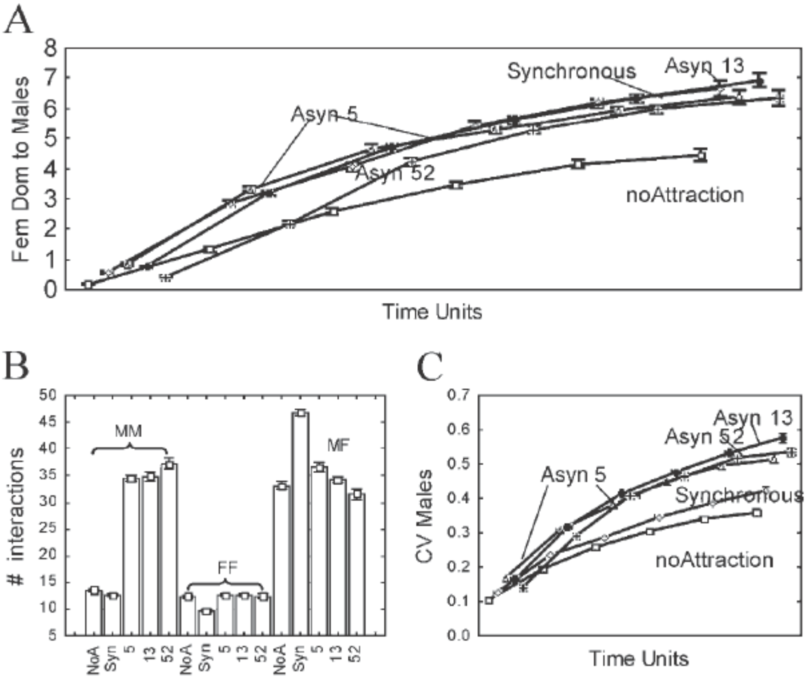

Figure 13. Effect of sexual attraction on (A) female dominance over males, (B) the frequency of interactions among and between the sexes, and (C) the differentiation of the dominance hierarchy of males. NoA: standard model without sexual attraction; Syn: synchronous attraction of females; AsynX: asynchronous attraction of females, with X (= 5, 13 or 52) indicating the length of the time interval until the next female becomes attractive.

If single females are attractive in turn, many males cluster close to such a female. Consequently, the frequency of male-male interactions markedly increases, while the frequency of interaction between the sexes and among females remains more or less the same as in the standard model without attraction (Fig. 13B). Due to the higher frequency of interactions among males, the male hierarchy differentiates more strongly than without attraction (Fig. 13C), and this causes certain males to become subordinate to certain females.

In contrast, when females are synchronously attractive, interactions between the sexes increase significantly when compared to the standard model, because several males can find a female (Fig. 13B). This leads to more opportunities for low ranking females to overcome high-ranking males during sexual attraction than at other times. As soon as this happens, dominance values of females increase to a larger degree than when females defeat lower-ranking agents. This is a consequence of the rule (built in the model) that unexpected outcomes of a conflict (e.g., when a lower-ranking agent defeats a higher-ranking one) have a stronger effect on the dominance values of both partners than expected outcomes. Thus, a higher percentage of interactions between the sexes will increase the probability of female victories over males and this will accelerate female dominance over them.

Summarising, our model shows that dominance style and the social relations between the sexes are closely interrelated with all kinds of factors, be they ecological (spatial distribution of food), morphological (sexual dimorphism), physiological (intensity of aggression) or ‘psychological’ (attraction to the opposite sex).

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