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Meetings of the Royal Botanical Society of The Netherlands

MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY AND THE SECTION FOR FERTILIZATION RESEARCH IN PLANTS ON 3 OCTOBER 1997

Analysis of Gibberellin-regulated Gene Expression in Anthers of Tomato

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Gibberellins (GAs) are endogenous plant growth regulators that are involved in the regulation of many aspects of plant growth and development, including seed germination, extension growth and flowering. An approach to understand GA action is to isolate genes which are regulated by GAs and use these genes as molecular markers for GA response and to determine the molecules involved in GA regulation.

We are investigating the role of GA in the promotion of anther development in the *gib-1* mutant of tomato. The phenotype of this GA deficient mutant includes dwarfism, failure to germinate and failure to flower normally. However, normal (wild-type) development can be restored by exogenously applied GAs. During flower development the anthers become developmentally arrested and are responsive to a single gibberellic acid (GA3) treatment. The developmental arrest occurs during the stage that the pollen mother cells are in the G_1 phase of the premeiotic interphase (Jacobsen & Olszewski, 1991, *Plant Physiol.* 97: 409–414).

Differential screening of a *gib-1* another cDNA library made 48 h after GA treatment resulted in the isolation of several GA-stimulated cDNAs (TGAS). The mRNAs of the corresponding cDNAs could be placed in two classes with respect to the kinetics of their accumulation patterns in the *gib-1* flowers. The class 1 consisted of genes that were increased in expression 8 h after GA3 treatment and were maximally abundant either 24 h or 48 h post-treatment, while the second class was not detected until 48 h post-treatment. However, anther development of the mutant can be rescued with a single application of at least 5 ng GA3/bud. It was shown by Northern blot analysis and *in situ* hybridization that a treatment with 50 ng GA3/bud restored the timing and localization of the gene expression as in wild-type. This was demonstrated for both classes.

In the untreated mutant the tapetum and pollen mother cell development is stopped. It was shown by scanning electron microscopy that there was also an arrest in the formation of interlocking hairs on the anthers. The interlocking hairs differentiate from epidermal cells, but were never formed on developmentally arrested anthers of the mutant. However, after GA treatment the formation of interlocking hairs was comparable to that in wild-type.

All cell types found in anthers of the wild-type are also present in the developmentally arrested *gib-1* anthers. Therefore, one possible role of GAs in tomato is that GAs are required for further differentiation of (some) these cell types. This differentiation presumably depends on cell size and/or the amount of nuclear DNA. Marker genes for cell expansion and DNA replication demonstrated an enhanced gene expression after GA-treatment by using Northern blot analysis. *In situ* hybridization with a histone H2B probe showed localized expression in cells of the anther during time. The hypothesis that GAs may be required for sustained cell elongation during anther development in tomato will be investigated further.

Cryo-FE-SEM Study on Inhibing Lettuce Seeds

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To study the structural mechanisms of seed germination and its inhibiting conditions, protocols for specimen preparation which include dehydration steps have to be avoided. For that reason, low temperature scanning electron microscopy (LTSEM) was used to investigate the structure of lettuce seeds during these processes. Lettuce seeds were prepared in various ways to compare normal germination with conditions which are inhibiting. These conditions were exposure to higher temperature (32°C instead

of 20°C), irradiation with far-red light or incubation in polyethylene glycol. After pretreatment, the (hydrated) seeds were mounted on copper holders and plunge-frozen in liquid nitrogen slush. The samples were transferred to an Oxford 1500 HP cryotransfer prechamber, which was mounted on a Hitachi SA4100 field emission SEM, fractured with a scalpel blade and etched for 5 min at −90°C and 10^{-4}Pa. After sputtercoating with platinum, they were examined at temperatures between −160°C and −196°C. In particular, the micropylar regions of the endosperm were investigated. Seeds which were conventionally prepared (3% glutaraldenhyde, ethanol dehydration, critical point drying) were used as a control.

The most striking result proved to be the absence of visible endosperm degradation prior to germination. Even after 15 h of imbibition at 20°C, i.e. just before germination, the surface of the endosperm appeared to be intact, nor did the inhibited seeds show cell wall degradation. In contrast, the seeds which were conventionally prepared for SEM showed endosperm surfaces with pits, cracks and depressed areas. After a few days of imbibition, thermo- or far-red inhibited seeds showed changes in the cell contents of both endosperm and embryo. Cells became filled with connected globular and oblong membraneous structures. They possibly represent accumulation of a metabolic intermediate. We can only speculate about the possible relationship between secondary dormancy and these changed cell components. PEG-imbibed seeds seemed to have not enough turgor; they did not show changes in the cellular structures.

We conclude that germination of lettuce seeds is caused by mechanical separation of endosperm cells rather than by enzymic breakdown. Previous structural studies which describe endosperm degradation prior to germination might have suffered from less optimal preparation and viewing conditions. Additional work with the same LTSEM-technique at higher magnifications and cytochemical analysis, using cryofixation and freeze-substitution, will provide more information about a possible local weakening of the endosperm and the nature of the cellular changes in secondary dormant seeds.

Influence of TIBA on Histogenesis and Morphogenesis of Microspore-derived Embryos of *Brassica napus* L. cv. Topas

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Microspore-derived embryos served as a model to study embryogenesis in plants. Microsporal embryogenesis is studied in *Brassica napus* L. cv. Topas with special attention, because this inbred is one of the most effective in producing microsporal embryos in the absence of growth regulators.

In order to investigate the influence of disturbance of auxin polar transport on the establishment of polarity and symmetry, 2,3,5-triiodobenzoic acid (TIBA) was added to the culture. Changes induced by TIBA were observed on morphological, anatomical and subcellular levels, applying light and scanning electron microscopical methods and immunocytochemistry. Normal globular embryos were formed in the presence of TIBA, but further development was affected. The hypocotyl thickened by irregularities in cell divisions and cell enlargement in the cortex. Instead of two cotyledon primordia, a rim of tissue surrounding the apical promeristem developed, giving rise to a single, collar-like cotyledon. There were no morphological changes observed in embryogenesis when TIBA was applied to embryos from the early heart-shaped stage onwards, indicating that an irreversible transition to bilateral symmetry had occurred.

Immunocytochemical research showed that TIBA caused disturbances in the organizaton of the microtubular cytoskeleton (MT). Because TIBA is an inhibitor of the polar transport of auxin, it is concluded that the changed distribution of auxin should be responsible for the changed organization of the MT cytoskeleton and hence for the changes in histogenesis. The regulation of the induction of bilateral symmetry expressed in the heart shape remains to be elucidated.

Flavonol Depleted Pollen tubes of *Petunia* Show Striking Alterations in Wall Structure Leading to Tube Disruption

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Pollen tubes of the flavonol depleted petunia transformant T17.02 are able to germinate and start growing *in vitro*, but eventually disrupt at the tip approximately 2 h after germination. Disruption of the tubes can be prevented by timely addition of

flavonols to the medium (Ylstra *et al.* 1994, *Plant J.* 6: 201–212). In order to establish possible points of flavonol impact, wild-type and flavonol-depleted pollen tubes were subjected to an extensive cytological and ultrastructural analysis. The results showed that before disruption of the flavonol-depleted pollen tubes the structure of the primary wall in the tip changed from layered to granular. Secretory vesicles at the tip still fuse with the wall but lose their capacity to melt into the wall and to form layers; instead they remain as dark, electron-dense granular structures surrounded by a light electron-translucent matrix that apparently is not able to sustain the wall's coherence and as a consequence the tube disrupts. No other cytological or ultrastructural differences between the transformant and the wild-type pollen tubes could be found. Even a morphometric analysis of abundance and distribution of endoplasmic reticulum, dictyosomes and mitochondria could not reveal any significant difference. It was concluded that flavonols act on cell wall precursors and interfere with a cross-linking system in the wall, possibly via extensins. Flavonol action on pollen tube walls is hypothesized to result from a co-evolution of secondary metabolites and flower development and to contribute to congruent pollination.

MEETING OF THE NETHERLANDS SOCIETY FOR PLANT CELL AND TISSUE CULTURE, WAGENINGEN, ON 13 NOVEMBER 1997

Proteinaceous Plant Growth Regulators

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Recently, the first peptides involved in plant development have been identified. Together with systemin and the avirulence factors, several peptides operational in different aspects of plant life have been identified. In animals hundreds of signalling peptides controlling growth and development have been found.

Plant signalling peptides, such as ENOD40 and a 2–3 kDA peptide synthesized by dividing tobacco protoplasts, are involved in hormonal controlled developmental processes. They are active at concentrations orders of magnitude lower than the classical plant hormones. Elucidation of the molecular mechanism of signalling peptide action and their relation to classical plant hormone action will be the subject of future studies.

Systemin represents a signalling peptide involved in systemic acquired resistance. The active peptide is proteolytically cleaved from a large precursor, as is the case for almost all signalling peptides in animals. In contrast, the ENOD40 peptide is not produced as part of a precursor protein. The regulation of the activity of peptide-signalling molecules is therefore a topic for further investigation. The avirulence factors, although not plant encoded, represent signalling peptides involved in the induction of plant defence mechanisms.

The notion that signalling peptides can be involved in various aspects of plant life leads to the hypothesis that in plants, as in animals, peptides are common growth regulators. Due to the low concentrations at which these peptides are active, it is likely that specific receptors exist in plants through which the signal is perceived and transduced. Over the last years, several receptor kinases have been isolated from plants. Among them are a large group of receptors containing leucine-rich repeats (LRR), which are thought to be involved in protein–protein interactions, and one having a TNF receptor resembling extracellular domain. The identification of the receptors and the elucidation of the signal transduction pathways are challenges for future years.

Elicitor-induced Tissue Competence for Auxins

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Wounding of plant tissues includes the destruction of cell compartments (vacuoles, vesicles, peroxisomes, plastids) which results in the release of catabolic enzymes (glucanases, peroxidases, phospholipases, lipoxygenases) that are present in these cell organelles. These enzymes cause the breakdown of specific cell structures (cell walls, cell membranes). Some breakdown products of the cell structures are known to be involved in plant-defence processes and are therefore elicitors. Our results show that a combined application of elicitor and auxin leads to a synergistic effect on auxin-induced rooting. Combination of elicitors with low auxin concentrations can lead to a threefold increase in the number of roots formed per explant. Elicitors alone (without auxin) did not induce rooting. Not all elicitors are active: salicylic acid (a systemic elicitor) leads to a reduction in auxin-induced rooting, coumaric acid and nicotinamide (no breakdown products of the cell wall or cell membrane)

did not enhance auxin-induced rooting on thin apple stem disks *in vitro*.

The mode of action of the active elicitors in root regeneration is currently under investigation in our laboratory. Possible explanations are: (1) The effect on auxin uptake and auxin transport. This is unlikely since our results indicate that these factors are not limiting auxin action. Moreover, preliminary results indicate that elicitors do not affect auxin uptake and transport. (2) The effect of elicitors on auxin metabolism. The elicitors might decelerate the rate of metabolic inactivation of auxins. In our metabolism experiment, however, we could not find any indication for this. (3) The elicitors might speed up the endogenous synthesis of auxins. GC-MS analysis showed that this is not the case. (4) The elicitors might enhance the sensitivity of the tissue for plant hormones. The fact that we found that subsequent application of elicitors and auxin leads to an even higher rooting response than their simultaneous application makes this explanation even more plausible. This is consistent with the situation in nature: in the plant elicitors are produced after wounding for defence purposes; its concomitant induction of tissue sensitivity to auxin subsequently results in the repair of the damaged tissue. Using cDNA-AFLP we are now isolating elicitor-induced genes involved in development of tissue competence.

Uridine, a Cell Division Factor in Pea Roots

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Nodule formation in pea roots, as induced by *Rhizobium* bacteria, starts with cell division in the inner cortex. Recently we have isolated and identified a factor from the central cylinder (stele) of pea roots which is capable of enhancing hormone-induced cell divisions in pea root explants (Smit *et al.* 1995, *Plant Mol. Biol.* **29**, 869–873). This factor was identified as uridine and was shown to be active at picomolar concentrations.

We further studied the specific hormone requirements for cortical cell divisions, and found that auxin alone was sufficient to initate cell proliferations in the inner cortex of cortical pea root explants. Addition of 10^{-12} M uridine together with auxin enhanced cell division in the inner cortex. In another bioassay, in which we studied the induction of lateral root primordia in pea root segments, we observed a similar effect of uridine. In this assay, addition of auxin together with 10^{-12} M uridine nearly doubled lateral root formation as compared to the addition of auxin alone. Other nucleosides showed some activity in both bioassays as well, but not as much as uridine.

As a working model we suggest that uridine acts as an auxin enhancer through changes either in the auxin concentration or in the auxin sensitivity of certain cortical cells. We tested this hypothesis by studying the effect of uridine on free auxin levels in pea roots. We found that addition of IAA and uridine for 24 h to cortical pea explants doubled the amount of free IAA compared to levels in only IAA-treated cortical pea segments.

Polyamines

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Polyamines occur widespreadly in plants. It is generally accepted that polyamines are associated with cell divisions and with active growth and metabolism. Much less is known about their involvement in regenerative processes. Nevertheless, polyamines are involved in the control of flowering, somatic embryogenesis, shoot development and rooting.

Focusing on rooting of poplar shoots *in vitro*, a correlation between putrescine accumulation and the initial stages of adventitious root formation has been shown. Exogenous application of polyamines also leads to changes in the rooting performance. Several hypotheses concerning the role of polyamines in the control of regeneration have been postulated: they could affect synthesis of macromolecules, membrane permeability, DNA conformation, gene expression or enzymatic activities.

While their precise physiological role remains unclear, polyamines should be considered as candidates for active regulators of plant growth. They clearly have a regulatory role distinct from a single nutritional requirement, even though their endogenous levels are about two orders of magnitude higher than those of the traditional plant hormones.

Lipochitooligosaccharides, a New Class of Plant Growth Regulators

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Lipochitooligosaccharides (LCOs) are a novel class of plant growth regulators usually consisting of a α-1,4-linked N-acetylglucosamine containing tetra- or pentasaccharide that is N-acylated with different long-chain fatty acids at the nonreducing glucosamine moiety. In legumes LCO signals secreted by rhizobia (so-called Nod factors) trigger the formation of nitrogen-fixing root nodules by initiating cell division at distinct sites.

We have developed a simplified procedure to synthesize LCOs and demonstrated that these synthetic glycolipid signals are efficient at extremely low concentrations to alleviate the requirement of the phytohormones auxin and cytokinin to sustain growth of cultured protplasts of the non-legume tobacco. A remarkable aspect of LCO action is the observation that these signals are still able to stimulate auxin-independent cell division of the tobacco cells in the subfemtomolecular range. The lack of correlation between this extremely low concentration of glycoplipid signal and the biological response suggests that the initial LCO stimulus might be amplified by the synthesis of another growth-promoting factor released from the cells into the medium. We isolated the mitogenic factor produced by LCO-treated tobacco protoplasts from the culture filtrate and showed that the mitogen is a peptide that fully mediates the growth promoting activities of the primary LCO signal. Furthermore, this peptide was shown to be antigenically related and functionally indistinguishable from a synthetic heptadecapeptide derived from region 2 of the tobacco homologue of the early nodulin gene ENOD40.

Fusicoccin

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Infection with the fungus *Fusicoccum amygdali* eventually kills the host plant, peach (*Prunus persica* (L.) St.) and almond (*Prunus amygdalus* St.) trees. The fungus excretes a diterpene glucoside called fusicoccin (FC) into the apoplast of the infected leaf and the toxin spreads via the transpiration stream throughout the leaf and plant. When FC reaches a guard cell pair it does not kill the cells, but instead it triggers a signalling pathway leading to a wide opening of the stomatal pore. The uncontrolled loss of water eventually results in the wilting of the infected leaf. Although the fungus is host-specific, FC is not and it affects a system common to most if not all plant cells. The intriguing thing about FC is that it interferes with most of the main plant hormones: it stimulates cell elongation (like auxin), it breaks seed dormancy (in synergy with GA and antagonizing ABA), it stimulates rhizogenesis (in synergy with auxin) and it stimulates ethylene production.

A few years ago we identified the receptor protein for FC as belonging to the family of so-called 14-3-3 proteins. since then, it has become clear that these 14-3-3 proteins are at the cross-point of a bewildering array of signalling and regulatory pathways. The best characterized target for FC action is the H^+-ATPase in the plasma membrane of plants. This enzyme plays a crucial role in all transport processes across the plasma membrane and moreover it is involved in the regulation of cytoplasmic pH. Here, I will report on the progress in our understanding how FC activates the H^+-ATPase and address the question whether FC has more target enzymes besides the ATPase. The latter is of interest in view of the wide range of effects that FC has.

The Relationship between Carrot Ep3 Endochitinases, Arabinogalactan Proteins (AGPs) and Embryogenesis

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Somatic embryogenesis of the carrot temperature sensitive mutant ts11 can be rescued by the addition of EP3 class IV endochitinases (De Jong *et al.* 1992, *Plant Cell* **4**, 425–433; Kragh *et al.* 1996, *Plant Mol. Biol.* **31**, 631–645). This suggests a role for EP3 endochitinases in somatic embryogenesis. Since no chitinaceous molecules had been found in plants we initiated work to identify a plant produced substrate for the EP3 endochitinases. We localized both the EP3 endochitinases and their mRNAs in developing seeds, suggesting that seeds may be a source of endochitinase substrates (van Hengel *et al.* in press, 1997). This led to the isolation of highly glycosylated seed AGPs that were shown by *in vitro* experiments to contain chitinase-sensitive oligosaccharides. The addition of untreated seed AGPs to carrot protoplasts derived from suspension cells increased the number of somatic embryos formed more than tenfold. The exact nature of the effect of AGPs on somatic embryogenesis remains to be elucidated, but cell tracking experiments have shown that the number of protoplasts that divide and retain a small cell size increases after the addition of AGPs. Addition of the EP3 endochitinases alone, or in combination with developing seed AGPs, results in a much lower number of somatic embryos as compared to the addition of AGPs alone. The addition of AGPs that had been reisolated after preincubation with the EP3 endochitinases resulted in an even higher number of somatic embryos formed when compared with untreated AGPs. These results suggest that EP3 endochitinases are required to activate GlcNac-containing AGPs, and simultaneously produce a low Mr compound inhibitory to somatic embryogenesis. These results are the first that demonstrate the existence of and identify biologically active plant-produced substrates for plant endochitinases.

MEETING OF THE SECTION FOR PLANT SYSTEMATICS ON 12 DECEMBER 1997

Tetrochidium (Euphorbiaceae) a Genus With and Without Petals?

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The genus *Tetrochidium* of the Euphorbiaceae–Crotonoideae has *c.* 20 species, 15 in tropical America and five in tropical Africa (Webster, 1994, *Ann. Miss. Bot. Gard.* **81**: 101). Its flowers were known to be apetalous till 1959 when J. Léonard described the new species *T. congolense* with petals in the female flowers (*Bull. Nat. Plantentuin Belg.* **29**: 197). This phenomenon was studied because it is unusual in Euphorbiaceae that in the same genus species are present with petals next to species which are apetalous.

The female flowers in African species of *Tetrochidium* are arranged in dichasia, which are well developed and bear (1–)3–5 flowers in *T. didymostemon* (Baill.) Pax & K.Hoffm., but are usually single-flowered in the other species. New material, representing a new species from the Cristal Mts in N. Gabon, helped to reveal the identity of the outer floral envelope, the calyx, in *T. congolense*. It is in fact an epicalyx formed by the bracts and bracteoles of the dichasium. The inner envelope, which is similar to the calyx in the apetalous species, is also the calyx and not a corolla of the female flower of *T. congolense*. *Tetrochidium* remains a genus with apetalous female flowers.

Phylogeny of the Subtribe Aleuritineae (Euphorbiaceae)

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According to the latest classification of the family Euphorbiaceae (Webster, 1994, *Ann. Miss Bot. Gard.* **88**: 33–144) the subtribe Aleuritinae belongs to the tribe Aleuritideae of subfamily Crotonoideae. The group was first established as a tribe by Hurusawa (1954, *J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot.* **6**: 209–342), after which Webster (1975, *Taxon* **24**: 593–601) reduced it to its present subtribal rank. The subtribe is either monotypic, consisting of the genus *Aleurites* only, or it is subdivided into three genera, following Airy Shaw (1967, *Kew Bull.* **20**: 393–395): *Aleurites*, *Reutealis* and *Vernicia*.

A phylogenetic analysis of the subtribe Aleuritinae was performed to answer the following questions:

- Is a subdivision into three genera necessary?
- Are the three genera monophyletic and do they possess apomorphies?
- Are the Aleuritinae a distinct, monophyletic unit or not?

The matrix used for the phylogenetic analysis included both vegetative and reproductive characters. The choice of the outgroup was difficult, because an obvious sister group was absent. Two genera which appear close to Aleuritinae according to the current classification of the family, namely *Borneodendron* and *Fahrenheitia*, appeared likely candidates. In order to ascertain their suitability as an outgroup a third unrelated genus was added, *Cheilosa* (subfamily Acalyphoideae).

The resulting cladogram showed that in addition to *Cheilosa* both *Borneodendron* and *Fahrenheitia* also acted as suitable outgroups. According to the obtained phylogeny the subtribe is monophyletic, and its present delimitation into three genera seems justified: the differences between the genera are small, but based on apomorphies and, moreover, the genera are monophyletic. In the cladogram the monotypic genus *Reutealis* splits off first, followed by a split into the genera *Aleurites* (two species) and *Vernicia* (three species, relationships among them not resolved).

Important Characters of and within *Baccaurea* (Euphorbiaceae)

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The genus *Baccaurea* (Euphorbiaceae) is placed in the subfamily Phyllanthoideae, tribe Antidesmeae, and subtribe Scepinae (Webster, 1994. *Ann. Miss. Bot. Gard.* **81**: 1–144). The same author circumscribed *Baccaurea* by the following characters: dioecious tree or shrub, pistillate disc absent, style bifid, staminate disc present and pistillate sepals deciduous. The first two characters are supported by my own observations. However, several characters are (partly) incorrect; not the style is bifid, but the stigma lobes are; only a part of *Baccaurea* has either a staminate disk or staminodes; this is subject of further study. Only a part of *Baccaurea* has deciduous pistillate sepals. In order to distinguish *Baccaurea* from neighbouring genera, namely *Aporusa* and *Maesobotrya*, further study is necessary. A unique character of *Baccaurea* appears to be stamens shorter than the sepals.

Within *Baccaurea* the following characters are constant: bark finely fissured; petiole relatively long; petiole apically pulvinate; stipules small and caducous; leaves usually alternate and papery; leaf margin entire; leaf apex rounded to acuminate; secondary venation pinnate; marginal leaf glands present; inflorescence a reduced thyrsus; flowers actinomorphic and valvate; flower pedicel with an abscission zone; pistillode present; fruit a berry; arillode present; stigma persistent.

Within *Baccaurea* the following characters are variable and important to classify species: stellate or simple hairs; leaves whorled or not; tertiary venation scalariform or reticulate; bark grey or brown; variation in indument of various parts of the plants; flower colour; inflorescences cauliflorous or axillary; extent of reduction in the male inflorescence; male flowers regularly dispersed over the inflorescence or clustered in the top; ovary with 2, 3 or 4 locules; fruit indehiscent, loculicidally, or loculicidally and septicidally dehiscent; fruit and arillode colour; fruit shape.

Phylogeny of the Malay species in the tribe Hippomaneae (Euphorbiaceae): Added Value for Local Floras

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A revision of the S.E. Asian (Malesian) Hippomaneae indicated that a new generic delimitation was inevitable. The new delimitation has been based on a phylogenetic analysis of the Malesian taxa and some S. American representatives. In particular, the genus *Sapium* became subdivided into several small genera, like the genus *Sebastiania*. Two new genera have to be described.

Normal phylogenetic procedures prescribe that only monophyletic groups may be analysed and no partial, paraphyletic groups such as the Malesian Hippomaneae. We think that our procedure is valid when the following rules are followed:

- Enter all taxa in the matrix, or
- when a group is insufficiently known or too large, subdivide the group into smaller groups which have unique characters (within the whole monophyletic group, thus in our case the whole of the Hippomaneae) or a unique combination of characters. Enter representative species in the matrix which cover the complete variation.
- The latter demand can only be fulfilled by somebody with expert knowledge of the whole group.
- After the analysis only distinguish those groups which have unique characters or a unique set of characters, preferably more than 1.
- Do not use the cladogram for classifications above the level of genus, for those purposes the complete monophyletic group has to be analysed.

Recognizing only those groups which have unique characters will ensure that these groups will also be found in larger analyses with more taxa. In this way floral treatments, especially for larger areas like Flora Malesiana, may apply phylogenetic analyses to a paraphyletic group and this will provide an added value to those floras.

Aspects of Reproductive Biology of *Mosannona* ined. (Annonaceae)

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Within the Annonaceae different strategies of beetle pollination exist, one of which is pollination by dynastine scarab beetles of the genus *Cyclocephala* which originated in different lineages within the family. Several morphological and non-morphological traits are associated with pollination by *Cyclocephala*. Among these are relatively large robust flowers, the formation of a pollination chamber at anthesis, the presence of nutritious tissue, protective structures preventing damage of reproductive organs, protogyny, fragrance and thermogenesis. Often, the presence of only a few of these traits, especially nutritious tissue and a pollination chamber, was sufficient to assume pollination by dynastine scarab beetles, this without confirmation by actual observations.

The genus *Mosannona* ined., which will be published shortly, exhibits many of the traits mentioned. Within this small genus one species from Mexico is known to be pollinated by *Cyclocephala*. We closely observed 37 flowers on four individuals of *M. raimondii* in Peru. We kept close track of the development from bud until flowers at anthesis. Shortly before anthesis a pollination chamber was formed. Nutritious tissue was present on the inner petals. There was no emission of fragrance and no thermogenesis. Visiting beetles were not observed, although these are abundantly present in the area as pollinators of species in other families. The lack of protogyny might point towards autogamy which, however, still has to be proved.

Do We Want a Nomenclatural Revolution?

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There is a call for a more effective nomenclatural system than is provided by the International Code of Botanical Nomenclature. Some leading taxonomists present the BioCode (Greuter, 1996, *Taxon* **45**: 349–372) as offering the solution. This new Code is proposed to be used for new names of all kinds of organisms, published from 2000 onwards, whereas the five Codes that are in use today, from 2000 onwards would continue to be in force only for names already existing on 1 January 2000.

The ambiregnal organisms provided the motive to consider harmonization of the Codes. At the Tokyo Congress (1993) a Special Committee was established 'to investigate all borderline problems between the biological Codes and all questions of harmonisation which were felt to be soluble'. Within 2 years, something appeared: not an inventory of problems and possible solutions, but the first draft of the BioCode! I do not think a BioCode for all organisms is necessary to solve the problems in nomenclature for a small group. By agreement it could be fixed which Code should be applied to names of ambiregnal organisms.

The principle of priority presents problems when an older synonym is found for a name that is in use. The proposed solution (already older than the BioCode ideas, and incorporated there): to produce Lists of Names in Current Use, and to grant a protected status to names in these lists. To achieve lists of good quality, I think one should at first restrict the period covered by such lists, e.g. one could start with the 1753–1850 period: this would cover the period from which the large majority of unused older synonyms emerge. As an alternative, one could introduce another solution: suppression of names that scarcely (if ever) have been in use, in favour of names that are in use.

When considering major problems under the Botanical Code, Hawksworth (1992, *Bot. J. Linn. Soc.* **109**: 543–567) presents an enormous simplification with respect to possible reasons why illegitimate names might have been published, and he suggests that the concept should be cancelled. The BioCode does not have the concept, that is in all present Codes, except the Zoological one.

The BioCode includes many new terms and even new concepts, if compared with the Botanical Code. I cannot imagine that it would constitute a simplification if from 2000 onwards every taxonomist would have to apply two Codes.

Phylogenetic Versus Linnaean Classifications

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Brummitt, in his last article (1997, *Taxon* **46**: 723–734), still refers to a discussion about the necessity of paraphyletic groups in a Linnaean classification. That is, in fact, not the issue; paraphyletic groups are imperative in Linnaean classifications for two reasons: (1) few ranks are used, which means that phylogenetic unequal groups receive the same rank. (2) if one part of a group is recognised and given a rank, then the remainder (mainly paraphyletic) also has to be recognized as a group with the same rank. Also his defence that they are natural groups, based on the fact that species may be paraphyletic groups of populations or specimens, is incorrect. Species may be paraphyletic due to a speciation event and that only takes place at the level of species not on higher levels.

The real topic should be what type of classification is preferred, a Linnaean or a phylogenetic classification. The main disadvantage of a Linnaean classification is that the criterion is recognition based on weighted characters. The latter is subjective and will always cause an unstable classification, because different researchers will give different weights to different characters and will thus create different classifications of the same group. A phylogenetic classification will use cladograms and the indicated relationships between the taxa as the classificatory criterion. This criterion is far less subjective and the resulting classifications will be more stable. Theoretical problems are that cladograms can only indicate split-ups and never split-offs and they cannot accommodate reticulate relations such as hybridization. More practical problems are that it is difficult and often impossible to create stable cladograms for taxa and that not many of those cladograms exist. This means that a phylogenetic classification of all species is still impossible.

The two types of classification look alike but are incompatible, therefore a simple compromise does not exist. Because of the practical problems with phylogenetic analyses the second option of Brummitt is supported: maintain the Linnaean classification, because it is practical, and in the meantime start with a phylogenetic classification with its own nomenclatural rules.

Morphological and Molecular Analysis of Endive, Chicory and their Wild Relatives (*Cichorium*; Asteraceae)

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As part of a monographic study of the genus *Cichorium*, the following results have been obtained. *Cichorium* L. ($2n=18$) is a small, monophyletic genus containing two widely cultivated species: *C. endivia* (endive) and *C. intybus* (witloof chicory and root chicory). A morphological analysis of the genus based on herbarium specimens and living collections revealed another four species: *C. calvum*, *C. bottae*, *C. divaricatum* and *C. spinosum*. Two species, *C. spinosum* and *C. bottae*, are morphologically easy to distinguish. *C. spinosum* is unique in having a spiny inflorescence, and *C. bottae* has the flower heads directly planted between the rosette leaves. The remaining four species *C. endivia*, *C. intybus*, *C. calvum* and *C. divaricatum*, however, form a morphologically complex group of species, differring in the maximum pappus length per head and the maximum plant height.

Apart from a morphological analysis, a phylogenetic analysis based on chloroplast DNA PCR RFLP data is currently undertaken to reveal the relationships between all *Cichorium* species. For this, a suitable outgroup for the genus *Cichorium* is needed. Unfortunately, the position of the genus *Cichorium* within the tribe Lactuceae differs in various phylogenetic studies based on morphological and molecular data. Therefore, a selection of nine possible outgroup genera for *Cichorium* (*Agoseris*, *Catananche*, *Chondrilla*, *Lactuca*, *Microseris*, *Prenanthes*, *Scolymus*, *Scorzonera* and *Taraxacum*) has been made and analysed using *Scolymus* as an outgroup for the whole tribe. About 12 different chloroplast regions were amplified and analysed with 10–15 restriction enzymes each (both 4- and 6-cutters). Unfortunately, much length variation was obtained, which complicates the restriction site analyses. Up to now, a total of 31 polymorphic characters were found and a phylogenetic analysis revealed 22 most parsimonious trees. A strict concensus tree (ci=0·76, 41 steps) of these 22 trees showed that *Cichorium* is a monophyletic genus indeed, but formed a polytomy for the other possible outgroup genera. In order to achieve more resolution within *Cichorium* and more insight into the best suitable outgroup for *Cichorium* a sequence analyse of the trnL(UAA)3′exon-trnF(GAA) intergenic spacer will be performed.

Evolution of Australian and New Zealand *Microseris* (Asteraceae) Based on Molecular Markers

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The allotetraploid species complex *Microseris lanceolata* and *M. scapigera* (Asteraceae, Cichorieae) is a variable group of perennial plants distributed over S. (E) Australia, Tasmania and New Zealand. According to morphological, cytological and molecular data, it arose in western North America by hybridization of an annual and a perennial diploid species followed by polyploidization and long-distance dispersal. Adaptive radiation resulted in various morphological types including a tuberous lowland and an alpine form, both self-incompatible, and a rare self-fertile form on the Australian main land, and self-compatible and -incompatible low- and highland forms on Tasmania and New Zealand. The chloroplast and nuclear DNA of 53 Australian and New Zealand populations has been investigated to reconstruct the evolution of the taxon.

A phylogenetic tree based on 58 HinfI, RsaI and TruII RFLPOs (restriction fragment length polymorphisms) and three trnL(UAA)-trnF(GAA) intergenic spacer length variants in the chloroplast DNA showed one group defined by three RFLPOs and the remaining accessions to be unresolved. The defined clade comprised all Australian tuberous and alpine plants and corresponded with the previously recognized species *M. lanceolata* while the remaining accessions were all members of *M. scapigera*. Within *M. lanceolata*, three subgroups reflect more the geographic distribution than morphological entities.

A principal component analysis of AFLPOs (amplified fragment length polymorphisms) in the nuclear genome showed the variation to be more continuous, suggesting a certain level of hybridization. Some support was found for geographic groups, and no discrimination was detected between the alpine and murnong type of *M. lanceolata*.

The chloroplast and nuclear data were congruent and showed that parallel adaptive evolution of different morphological types within *M. lanceolata* might have occurred.

Phylogeny of *Coelogyne* Lindl. (Orchidaceae) Based on Morphology and cpDNA RFLP Data

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Coelogynes are epiphytic orchids, growing in lowland and montane rain forests and secondary vegetations in S.E. Asia. Approximately 200 different species are recognized currently. Using the classification of Pfitzer & Kraenzlin (in A. Engler, 1907, *Pflanzenr. IV.*50.ii, Wilhelm Engelmann, Leipzig), these species are grouped into 14 different sections on the basis of some morphological key characters and geographical

distribution patterns. However, numerous later taxonomists have proposed different section subdivisions. This illustrates clearly the problematic delimitation of groups within the genus. It is not clear which subdivision of *Coelogyne* really reflects the evolutionary history of the species within the genus. To answer this question phylogenetic analyses are conducted with morphological and molecular data.

RFLP's of cpDNA are currently being collected. Data collected so far include digestions of the trnT–trnL intergenic spacer, trnL intron, trnL–trnF intergenic spacer, trnS–psaA, atpB–rbcL, petA–psbE and trnC–trnD intron with 20 different 6- and 4-base restriction endonucleases. Both restriction sites and length mutations within these regions were scored. All characters were treated as unordered in the cladistic analyses.

The phylogeny of 27 species from 10 different sections within *Coelogyne* was analysed with the help of 24 molecular characters. A heuristic search with PAUP, stepwise addition (100 replicates) produced 1794 MPTs (length: 46 steps; consistency index: 0.54). The strict consensus tree proved to be partly informative on the ingroup level. Collection of the complete sequences of the trnL–trnF intergenic spacer and of the nuclear ITS regions is planned to enlarge the molecular dataset.

