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Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FOR FERTILIZATION RESEARCH IN PLANTS ON 1 OCTOBER 1993

Interspecific hybridization of *Alstroemeria*

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Alstroemeria is an important ornamental crop grown in The Netherlands for cut flowers. The *Alstroemeria* varieties are developed by breeding companies using interspecific crosses. The natural habitat of *Alstroemeria* species is South America, mainly Chile and Brazil.

During interspecific hybridization, crossing barriers were found between Chilean and Brazilean species, and among Chilean species. A normal sexual reproduction process in *Alstroemeria* in compatible crosses in comparison with the abnormalities found in incompatible crosses was presented. Results from a diallel cross revealed that the crossing barriers are mainly of a post-fertilization nature, although inhibition of pollen tubes in the style is also found.

Embryo abortion takes place in various stages of seed development resulting in different sizes of shrivelled seeds. All these seeds lacked a cellular endosperm, which was abundantly present in normal round seeds.

A method to overcome the post-fertilization crossing barrier was developed by applying an *in vitro* ovule culture. Two days after pollination, the ovules were dissected out of the ovaries and were cultured in a hormone-free MS medium containing sucrose. After transferring to another MS medium with less sucrose the ovules germinated and produced young seedlings. The plants resulting of this ovule culture were hydrids.

In the first 2 weeks of the *in vitro* culture, the ovules grew abundantly. Histological investigation of these ovules revealed that the inner and outer integuments produce a tissue proliferation whereas inside the embryo grew without the development of cellular endosperm. Depending on the cross combinations used for the application of the ovule culture, undifferentiated callus and root/shootlike callus are also developed. Plants could be regenerated from this callus using shoot- and root-inducing hormones (J. Buitendijk, in press). The origin of this callus is related to the shape of the embryos in the incompatible cross combinations.

Future research will focus on the intercrossability of the *Alstroemeria* species with or without the use of ovule culture. A reliable species identification system is also required using morphological, biochemical and/or molecular markers.

Pistel-specific Genes from Potato

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Little research has been done on the molecular details of a successful pollen tube–pistil interaction. To elucidate this cell–cell interaction between pollen tube and pistil a cDNA library of cross-pollinated pistils of *Solanum tuberosum* was screened using two different techniques, i.e. a differential screening and cold-plaque screening (Hodge R. *et al.* (1992): *Plant Journal* **2**: 257–260). Differential screening resulted in the isolation of several pistel-specific genes. One of these genes, STS14, is a single copy gene that is only expressed in the pistil. Expression levels in pollinated and unpollinated pistils are equal. Homologous transcripts have been found in pistils of *Petunia hybrida, Nicotiana tabacum* and *Brassica oleraceae*. Sequence analysis of the partial STS14 cDNA clone revealed that the C-terminal part of the coding region has 43% amino acid identity with pathogenesis-related proteins from tobacco (PR-1). To complete the entire sequence a genomic clone (STSg14) was isolated and sequenced. STSg14 has an open reading frame lacking any introns encoding a protein of 207 amino acids. *In situ* hybridization experiments resulted in the localization of STS14 in the cortex and stigmatic tissue of the pistil. No STS14 transcripts were detected in the transmitting tissue.

A cold-plaque screening resulted in the isolation of more than 100 cold-plaques. Northern blot analysis of a selection from these cDNA clones resulted in six different pistil-specific genes. One of these genes showed increased expression in pistils after pollination. This gene encodes isoflavone reductase (IFR), an enzyme that is involved in phytoalexin production in alfalfa. The function of this gene in potato pistils will be analysed.

Immunocytochemical Localization of Protein Phosphorylation and Expression of Heat Shock Proteins in Cultured Microspores and Pollen of *Brassica napus* L.

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Embryogenesis was induced in isolated microscopes and pollen of *Brassica napus* by culture at elevated temperature (32°C) for at least 8 h. Only isolated cells, cells cultured under non-embryogenic conditions (18°C) and embryogenic cultures were compared with respect to the phosphorylation of proteins and the expression of heat shock proteins (HSPs) 68, 70 and 72/73.

Phosphorylation was detected on cryosections and polyethylene glycol (PEG) embedded sections by the monoclonal MPM2, known to identify cell cycle related phosphorylation. MPM2 was visualized by goat-anti-mouse antibody labelled with FITC. It was found that cryosections showed a strong signal in the nuclei and/or in the cytoplasm depending on the stage of the cell cycle but independent of the culture conditions. PEG sections exhibited comparable localization at interphase but reduced or absence of labelling during microspore and pollen mitosis. It is concluded that PEG embedding procedures caused a loss of epitopes present during mitosis. A relation between phosphorylation and embryogenesis was not distinguished.

HSPs were localized immunocytochemically, too. HSP 68 was exclusively found in organelles which also stained with DAPI, presumably mitochondria, irrespective of the stage of the cell cycle or culture conditions. HSP 70 was always found in microspores and pollen but showed a clear heat-induced increase in concentration in those nuclei, from which it is known that they synthesize DNA (Binarova *et al.* 1993, *TAG*, **87**: 9–16). It was concluded that HSP 70 is related to this nuclear DNA synthesis. This is in agreement with the DnaK function suggested for HSP 70. HSP 72/73 exhibited heat-induced increase in expression but a relation to either nuclear DNA synthesis or to induction of embryogenesis was not observed.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 29 OCTOBER 1993

Pollen Morphology of *Amorphophallus* (Araceae)

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Amorphophallus comprises approximately 120 species, which occur in the tropics of the Old World. The genus is currently being revised. The aim of the present pollen study is to provide an additional set of characters for a phylogenetic analysis. Up till now more than 100 species have been studied with light microscopy and scanning electron microscopy. It appeared that the standard method for preparation of pollen (acetolysis) has a destructive influence on the outer layer of the exine. The ornamentation (sexine) disappears completely in most samples, whereas the inner layer (nexine) suffers very little. Therefore, the samples were studied untreated (living material), or boiled in water (herbarium material). Pollen grain size is between 27 and 93 (av. 53) µm. Grain shape is spherical to ellipsoidal. The grains look inaperturate, but a cryptoaperturate condition (e.g. the presence of a locally thickened intine: oncus) cannot be excluded. A wide range of ornamentation types exists, including psilate, echinate, verrucate, areolate, striate, and various intermediates. Striate pollen is nearly always ellipsoidal. The other ornamentation types are usually associated with spherical shape. An infratectal layer is indistinct, or possibly absent.

Besides disappearing during acetolysis, the sexine is aberrant in several other aspects. The ornamentation pattern seems to originate by cracking of the outer exine, rather than by differentiated sporopollenin deposition. In the few species with a ± acetolysis-resistant sexine, the sexine elements remain colourless after acetolysis, while the nexine stains yellow-brown. These results suggest that the nature (e.g. chemical composition) of the sexine of *Amorphophallus* pollen differs from that of most other angiosperm sexines. Transmission electron microscopy must be applied to solve questions as to the stratification, cryptoaperturate condition and ontogeny of the pollen wall.

Evolution of Microsporangium Numbers in *Microseris* (Asteraceae: Lactuceae)

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Meristic characters (number of organs) of Angiosperms are often stable within species and higher

taxonomic levels. Little is known about the developmental mechanisms that lead to such invariant phenotypes. We have studied the number of microsporangia per anther, which is four in most angiosperm species. One of the genera in which a reduction of microsporangia to two has occurred is *Microseris*. In order to reconstruct the evolution of this character, we determined the number of microsporangia per stamen in serial sections of heads from all 13 species of *Microseris*, its close relative *Uropappus lindleyi* and the two allopolyploid species of *Stebbinsoseris*. Four *Microseris* species, three diploid and one tetraploid, have two microsporangia per stamen, all other species investigated have four. The most parsimonious assumption is that the disporangiate condition is derived, and arose once in the evolution of *Microseris*. One tetraploid species derived from a bisporangiate and a tetrasporangiate diploid ancestor, has four microsporangia. Another tetraploid species derived from a di- and a tetrasporangiate ancestor has two microsporangia per anther. The inheritance of the number of microsporangia per stamen in crosses between *M. bigelovii* (disporangiate) and *M. douglasii* (tetrasporangiate) was determined. Segregation of microsporangium number per stamen in F2s derived from these crosses is continuous rather than in discrete classes. The average number of microsporangia per stamen in the F2 plants ranges from 2·0 to 4·0. There is a predominance of tetrasporangiate stamens in the F1 and in most F2 plants. The observed pattern of inheritance suggests a major gene with dominance and quantitative modifiers. The inheritance is independent from another meristic character, the number of pappus parts per fruit, that segregates in the same F2.

A Method for the Computerized Analysis of Organelle Movement.

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Advanced microscopical techniques have revealed a highly individual movement of organelles, previously indicated as cytoplasmic streaming. We have developed a method to analyse these organelle movements using a computer-based image analysis system (VIDAS, Zeiss/Kontron, Eching, Germany).

Pollen tubes of *Nicotiana tabacum*, and root hairs of *Limnobium stoloniferum* were recorded on videotape. Distint images were displayed through a framegrabber and digitized. The chosen time interval between two images was 0·240 s. Stored images could be displayed either separately or in sequence at various speeds. The longitudinal axis of the tubular shaped cells was declared the *x*-axis with the tip-most point as zero reference. Organelles seen in at least six images were selected. Their centre was marked on the computer screen with the cursor. The marks were used by the VIDAS program to calculate the position in the cell (*x*- and *y*-coordinates), distance, velocity and angle of displacement. Data were collected in files per organelle and used for mathematical and statistical analysis.

This method provides quantitative information on organelle trajectories for a broad selection of organelles, sampled at fixed time intervals with known errors. The organelle trajectories were characterized by the linearity ratio, i.e. the total travelled distance divided by the distance between first and last recorded position. The linearity ratio ranged from 1·0 to 43·0 for 64 organelles measured in the tip of a pollen tube in 1 min. The error for the velocity is determined by the accuracy of marking the centre, the width of the screen pixels at a given enlargement and the time interval. The error was $\pm 0{\cdot}5\ \mu m\ s^{-1}$ for the data shown. Six root hairs of *Limnobium stoloniferum* were measured using this method and by clocking organelles with a stopwatch. The average speed did not differ significantly between the two methods.

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE ON 26 NOVEMBER 1993

Physiological Disorders and Plant Cancer in *In Vitro* Cultures

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A comparative investigation of *habituation* (hormone independence) in plant calli and of *vitrification* (malformations associated to hyperhydricity) of shoots under vegetative multiplication *in vitro*, has led us to establish a reciprocal relationship between both phenomena. Some fully habituated callus lines have (apparently irreversibly) lost the capacity to form primary meristems, thus their organic totipotency. Such lines exhibit many characteristics of vitrified tissues but also of animal cancerous cells. Conversely, vitrified shoots under micropropagation may become habituated, with many characteristics similar

to habituated calli. Progressive reduction of cell–cell adhesion inducing shoot breakability (friability is the term used for calli) leads to apex necrosis. This may represent cancerization at the whole plant level resulting in whole plant death through abandoning of the meristems, just as arrest (death) of the whole animal (human) being is caused by heart arrest. The surviving cells in culture are further incapable of organizing themselves into primary meristems. The irreversible loss of organic totipotency thus may represent the typical plant cancer trait, both at the cellular and organismic level, at the end of neoplastic progressions in the absence of pathogens (Gaspar, T. *et al.* 1991, *Physiol. Plant.* **83:** 696–701).

Habituated non-organogenic cells and vitrified shoots share, besides hyperhydricity, other common biochemical characteristics such as deficiency in tetrapyrrole-containing compounds and hypolignification. Some of them, for instance a deviation of nitrogen metabolism leading to accumulation of glutamate, proline and polyamines (Le Dily, F. *et al.* 1993, *Plant Cell Tissue Organ Culture* **35:** 69–74), may be interpreted as responses to one or several stresses and give the basis for an explanation of hormonal autonomy and the appearance of necroses.

Somaclonal Variation and Identification in Tissue-Cultured Plants

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Plant originating from adventitious meristems by organogenesis or embryogenesis may show mutations (=somaclonal variation, SV). We have examined two methods to measure the extent of SV in a population of regenerated plants. By such methods it will be possible to select regeneration protocols and cultivars that have the lowest SV. Our research was done in *Begonia* × *elatior* cv. Schwabenland Red.

In the first assay we measured the spread of variation in a quantitative trait, viz. leaf shape (G.-J. de Klerk *et al.* 1990, *Acta Bot. Neerl.* **39:** 145–151). Plants originating from callus, especially long-term cultures, showed a higher spread than plants directly regenerated on leaf explants. After mutagenesis with nitrosomethylurea a dose–response curve was obtained plotting the spread in variation against the concentration of the mutagens. The maximum spread was the same as found after regeneration from the long-term callus cultures. However, the spread was reduced in plants that originated by direct regeneration from the first generation of callus regenerants, albeit still significantly higher than in the direct regenerants. This indicates that the observed variation was not totally 'inherited', and it demonstrates the limits of the assay.

In the second assay we determined RAPD patterns of the DNA of the regenerated plants. We only found a few changes in the banding patterns of the plants from long-term callus. Plants which showed obvious phenotypic changes did not show a corresponding extent of change in RAPD pattern. So RAPD analysis is not suitable for the identification of SV.

This second assay, however, proved to be very suitable for the identification of cultivars. We tested the method for several begonia cultivars and we were able to discriminate between colour cultivars as 'Schwabenland Red' and 'White'. Preliminary experiments with very diverse plant material (callus, bulb, flower, *in-vivo* or *in-vitro* leaves) from several species and cultivars of lily, cucumber, tulip and anthurium were successful. The RAPD assay can be very useful for, e.g. protection of breeders' rights, and control against mistakes or misconduct.

A New Approach for Direct Embryogenesis in Plants

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At the Institute of Genetic Engineering, a new system for direct somatic embryogenesis has been developed in the model species alfalfa. We assume that omission of the callus phase during the process of regeneration will lead to avoidance or the diminishing of somaclonal variation.

Leaf explants were cut into small pieces, washed and cultivated on liquid B5-medium containing 2,4-D. The directly formed embryos were allowed to develop by treatment with polyethyleneglycol (PEG) and abscisic acid (ABA). The conversion into whole plants was realized by cultivation of singular embryos on MS-medium, containing gibberellic acid (GA_3) and 3-indoleacetic acid (IAA). An inverse correlation between the ABA content and the ability to form embryos was found in embryogenic and non-embryogenic donor material.

The initial stage of this procedure for alfalfa was also shown to work for sunflower, tomato, acacia, carnation and maize.

Hormonal Regulation of Apical Dominance in Microcuttings of *Rosa hybrida*

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Outgrowth of axillary buds can be inhibited by domination from an actively growing apical meristem. Hormonal studies suggest that apically produced auxin indirectly inhibits outgrowth of lateral

buds, whereas cytokinins promote outgrowth. To study the effects of different plant hormones on the maintenance and breaking of apical dominance *in vitro* independently from the presence of an apical meristem, we used isolated axillary buds (single-nodes) from microcuttings of rose cultivars 'Madelon' and 'Motrea'.

Positional effects were evident. With the apex removed, single-nodes from original positions closest to the apex sprouted faster and to a higher extent than single nodes from positions further down the stem, when placed on basal medium. Outgrowth could be inhibited by adding auxin to the medium. An amount of 0·1 μM 3-indolebutyric acid (IBA) was sufficient to inhibit sprouting of isolated single-nodes. Simultaneous addition of the cytokinin 6-benzylaminopurine (BAP) reduced the inhibitory effect of auxin, thus confirming earlier studies. However, in contrast to what is generally assumed, not the ratio of cytokinin:auxin but the absolute concentration of cytokinin was apparently the most important factor which affected the outgrowth of the isolated buds.

After breaking apical dominance, the correlative inhibition could be re-installed by adding IBA during the first 5 days after removal of the apex; there was no inhibitory effect of IBA on bud outgrowth when added after day 6. Pulse experiments with IBA suggest that the most sensitive period for IBA lies between 24 and 72 h after removal of the apex.

It was also possible to retard BAP-stimulated outgrowth of 'single nodes' using abscisic acid (ABA) instead of IBA. This suggests that ABA may also be involved in the regulation of apical dominance.

Regulation of Rooting in Microcuttings of *Malus*

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Rooting of microcuttings is a crucial step in micropropagation of many crops. It can be improved by optimizing the capability of microcuttings to respond to the rhizogenic signal. Various conditions during the propagation phase affected the rooting capability of microcuttings of *Malus*, a.o. the dormancy-status, the occurrence of vitrification, the concentration of hormones during propagation, and the time after subculturing at which the microcuttings had been harvested.

Rooting can also be improved by adapting the rooting treatment. In the rooting process, various phases can be distinguished: (i) dedifferentiation; (ii) the formation of a root meristemoid; and (iii) outgrowth of the root meristemoid to a primordium and subsequently to a root (cf. the phases in shoot regeneration, Christianson & Warnick, 1983, *Dev. Biol.* **95**: 288–293). It is likely that the various phases have different hormone requirements. We established the timing of the various phases by giving pulses with 6-benzylaminopurine (BAP) during the rooting treatment assuming that the second phase was specifically inhibited by BAP. The timing obtained in this way was confirmed by pulses given with auxin or with the anti-auxin 2-(p-chlorophenoxy)isobutyric acid.

The Use of Cyclodextrins in Plant Cell Biotechnology

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Cyclodextrins are cyclic oligosaccharides consisting of six (α), seven (β) or eight (γ) glycopyranose units. Cyclodextrins should be regarded as torus like rings with a polar outside and an apolar cavity. Due to the hydrophilic outside, the cyclodextrins are soluble in water. Dissolved in water, the apolar cavities of the cyclodextrins provide a hydrophobic matrix and are able to form complexes with a variety of guest molecules. Besides α-, β- and γ-cyclodextrin, several derivatives have been synthesized.

In bioconversion experiments using plant cell cultures, the efficiency and product yield are often limited by the water-solubility of the substrates. Organic solvents cannot be used as a result of decrease of cell viability. A new approach to solve this problem has been developed in our laboratory by carrying out bioconversions in the presence of cyclodextrins. The applicability of cyclodextrins in plant cell biotechnology has been demonstrated for the first time by the regiospecific ortho-hydroxylation of 17β-oestradiol into the catechol 4-hydroxyoestradiol by a phenol-oxidase from *in vitro* grown cells of *Mucuna pruriens*. Complexation with β-cyclodextrin enhanced the solubility of β-oestradiol from 12 μM to 660 μM.

Cell suspension cultures of *Podophyllum hexandrum* accumulate the cytotoxic lignan podophyllotoxin. β-Cyclodextrin has been used in order to facilitate feeding of the poorly water-soluble biosynthetic precursor coniferyl alcohol at a concentration of 3 mM to these cultures. The substrate feeding resulted in *c.* 6-fold enhanced podophyllotoxin accumulation.

Furthermore, the glucosylation of podophyllotoxin into its corresponding glucoside by cell cultures derived from *Linum flavum* has been investigated. The solubility of podophyllotoxin could be increased easily by the complexation with β-, γ-, dimethyl-β- and hydroxypropyl-β-cyclodextrin. Dimethyl-β-cyclodextrin met our needs the best and the solubility of the substrate podophyllotoxin could be enhanced

from 0·15 to 1·92 mM using a podophyllotoxin/cyclodextrin ratio of 1:1. A high glycosylation rate of 0·51 mmol l^{-1} suspension day^{-1} was calculated for the *L. flavum* cells growing in a medium that included the podophyllotoxin/dimethyl-β-cyclodextrin complex at a final concentration of 1·35 mM.

Finally, the *L. flavum* cell suspensions were fed with β-cyclodextrin-complexed coniferyl alcohol in order to study its glucosylation into coniferin. Endogenous coniferin levels were enhanced from 4·0% to 6·5% on a dry weight basis by this substrate feeding. It is concluded that cyclodextrins can be applied successfully in plant cell biotechnology because of their solubility action and non-toxicity to plant cells, leading to an extension of bioconversion possibilities.

MEETINGS OF THE SECTION FOR VEGETATION RESEARCH ON 4 NOVEMBER 1993

LONG-TERM VEGETATION CHANGES IN RELATION TO WILD HERBIVORES IN COASTAL ECOSYSTEMS

The meeting was arranged in cooperation with The Netherlands Ecological Society, on the initiative of the Centre for Ecological and Evolutionary Studies, University of Groningen.

Half a Century of Vegetation Change on the West Frisian Islands

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Due to their rich diversity in flora, vegetation and fauna the West Frisian Islands (the Dutch Wadden islands) are the most important wildlife area of The Netherlands. Such is not the consequence of minimal disturbance by man. On the contrary, the dune area of the islands has been recklessly exploited by man since time immemorial, and it was devastated till around the turn of the last century. The high diversity mentioned above is largely due to a strong small-scale habitat differentiation, resulting in short-distance gradients such as salt–fresh, sand–silt, high–low, dry–wet, rich vs. poor in lime, old–young. This effect is reinforced by the overall low lime content of the dune sand (in contrast to the southern mainland dunes which are rich in lime).

The vegetation changes on the islands are partly a natural process due to: (i) alternating periods of accrescent and abrasion coasts; and (ii) decalcification and acidification brought about by the precipitation balance. On the other hand, changes are the result of human impact; these may have either a positive or a negative effect on flora and vegetation.

On most islands, cattle, sheep and goats freely roamed about until *c.* 1900. After the government stopped this practice, farmers were compensated by drainage and cultivation of wet dune slacks; moreover, coniferous forest has been planted on up to 12% of the surface of the dune area. The loss of hygrosere communities was partly compensated by the effect of accrescent coasts; new wet primary dune valleys originated in the younger dune system near the coast. However, since about 1940 the phreatic level is decreasing over 1–1·5 m, due to (i) deep drainage in adjacent cultivated polders: (ii) the larger evaporation of coniferous afforestation compared to that of herb vegetation; and (iii) drinking-water extraction.

The succession from pioneer stage, preponderant in previous centuries, to the terminal dune heath communities (*Salici–Empetretum, Empetro–Ericetum tetralicis*) has been taken about half a century after the exploitation of the dunes stopped.

During the last decades, spontaneous establishing of trees and shrubs in dune valleys has considerably increased. The causes of it are complex: (i) decreased rabbit grazing due to myxomatosis since 1957; (ii) a series of wet years since 1983; (iii) the impact of recent nearby plantations acting as diaspore sources (vicinism); (iv) pollution by precipitation. The pollution factor is obvious by the strong decrease of terrestrial lichens in the *Violo–Corynephoretum.* Moreover, the introduced neophyte moss *Campylopus introflexus* is becoming a pest.

The development of coniferous afforestation has induced controversy: on the one hand, a tendency to stimulate deciduous forest; on the other, the conservation of pure coniferous forest, since a number of species native to natural coniferous forests have spontaneously established (neophytes).

The pioneer communities of wet dune slacks, e.g. the *Samolo-Littorelletum* and the *Junco baltici–Schoenetum nigricantis*, have a high conservation value. They require seepage of base-rich (lime-rich) ground water. Such communities are seriously threatened by a decrease in phreatic level, the more so when the management results in change to a rainwater regime.

Whereas the overexploitation in previous centuries has lead to a preponderance of pioneer communities,

the managing policy of the last 50 years has brought about the opposite: a strong prevalence of terminal stages. For the sake of optimal diversity the contemporaneous nature management rightly aims at a balance between too much and too few dynamics.

In the salt-marsh (halosere), the natural succession is much less spectacular than that in xero- and hygrosere. The spectacular development of salt-marsh upon the large former sandflats on Terschelling and Schiermonnikoog is largely the result of human impact, viz. by the construction of sand dams which prevent the high energy flooding by the North Sea, but admit the low energy flooding by the Waddensea, resulting in silt deposit.

Traditionally, vegetation succession in salt-marshes has been inferred from the zonation of the vegetation. Long-term monitoring of the halosere succession on the Boschplaat, Terschelling revealed, however, that the changes in vegetation did not correspond to the zonation scheme. On such flats, the primary abiotic difference between low (submergence) and high (emergence) salt-marsh are not overruled by succession. Different abiotic factors control the changes in the distinguished zones and give the vegetation development the character of a primary allogenic succession. In the low salt-marsh the accretion of silt and the increasing aeration of the soil preponderate. In the middle high salt-marsh the master factors are consecutive silt accumulation on the formerly sandy beachplain and an increasing stronger drainage by an expanding creek system. In the high salt-marsh the prevailing factors are probably the consolidation by the vegetation of blowing sand, followed by the deposition of silt which counteracts desalination. In the upper high salt-marsh the succession is mainly determined by the increase in tidal drift material washed ashore.

Soil-borne Pathogens and Parasites and their Role in Changes of Coastal foredune Vegetation

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In coastal foredune vegetation, the successional gradient from the beach towards the inland dune area is correlated with changes in both abiotic and biotic factors. From the beach towards the inner dunes, the deposition of windblown sand and salt diminishes. In stabilized dunes, organic matter and nutrients accumulate and calcium carbonate leaches from the top soil. There is interspecific competition for resources and the nature of limited resources may be variable along the vegetation gradient, shifting from nutrient limitation in pioneer stages towards light limitation when shrubs invade. Herbivores, especially rabbits, can have a large impact on the development of the vegetation and animal diseases, such as myxomatosis, affect vegetation development in dunes.

Gradually, the role of soil micro-organisms in vegetation processes becomes more and more apparent. Free-living nitrogen-fixing bacteria and vesicular–arbuscular mycorrhizae are supposed to enhance colonization abilities of various foredune plant species. Soil-borne pathogens (fungi) and parasites (nematodes) were found to be involved in the degeneration of *Ammophila arenaria* (marram grass) and *Hippophaë rhamnoides* (sea buckthorn), two plant species that dominate coastal foredunes in Europe. Young dunes also contain large amounts of soil micro-arthropods compared with the expected food sources in these systems. Their effect on foredune ecology needs to be studied.

The role of pathogenic and parasitic micro-organisms will be the main theme for this presentation (cf. Van der Putten, W. H. (1993). In: Miles, J. & Walton, D. W. H. (eds.): *Primary Succession on Land.* Blackwell Scientific, Oxford, pp. 273–281; Van der Putten, W. H. *et al.* (1993): *Nature* **362:** 53–55; Zoon, F. C. *et al.* (1993): *Fundam. Appl. Nemotol.* **16:** 247–258). Recently, it appeared that soil-borne pathogens and parasites of *A. arenaria* did not affect *Festuca rubra* ssp. *arenaria, Carex arenaria, Elymus athericus*, which succeed *A. arenaria*. In earlier studies, *H. rhamnoides*, was found to be reduced less in soil from the root zone of degenerated *A. arenaria*, than in soil from its own root zone. At that moment, it was concluded that nematodes, which were present in the root zone of degenerated *H. rhamnoides*, could be involved in its degeneration. Now we know that the root zone of *A. arenaria* also contains soil pathogens, we may as well conclude that *H. rhamnoides* is relatively tolerant for the pathogens of *A. arenaria*. A large number of soil micro-organisms may be involved in the growth reduction of the plants and there may be a number of mechanisms contributing. The micro-organisms may act as sinks for assimilates, they may change the uptake capacity of the roots by injuring root hairs or by causing stunted root growth, and the vascular system of the plants may be disturbed, resulting in reduction of the transport process. Several of these phenomena have been observed in our experimental plants.

How may vegetation processes be influenced? Most likely, involvement in interactions among different biotic levels might clarify the role of specific soil pathogens and parasites in succession. Preliminary studies have shown that the specific micro-organisms affect the competitive ability of their host negatively. In a successional gradient, therefore, a preceding plant species will have a disadvantage as compared to its successor. Long-term replacement

experiments in the absence or presence of pathogens and parasites should be done to provide further information on the role of these micro-organisms in foredune vegetation succession.

In my opinion, integration of long-term vegetation studies, extensive field work on species and/or genotypes, and experiments in the field as well as under more controlled conditions in relation to soil micro-organisms is highly important to understand processes on the level of individual species, communities and ecosystems. When the importance of well-defined long-term studies is recognized, a strategy should be defined by both scientists and grant-supplying organizations to support such initiatives.

Vegetation Mapping of the East Frisian Islands

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The intention of this project is the investigation of vegetation of the inhabited and uninhabited East Frisian Islands in order to achieve a complete floristic and plant-sociological presentation. The results of this exploration will be association tables and maps, supported by investigations on soil and water under physical and hydrochemical aspects. On this data, a comparison of the vegetation and landscape development can be worked out, regarding aspects such as population dynamics and succession processes.

The investigations are based on vegetation maps and plant-sociological relevés on a fine level made by Tüxen *et al.* (1929–49) which are gathered in the archive Tüxen (Institute of Geobotany-University of Hannover); see also Pott, R. (1991): Vegatationskartierung der Ostfriesischen Inseln. In: Haseler, V. & Janisch, P. (ed.), *Bibliographie und Informationssysteme der Universität Oldenburg*. 61–63, Oldenburg Verlag; Hobohm, C. (1991): *Die Vegetation von Norderney*. Diss. Univ. Hannover, 150 S. u. Anhang. Fine stratigraphic peat examinations and pollen-analytical investigations in suitable organogenic deposits in recent and subfossil dune valleys will provide knowledge about competition and succession biology of special plant communities.

The combination of all methods described above makes it possible to show the development and change of form, vegetation and landscape concerning anthropo-zoogenic influences over a defined period of about 50 years (documentation of changes in relief, such as succession of dune vegetation, ageing of dunes, parabolization, development of settling and cultivation, tourism, rabbit population development, dune protection, etc.).

General issues

Floristic and plant sociological investigations on the vegetation of all islands.

Immigration of plant species and their distribution (floral gradient from east to west).

Documentations of changes of the islands' positions in the last 50 years (formation of dunes and the development of salt-marshes).

Representation of changes in relief of all islands (changes of dune and dune valley morphology in space and time).

Changes in vegetation and characteristic landscape of the islands under anthropo-zoogenic influences.

Special changes due to mass tourism development during the last 50 years (sinking of the ground water level and the sealing up of the soil).

Problems of the ruderalization comparing urbanized and rural islands.

Rating the influence of wild rabbits (comparison of islands with or without rabbits).

Actual vegetation mapping.

Special issues on vegetational dynamic and successional processes

Mapping of cryptogamic communities and special mapping in the area of dunes and salt-marshes if changes of vegetation are observed.

Succession models of the xeroseries and coastal dunes.

The haloseries in the salt-marshes.

The hygroseries in the area of wet dune valleys.

Investigation of local succession processes.

How do soil acidification processes influence the successional processes?

Influence of intensive and extensive grazing.

Influence of fertilizing effects (e.g. aerial depositions).

Changes in substance composition of soil and water on the vegetation; biotic and abiotic phenomena.

Questions on the co-evolution of nitrophytic woodland associations (e.g. *Sambucus nigra* shrubs).

Investigations of vegetation history characterizing the vegetation and landscape development processes of the islands

Pollen-analytical investigations in organic deposits in dune valleys in order to examine the vegetation development of the last centuries.

Fine stratigraphical analysis of macro-fossils in suitable peats to reconstruct the local vegetation succession.

Prospection of former dune valley peats covered by sand to date the time of dune formation.

Specific questions on islands

Changes in agriculture.

Abandonment of farming.

Fixing and documentation of areas which are worth preserving.

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Plant Species Composition, Plant Production, Compartmentation of Nitrogen and Geese Grazing in a Successional Gradient on Salt-Marshes

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The Wadden Sea island of Schiermonnikoog moves gradually from west to east. The result is that the salt-marsh area spreads towards the east. Hence, a chronosequence of primary succession starting on sand can be found including development stages ageing from a few years to 100 years (De Leeuw, J. *et al.* (1993): *Acta Bot. Neerl.* **42:** 435–445). The successional series on the higher salt-marsh features *Platago maritima, Armeria maritima, Festuca rubra* and *Elymus athericus*, on the mid salt-marsh *Plantago maritima, Festuca rubra, Artemisia maritima* and *Elymus athericus*, and on the lower salt-marsh *Puccinellia maritima, Limonium vulgare, Atriplex portulacoides* and *Juncus gerardii* (Olff, H. (1992): *Mechanisms of vegetation succession.* Thesis, University of Groningen).

The thickness of the sediment layer, determined by the frequency of inundation, increases rapidly in the chronosequence of the lower salt-marsh, and slowly on the higher salt-marsh. The soil N pool is positively correlated to the thickness of the sediment layer. The N mineralization rate gradually increases in the successional series of both higher and lower salt-marsh, which might be related to an increase of the amount of organic matter. The relative nitrogen turnover (percentage N mineralization of soil N pool) decreases from 20–70% in the young salt-marsh to 5% in the older salt-marsh.

The net increase of living plant material in spring is positively correlated to the N mineralization rate, except for the oldest part of the higher salt-marsh. It is suggested that the dense layer of litter of the *Elymus athericus* stand prevents early growth through shading (Bakker, J. P. *et al.* (1993): *Hydrobiologia* **265:** 73–95). Despite the net increase of living plant material on the lower salt-marsh, the aerial fresh biomass does hardly increase during succession, suggesting a quick decay of plant material, particularly in the lower salt-marsh.

Brent geese (*Branta bernicla*) forage the lower salt-marsh in the whole successional series. They prefer, however, the stage of 25 years old, including the mid salt-marsh part. Some relationships seems to exist between the tissue N concentration of living plant parts and the grazing intensity on the lower salt-marsh. However, the extent to which geese themselves increase tissue N-concentration by the quick recycling of nitrogen from their droppings should be studied. The impact of geese (and rabbits and hares) in spring results in higher percentages of light reaching the soil in grazed than in excluded sites. These differences are smaller in September after the growing season.

Changes in the Vegetation of a Wet Dune Slack over a 30-year Period in Relation to Changes in the Hydrological System

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Recent studies on dune slack vegetation in The Netherlands have shown that calciphilous dune slack species have become increasingly rare during the last 20–30 years, even in dune areas with a high lime content (Van Dijk & Grootjans (1993): *Hydrobiologia* **265:** 281–304). Among the species most affected are *Littorella uniflora, Schoenus nigricans, Liparis loeselii, Epipactis palustris, Dactylorhiza incarnata*, and *Parnassia palustris*. These species can still be found on several Wadden Sea islands in (different) pioneer stages in mainly three types of dune slacks: (i) primary beachplains; (ii) secondary, blown out, slacks; (iii) slacks in seepage areas of the main hydrological system of an island.

Most of the endangered calciphilous species are restricted to habitats with a low nutrient availability. A high pH and regular flooding (Lammerts *et al.* (1992): *Coastal Dunes:* 265–272). Later successional stages are characterized by a rapid build-up of organic material and associated acidification of the top soil. The higher availability of nutrients stimulates the growth of more productive plant species, such as *Calamagrostis epigejos* and *Carex nigra* and the low productive pioneer species disappear. The rate of organic matter accumulation in dune slacks shows marked differences between slacks. Some slacks start to accumulate organic matter as soon as plants are established, but in others pioneer species persist for decades and almost no accumulation of organic matter takes place. Nowadays dune slacks with a low rate of organic matter accumulation are extremely rare along the Dutch coast, but historical evidence suggests that several of such dune slack types have existed on the Wadden Sea islands, one example being an old dune slack complex ('Kapenglop') situated in the centre of the island of Schiermonnikoog. Calciphilous pioneer species, such as *Schoenus nigricans, Dactylorhiza incarnata, Epipactis palustris* and *Pedicularis palustris*, have existed here for decades. Practically all pioneer species disappeared between 1977 and 1983 (Grootjans *et al.* (1991) *Acta Bot. Neerl.* **37**: 265–278) and a vegetation analysis over a 30-year period indicated a marked acidification and a decrease in highest water levels. These changes were caused by changes in the local hydrological system (groundwater abstraction on behalf of the public water supply and drainage) and not by decalcification of the top soil during the period.

In order to study the hydrological system that is responsible for the long-term existence of nutrient-poor and alkaline conditions in the rooting zone, a hydrological modelling of the area was carried out and the simulated flow line pattern was compared with the results of a hydrochemical facies analysis of the groundwater. The hydrochemical evolution of the dune water along the flow lines was interpreted from 250 water samples, most of them obtained from minifilters installed in deep borings to a depth of 24 m below the surface. We found a highly reduced groundwater facies underneath the dune slack, which was characterized by very low values of SO_4^{2-} and very high contents of Ca^{2+} and HCO_3^-. This was interpreted as outflowing groundwater. A detailed analysis of the inflowing groundwater in the seepage zone of the slack revealed that this water was moderately rich in Ca^{2+} and HCO_3^- and had fairly low values of SO_4^{-2}.

A simulation of the groundwater flow pattern showed that the inflow of seepage water was only possible when the slack was flooded. Under such conditions groundwater can proceed as surface water and is depleted in CO_2, Ca^{2+}, PO_4^{3-} and NH_4^+ by aeration, biological uptake and (co)precipitation (Stuyfzand (1993): Thesis, University of Amsterdam). In the infiltration part of the slack the water passes through the anaerobic slack bottom layer with CO_2 producing plant roots and creates a water type which is highly reduced and is aggressive toward calcareous substrates in the subsoil. It has also higher phosphate levels due to phosphate mobilization in the anoxic slack layer.

The interactions between calciphilous vegetation types and the discharge of calcareous groundwater are easily disturbed by even small changes in the local hydrological conditions. The result is a rapid acidification and the decline of endangered dune slack species.

It would be very interesting to test the hypothesis that in undisturbed seepage slacks the build-up of organic material is retarded by interactions between microbial mats (that keep anoxic conditions predominant in the top soil (Visscher (1992): Thesis, University of Groningen), phanerogams that leak oxygen from their roots, and marsh plants and shrubs that accumulate nutrient in the rooting zone. Furthermore, grazing by rabbits appears to be important in early successional stages. This may also be an important mechanism to keep the accumulation or organic matter at a low level.

Herbivores and Ecosystem Function

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The text-book view that 'the world is green, so herbivores cannot be important in ecosystem function' (Hairston, N. G. *et al.* (1960): *Am. Nat.* **44**, 421–425) has been thoroughly refuted by long-term experimental work involving the exclusion of vertebrate herbivores using fences (Crawley, M. J. (1983): *Herbivory*. Blackwell Scientific Publications, Oxford) and invertebrate herbivores using chemical pesticides (Crawley, M. J. (1989): *Annual Review of Entomology,* **34**, 531–564). The impact of excluding small, sessile, relatively monophagous herbivores like insects tends to be more subtle, and to take longer to manifest itself (typically 5–10 years) than does the exclusion of large, mobile polyphagous species (like deer, rabbits, sheep or cattle). Erecting a fence generally produced a substantial visual effect within a single growing season (plants grow taller, the rates of flowering and seed set are increased, new seedling of previously excluded species become established; Crawley, M. J. (1990): *J. Appl. Ecol.* **27**, 803–820).

An important issue involves the extent to which herbivores can affect ecosystem-level functions like primary productivity, rates of nutrient cycling, standing crop biomass or species richness. Likewise, there

is some debate about the extent to which herbivores could be said to be important if they affect none of these high-level system properties, but have other, no less conspicuous, impacts (e.g. they might change the identity of the dominant plant species without any impact on biomass or productivity; Pacala, S. W. & Crawley, M. J. (1992): *Am. Nat.* **40,** 243–260).

The impact of specialist insect herbivores on plant population dynamics has been demonstrated in spectacular fashion by numerous successful cases of biological weed control in which an introduced insect has eliminated what had previously been impenetrable thickets of weed (Crawley, M. J. (1989): *Biocontrol News and Information* **10,** 213–223). The difficulty in generalizing from these cases of biocontrol is that both the insect and the weed are usually alien species growing in a foreign environment where they have no history of adaptation. The plants have often become abundant in the first place because of mismanagement of the habitat by humans (e.g. overgrazing, clear-felling, eutrophication, urban dereliction). The insect herbivores introduced for weed control have been carefully screened to ensure that they are introduced into the new environment without any of their predators, parasites or diseases. There are remarkably few clear-cut examples of a native insect herbivore having a profound impact on the population dynamics of a native plant. Where there have been long-term studies of native insect–plant systems (e.g. ragwort and cinnabar moth, studied in Meijendel by Van der Meijden, E. (1979): *J. Ecol.* **67:** 131–153; and at Silwood Park by Crawley, M. J. & Gillman, M. P. (1989): *J. Anim. Ecol.* **58,** 1035–1050), these studies have both shown extreme assymmetry in the interaction; the insect is food-limited and fluctuations in its abundance are driven largely by competition for fluctuating plant resources, but feeding by the insect has little if any impact on the population dynamics of the plant (its recruitment is regulated by external factors such as rainfall and microsite availability). It now seems likely that it is the less conspicuous insect herbivores (like root-feeders and stem borers) that have a measurable impact on ragwort populations (e.g. the root-feeding beetle *Longitarsus jacobaeae* increases mortality suffered by the rosette stage; McEvoy, P. B. & Rudd, N. T. (1993): *Ecological Applications* **3,** 682–698).

The other important role of herbivores (especially, the large, mobile, polyphagous species) is in altering the physical structure of the plant community and in providing the background level of physical soil disturbance in which seedling recruitment can occur (Lubchenko, J. (1978): *Am. Nat.* **112,** 23–39). In the case of ragwort, for example, long-term removal of the cinnabar moths by hand-picking the caterpillars led, not to the increase in plant numbers that might have been expected, but rather to their decline to extinction. The reason was that the main effect of cinnabar caterpillar feeding was to reduce seed production by ragwort (often reducing it to zero), so the hand-cleared plants produced large seed crops while the control plants were defoliated and stripped of their young flower heads. The key point is that the probability of death of a ragwort plant depends upon its seed production in the previous summer. Plants that produce a lot of seeds are much more likely to die during the winter than plants of a similar size which produced little or no seed (Gillman, M. P. & Crawley, M. J. (1990): *Funct. Ecol.* **4,** 585–589). Thus, the caterpillar removal *increased* the death rate of the experimental plants while the plants with caterpillars produced much less seed and had much higher over-winter survival. The experiment was carried out inside rabbit-proof fences, so the grass grew long during the course of the work, and this meant that suitable microsites for seedling recruitment by ragwort were no longer available. This in turn meant that the extra seed produced by the caterpillar-free plants did not lead to the appearance of new recruits. The only ragwort plants left at the end of the experiment were the survivors of the original cohort of individuals present at the beginning. These were the plants which had continued to be attacked and defoliated by cinnabar moths; the insect-free plants had long-since reproduced themselves to death without leaving any progeny.

This example highlights the fact that without rabbits to maintain a short turf in which the rosette-forming ragwort juveniles can survive, and to create the open sandy gaps in the sward by their digging and scraping in which ragwort seedlings can become established, then neither ragwort nor cinnabar moth could persist. In many systems, the actions of keystone herbivores (like rabbits in this example) determine the nature and the outcome of interactions between quite unrelated herbivores and their host plants. It is salutary to bear in mind, however, that most of the examples of keystone herbivores come from systems in which humans have eliminated or greatly reduced the population densities of natural enemies. We have very few examples of keystone herbivores from pristine systems where top predators like wolves are present at naturally determined densities. The examples that we do have tend to come from large, inhospitable regions like the Canadian arctic (e.g. snow-shoe hares and lynx; Keith, L. B. (1963): *Wildlife's Ten-Year Cycle.* University of Wisconsin Press, Madison). We shall only discover the role of herbivores in pristine ecosystems by long-term observation, experiment and modelling.

Those charged with the management and conservation of habitats should be aware that changes in the grazing regime are likely to have far-reaching and long term consequences, not only for the plant

community but also for the distribution and abundance of the other animals that live in the habitat. The changes may be profound, and may be extremely difficult to reverse if habitat management objectives were subsequently to change (Pacala & Crawley, *loc. cit.*). For example, the introduction of domestic livestock onto salt-marsh is likely to lead to a reduction in plant species richness. this prediction is based on one of the best-documented empirical generalizations in ecology: for a given system, a management-imposed increase in primary productivity will be associated with a reduction in plant diversity. The prediction is somewhat counter-intuitive, in that comparisons between different habitats often show that reduced biomass is associated with *increased* biodiversity. In a newly grazed salt-marsh, however, productivity would increase under grazing despite a reduction in sward height, becauseof a reduction in the mean age of plant tissues (young, regrowth leaves have higher rates of net photosynthesis) and an increase in the rate of nitrogen cycling (dung and urine are associated with higher rates of mineralization than many kinds of dead plant tissue). The reduction in biomass would be associated with a reduction in plant species richness because it is only a subset of the original salt-marsh species that would remain competitive under these new circumstances (e.g. the grazing-tolerant species with high powers of regrowth, or the grazing-resistant species which are unpalatable to the herbivores). If, in the longer term, plant species diversity were to recover its original levels, this would probably be due to the invasion of weedy species and would almost certainly be symptomatic of over-grazing (e.g. the weeds would colonize eutrophic patches or bare ground caused by trampling or poaching).

While the impact of invertebrate herbivores is often subtle, the impact of domestic livestock is generally profound. Experience has shown that most of the processes involving large, polyphagous herbivores exhibit strong hysteresis: the changes caused by the introduction of livestock are much more difficult to reverse than they are to initiate.

Disturbance, Herbivory and Resilience of Tundra Plant Communities

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Northern terrestrial ecosystems in spite of their overall low net above-ground primary production support large populations of herbivores (Jefferies, R. L. *et al.* (1992): Tundra grazing systems and climatic change. In: Chapin, F. S. *et al.* (eds) *Arctic Ecosystems in a Changing Climate, An Ecophysiological Perspective.* Academic Press: 391–412). The availability and food quality of forage species, together with the different digestive physiologies and gut morphologies of herbivores, set limits on the ability of animals to effectively exploit alternative forage types in order to meet requirements for growth and reproduction.

A continuum of plant responses to herbivory may be recognized. Early successional plant communities of disturbed environments, which are often characterized by high rates of nitrogen turnover, are a prime source of forage. The growth habits of the plants, their nutritional quality and the foraging behaviour of the herbivores result in strong positive feedbacks between the plants and animals (Jefferies, R. L. (1988): Vegetational mosaics, plant–animal interactions and resources for plant growth. In: Gottlieb, L. D. and Jain, S. K. (eds): *Plant Evolutionary Biology*, Chapman and Hall: 340–361). Herbivores may accelerate plant succession (Pastor, J. & Naiman, R. L. (1992): *Am. Nat.* **139:** 690–705) or 'reset the successional clock' (Hik, D. S. *et al.* (1992): *J. Ecol.* **80:** 395–406) depending on their foraging behaviour and the type of plant community. Within other plant communities seasonal pulses in the availability of high-quality food sources may lead to intense foraging and a temporary loss in species abundance, thereby producing a vegetational mosaic as forage at alternative sites is successively utilized (Jefferies, R. L. *et al.* (1992): *op. cit.*).

In recent years herbivores such as lesser snow geese have increased dramatically in numbers, probably as a result of changes in winter food supplies and in hunting pressure brought about by conservation and farming interests. In addition, the prevailing weather in spring during the last decade has delayed the northward migration of these birds. As a consequence, intense foraging by large numbers of birds at southern arctic staging sites on the coasts of Hudson Bay in spring has initiated a sequence of changes that has led to the destruction of coastal summer grazing pastures via a positive feedback mechanism similar to the process of desertification (Kerbes, R. H. *et al.* (1990): *J. Appl. Ecol.* **27:** 242–258, and Strivastava, D. S. (1993): *The role of lesser snow geese in positive, degenerative feedback processes resulting in destruction of salt-marsh swards*; M.Sc. thesis, University of Toronto). This had resulted in marked asynchrony between the re-establishment of vegetative swards following habitat destruction and the life expectancy of cohorts of lesser snow geese. The depletion of primary sources of forage has had adverse effects on gosling size and survivorship (Cooch, E. G. *et al.* (1991): *J. Anim. Ecol.* **60:** 483–496 and Williams, T. D. *et al.* (1993): *J. Anim. Ecol.* **62:** 766–777) which in turn has affected adult size, survivorship and fecundity.

Feedback processes initiated by herbivores influence not only the structure and composition of plant

communities but also the biomass of each trophic level in these ecosystems.

Interdependence of *Branta* geese and Saltmarsh Vegetations in the Wadden Sea

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The spring flush of highly nutritious and easily digestible growth of the early successional salt-marsh communities is intensively harvested by staging geese preparing for migration to the high Arctic breeding grounds. On our study island Schiermonnikoog both barnacle (*Branta leucopsis*) and brent (*B. bernicla*) geese succeed one another in the course of the season with a partial overlap resulting in exploitation periods of up to 12 weeks in some vegetation zones. The heavy offtake prolongs the explosive phase of vegetative growth extending the time window of exploitation by the geese (Ydenberg, R. C. & Prins, H. H. T. (1981): *J. Appl. Ecol.* **18:** 443–453). Recent work shows that a growth enhancement due to the nutrient inputs from goose droppings as has been demonstrated elsewhere applies here as well (Bazely, D. R., McCleery, R. H. & Prins, H. H. T. (in press)), and current work is aimed at detecting effects on the vegetation over a period transcending the within-season effects studied so far.

Geese at this season are dependent on a limited set of plant species where nutrient-rich new growth can be harvested efficiently. Detailed work (Prop, J. & Deerenberg, C. (1991): *Oecologia* **87:** 19–28) on diet assembly in brent geese shows that inclusion of *Plantago* and *Triglochin* (that can be harvested by large bite-size) is crucial to the achievement of body condition providing adequate reserves for both migration and breeding. This critical resource is distributed patchily on the merse and detailed analysis of flock passage (combining ciné-film with stereophotography of the blades on offer both before and after massive waves of exploitation) reveals that these patches are rapidly depleted, a considerable segment of the flock obtaining none whatever. Under these circumstances of intense competition behavioural observations on marked brent, coupled with analysis of diet (via droppings of that individual), showed that females whose mate enjoyed a high dominance score (i.e. won most interactions) achieved diets with the highest levels of *Plantago* and *Triglochin* and concomitantly the most rapid rate of fattening, as established by intensive visual monitoring of the abdominal profile by referring to a set of profiles. This index was calibrated with birds caught and weighed locally and categorized subsequently by referring to a set of profile drawings whenever a ringed individual was spotted from the observation tower. Mean values cluster within 5% of actual body mass, ranging from 1200 to 1600 g in this period.

Brent goose counts show this site is already used to capacity, numbers being constant over the past 20 years despite a continuous rise of the world population of the subspecies concerned, the surplus spilling over onto the marsh fringing the adjacent mainland where a disproportionately steep rise has been documented over the same period. This situation parallels that documented for the nearby island Terschelling (Ebbinge, B. C. (1992): *Ardea* **80:** 203–228) where brent numbers likewise have tended to plateau during the last two decades. Indirect evidence that the food supply is limiting has accrued from small-scale experiments where growth was enhanced by applying fertilizers (Teunissen, W., Spaans, B. & Drent, R. (1985): *Ardea* **73:** 109–119) resulting in sharp increases in goose visitation. Observed mortality and production figures show that the brent utilizing the island show a positive balance from year to year, hence active exclusion of some individuals is implied. Direct observation of return rates of birds marked on the island confirm that pairs with low dominance status are least likely to return in subsequent seasons. Future work will include radio-telemetry to study movement of individuals in greater detail in a vegetation grid.

Return rates of goose flocks to utilize regrowth (Prop, J. (1991): *Ardea* **79:** 331–342) are so timed as to maximize the net energy intake given the constraint of habitat saturation implied by the census data. More work at the individual level is aimed at elucidating the mechanism the geese rely on to achieve this dose match.

The Importance of Long-term Studies in Ecology

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Because science is expensive today it is continuously judged by the fund-providing authorities. Peers and citation indexes are popular instruments for such judgements. Not surprisingly, individual scientists, departments and institutes anticipate on these judgements by making a selection of research themes and objects that enable them to meet the required standards. The most important standard, the quality of research, has not changed. Today's selection, however, is biased by opportunism. It is driven by the wish of fast production of results, i.e. publishable papers. The present-day system of doctoral research by Ph.D. students during 4-year periods in which at least four to five papers should be finished contribute to that selection. In the extreme situation such selection might lead to neglecting the study of:

(1) extremely exciting 'high-risk' topics;
(2) very complex questions;
(3) long-term processes.

Most universities now allot structural Ph.D.positions to research groups. I hope that these groups dare to 'spend' a fair proportion of these positions on the really exciting questions in their field of research.

As to the second aspect, the complex questions, scientists can and should work together to tackle these questions. I think that it was a wise decision of the National Science Foundation (NWO) to introduce the so-called program grants alongside the project grants. Of course cooperation is not a goal in itself, although one sometimes has that impression from scientific boards or managers. Cooperation is sometimes a necessary tool to answer questions that need the expertise of a team.

It is the third aspect that really worries me. In some fields of science long-term studies are essential because we are dealing with processes that cannot yet be simulated by short-term experiments because much essential basic information is still lacking. One example is the quantitative and qualitative effect of environmental changes on life on earth. Especially in this era of accelerating environmental change long-term studies are needed.

It was a lucky choice of the organizers of this symposium on the occasion of the fortieth anniversary of the Zoological Laboratory of the University of Groningen to choose 'Long-term changes in coastal ecosystems' as the central theme. It provides the opportunity to reflect on the meaning of long-term studies in ecology.

Stating really important new ecological questions needs a solid basis of information that allows us to pinpoint phenomena or patterns that cannot be explained by existing theory. The elegant experiments on the influence of micro-organisms on plant growth that provide an explanation for yet unexplained aspects of vegetation succession by Van der Putten (this symposium) is a good illustration of this statement. Van der Putten's study finds its basis in many years of ecological research on patterns in the spatial and temporal change of vegetation composition at the former biological field station 'Weevers' Duin'. That study provided a number of unanswered questions: the inexplicable pattern of disappearance of some plant species. Oremus and Van der Putten could demonstrate that micro-organisms are responsible and have a different effect on different plant species, and thus may cause specific patterns in vegetation succession. I expect that several researchers all over the world will not start to test these ideas on the same system elsewhere or on other systems. Although such research is rather duplicative and affirmative than moving the frontiers of our knowledge, it may result within a short time in publishable papers. The only positive contribution of this latter group of papers is that they eventually determine the generality of new ideas, but they do not generate new ideas themselves.

The long-term studies on geese and salt-marshes, and their interdependence, carried out by the animal and plant ecology groups at the University of Groningen provide more examples of the scientific importance of long-term studies. They show that abiotic processes are usually much slower in affecting the environment than biotic processes like grazing, and that at least a number of biotic effects seem to be reversible. This combined study is one of the few in the world in which both herbivores and plants are studied with equal attention. The results on their interdependence are fascinating. I will not repeat them here as there are reports on the preceding pages. What this study also demonstrates is that it provides a continuous flow of important questions suitable for doctoral research programs. The general study profits from the PhD studies. At the same time the students profit from the wealth of background information that is available.

Probably one of the best illustrations of the importance of long-term studies is Den Boer's work on the distribution and abundance of carabid beetles. His study not only forms an excellent contribution to our knowledge on the population dynamics of insects, it also led to a general theory applicable to all kinds of organisms: the 'Spreading of Risk' theory. The work also contributed to the theory of metapopulation dynamics. Apart from these contributions to science the study provides the scientific basis for the conservation of nature in scattered habitats. Without long time-series of data, combined with experiments on dispersal, this major contributions to ecology would have been impossible.

I certainly do not intend to make a single-minded plea for long-term studies in general. They usually require much manpower for a long period. Consequently, one should be absolutely sure that the specific problem can only be solved by a long-term study. The ultimate success of a study depends to a large extent on the accuracy of stating the problem and on the careful selection of a specific procedure to solve it. These aspects are important for all studies, but they are crucial for the success of long-term studies.

I would like to call the attention of the Ministries of Education and of Agriculture, The Netherlands Organization of Scientific Research (NWO) and The Royal Netherlands Academy of Sciences (KNAW) to the importance of long-term studies in ecology. The present-day system of funding research is not conducive to starting or continuing long-term studies.