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MEETING OF THE SECTION FOR FERTILIZATION RESEARCH IN PLANTS ON 24 FEBRUARY 1995

Strategies to Elucidate the Function of the Pollen-specific Gene NTP303 of Tobacco

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Plant sexual reproduction is a process which depends on the co-ordinate development and activity of the male and female gametophyte. In flowering plants, the male gametophyte or pollen is produced within the anther and its development is dependent on the provision of nutrients and other components from the tissues of the sporophyte as well as on an extensive genetic programme within the pollen grain itself.

The analysis of the molecular basis of pollen development and its role in fertilization will improve our understanding of plant reproduction. By differential screening we have isolated a pollen-specific gene ntp303. This gene has been shown to be conserved in dicotyledonous and monocotyledonous species and is transcribed during the late phase of pollen development in the anther and during pollen tube growth in the style. Although the characteristics of the ntp303 gene suggest a function of the protein within the fertilization process, the sequence analysis did not reveal any clues about a possible function. Therefore, we developed a research programme for functional analysis. This research is based on two strategies, the establishment of a function–localization relationship and loss of function analysis.

For the study of the NTP303 protein *in vivo* and *in vitro*, we raised polyclonal antibodies against recombinant proteins encoded by ntp303 sequences. The coding region of ntp303 was split in two parts, one encoding the 24 kD carboxy terminal part and another encoding the 42 kD amino terminal part. These parts were individually cloned into an inducible expression vector with a hexameric histidine tag and overexpressed in *E. coli*. The recombinant protein was isolated with the aid of immobilized metal affinity chromatography and gel electrophoresis. Polyacrylamide slices containing the recombinant proteins were used to immunize rabbits. Western blot analysis showed a high specificity of the polyclonal antibodies to the recombinant protein. Currently, we are describing the appearance of the NTP303 protein by Western blotting and immunolocalization.

A loss of function analysis is carried out by antisense inhibition and co-suppression. The analysis of transformants in which the sense and antisense gene construct of ntp303 is integrated is still in progress.

Function Analysis of Pistil-specific Extensin-like Proteins in the Progamic Phase of Tobacco

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We are interested in studying pistil development and pollen–pistil interaction within self-compatible species. Several pistil-specific genes of tobacco have been identified and characterized. One group of genes is shown to be extensin-like (Goldman, M.H.S. *et al.* 1992, *Plant Cell*, **4**: 1041–1051). These genes could be divided into three Classes on the basis of their temporal and spatial expression pattern and their deduced amino acid sequence. The Class II and Class III genes are pistil-specific, whereas the Class I genes are flower-specific. Pollination experiments show that their expression is modulated by pollination.

Sequence analysis of the cDNA clones corresponding to Class III revealed that they encode for proteins with a proline-rich domain, containing a few repetitions of the pentapeptide Ser-Pro_4, which is a typical motif of extensins. These pistil-specific extensin-like proteins (PELPs) are highly basic. One of these clones, pMG15, contains a hydrophobic N-terminus characteristic of a signal peptide, suggesting that the corresponding protein enters the secretion pathway and is extracellularly localized. *In situ* hybridization experiments show that the Class III mRNA is specifically localized in the cells of the transmitting tissue of the style. However, the possible function of these genes in pistil development, pollination and pollen–pistil interactions is still unknown.

To localize extensins in general during tobacco pistil development, we have used a specific polyclonal antibody raised against soybean seedcoat extensin (kindly provided by Dr J. Varner). By indirect immunofluorescence, we were able to show the presence of extensins and/or extensin-like proteins in the cell walls and intercellular matrix of the transmitting

tissue, and in the secretory zone of the stigma at stage 7 of flower development (Goldberg, R.B. 1988, *Science*, **240**: 1460–1467). At stage 11 of flower development, just before anthesis, extensins and/or extensin-like proteins are also recognized in the cell walls of the cortex cells. However, to study the specific accumulation of the PELP proteins, and to distinguish them from extensins that are commonly present in all plant tissues, specific antibodies against PELPs must be produced. Therefore, the coding region for the non-proline rich domain of the pMG15-PELP was cloned as a fusion protein with DHFRS into the expression vector pQE30. Expression of this construct in *E. coli* produces a protein with the predicted size of approximately 43 kD. This fusion protein was purified and polyclonal antibodies were raised against it. Western-blot analysis shows that the antibodies specifically recognize the protein expressed *in vitro*, and a tobacco pistil protein from total extracts. Moreover, no cross-reactivity was found with total protein extracts of leaf, stem and young buds. Currently, we are in the process of localizing PELP proteins in tobacco pistils by immunocytochemistry.

A second approach to understand the function of PELPs is to inhibit their expression by anti-sense approach. PELP as RNA will be transcribed under control of the PELP promoter, to be absolutely sure that the anti-sense RNA is expressed at the same time and the same place during plant development. To this end, we screened a tobacco genomic library and isolated the genomic clone corresponding to cDNA clone pMG15. The sequence analysis of the genomic subclones is in progress.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 28 APRIL 1995

Advantages of High-Resolution SEM over TEM in Ultrastructure Research

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In recent years more and more groups have changed from TEM to high-resolution SEM for ultrastructural studies. Although excellent preparation techniques have existed for over 10 years (Tanaka, K. & Mitsushima, A. 1984, *J. Microsc.* **133**: 213–222), the small number of high-resolution SEMs available was the main reason for their limited application.

The advantages of using SEM instead of TEM for ultrastructure research are obvious when studying irregularly (non-spherically) shaped structures in the cell, such as ER, SER-vacuole (-formation) complexes and the different elements of the cytoskeleton. The technique can also show flat but irregular structures that lie just beyond, or even in line with the plane of sectioning, like the 2D-distribution of plasmodesmata or Golgi vesicles on a plasma membrane against a cell wall. In addition to this, tracing rare structures, e.g. the two tiny sperm nuclei inside an embryo sac, is much easier using SEM than in a series of sections for a TEM.

So far, the development of heavy metal histochemistry for specific subcellular structures in the SEM has been mainly focused on immunogold-labelling of cytoskeletal elements and chromosomes. The gold can be traced using a backscattered-electrons detector in the SEM. Apart from these labels, selective heavy metal stains for different membranes and cell wall components, still the domain of the TEM, can be converted for use in the SEM.

A disadvantage of the technique is the large number of intact spherical structures beyond the plane of fracturing that accordingly cannot be identified and distinguished from each other; for example, plastids, mitochondria and vesicles.

Semi-automatic Measurements of Organelle Trajectories in Pollen Tubes Using Videomicroscopy and Image Analysis

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A living pollen tube is a highly dynamic system with individually moving organelles. Previous research in our laboratory on freeze-fixed–freeze-substituted tobacco pollen tubes revealed a quantitatively specific accumulation of secretory vesicles in the tip and an apical zonation of dictyosomes and coated pits (Derksen, T.J. *et al.* 1995, *Protoplasma*, in press). Two hypotheses were postulated on how accumulations of organelles and organelle movement can coexist. Accumulations of organelles may occur as an adventitious response on the structure of the actin skeleton or may occur on the level of specific regulation of actin–myosin interactions.

Pollen tubes of *Nicotiana tabacum* were recorded on video tape using VEC-DIC microscopy. Time-lapse series of digital images from these recordings

were analysed in the VIDAS image and data analysis system. Particle centres were indicated interactively, and the pixel coordinates were used to calculate the position and initial velocity in the cell, relative to the tip of the tube.

A minimum of 50 organelles per recording was measured for reliable statistical analyses. Reconstructed trajectories clearly show the many differences in the pattern of movement of individual organelles. The initial velocities are not normally distributed. A highly specific correlation of 0·40 (P=0·0001) was found between the initial velocity and the position from the tip. In general, the velocity of an organelle increases if its position is further away from the tip. The velocity of organelles moving in a directed way (persistence in increment along the x-axis for more than 0·5 μm) had a correlation coefficient of 0·25 (P=0·0001) with the y-axis. This means that organelles that move in a directed way have in general a higher velocity near the wall than in the centre of the tube. The quadratic quotient, *Qr* (Jarosch, R. 1956, *Protoplasma* **67**: 478–486), which describes both randomness and velocity of moving organelles, was 3·04 in an example tip and 3·8 in the vacuolated part of another tube. In general, the *Qr* increased with distance from the tip.

Towards Receptor-mediated Endocytosis in Plants

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Receptor-mediated endocytosis (RME) is a well-established phenomenon in mammalian cells. It includes the following steps: (i) a high affinity binding ('trapping') of extracellular ligands by transmembrane receptors located in the plasma membrane (PM); (ii) an aggregation ('clustering') of ligand-receptor complexes in clathrin-coated pits; (iii) an internalization of the ligand-receptor complexes through formation of a clathrin-coated vesicle (CCV); (iv) dissociation of the CCV coat polypeptides and fusion of the endocytic vesicle with a low pH-compartment whereby receptor and ligand dissociate; (v) vesicle-mediated recycling of the receptor to the cell surface; (vi) degradation (but not always!) of the ligand in the lysosome.

Although there is now a significant body of evidence for the occurrence of endocytosis in plants, in the case of RME it is entirely circumstantial. RME encompasses molecular interactions at both surfaces of the PM. Therefore, we have decided to provide answers to the following two questions: (i) Do adaptor complexes exist in plant CCV, and if so are there two types (one for PM-derived CCV, one for Golgi-derived CCV)? (ii) Can one visualize the binding of specific ligands at the surface of the PM and actually follow their internalization?

By isolating CCV under low proteolysis conditions and using monoclonal antibodies directed against the β(β′) adaptins from bovine brain we have been able to detect an equivalent polypeptide in plant CCV. Southern blotting with a human fibroblastic β-adaptin cDNA-clone suggests that, in contrast to mammals, but in line with results obtained in *Drosophila*, plants have only a single β-adaptin gene. The plant β-adaptin has properties more like the Golgi associated β-adaptin of mammalian cells, but can also be detected immunologically *in vitro* and *in situ* at the plant PM. The targeting function of plant adaptor complexes thus seems to be a property of α- or γ-adaptin rather than β(β′)-adaptin equivalents.

In order to visualize ligand binding at the cell surface, protoplasts were prepared under conditions of low proteolysis and subjected to an immunological method (SEIG-EPOM—silver enhanced immunogold as viewed by epipolarization microscopy). With this very sensitive procedure we have been able to demonstrate the presence of the auxin binding protein (ABP) at the surface of maize coleoptile protoplasts. ABP clusters in an auxin-specific fashion when the temperature of the protoplast is raised from 4°C to 25°C. Evidence for the internalization of ABP has yet to be provided.

Scanning Electron Microscopy of Premitotic Cells in Leaf Explants

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Cell division induced by wounding, in mature highly vacuolated epidermal cells (diameters 50–100 × 30 μm) of *Nautilocalyx lynchii* explants, is an articulated process. It can be divided into a relatively long-lasting phase preparatory to the mitosis, and cytokinesis. This study aimed at testing the visualization of the preparatory phase by use of scanning electron microscopy. The explants were studied previously by means of various other methods (Venverloo, C.J. 1990, *Protoplasma* **155**: 85–94).

Explants from the leaf midrib were cultured for 3–4 days on an agar medium on a microscope slide. The explants were fixed (for 3 h in a solution of 1% glutaraldehyde and 2% paraformaldehyde in 0·1 M sodium cacodylate buffer, pH 7·2, at room temperature), dehydrated, and critical-point dried. Epidermal cells were dry-cleaved using Scotch Magic Tape. The explants were sputter-coated with gold and examined in a Jeol JSM 6400 scanning electron microscope.

The resulting images had a large field of depth. The protoplasts in the cleaved epidermal cells were viewed from within the vacuole to the tonoplast, with some minor damage of broken transvacuolar strands. All characteristic features of the preparatory phase were found: the formation of cortical cytoplasmic strands and ridges, the flattened, peripheral nucleus becoming globular, the migration of the nucleus through cytoplasmic strands to the cell centre, the formation of many strands radiating from the nucleus, and nuclear suspension in the phragmosome and, finally, a cytoplasmic diaphragm crossing the vacuole in the division plane. The results also showed that only in the preparatory phase besides the thick cytoplasmic strands with broadened bases at the site of attachment, thin cytoplasmic strands with a constant diameter were present. The neighbouring cortical attachment sites of the cytoplasmic strands were interconnected by ridges.

The Endoplasmic Reticulum in Living Cells

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Thin cisternae of the endoplasmic reticulum (ER) are beyond the resolution of the conventional light microscope (LM) but become visible by video-enhanced contrast bright-field, phase contrast and ultraviolet microscopy. Organization and dynamic behaviour of the ER therefore can be analysed in the living cell by means of micro-cinematography (Lichtscheidl & Url 1990, *Protoplasma* **157**: 203–215). In onion inner epidermal cells, thin tubules and lamellae form a net of polygonal meshes in the peripheral cytoplasm. This net seems stretched in transvacuolar cytoplasmic strands: most of the still interconnected tubules run parallel. Several types of ER movement occur in the thin peripheral cytoplasm. (i) Tubules elongate, growing out from lamellae or from tubules of the net. Flat sheets may also arise from various elements of the ER. As a result, the density of the net increases. In many cases, organelles are observed at the tip of growing tubules and lamellae. On contact with other membranes, the newly formed tubules and lamellae fuse and form a new mesh. These connections may be very stable, thus hindering continuous motion of other organelles which may become repulsed. (ii) In many instances, however, the junctions are mobile so that one tubule can glide along the other. Meshes thereby transform: they may widen or shrink and finally disappear, causing decomposition of the net. Similar ER movements have been observed in animal cultured cells, but their velocity is much lower (Terasaki 1990, *Cell Motil. Cytoskel.* **15**: 71–75). Despite these constant movements, in plant cells parts of the ER are found in the cortical cytoplasm, where they appear fixed and immobile, as if they were anchored to their positions. Organelles seem to represent such anchor sites in the inner cytoplasm.

Dynamics of a Spectrin-like Protein in Root Hairs after Stimulation of the IP_3 Signal Transduction Pathway

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In higher plants we have found a protein that is recognized by anti-red blood cell spectrin. The protein is accumulated in tips of growing root hairs of *Vicia sativa* L.. Nod factors, which are lipo-chito-oligosaccharides from *Rhizobium* bacteria, are able to induce root hair deformation in legumes as a first step in the nodulation process. Only when root hairs of *Vicia* have almost or just reached their mature size, do they respond to *Rhizobium* Nod factors. These susceptible root hairs have lost the polarly organized cytoplasm, which is a characteristic of the non-deforming, growing root hairs. Also the amount of the spectrin-like antigen is markedly reduced in the tips of susceptible hairs. Application of Nod factor results in a swelling of the root hair tip, accompanied by an accumulation of the spectrin-like antigen in the swelling. Root hair deformation proceeds with a polarly organized outgrowth from this swelling, also containing the spectrin-like epitope at its growing tip. When LiCl is applied to *Vicia* or *Medicago* plants, root hair deformation is induced in the susceptible root hairs, whereas other monovalent cations as Na^+, Rb^+ or K^+ are inactive. A treatment with 20 mM Li^+ for only 5 min is sufficient to start the deformation process. Li^+ mimics Nod factor; both induce deformation in the same root hairs, accompanied by the accumulation of a spectrin-like antigen in the tips of deforming hairs. Furthermore, both Nod factor and Li^+ are unable to induce root hair deformation when plants have been grown in the presence of NH_4NO_3. Lithium also induces root hair deformation in the non-legume *Lycopersicon esculentum* L., whereas Nod factors are unable to induce this response. However, growth in the presence of NH_4NO_3 does not alter the deformation response in these tomato root hairs. Lithium is an uncompetitive inhibitor of plant myo-inositol monophosphatases (Gillaspy *et al.*, subm. *Plant Cell*).

Assuming that this enzyme is a target of Li^+ in the root hairs, a changed inositol metabolism could trigger a signal transduction pathway that causes root hair deformation.

Structural Adaptations to Seed Germination

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Seeds are subjected to conflicting demands. Larger embryos have better opportunities in seedling establishment, but the development of larger seeds will be at the expense of the number of seeds produced, and may have disadvantageous consequences for their dispersal. So, strong mechanical protection by the sclerotic layers necessary to protect the seed, for instance during the passage of the alimentary tract of their dispersers, may confront the embryo with problems of how to rupture the seed coat during germination. In contrast to our knowledge of the relation between seed structure and dispersal, there is scanty information on the structural adaptations of seeds to germination. This is true not only for the way seeds imbibe water, but also for the way in which the seed coat dehisces during germination.

It is generally assumed that as a result of swelling of the endosperm and/or stretching of the embryo the seed coat bursts or ruptures. In seeds with non, or poorly sclerified testas, the seed coat may rupture irregularly, mostly starting from the micropylar end. Shape of the seed and size and orientation of the embryo and its cotyledons may determine the number, course and location of the splits. Flattened seeds mostly have the cotyledons parallel to the flattened sides and rupture along the borders, campylotropous seeds often split along the median. In seeds of, for example, many Leguminosae, Convolvulaceae and Cannaceae, the initially hard seed coat is softened during imbibition.

However, especially in seeds with thick, lignified testas, the embryo may encounter serious problems in penetrating or rupturing the tough seed coat. These seeds show different adaptations and the seed coat often splits along predetermined rupture zones. The rupture of the seed coat may be across cells or between cells along the middle lamellae. Rupture zones show a structure deviating from the remaining part of the seed coat. This may be (i) the presence of a thin-walled parenchymous layer as in the micropylar collar of Zingiberales; (ii) a change of cell shape from puzzle-piece-like with undulated anticlinals to polygonal with straight anticlinals near the rupture zone (Begoniaceae, Cactaceae, Caryophyllaceae); or (iii) a change in the orientation of testal cells, for instance from crosswise in the seed coat to longitudinal fibres along the rupture zone.

Seeds with predetermined rupture zones may open in different ways. (i) Seeds dehisce with one or more valves. Many taxa in the Gymnosperms, Magnoliaceae, Annonaceae, Calycanthaceae a.o. dehisce with two valves along the raphal and antiraphal sides. (ii) Seeds open with a flap. In Melastomataceae, the raphal part of the testa is lifted like a flap. The rupture zone is situated between the exotegmic layer of the raphe and the exotestal layer of the remaining part of the seed coat. In some taxa of the related Lythraceae the valve is of antiraphal origin. (iii) Seeds open with an operculum or plug, and the micropylar part is released by circumcisile dehiscence. Opercula are best known from the monocot families of Zingeberales and Commelinaceae, plugs from, for example, Bromeliaceae, Philydraceae, Pontederiaceae and Typhaceae. Comparable structures are known from about 20 families of the dicotyledons.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON 10 MAY 1995

Vegetation Succession in Lakes of West Connemara, Ireland: Comparing Predicted and Actual Changes

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Changes in vegetation of lakes and wetlands have been investigated over a period of 18 years. It is assumed that changes in vegetation are related to changes in agricultural land use, after Ireland joined the European Community (EC) in 1975. This has resulted in increased phosphate levels in surface waters. Fieldwork has been carried out in 1975, 1988 and 1993. Multivariate techniques have been used to relate changes in vegetation to changes in environmental factors. With the use of a Markovian chain model the vegetation development is projected into the future. Projections based on vegetation dynamics

between 1975 and 1988 are compared with actually observed changes in the vegetation in 1993.

The vegetation dynamics appear quite stable on a regional scale with a slight change towards a wetter vegetation. On a local scale vegetation was quite dynamic. A continuous decline in species diversity was noted as well as an overall increase of phosphate level, especially in lakes in the coastal zone where most human activity is concentrated. However, only minor changes in vegetation could be attributed to this increase of phosphate. Major changes were a result of fluctuations in water level. These changes coincide with periods of dryer and wetter climate. Because of the fluctuating nature of these changes predicted vegetation change did deviate from the observed change.

Micropatterns in a *Festuca rubra*-dominated Salt-marsh Vegetation Created by Sheep Grazing

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The creation of a micropattern in a sheep-grazed salt-marsh vegetation was studied by describing the micropattern and analysing the interaction between vegetation and sheep. The micropattern was formed by a variation of short and a tall-growing *Festuca rubra* vegetation on a scale of some square decimeters. The study was carried out in a middle salt-marsh on the west coast of Schleswig-Holstein (Germany). A long-term experiment was started here in 1988 to study the effects of different grazing intensities on the salt-marsh vegetation.

The micropattern was mapped at five different grazing intensities in plots of 2×10^2 in May 1993 and in May 1994. A micropattern was found in the low and moderately grazed paddocks in both years. No micropattern was found at a high grazing intensity (3·4 SU ha^{-1}, 1 SU=*c.* 3 sheep) or when grazing was excluded. Variation in micropattern was maximal at a grazing intensity of 1 SU ha^{-1}. The micropattern was not stable from year to year. In 1993 a relationship was found between the micropattern and small-scale elevational differences.

In May and September 1993 the short and tall-growing vegetation were described in more detail in the paddock of 1 SU ha^{-1}. The tall vegetation had a higher above-ground biomass than the short vegetation due to a higher amount of dead biomass and to a higher amount of living biomass in May and September 1993, respectively. Contrary to what we expected, no relevant differences were found between the percentages crude protein and *in vitro* digestibility of green leaves of the short and tall vegetation. The grazing intensities of individual sprouts proved to be very low. A demographic study of plant tissue turn-over and individual sprouts did not reveal a causality for the micropattern. The micropattern could not be explained by differences in productivity or by the selectivity of the grazing sheep.

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Vegetation Development in a Dune Grassland under Hayfield Management; 15 years after recovery of the groundwater level

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The Amsterdam Waterwork Dunes are situated in the calcareous Younger Dunes south-west of Haarlem. Before their utilization for water catchment (since 1851) the dune slacks were moist, sometimes marshy grasslands.

The Groot Zwarteveld is a former farmland in this area with deeply decalcified soil. The field of 20 ha has been used for grazing cattle and for agriculture. After the groundwater level dropped by 4 m, agricultural activities had to be terminated at the beginning of the twentieth century. Since approximately 1975 the groundwater level has been raised again to almost its former level as a result of infiltration with pre-purified water from the river Rhine. Also, from this time the Amsterdam Water Supply started to manage the Groot Zwarteveld as a hayfield by mowing every year in September. Dry parts are mown every 2 years.

Since the restoration of the groundwater economy, a number of plant species characteristic of dune slacks but also of peat-moors have established. Some of these were not known from the Younger Dunes at all or have not been seen for some time. From floristic inventories in 1935 (dry), 1972 (dry–moist, nutrient-rich) and 1993 (dry–wet, nutrient-poor) we have already observed a gradual increase in the number of species from 151 to 309. In a comparative vegetation study, 29 sites were investigated in 1985 and 1993. These sites represent the different abiotic conditions in the Groot Zwarteveld and describe about 5 ha (40%) of the mown area.

Indications of plant species for the abiotic conditions were determined on the basis of the phreatophyte categories (Londo, 1988 *Dutch Phreatophytes.*

Pudoc, Wageningen) and the acidity and nutrient classes according to Runhaar *et al.* 1987, *Gorteria* **13**: 276–359. Most of the observed species belong to the aphreatophytes in both years and indicate weak acid to basic soil conditions. Only in a few fields, with *Sphagnum* species and ferns, were more acid soils present. Between 1985 and 1993 a shift towards nutrient poverty can be observed. In 1993 most plant species indicate (moderate) nutrient-poor conditions.

Ecological gradient analysis has been made by using the ordination program CANOCO. The pseudo environmental variable 'time' was used to execute a redundancy analysis (RDA). In the diagram the 58 relevés are ordinated into four clusters. Two clusters represent the relevés with moist or wet soil conditions in 1985 and 1993, respectively, and the other two correspond with dry conditions in 1985 and 1993. All mown areas, both the dry and the moist or wet, shift along the time axis, indicating the progress of a dynamic process. Two areas have not been mown. These almost do not move along the time axis. From the ordination diagram it can also be derived that the clusters of dry and moist sites diverge with time. This means that the species composition of the dry and moist or wet sites indicate a differentiation process.

The ordination biplot of species and relevés confirms the increasing nutrient poverty. Rare and typical species of dune slacks and hayfields, such as *Dactylorhiza majalis* ssp. *praetermissa*, *Rhinanthus minor* and *Carex caryophyllea* are particularly strongly correlated with time in the moist or wet sites. High grasses such as *Calamagrostis epigejos* and ruderals decrease in time.

Beech and Beechwoods in The Netherlands

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The beech (*Fagus sylvatica*) reached The Netherlands and England in the early Subboreal. Only in early Subatlanticum did it reach dominance, many places having 5–35% or more *Fagus* pollen, even without a correction factor of 4 because of underrepresentation. The expansion was much slower than the change in climate, owing to slow dissemination. The latter was illustrated by mapping the entire offspring (235) of a single old beech, which was limited to 50 m. After 800, oak was favoured by coppicing, charcoal burning, timber growing and swine pasture. Clearings for agriculture increased.

Beech naturally dominates in the associations Fago-Quercetum, Luzulo-Fagetum, Milio-Fagetum, Melico-Fagetum and Carici-Fagetum. Maps of potential distribution have been drawn. The litter layer decreases in the mentioned sequence from 6 cm (impoverished) to nearly 0, with a pronounced shift from the resistant H-layer to the F-layer in decomposition.

Recovery and retrophication of degraded heathland to Fago-Quercetum takes some centuries. Any action of man—input or output by eutrophication, ploughing, planting, grazing, etc.—counteracts the natural succession. Coppicing changes tree and shrub layer and rejuvenation, but the herb layer is less affected by several management variants.

Potential natural vegetation (PNV) is the (semi-)natural vegetation after one tree generation, if left untouched. In old woodland this can equal the actual situation. Several 'objective' classifications tend to overestimate disturbance indicators and to neglect scarce character species and other identifiers of an association like fringe plants, *Rubus* species, weeds and species of microhabitats. If well designed, PNV-types will also have specific soil and litter profiles, chemical properties, fauna elements like ants and nematodes, tree growth rates and diseases.

Field names with beech, viz. the saxon boek and the Frankish hees, yielded extra information. The 480+390 names correspond very well with the present distribution of beech: mainly in the southern and eastern flora districts, decreasing to the north and not or scarcely in the clay, peat and coastal districts. Hees names dominate in the Frankish south, but are missing in the formerly Frisian west. The distribution of boek and hees names over PNV-types agrees with that of actual beech. Birch names are mainly confined to dune and marsh communities and Betulo-Quercetum; oak names are intermediate.

The suffix behind boek and hees points mainly (50% and 65%) to a natural environment like wood or hill, whereas with oak and even more birch, house-, road- and cultivated-land names dominate. Hees names appear to be the oldest, going back to 777, followed by boek. Oak and birch names start low, but dominate after 1600, the beginning of our Golden Age. This all points to a shift of beech–oak–birch (–heathland, not investigated) owing to human influence. In 35% of the country beech would be the dominant tree in natural woodland.

The Use of the Potential Natural Vegetation in Forest Management

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Forest (nature) reserves. In nature reserves the management should create room for the natural elements and conditions for the natural processes to take place. Human-caused deflections should be corrected. Species that are not part of the potential natural vegetation (PNV) must be removed. In order

to create the potential natural structure (PNS) of the system (closed canopy forest/degradation phases/ open areas) the potential natural dynamics (PND) must be reintroduced.

Multifunctional forests. If a combination of nature protection and timber production is the objective, exploitation activities reduce the naturalness of the forest by: extraction of biomass, limitation of tree sizes and ages, reduction of dead wood, manipulation of tree species and of abundance and distribution of game. Degradation phases and open areas in particular conflict with the timber production objective. Species dependent on dead wood or light and heat are therefore limited. If exotic tree species are used the forest cannot be called 'natural'. Therefore, if nature is one of the objectives, zoning must be applied. In one zone there should be no exploitation, another zone can be exploited. The latter should at least consist of the species of the potential natural vegetation.

Production forests. In production forests the fastest growing tree species are the most interesting, they might even be exotic species. Knowledge of the potential natural vegetation helps to indicate the growth potential of specific localities.

New forests. Afforestation is an expensive form of creating forest. In addition, afforestation creates poor natural environments and landscape. Natural processes that lead to forest mosaics are the cheapest way to produce timber, and produce the best nature and landscape. If the species of the potential natural vegetation are not present as seed source, they should be reintroduced; however, not in the abundance practised in classical forestry, but in numbers of approximately one per ha.

