

Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FOR FERTILIZATION RESEARCH IN PLANTS ON 16 FEBRUARY 1990

Placental Pollination in *Lilium longiflorum*

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Cut-style pollination was carried out in lily to achieve interspecific crosses. Although there was abundant pollen tube growth, penetration of the ovules was rare. Using intraspecific compatible pollen and the cut-style pollination method, the percentage of ovules with a pollen tube in the micropyle was also low (about 8%, compared with about 85% when pollinated on the stigma). Placental pollination within the species *Lilium longiflorum* was used to study and influence this process. With this method not only the pollen grains or tubes could be manipulated, but also the pistil material, i.e. a placenta with ovules and a part of the ovary wall. After placental pollination, the pollen germinated. Part of the pollen tubes grew in between the ovules towards the inner integument which has formed the micropyle. Growth along the inner integuments and circling of pollen tubes was observed using scanning electron microscopy. Only a small percentage of the total number of ovules was penetrated by a pollen tube, comparable with that observed after cut-style pollination. This percentage fluctuated strongly throughout the year and between experiments. The variation was much larger compared with the results of cut-style pollination. In attempts to find an explanation for this low percentage, variations were carried out, e.g. various ages of flowers, various tissue culture media, using a medium to promote pollen tube growth, various plant hormones, and pre-germinated pollen, but the percentage of penetration remained low. Sperm cell formation did take place in about one-third of the pollen tubes. Embryos (as a proof of fertilization) were hard to detect because they remained small, therefore the medium probably needs some modification.

Gametoplasts in Perennial Ryegrass (*Lolium perenne* L.)

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For specific approaches in genetic manipulations with perennial ryegrass, the gametes may provide an

alternative to somatic cells. For techniques like fusion and direct gene transfer, gametoplasts (protoplasts of gametes) should then be isolated.

Attempts were made to isolate embryo sacs employing various enzyme mixtures differing in concentration and cellulase/pectinase ratio. After incubation only protoplasts could be recovered, indicating that the digestion was too severe. Subsequently, different osmolalities and pH values were tested. At an optimal osmolality of 360 mOsmol/kg H₂O using 9% sucrose and a pH of 5.0, embryo sacs could be isolated as entities by a simple squash technique. Unfortunately the embryo sacs were not viable.

A sperm cell isolation procedure was developed for the tricellular pollen from perennial ryegrass. Anthers which were collected just before anthesis from fresh cut spikes were squashed in modified BK medium (Brewbaker & Kwack, *Am. J. Bot.* 1963, **50**: 747–858), 10% sucrose, pH 6.0. Subsequently, a modified RY medium (Yamada, Zhi-Qi & Ding-Tai, *Plant Cell Rep.* 1986, **5**: 85–88), 20% sucrose, pH 6.0 was added. After a purification step on a Percoll gradient, consisting of 10 and 35% Percoll diluted in RY medium, the viable male gametoplasts were counted to determine the yield. By adding the anti-oxidants vitamins C, E and calf serum, which contains, among others, superoxide dismutase activity, the male gametoplasts remained viable until 48 h after isolation. Under the conditions established so far, the yield of viable male gametoplasts was 10–15% with respect to the original number of pollen grains.

Partial Characterization of Two Pollen-Specific cDNA Clones

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Pollen grains of tobacco contain a stock of presynthesized mRNA. Probably these mRNAs are translated during germination and pollen tube growth. In order to study the regulatory mechanism of gene expression in pollen, a pollen-specific gene has to be isolated. To achieve this, a cDNA library against the total poly(A)-RNA pool of mature tobacco pollen was constructed. The library has been differentially screened with labelled cDNA synthesized from leaf or pollen-poly(A)-RNA, respectively. This resulted in the isolation of different pollen-specific cDNA clones.

Two clones ($\lambda 202$ and $\lambda 303$) were analysed by Northern blot hybridization. The clones are pollen-specific because no signal appeared on blots containing RNA from different parts of the plant and of the flower. Pollen from *Solanum* sp., as well as pollen from *Digitalis purpurea* contain the mRNAs corresponding to both clones.

The expression of the genes coding for $\lambda 202$ and $\lambda 303$ starts after pollen mitosis. The mRNA-level of $\lambda 202$ and $\lambda 303$ rises thereafter. This fact, together with the presence of the $\lambda 202$ and $\lambda 303$ transcripts in a broad range of plant species, suggests an important role for both mRNAs during pollen development and/or germination. Preliminary results from Northern blot analysis of RNA from germinating pollen show that $\lambda 202$ is still expressed during pollen tube growth.

Because both clones are regulated in the same fashion a similar expression regulation mechanism is presumed. To prove this the genes coding for $\lambda 202$ and $\lambda 303$ will be isolated and studied for sequence homologies in 5' and/or 3' (up/downstream) non-coding regions. These homologous areas will then be used in promoter studies.

Expression in Pollen of GUS Reporter Gene Constructs

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For pollen transformation, a gene construct which is expressed in pollen and of which the activity can easily be determined is required. A good candidate is the β -glucuronidase reporter gene. We analysed the

expression of a 35S-GUS gene construct histochemically in the anthers and pollen of various transgenic and untransformed Solanaceae. These studies showed that an endogenous GUS gene was expressed in a temporal and spatial specific manner in the tapetum and pollen of untransformed plants (Plegt & Bino, *Mol. Gen. Genet.* 1989, 216: 321-327): both transgenic and untransformed exhibited GUS activity. From studies with vegetative cells it became apparent that endogenous GUS activity had a lower pH optimum (between pH 5 and 6) than the chimeric GUS gene product (between pH 6.5 and 7.5). This difference in pH optimum allowed a distinction between endogenous and transgenic GUS activity to be made. With the histochemical assay, at pH 7.5, no GUS activity was visible in pollen of plants transformed with the 35S-GUS construct and in pollen of untransformed plants.

We also analysed GUS activity in pollen of *Petunia hybrida* transformed with a GUS gene driven by a pollen-specific chalcone flavone isomerase promoter (van Tunen, *et al. Plant Mol. Biol.* 1989, 12: 539-551). With the histochemical assay, transgenic activity of this PA2-GUS construct was observed at pH 7.5, while no activity was found in pollen of the negative control.

Whereas no activity of the 35S-GUS construct was observed in pollen of tobacco by means of the histochemical assay, a low GUS activity could be measured in pollen extracts with a fluorimetric assay. In vegetative tissues, transgenic GUS activity could be increased by treating the extract with Sephadex G50. Apparently, the column inhibited intrinsic quenching of transgenic GUS activity. Effects of Sephadex G50 on pollen extracts are now under study.

MEETING OF THE NETHERLANDS SOCIETY FOR VEGETATION RESEARCH ON 25 APRIL 1990

The Vegetation of the Westernmost tip of the Island of Java (Indonesia), 100 Years after the 1883 Krakatoa Eruption

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Ujung Kulon is a peninsula, situated on the westernmost tip of the island of Java (Indonesia). The area covers some 30 000 ha. In 1883, the region suffered severely from the tsunamis and ash rains that accompanied the notorious eruption of the nearby volcano Krakatoa. Soon afterwards, the peninsula was deserted completely. At present, Ujung Kulon is a nature reserve, renowned for its population of Javan rhinoceros (*Rhinoceros sondaicus*).

During the period 1981-1983, a survey was carried out in Ujung Kulon, initiated by the World Wildlife Fund. A broad landscape-ecological approach was chosen (Zonneveld, I.S., *Land Evaluation and Land(scape) Science*, 1979. ITC, Enschede). To study the aspect of vegetation, the phyto-sociological approach was used. The results have been published by Hommel (*Landscape-ecology of Ujung Kulon (West Java, Indonesia)*, Thesis, Wageningen Agricultural University, 1987).

The present pattern of vegetation types is shown on a landscape-ecology map (scale 1:75 000). The pattern is mainly correlated with differences in climate (former) land use, and edaphic factors.

The variation of climate-related vegetation zones is closely correlated with altitude. The climatological

gradient is strongly reinforced by the so-called telescope effect: the condensed, 'telescoped' vegetation zoning of mountainous islands in the tropics.

The predominantly semi-deciduous vegetation types of the lowlands show an intricate mosaic of young secondary growth, older secondary forest and remnants of primary forest. Interestingly, relatively young formations, e.g. the extensive rattan shrublands in the interior, indicate the location of the fields deserted in or shortly after 1883, and the coppice-like forests of the coastal plain, indicate the former presence of man-made savannas.

One major conclusion of the study is that the arguments against the use of the phyto-sociological approach in the forests of the humid tropics are invalid. On the contrary, the advantages above a physiognomic approach are great. The plant communities show close correlations with edaphic and climatological conditions, and may readily be interpreted in terms of succession and geographical position of the plant. The significance of such an interpretation for nature conservation is self-evident.

Large Herbivores, Carrying Capacity and Stability in East Africa

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Animal census data from Lake Manyara National Park in northern Tanzania were collated from different published and unpublished sources with the aim of discovering what the population fluctuations were over the several decades, whether a community of ungulates can attain stability, and whether epidemics in animals cause disturbances in the vegetation.

The data refer to large mammalian herbivores, which are individually heavier than 20 kg, of which the numbers were counted in nine different years between 1959 and 1984. The total biomass of these herbivores comprised mainly African buffalo and African elephant. Five functional groups of herbivores were distinguished (buffalo, 'elephant-as-grazer', 'elephant-as-browser', 'other grazers', and 'other browsers'). Biomass data were transferred into consumption pressures under the assumption that daily food intake equals 2.5% of body mass. The pressures of all these groups were constant over time with the exception of that by buffalo. Buffalo numbers increased from the last outbreak of rinderpest in 1959. There was no correlation between herbivore biomass and rainfall fluctuations.

Individual species showed large fluctuations in their numbers, and thus their consumption pressures on the vegetation. Many of these fluctuations were caused by diseases. However, within the total herbivore assemblage the different species compensated for the

fluctuations of the other species. This resulted in an overall constancy of herbivore biomass, and, thus, the carrying capacity of the system has to be viewed on the level of all species combined and not on that of the individual species. This view is supported by the result that size of the stability index showed that the system of herbivore species was stable.

Vegetation and Vegetation Management in the Nepalese Middle Mountains

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In the Nepalese middle mountains the use by local people of land without any agricultural value causes strong degradation of vegetation and increased soil erosion. This land is essential for the supply of fodder, firewood, timber and litter (compost) which are getting scarce in many areas. People have to go long distances to obtain these forest products, while on the degraded land near the villages plants with no use are dominant and only some grazing is possible. An increasing population becomes dependent on a declining source.

The main aim of our study is to indicate which alternatives are available or can be developed for the present management of land not used for agriculture. Improved management should be in the villagers' interest primarily. Their attitudes determine the possibilities for change. To reach this aim the following questions have to be answered:

What ecological factors determine the species composition and vegetation structure in the Nepalese Middle Mountains?

What role does vegetation play within the agro-ecosystems in Nepal?

What are the effects of present management on the species composition and structure of vegetation?

Different aspects of the landscape in a study area north of Kathmandu were sampled (ecosystem descriptions; soil profile analysis; questionnaires) to study relationships between different elements of the middle mountains agro-ecosystems. A classification of vegetation types and vegetation structure types was made.

Climate in relation to altitude is the major factor determining species composition. All species have their species-specific limited altitude range. Furthermore, north and south facing slopes differ enormously in the amount of radiation received which also makes the soil moisture conditions differ. Soil factors determined by mother-rock could not be related to differences in species composition. From site to site, humic layers and A-horizons differ enormously. These differences are related to differences in microclimate and management. Differences in exploitation on

pressure from site to site are causing a sequence of structure types from forest to grassland. Species composition is related to these differences in structure.

Many indigenous species are mentioned by the villagers as being used for their supply of fodder, firewood and timber. Appearance of these species within certain vegetation types gives an indication which of these types are valuable for the people. In the final report the form of management needed to improve the habitat conditions for valuable species will be indicated in a qualitative way for each vegetation.

Ecology and Exploitation of Kalahari Devil's Claw (*Harpagophytum procumbens*) in Botswana

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During the last 50 years the increase in cattle numbers has caused severe changes in the natural vegetation of Botswana, especially in the equilibrium between woody plants and grasses. Large areas are covered by a dense thorn vegetation (Tolsma, D.J. *On the ecology of savanna ecosystems in south-eastern Botswana*. Thesis, Free University Amsterdam, 1989). However, the disappearance of the grass layer also gave the opportunity for other, less palatable herb species to increase in density, annuals as well as perennials. One of the perennial species which occurs in overgrazed areas is Kalahari devil's claw or the grapple plant (*Harpagophytum procumbens* DC., *Pedaliaceae*). This species occurs in the Kalahari region (Botswana, Namibia, northern Transvaal and western Zimbabwe). The storage tubers of the plant are harvested and used as a medicine, locally as well as in western Europe (mainly for rheumatism).

In 1982, the Ministry of Agriculture of Botswana requested the National Institute for Development Research (NIR) of the University of Botswana to study the ecology and the effects of harvesting on the population biology of this species. Research has been carried out on the occurrence of the plant, germination, different harvesting methods, effect of harvesting on the population dynamics, regrowth of tubers, occurrence and content of the active ingredient (harpagoside) in the tubers (Ernst, W.H.O. *et al. J. Trop. Ecol.* 1988, 4: 185–198; *Grapple Plant Project Reports, 1984–1987*, N.I.R. Research Notes, University of Botswana).

Some of the results of importance to the harvesting and survival of the plant are: plants with a parent tuber with a diameter of less than 3 cm have hardly any storage tubers, so these plants should not have been harvested; and a parent tuber will only produce new storage tubers after harvesting if it is left intact, together with the vertical roots.

From the results of the cultivation experiments it can be concluded that the plant is a valuable addition to the more traditional crops. As distinct from these traditional crops, devil's claw is a perennial and therefore will be less affected by long drought periods.

Soil Moisture Regime as the Major Factor Determining Vegetation Stature on Ultramafic Soils in Palawan, The Philippines, and Sabah, Malaysia

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The stature of vegetation on ultramafic soils is often strongly reduced compared to that of adjacent sites on other substrates. This phenomenon is often ascribed to the specific chemistry of ultramafic soils, namely high Mg/Ca ratios, low K contents and possibly toxic levels of Ni, Cr or Co.

Forests and soils from Mt Bloomfield, a low coastal ultramafic mountain in Palawan, western Philippines, were studied in the rainy season of 1986 by a joint expedition of the Universities of Stirling and Sheffield, the Free University of Amsterdam, and the National Museum of Manila. The sites were revisited during the 1987 dry season by members of the latter two institutes.

Forest on sedimentary rock was of higher stature and basal area than that on ultramafic rocks whereas canopy height decreased rapidly between 50 m (bottom of ultramafic slope) and 170 m (top of knoll). Tree height was not related to any soil chemical parameter but showed a distinct relationship with soil depth and hence plant-available water. Soils with well-developed forest still contained moisture below 50 cm depth whilst soils with stunted vegetation had dried out almost completely at the end of the dry season. Differences in leaf anatomical and stomatal characteristics between vegetation types supported the hydrological explanation of stunting in this particular case.

Additional support comes from the observation that on Mt Silam, an ultramafic mountain (884 m) in Sabah, high-stature forest is found below the cloud cap with the forest within the clouds becoming increasingly stunted (Proctor, J. *et al. J. Ecol.* 1988, 76: 320–340). Measurements of soil moisture below and within the cloud zone on Mt Silam suggested the soil under stunted forest always to be close to saturation and rather acid. Bacterial counts and soil extraction tests indicated a shortage of nitrifying bacteria and soluble nitrogen compounds in the cloud forest soil, suggesting nitrogen deficiency as a possible cause of the reduced height of upper montane forest (cf. Marrs, R. *et al. J. Ecol.* 1988, 76: 466–482).

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 18 MAY 1990

In vitro Pollination and Fertilization in *Zea mays* L.

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In order to analyse gamete fusion and early embryogenesis in the maize inbred line A188, the progamic phase was shortened artificially. Excised pistillate flowers with cut silks were hand pollinated. Pollen germination and pollen tube growth in silks and ovaries were visualized with fluorescence microscopy after sodium hydroxide treatment and staining with Water blue. The penetration of pollen tubes into embryo sacs and the fate of the sperm were investigated after fixation, embedding in Spurr's resin, and semi-thin sectioning of ovaries.

It was found that up to 100% of fresh pollen germinated after being hand transmitted onto the surface of the shortened silks (1–2 cm), provided pollen did not come into contact with the wound. Pollen germinated within 10 min. The rate of pollen tube growth in the silk was approximately 0.5 cm/h. The first pollen tubes penetrated the ovarian cavity after 140 min and grew along the integuments towards the micropyle. The first tubes were found in the micropyle at 255 min. At 300 min we repeatedly observed penetration of pollen tubes in synergids and at 360 min fertilization had always taken place. Light microscopy revealed the simultaneous fusion of sperm nuclei with the egg cell nucleus and the partly fused polar nuclei.

In all, hand pollination on cut silks under controlled *in-vitro* conditions shortened the progamic phase from 21 h to about 4 h and enabled the timing and analysis of fertilization.

Growth, Wood Anatomy and Water Transport Capacity in Douglas Fir (*Pseudotsuga menziesii* (Mirb.) Franco) in Kootwijk and Garderen

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Growth analysis at six height levels in the stem of five, and at breast height of 20 Douglas firs in each of the two *c.* 30-year-old stands of the ACIFORN-project (ACIdification of the FORest in The Netherlands) showed no recent decline in radial or height growth. Growth was strongly influenced by weather conditions, especially warm and dry summers which are

reflected as small growth rings. Basal area was significantly and positively related to total wood volume. The amount of sapwood in the basal area was positively related to the diameter of the trunk at breast height.

Moisture content in the sapwood was similar to that in other softwood species. Differences between trees in average sapwood moisture content at breast height were negatively correlated to the relative area of sapwood. The moisture content in the heartwood varied only slightly from the fibre saturation point of *c.* 30%.

The moisture content in sapwood growth rings depends on the amount of earlywood in the ring and was somewhat lower than the maximal moisture content, calculated from the amount of cell wall material in a ring. This implies that part of the tracheids are embolized and do not take part in long distance water transport.

Within trees, sapwood moisture content at breast height is lower than in or just below crown, probably partly as a consequence of a higher earlywood percentage in rings higher in the stem.

The parameters investigated do not indicate that the sampled trees are exposed to stresses other than those caused by natural variation in weather conditions and social position.

Organization of Cytoskeletal Elements in Pollen Tube Subprotoplasts and Their Relation to Outgrowth

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Subprotoplasts derived from *Nicotiana tabacum* pollen tubes can regenerate a cell wall and are able to grow. However, in contrast to pollen tubes, these subprotoplasts do not show tip growth. As the cytoskeleton plays an important role in cell growth, the abnormal growth of pollen tube subprotoplasts may be caused by the aberrant cytoskeletal organization. Actin filaments and microtubules were studied in subprotoplasts just before and during the first stages of growth, by means of fluorescence microscopy and electron microscopy. Actin filaments were initially organized into cortical arrays connecting two foci. Upon growth, the actin filaments became organized into an orientation transverse to the direction of growth. Cortical microtubules in both non-growing and growing subprotoplasts were undulating and organized into circular arrays, showing no relation to site or direction of growth. Thus the organization of the actin filaments, but especially that of the microtubules, does not resemble cytoskeletal

organization with pollen tubes, where these cytoskeletal elements co-distribute and are oriented in a net axial orientation. Derksen & Traas (In: Willemsse, M.T.M. and van Went, J.L. eds. *Proc. 8th Int. Symp. Sexual reproduction in seed plants, ferns and mosses, 20-24 August 1984*. 1984 64-70. Pudoc, Wageningen) showed that the organization of microtubules depends, at least partly, on an interaction with actin filaments. Therefore, the abnormal growth of the subprotoplasts is probably the result of a distorted microtubular organization, which may have been caused by the absence of an interaction with the actin filaments.

Structure and Degradation of Epicuticular Wax of Douglas Fir in Relation to Needle Age and Air Pollution

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Changes in epicuticular wax morphology of *Pseudotsuga menziesii* needles were studied with SEM throughout the growing season in 1989 and older needles in 20 trees from two sites, Kootwijk and Garderen, in The Netherlands. Fusion of crystalline wax rods leading to a reticulate structure and ultimately to degradation of the regular three-dimensional porous structure started several weeks after bud break and reached a high level at the end of

the first growing season. The increase in amorphous (solid) wax showed a similar, but slightly slower development. In 1- and 2-year-old needles the degradation of crystalline wax and increase in amorphous wax had progressed only slightly further. The rates of change in crystalline wax morphology did not differ significantly between sun-exposed and shaded needles, between sites, or between the two tree-vitality classes included in the samples. The development of epiphyllous fungi and the occurrence of cracks in the wax layers was also studied and appeared to be needle-age dependent.

The effects of NH_3 on needle wax morphology were studied in 4-year-old saplings from open-top fumigation experiments (controls subjected to either ambient or filtered air; three levels of NH_3 : 25, 45 and $100 \mu\text{g m}^{-3}$). Short term fumigation (c. 5 weeks) did not affect wax morphology in 1989 needles, but 1-year-old needles which had been exposed to different NH_3 concentrations from budbreak onwards showed a severe degradation of the crystalline wax, regardless of the concentration used. In 2-year-old needles the effect of NH_3 could not be traced and seemed to be overshadowed by the natural ageing process. From the fumigation experiments it could also be concluded that ambient O_3 , SO_2 and NO_x levels did not affect epicuticular wax morphology.

It is suggested that the variation recorded for the two forest stands does not show effects of local pollution levels but depends on needle age in combination with other natural factors.

COMBINED MEETING OF THE SECTIONS FOR PLANT SYSTEMATICS AND GEOGRAPHY, FOR FLORISTIC RESEARCH IN THE NETHERLANDS AND FOR THE PROTECTION OF THE WILD FLORA, ON 27 APRIL 1990

Population Biology of the Marsh Gentian (*Gentiana pneumonanthe* L.) and its Use for Nature Management

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This study, which is made possible by a grant from the Prins Bernhard Fund, deals with the effects of different management regimes on the population ecology of the Marsh Gentian (*Gentiana pneumonanthe*).

Populations of this species can be divided into two types, differing in demography. First, there are populations characterized by a high turnover of individuals

and a high annual recruitment of seedlings. This type occurs in heathlands with relatively high dynamics due to sod-cutting, trampling or later mowing. The second type consists mostly of surviving flowering plants and shows hardly any seedling recruitment. This type is commonly found in *Molinia*-dominated, unmanaged heathlands and early-mown, unfertilized grasslands. In grasslands, mowing late in the season is the most favourable form of management. Early mowing increases the risk of losing or reducing seed crops, because regeneration to the fruiting stage is becoming more dependent on the weather in the autumn. Besides, in this regime there is no possibility for survival of the Alcon blue (*Maculinea alcon*). Moreover, these circumstances seem to favour the moss layer, which inhibits germination.

In heathlands, small-scale cutting of sods is the best form of management. Care has to be taken not to

remove all the flowering plants, because these are very important for new input of seeds as there is hardly any seed bank present.

Grazing increases the number of sites suitable for germination. However, when there is a small starting population, the risk of a total destruction of the seed crop is very high.

Small populations of the Marsh Gentian show an increased degree of inbreeding, caused by self-pollination. We have found that the progeny of individuals in small populations has a reduced growth compared with that of large populations, which is probably linked to inbreeding. If, by a changed management treatment the ecological circumstances for the species improve, there is a possibility that the progeny (the seedlings) are not fit enough to respond. Input of new individuals with a broader spectrum of genetical variation, for example, may then become necessary.

Fluctuations in Population Sizes and Reproductive Success in some *Gentianella* Species in The Netherlands

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In The Netherlands, the *Gentianella* species *G. amarella*, *G. campestris*, *G. germanica* and *G. ciliata* are rare and for the greater part endangered (IUCN category, 2,1,1, and 1 respectively). We are studying these species to establish the relation between the (often small) size of the populations and the population dynamics, reproductive parameters (capacity, success) and population genetics, and the pattern of genetic variation.

In some plots of *G. amarella* and *G. campestris* strong density-dependent plant development and fecundity is recorded. Density exceeds 500 reproducing individuals per m² in *G. amarella*. These plants (up to about 5 cm tall) produce a mean of two flowers per individual; in contrast, specimens from low densities, which reach a height of about 8 cm, have a mean of seven flowers. Preliminary demographic data on two successive years from 17 permanent plots show drastic fluctuations in number, density, and fecundity. In all plots except one, the decline of the numbers ranges from 50 to 100%. This is mainly caused by the dry spring and summer season of 1989.

Calculated seed input into the seed bank in 1989 is less than 0.1 compared to 1988 production. One *G. amarella* population increased in number with over 60%, as the obvious effect of a change in management: a dense cover of dominant *Calamagrostis* was mown.

This strong positive effect indicates the perspectives for future developments in some nature reserves.

G. germanica and *G. ciliata* showed similar fluctuations. The latter species did not produce any seeds in the only Dutch population left. Pollination experiments were carried out: *G. amarella* and *G. germanica* are self-compatible and are probably almost 100% selfers in nature. The primary genetical analyses, showing hardly any variation, are in conformity with this mode of reproduction.

Based on preliminary morphological and life strategy characters, it was suggested that a continued study could conclude to change the taxonomic position of *G. ciliata* and to move the species from the genus *Gentianella* (back) into the genus *Gentiana*.

Effects of Population Size and Density on Reproduction in *Phyteuma nigrum* F.W. Schmidt

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The problems faced by small and/or isolated plant populations can be numerous. The individuals might have suffered from loss of genetic variation due to genetic drift or insufficient gene flow between populations. The environment might have become hostile for the species so that it cannot complete its life-cycle. Furthermore, small isolated populations are more vulnerable to stochastic events. All these factors can lead to a rapid extinction of local populations.

Phyteuma nigrum F.W. Schmidt is a herbaceous perennial, often found in woodlands, but also occurring in meadows and along road verges. In The Netherlands several populations are declining. The species is insect-pollinated and needs a vector to set seed. The role of the reproductive phase for the future perspectives of some of these small populations was investigated. For this purpose, we compared pollination ecology, reproductive output and seed quality from populations of different size and origin.

It was concluded that the bumblebee species *Bombus pratorum* and *B. jonellus* were the most important pollinators in the populations under study in 1989. A strong positive correlation between male flowering density and bumblebee visiting intensity was found. Although seed-setting percentages did not differ significantly between small and large populations, the potential for reproduction appeared to be much lower in small populations. This was mainly caused by poor ovule development. Whether this is caused by inbreeding or by environmental conditions is not clear yet. No clear relationship between population size and seed weight or germination percentages existed. Some characteristics, e.g. corolla colour and spike length, differed between populations of different origin.

Genetic Erosion in *Salvia pratensis* and *Scabiosa columbaria*

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The aim of conservation biology is to increase knowledge of both biology of threatened species as well as knowledge about the underlying mechanisms of extinction. One of these mechanisms, genetic erosion, is closely related to population size. Small populations will, as a result of genetic drift and inbreeding, have decreased levels of genetic variation, resulting in decreased individual fitness and increased extinction chances. We tested this idea in two threatened species, *Salvia pratensis* and *Scabiosa columbaria*. There appeared to be a significant correlation between population size and both proportion of polymorphic loci (*Sp*: $r=0.921$; *Sc*: $r=0.703$) and mean number of alleles (*Sp*: $r=0.841$; *Sc*: $r=0.773$). The correlation between population size and morphological variation was also significant for both species.

It appeared that plants from large populations of *Salvia* were growing faster than those from small populations, when grown in a greenhouse. Whether a lower growth rate leads to decreased reproduction and, if so, to increased extinction chance is the subject of further research. Artificial inbreeding in *Salvia* by means of one generation of selfing, resulted in an inbreeding depression of approximately 20% in seed weight and 25% in percentage germination, indicating that this species is highly sensitive to inbreeding. Pollinating with pollen from another population resulted in increased seed weight, as compared to pollinating with pollen from the own population, in small populations, but not in large ones. Whether this is a result of higher inbreeding level in small populations or of outbreeding depression in large populations is still unknown.

Variation and Rarity of Threatened Microspecies of the Sections *Celtica* and *Palustria* of *Taraxacum* in The Netherlands

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In the Netherlands and neighbouring countries, many microspecies of the sections *Celtica* and *Palustria* of

Taraxacum have become rare and threatened and are placed on Red Lists. A number of them have already died out. The phenomenon is little known and hardly mentioned in the literature. To a large extent this ignorance is caused by the difficult microsystematics of *Taraxacum*. The sections *Celtica* and *Palustria* are characteristic for extensively managed wet grasslands and these grasslands have become rare in large parts of Europe. In general the *Palustria* are ecologically more vulnerable than the *Celtica*. Compared with the very common *Ruderalia* from intensively managed heavily fertilized grasslands, the *Celtica* and *Palustria* have a lower relative growth rate and reproduction capacity. The smaller plants have less competitive ability and are adapted to less productive ecosystems than most of the *Ruderalia*. The difficult systematics of the microspecies is caused by asexual agamosperous reproduction. This means that unfertilized egg cells give rise to new individuals so that populations of microspecies of *Taraxacum* consist of clones.

It is generally assumed that the decline of the number of populations and individuals results in a process of declining genetical variation. This theory is tested in populations of rare microspecies of the sections *Celtica* and *Palustria*. From investigations of proteins (isozymes) it appeared that most populations consist of one clone, only rarely are there two, in one case four clones have been found. If there are several clones, one is dominating which is always the same in the same microspecies. From investigations of a small number of populations of very common microspecies of the section *Ruderalia*, it becomes clear that these populations also consist of one or a small number of clones. So for the time being the conclusion is that the difference between the populations of the rare microspecies of the *Celtica* and the *Palustria*, and the abundant ones of the *Ruderalia*, is not the clonal structure of the microspecies, but probably a combination of the ecological preference and amplitude of individual microspecies and a far greater number of microspecies in the *Ruderalia* (200) than in the *Celtica* (10) or *Palustria* (7).