1 - Introduction and Sustainability Overview

1.1
One small step for carbon conservation, one giant leap for photosynthesis: the initial steps into the C3-C4 (and CAM) evolutionary divide

R Sage (University of Toronto)

C_4 and CAM photosynthesis are classic examples of complex trait evolution, where a multitude of structural and physiological changes occurred to create a radically new biological technology. As such, the evolutionary distance between the ancestral state (C_3 photosynthesis) and the derived state (C_4 or CAM photosynthesis) appears great, and the means by which the evolutionary gap is bridged is considered a mystery. The solution to the mystery of complex trait evolution often lies embedded in the last species in the ancestral lineage, who form the first link in the bridge across the evolutionary divide. These initial links are the small steps that make everything that follows possible, and may explain why certain lineages can evolve C_4 or CAM photosynthesis (often multiple times), while so many others cannot. In the case of C_4 evolution, the earliest stages appear to enhance the scavenging of photorespiratory carbon, while in CAM photosynthesis, the earliest stages may involve the scavenging of respiratory carbon. Recent phylogenetic work with genera containing C_4 species has identified a number of the immediate C_3 ancestors to the C_3-C_4 intermediate and C_4 lineages. Work with a number of these immediate C_3 ancestors shows close vein spacing is a common trait. Close vein spacing establishes the potential for photorespiratory scavenging through enhanced glycine decarboxylase expression in the bundle sheath tissue. Consistently, we find that these C_3 ancestors localize mitochondria around the inner wall of the bundle sheath cells, possibly as a means of enhancing photorespiratory scavenging. Such small developments in the C_3 ancestors may facilitate the subsequent localization of glycine decarboxylase in the bundle sheath cell, which is considered the key early step in the evolution of C_4 photosynthesis.

1.2
Imitation and invention: The route to C4 rice

J.E. Sheehy, M.J.A. Dionora, A.B. Ferrer, P.P. Pablico, A.E. Mabilangan (IRRI) and P.L. Mitchell (University of Sheffield)

We will approach the subject of photosynthesis from an agricultural perspective and in particular down from crops to molecules. Over the next 50 years, the population of the world will increase by about 50%, climate change will likely result in more extreme variations in weather and cause adverse shifts in the world's existing climatic patterns, water scarcity will grow, and the demand for biofuels will result in competition between grain for fuel and grain for food, resulting in price increases. Currently, a billion people live on less than a dollar a day and spend half their income on food, 854 million people are hungry and each day about 25000 people die from hunger-related causes. Research at international agricultural research institutes has been shaped by those problems. In rice, plant breeding seems to have exploited all of the intrinsic high yield-linked genes and yield potential has reached the limit. Theoretical models suggest that, for rice, further increases in yield potential can be achieved only by converting it from a C3 to a C4 plant. This will require the integration of efforts from those engaged in fundamental and applied research. All forms of research require a funding flow and it is only in the more affluent societies that pure curiosity-driven research can prosper. Curiosity and demand for products are the engines of research and one form of research can stimulate the other. However, funding mechanisms to integrate the research required for C4 rice, across national and disciplinary boundaries, are almost non-existent. Skepticism concerning such a project exists. Questions arise: Is it feasible and could it really deliver a quantum increase in yield, water-use efficiency and nitrogen-use efficiency simultaneously? In this paper, I will address those concerns and, I hope, demonstrate that not only is constructing C4 rice a solvable problem, but that it can be done on a time scale relevant to food security during the next half century.

1 - Biofuels and Global C sequestration

1.1
Why C4 perennials appear the ideal biofuel feedstock crops for the temperate zone

S Long (University of Illinois)

C4 plants with their higher potential efficiencies of water, nitrogen and intercepted light energy are well suited as biofuel feedstocks. At present maize and sugar cane are the two major global sources of biofuels, using starch and sucrose. However, even if the entire maize crop of the US was used for ethanol production it would only replace about 25% of current US gasoline use. While energy security has motivated interest in biofuels, in the longer term mitigation of global change is likely to become the dominant driver. Existing food crops are poorly suited for this mission. They require many energy-intensive inputs that decrease their value in offsetting CO_2 emissions. Ability to digest cellulose and hemicelluloses to monosaccharides opens the use of a much wide range of feedstocks. C4 rhizomatous perennials with the prairie life-form appear well suited as sustainable supplies of lingo-cellulose. Their extensive root and rhizome systems give them high interception efficiencies of water and nutrients, and allow far more rapid canopy development in the spring than is possible with annuals. Efficient retranslocation of nutrients to the root system in the autumn, and prior to harvest of the biomass, minimizes the need for fertilizers. These advantages are exemplified by the bioenergy crop, Miscanthus x giganteus. We will show that this plant in the Midwest can achieve annual solar energy conversion efficiencies of about 3%, yielding sufficient biomass feedstock to replace 25% of current gasoline use in the US on a fraction of the land required to achieve the same with maize, and with a fraction of the resource requirement. Using comparative physiology, gene sequences and gene expression we will also show how this remarkable plant is able to maintain photosynthetically competent leaves at much lower temperatures than maize, allowing it a much longer growing season than other C4 crops

1.2
Optimizing nitrogen allocation in *Miscanthus gigantus* for higher biomass production. A theoretical study

X Zhu (University of Illinois at Urbana Champaign), F Miguez (UIUC)

Miscanthus gigantus is a perennial grass with an NADP-ME C4 photosynthetic pathway which can routinely reach a dry biomass yield of 20-30 tons per hectare. Due to its high productivity, it is chosen as one of the elite energy crops to generate biomass for cellulosic biofuel production. Unlike common food crops such as rice, which reaches its high grain yield production through extensive breeding, Miscanthus has remained in its native state with no improvement through breeding or genetic engineering yet. To facilitate improvement of productivity of this crop, we have developed a mechanistic model of Miscanthus photosynthetic productivity, which can effectively predict the growth and development of Miscanthus. In this study, we combined this model and an evolutionary algorithm to explore the potential of modifying nitrogen allocation among photosynthetic enzymes between sunlit and shaded leaves to gain higher canopy photosynthetic CO_2 uptake rate. Depending on conditions, we assume control of C4 photosynthesis resides between Rubisco, PEPC, PPDK, and ATP-NADPH regeneration. Results showed that if Rubisco with higher catalytic number can be transformed into C4 plants, less nitrogen investment would be required to achieve the same canopy photosynthesis. With the same nitrogen investment, the total canopy photosynthesis can be dramatically increased if we replace current C4 Rubisco with one having higher catalytic number and re-optimize nitrogen allocation among photosynthesis enzymes. The optimized nitrogen allocation among PEPC, PPDK, Rubisco, and proteins in the light reactions were identified for a typical Miscanthus canopy at different temperature. The potential routes and hurdles to realize the identified optimal distribution are discussed.

1.3
Can we improve digestibility of C4 plants for biofuel feedstocks?

Robert. T. Furbank (CSIRO), Rosemary White (CSIRO), Susanne von Caemmerer (RSBS ANU)

Current technologies for conversion of biomass to fuel are focussed on either the conversion of non-structural carbohydrate to ethanol (using sugarcane-derived sucrose or maize starch) or conversion of cellulose to ethanol. The latter process is currently being trialled commercially across the USA and Europe and is becoming economically more attractive through the availability of appropriate enzymes and micro-organisms to digest biomass. C4 plants such as Panicum virgatum and Miscanthus sp. are favoured feedstocks due to their high growth rates and yields per Ha and their high water and N-use efficiency. C4 plants, however, due to their highly thickened bundle sheath and vasculature are poorly digestible by microorganisms. Here we explore the possibilities for improving biomass quality while retaining bundle sheath resistance to CO2 diffusion and discuss new technologies to screen for cell wall composition and plant performance. The potential use of FTIR imaging microscopy, digital growth imaging and carbon isotope discrimination as screening tools is presented.

1.4
C_4 pathway evolution and its ecological consequences in *Alloteropsis semialata*

C Osborne (University of Sheffield), D Ibrahim (University of Sheffield), M Gilbert (Rhodes University), B Ripley (Rhodes University)

Although half of today's grass species utilize C_4 photosynthesis, the ancient origins of this pathway in C_4 grass lineages hinder direct evaluation of its ecological costs and benefits. We have addressed this problem by investigating the physiological ecology of Alloteropsis semialata, a unique grass species with co-occurring C_3 and C_4 subspecies. Molecular phylogenetic analyses indicate that the C_3 subspecies represents an evolutionary reversion from a C_4 ancestor. Controlled environment and common garden experiments demonstrate a photosynthetic and growth advantage for the C_4 subspecies over the C_3 type at temperatures above 15 ºC. However, during drought events this advantage is lost because the C_4 subspecies shows greater susceptibility to metabolic limitations imposed by water deficit. The C_4 subspecies also suffers from freezing-induced mortality of its leaves during winter, which the C_3 subspecies avoids via cold acclimation. Crucially, CO_2, nitrogen and fire manipulation experiments suggest that photosynthetic pathway in this species is linked with ecological strategy. The C_3 subspecies favours canopy development, whilst the C_4 diverts greater resources to sexual reproduction and storage, allowing more rapid regrowth after fire. Together, these data suggest that C_4 photosynthesis in A. semialata is advantageous in warm, high rainfall climates characterised by frequent fires, but confers a cost of greater sensitivity to frost and drought events. We hypothesise that evolutionary loss of the C_4 pathway in A. semialata has been an important determinant of its range expansion from the subtropics into cooler, drier, and less fire-prone areas of southern Africa.

1.5
C4 photosynthesis at regional- and global-scales

J Berry (Stanford), Christopher Still (UC Santa Barbara), Brent Helliker (University of Philidelphia), Ulli Seibt (Stanford and Cambridge), Neil Suits (Colorado State).

Abstract not submitted

1 - Genetic, Molecular and Biochemical Correlates for C4 and CAM (1)

1.1
Isolation and characterization of mutants of ice plant, *Mesembryanthemum crystallinum*, defective in Crassulacean acid metabolism

J Cushman (University of Nevada), Sakae Agarie (Saga University), Rebecca Albion (University of Nevada), Richard French (University of Newcastle), Stewart M. Elliot (University of Newcastle), Tahar Taybi (University of Newcastle), Anne M. Borland (University of Newcastle)

Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that requires reciprocal carbon flux from storage carbohydrates to carboxylic acids during the night and acids to carbohydrates during the day. Genetic dissection of regulatory and metabolic attributes of CAM has been limited by the difficulty of identifying a reliable phenotype for mutant screening. We have developed a novel and simple method to screen large numbers of the inducible CAM species Mesembryanthemum crystallinum using pH as an indicator to detect mutants with limited nocturnal acidification within leaf tissues following the imposition of salinity. The isolated CAM-defective mutants showed negligible net dark CO_2 uptake compared to wild type plants following the imposition of salinity. The carbon-isotope ratios of seed collected from 4-month-old plants indicated that C_3 photosynthesis made a greater contribution to seed production in the mutants compared to wild type. The mutants also had substantially reduced seed set and weight relative to wild type. CAM-defective mutants were deficient in leaf starch suggesting substrate limitation of nocturnal C_4 acid formation. The restoration of nocturnal acidification by feeding detached leaves with glucose or sucrose supported this hypothesis. CAM-defective mutants were found to be deficient in plastidic phosphoglucomutase (EC 5.4.2.2), an enzyme of glycolysis/gluconeogenesis that is responsible for the interconversion of glucose-1-phosphate and glucose-6-phosphate. The CAM-defective mutants described here constitute important models for exploring regulatory features and metabolic consequences of CAM.

1.2
Cloning, localisation and expression analysis of sugar transporters in pineapple

E Antony (Water Treatment Centre, India), T Taybi (Newcastle University), A Smith (Oxford University), I Moore (Oxford University), A Borland (University of Newcastle Upon Tyne)

In *Ananas comosus* , storage of soluble sugars in the central vacuole during the daytime and their remobilisation at night is required to provide carbon skeletons for nocturnal CO_2 fixation in this CAM species, but soluble sugars must also be exported to support growth processes in heterotrophic tissues. To begin to address how vacuolar sugar storage and assimilate partitioning is regulated in pineapple, we used degenerate PCR and cDNA library screening to clone four candidate sugar transporters from the leaves of this species. Subcellular localisation of three of the putative transporters isolated was investigated via fluorescent protein tagging (YFP) and colocalisation with subcellular markers by transient expression in tobacco epidermal cells. We identified a putative hexose transporter (*Ac2.1*) and a putative inositol transporter (*Ac3.1*) that were localised to the tonoplast, as well as a sucrose transporter (*Ac6.1*) that localised to pre-vacuolar compartments. We also identified a cDNA (*Ac4.1*) with similarity to a recently identified tonoplast hexose transporter in *Arabidopsis*. Analyses of transcript abundance indicated that *Ac2.1* was more highly expressed in fruits compared to leaves of pineapple, whilst transcripts of *Ac3.1*, *Ac4.1* and *Ac6.1* were most abundant in leaves. Transcript abundance of *Ac3.1*, the putative inositol transporter, showed day–night changes comparable to those found in the facultative CAM species *Mesembryanthemum crystallinum*. We have overexpressed three of the pineapple sugar transporters in *Arabidopsis thaliana* under control of the 35S CaMV promoter. The *Arabidopsis* transformants show interesting phenotypes in terms of growth and development that await further characterisation.

1.3
C_4 photosynthetic isotope exchange in NAD-ME and NADP-ME type grasses.

A Cousins (Australian National University), M Badger (Australian National University), S von Caemmerer (Australian National University)

Monitoring photosynthetic isotope exchange is an important tool for predicting the influence of plant communities on the global carbon cycle in response to climate change. C_4 grasses play an important role in the global carbon cycle but their contribution to the isotopic composition of atmospheric CO_2 is not well understood. We have conducted instantaneous online measurements of $^{13}CO_2$ (D (Delta)^{13}C) and C^{18}OO (D^{18}O) isotope exchange in 5 NAD-ME and 7 NADP-ME C_4 grasses to investigate the difference in photosynthetic CO_2 isotopic fractionation in these subgroups. As previously reported, the isotope composition of the leaf material (D^{13}C) was enriched in ^{13}C in the NADP-ME compared to the NAD-ME plants. However, D^{13}C was similar between subtypes at both high and low light. D^{13}C increased at low compared to high light in both subtypes. This suggests differences in leaf d^{13}C between the NAD-ME and NADP-ME subtypes is not caused by photosynthetic isotope fractionation and leaf d^{13}C is not a good indicator of bundle sheath leakiness. This raises the question whether respiratory $^{13}CO_2$ fractionation may influence leaf d^{13}C. The oxygen isotope composition of CO_2 is determined by the isotope composition of leaf water at the site of evaporation (d_e) and carbonic anhydrase activity (CA). Based on estimates of d_e we found that D^{18}O was lower than predicted from leaf CA activities in all plants and there was no difference in D^{18}O and CA activity between subtypes. Low D^{18}O in these C_4 grasses are similar to D^{18}O measured in C_4 dicots which containing 4 times the leaf CA activity suggesting that leaf CA activity is not a predictor of D^{18}O in C_4 plants.

1.4
Evolution of mesophyll and bundle-sheath specific gene expression in the genus *Flaveria* - a case study with phosphoenolpyruvate carboxylase and glycine decarboxylase

P Westhoff (Heinrich-Heine-Universitaet Duesseldorf)

Abstract not submitted

1.5
Mutational and Structural Analyses of Regulatory Properties of Phosphoenolpyruvate Carboxylase (PEPC) for C4 Photosynthesis: Molecular basis for pH-dependent regulation

K Izui (Kinki University), H Matsumura (Osaka University), H Tokunaga (Kinki University), T Ogawa (Osaka University), T Maeda (Osaka University), T Inoue (Osaka University), Y Kai (Osaka University), T Endo (Kyoto University), Y Mihara (Kyoto University), T Furumoto (Hiroshima University), K Yokosho (Kinki University), M Akita (Kinki University)

PEPC plays a pivotal role in C4 and CAM photosynthesis. Based on 3D structure of PEPCs from *E. coli* and *Z. mays*, the locations of catalytic- and regulatory sites, and the flexible loops are first overviewed. This talk focuses on the following two topics. (1) Mutationally altered maize C4-form PEPC with higher affinity to PEP and diminished sensitivity to malate (MA)-inhibition was successfully prepared by simultaneous replacement of amino acid residues (S780A/K835A). Ser780 is a hallmark of C4-form PEPC and K835 participates in MA-binding. Expression plasmids were constructed and introduced to tobacco to evaluate its functional efficiency in a “C3 environments”. (2) MA-inhibition is known to be pH-dependent in higher plant PEPCs. PEPC shows MA-sensitivity at pH 7, but not at pH 8. Recent success in X-ray crfystallographic analysis of recombinant maize PEPC with intact *N*-terminal revealed the structres at both pHs. The essential loop (639-651) swinging between catalytic site and MA-inhibition site is fixed to the inactive form presumably through ionic interaction of H653 with E134 and E140 at pH 7, while this interaction is lost at pH 8. This assumption was verified by a series of site-directed mutagenesis and H653 was concluded to be a key residue involved in the pH-dependent regulation. A clue to the reason for the lack of

this pH-dependency in *E. coli* PEPC was also obtained. Since E134 and E140 are resided on one of the plant-specific mobile loops (124-140), this loop seems to have been acquired for pH-dependent regulation during evolution in plants.

1.6
The gdcsPA promoter from *Flaveria trinervia* – *Cis*-regulatory determinants for bundle-sheath-specific gene expression

U Gowik (Heinrich-Heine-Universität Düsseldorf), C Wiludda (Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität Düsseldorf), S Engelmann (Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität Düsseldorf), J Burscheidt (Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität Düsseldorf), H Bauwe (Institut für Biowissenschaften,

The multi-enzyme complex glycine-decarboxylase (GDC) is involved in the process of photorespiration in plants which leads to the release of CO_2 during the conversion of glycine to serine. In C3 plants photorespiration occurs in all photosynthetically active tissues of the leaf due to the presence of the GDC and the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) as the initial enzyme of the respiratory pathway in these cells. By contrast in C4 plants Rubisco is restricted to bundle-sheath cells of leaves as the result of concentrating CO_2 in this special cell type. Therefore GDC can also only be found in bundle-sheath but not mesophyll cells of C4 leaves.
We are working with the promoter of the gene encoding the P-subunit of the GDC (gdcsPA) from the C4 plant *Flaveria trinervia* (Ft), thus called gdcsPA-Ft. Surprisingly the gdcsPA-Ft promoter is only active in the vascular tissues and the cell layer surrounding the vascularity in leaves of transgenic *Arabidopsis thaliana* plants (C3) indicating that this C3 plant might have bundle-sheath cells like C4 plants. We are interested in identifying the *cis*-regulatory elements of the gdcsPA-Ft promoter being necessary for the bundle-sheath-specific activity in *A. thaliana* (C3) and *F. bidentis* (C4). We also want to use the gdcsPA-Ft promoter as a molecular tool to label bundle-sheath cells of *A. thaliana* with the green fluorescent protein (GFP) as a vital reporter to analyze the function of these cells in C3 plants in more detail.

1.7
Biotechnological approaches to improve C3-photosynthesis: establishment of a complete glycolate pathway in C3 chloroplasts and overexpression of C4 single enzymes in *A. thaliana*

V Maurino (University of Cologne), H Fahnenstich (Institute of Botany, University of Cologne, Germany), M Saigo (CEFOBI, University of Rosario, Argentina), M Drincovich (CEFOBI, University of Rosario, Argentina), U Flügge (Institute of Botany, University of Cologne, Germany)

The photosynthetic cycle of C4-plants provides a CO2 pump, which leads to increased CO2/O2 ratio at the site of Rubisco and thus results in a decreased oxygenase activity. In this way, C4-plants display a high photosynthetic capacity. By introducing a complete glycolate catabolic cycles into C3-chloroplasts, it is attempted to create an autoregulatory cycle which results in an attenuation of photorespiration and an expected improvement in the efficiency of CO2 assimilation. Arabidopsis thaliana was used to set up and characterized the novel pathway. Other approach to improve C3-photosynthesis is the introduction of single C4-activities in C3-plants, expecting that it will led to the desired redirection of fluxes. C4-NADP-ME was overexpressed in A. thaliana leading to 6 to 30 fold higher activities. There were no beneficial impacts in the photosynthetic performance in the transgenic plants. However, plants growing in short days displayed a pale green phenotype. In these conditions, MEm plants have a decreased fresh weight/area ratio and thinner leaf sections. No differences in morphology and development were evident in long days. Analysis of data showed that MEm transgenic plants entered dark induced senescence more rapidly due to an accelerated starvation caused by extremely low levels of malate and fumarate. Thus, in prolonged darkness these metabolites are consumed faster than in the wild-type and, as a consequence, MEm plants enter irreversible senescence more rapidly. In addition, our data point out that malate and fumarate are important form of fixed carbon that can be rapidly metabolized at least in Arabidopsis thaliana.

1.8
Relocation of photorespiratory CO_2 release from the mesophyll to the bundle sheath of a C3 plant

H Bauwe (University of Rostock), N Engel (University of Rostock), N Qu (University of Rostock)

Photorespiration was one of the selective pressures that resulted in the evolution of C4 photosynthesis. As inferred from the analysis of C3-C4 intermediate plants, the relocation of photorespiratory CO_2 release by glycine decarboxylase (GDC) has been an early and very important event in this evolutionary multi-step process. We want to construct an artificial 'C3' plant where glycine is not decarboxylated in the mesophyll (the original C3 location), but only in the bundle-sheath (corresponding to the C3-C4 and C4 situation). To this end, we have isolated Arabidopsis GDC null and knockdown mutants and are now complementing these plants with GDC genes from a C3-C4 intermediate plant. In short, a complete knockout of GDC activity is lethal even under 0.9% CO_2, indicating a non-replaceable function of GDC in vital non-photorespiratory processes (one-carbon metabolism). In contrast, partial knockout of GDC activity results in features as they are also typical for other photorespiratory mutants. Full complementation of one of these GDC mutants was achieved by overexpression of the corresponding bundle-sheath specific gene from a C3-C4 intermediate plant. Features of these non-complemented and complemented mutants will be described and discussed.

1 - Environmental physiology : C4 and CAM

1.1
Environmental and developmental control of CAM in C3-CAM species

K Winter (STRI, Panama)

The capacity to developmentally induce CAM and to upregulate CAM expression in response to drought stress was studied in a range of species considered to exhibit CAM either facultatively or constitutively. Twenty four-hour net CO2 exchange of whole shoots was measured continuously during the early growth of plants or throughout their entire life cycle. The results shed new light on the nature of facultative and obligate CAM.

1.2
Consequences of photosynthetic pathway evolution for plant-climate interactions: a test using C_3 and C_4 subspecies of *Alloteropsis semialata*

D Ibrahim (University of Sheffield), C Osborne (University of Sheffield), T Burke (University of Sheffield), M Gilbert (Rhodes University), B Ripley (Rhodes University)

The only plant known to have both C_3 and C_4 subspecies is the grass *Alloteropsis semialata*. This species therefore offers a unique opportunity to test the ecological consequences of photosynthetic pathway evolution. We used a molecular phylogeny to determine the relationship between the subspecies, and established a common garden experiment in South Africa to investigate how photosynthetic pathway modifies plant-climate interactions. Sequencing of the chloroplast gene ndhF confirmed a close relationship between the C_3 and C_4 subspecies, and suggested that the physiology of the C_3 subspecies represents a reversion from the C_4 pathway. The common garden experiment tested the hypothesis that the C_4 subspecies has a photosynthetic and growth advantage over the C_3 subspecies during periods of high soil water availability in summer, but that this advantage is reversed in winter. We applied irrigation and natural rainfall treatments throughout two growing seasons, and measured aboveground biomass and gas exchange. The C_4 subspecies had the anticipated photosynthetic and growth advantages over the C_3 subspecies during wet conditions in summer. However, these were lost during periodic natural drought events. As expected, the C_3 subspecies achieved a photosynthetic advantage over the C_4 subspecies during winter, but this was not due to higher C_3 photosynthetic efficiency at low temperatures. Instead, winter frost killed all of the C_4 leaves, while photosynthesis continued in the freezing-resistant C_3 subspecies. This study demonstrates that climatic extremes such as drought and frost significantly modify the seasonal advantages of C_3 and C_4 photosynthesis in relation to temperature and water availability.

1.3
Functional significance of C_3-C_4 intermediate traits in *Heliotropium* L. (Boraginaceae): gas exchange perspectives

P Vogan (University of Toronto), M Frohlich (Natural History Museum, London), R Sage (University of Toronto)

C_3-C_4 intermediate traits have been identified and described in over thirty species from twelve genera. Here, we demonstrate for the first time the presence of species exhibiting C_3-C_4 intermediacy in Heliotropium, a genus with over 100 C_3 and 150 C_4 species. CO_2 compensation points (G) and photosynthetic water-use efficiencies (WUE) were intermediate between C_3 and C_4 values in three species of Heliotropium: H. convolvulaceum, H. racemosum, and H. greggii. We also determined that H. procumbens is a weak C_3-C_4 intermediate based on a slight reduction in G compared to C_3 Heliotropium species. The intermediate species H. convolvulaceum, H. greggii and H. racemosum exhibited over 50% enhancement of net CO_2 assimilation rates at sub-ambient CO_2 levels due to a reduction in photorespiration; however, no difference in stomatal conductance between C_3 and C_3-C_4 species was observed. We also assessed the response of ? to O_2 concentration for these species. The three strongly intermediate species exhibited similar curvilinear responses of G to O_2, characteristic of C_3-C_4 photosynthesis. Recent phylogenies indicate that H. convolvulaceum, H. greggii and H. racemosum attach above the C_3 species in Heliotropium section Orthostachys and at the base of the clade(s) containing C_4 species. Thus, the hypothesis that the photosynthetic characteristics in these three species represent true evolutionary intermediacy is supported. These results establish Heliotropium as a valuable new system for studying the evolutionary transition from C_3 to C_4 photosynthesis, and may also be employed to introduce C_4 anatomical and biochemical traits into important C_3 crops such as rice.

1.4
Clusia: Holy grail and enigma

U Luettge (Technische Universitaet Darmstadt)

Plants of a clone of *C. minor* were acclimated for performance of C_3-photosynthesis and CAM, respectively. Photosynthesis and photorespiration were recorded on-line by measurements of gas exchange, chlorophyll fluorescence imaging and application of pulses of air with 1 % O_2 for the establishment of non-photorespiratory conditions. In the C_3-mode photorespiration was constant over the entire light period. In the CAM-mode it depended on the CAM phases. Chlorophyll fluorescence imaging allowed calculation of spatiotemporal heterogeneity of the apparent rate of photosynthetic energy use (FPSII). In the C_3-mode heterogeneity was low and constant under 21 % O_2. Under 1 % O_2 heterogeneity was high. This showed that the particular energy demand of photorespiration was synchronizing the energy demand over the leaves. In the CAM-mode heterogeneity was more dependent on the CAM phases than on photorespiration, and it was particularly high in the transitions between phases when local differences in internal CO_2 concentrations are building up in the leaves. CO_2 is considered as a synchronizing internal signal. Under constant external conditions circadian rhythmicity occurs in both modes. However, it is strongly dampened and lost after only a few periods and more quickly so in the C_3- than in the CAM-mode. This is in contrast to the obligate CAM plant *Kalanchoë daigremontiana* Hamet et Perrier where endogenous oscillations of CO_2 gas exchange pertain for very many periods. *C. minor* is an extremely flexible plant using rapidly reversible changes between the two modes of photosynthesis in eco-physiological adaptations. It is an interesting question for a discussion during the meeting if endogenous circadian rhythmicity, which is thought to be important for anticipation of and preparedness for rhythmically changing conditions, is not a hindrance for such flexibility and thus the rapid dampening of the rhythmicity is an advantage for *C. minor*. An intriguing observation was that during endogenous oscillations the C_3-mode was kept as such as it was revealed when the plants were returned to an external dark/light rhythm after endogenous oscillations had ceased. Conversely, the CAM mode was not maintained during the circadian oscillations and plants had returned to the C_3-mode during the constant conditions. There is an interesting analogy to this in *K. daigremontiana* where the malic acid rhythm is highly dampened during the first 3 to 5 endogenous periods although in this plant CO_2-gas exchange rhythmicity is kept unaltered. In both cases it appears that the C_4-malate rhythm is dampened and is lost together with rhythmicity in *Clusia* and by being replaced by a more C_3-like metabolic rhythmicity in *Kalanchoë*. This is a second point of interest for a discussion in the meeting, because many current molecular studies only cover the first two endogenous circadian periods, were inclusion of coverage of later periods may bear out interesting surprises. In both modes FPSII and its heterogeneity do not show any oscillations in constant light at 21 % O_2. Oscillations in FPSII and heterogeneity over the leaves are observed at 1 % O_2. This confirms that photorespiration stabilizes and synchronizes energy use over the leaves.

1.5
Oxygen isotope composition of CAM and C_3 *Clusia* species growing in a common garden in Panama

L Cernusak (Smithsonian Tropical Research Institute), K Winter (Smithsonian Tropical Research Institute), M Mejia-Chang (University of Cambridge), H Griffiths (University of Cambridge)

We measured oxygen isotope composition of leaf and branch dry matter in CAM and C_3 *Clusia* species growing in a common garden in Panama. We hypothesized that the CAM species would show lower $\delta^{18}O$ in leaf and branch dry matter, resulting from lower leaf water $\delta^{18}O$, because the CAM photosynthetic pathway is associated with stomatal opening at night, when relative humidity is high. Leaf dry matter $\delta^{13}C$ and $\delta^{18}O$ for *C. uvitana* and *C. rosea*, two CAM species, averaged –20.1‰ and +24.0‰, respectively. Values for a group of 17 C_3 and weak CAM species averaged –25.7‰ and +24.6‰, respectively. Thus a decrease of 5.6‰ in average $\delta^{13}C$ of leaf dry matter between the CAM and C_3/weak CAM groups was accompanied by an increase of only 0.6‰ in $\delta^{18}O$. Values for branch dry matter for the two groups followed a similar trend: *C. uvitana* and *C. rosea* showed average $\delta^{13}C$ and $\delta^{18}O$ of –17.5‰ and +19.8‰, respectively; whereas the C_3 and weak CAM species showed average $\delta^{13}C$ and $\delta^{18}O$ of -25.1‰ and 20.4‰, respectively. In this case a decrease of 7.6‰ in $\delta^{13}C$ between CAM and C_3/weak CAM species was accompanied by an increase of only 0.6‰ in $\delta^{18}O$. Diel measurements of leaf gas exchange and leaf water $\delta^{18}O$ during the dry season and wet season revealed that the *Clusia* leaf water system is dominated by non-steady state dynamics as a result of generally low stomatal conductance and high leaf water content. The net result is that only very subtle shifts in leaf water oxygen isotope enrichment can be detected between CAM and C_3 *Clusia* species growing in the same environment. In conclusion, our observations failed to support our hypothesis that CAM *Clusia* species should have significantly lower $\delta^{18}O$ than C_3 *Clusia* species.

1.6
Nocturnal sap flux in the C3-CAM species, *Clusia minor*

A Herrera (Universidad Central de Venezuela), C Ballestrini (Universidad Central de Venezuela), W Tezara (Universidad Central de Venezuela)

In order to gain knowledge on the water relations of the C3-CAM species Clusia minor, we followed for two years the seasonal changes in parameters of water relationships and sap flux velocity in one single tree growing in Caracas. Leaf water, osmotic and turgor potential remained relatively high throughout the seasons, water potential decreasing 0.7 MPa at the most, and responded rapidly to rainfall. Nocturnal acid accumulation was nil under extreme drought or after frequent and heavy rains and increased with drought. Estimated malate concentration contributed up to 62 % of the value of osmotic potential. Sap flux velocity also responded markedly to rains, not so much in magnitude as in daily pattern. During the dry season, V was highest during phase III of the daily CAM cycle; as drought progressed, the highest V was found at the end of phase II and on phase IV, and a small night-time flux during phase I was observed. The V decreased with the beginning of rains, when nocturnal sap flux was observed only between 1400 and 1730 h, and regained high values in the rainy season in an inverse pattern of highest V during phase I. There was a strong linear relationship between the proportion of the integrated sap flux courses corresponding to the night and nocturnal acid accumulation. We conclude that a lower osmotic potential together with nocturnal stomatal aperture helps explain increased nocturnal sap flux in the CAM stage of the tree of *C. minor*.

1.7
Does Crassulacean acid metabolism enable an enhanced capacity for sunfleck use in net daily carbon gain?

J Skillman (California State University), K Winter (Smithsonian Tropical Research Institute)

Previous studies suggest understory tropical CAM plants make greater use of sunflecks for C gain than co-occurring C3 species. This CAM-enhanced capacity for sunfleck use may compensate for the low quantum yield (QY) assumed for CAM. This could help explain the persistence of CAM species on the floor of tropical rainforests among vegetation generally dominated by C3 species. We tested the CAM-enhanced sunfleck use hypothesis. Comparative 24-hr foliar gas exchange measurements were made on six different Panamanian species native to the rainforest understory. Measurements were made under low light plus or minus controlled sunfleck treatments. Sunfleck-use was quantified as the ratio of net 24-hr C gain in the sunfleck treatment against that without sunflecks. Among the test species was *Aechmea magdalenae*, a terrestrial understory Bromeliad that is constitutitively CAM. All other species were C3. Daily light dosages during the 12-hr day were 0.4 and 2.2 mols photons / m^2 without and with sunflecks, respectively. Across all species, net 24-hr C gain in the absence of sunflecks ranged between 12 and 20 mmol CO_2 / m^2. The sunfleck-use for *Aechmea magdalenae* (147±8 %, n=3) was greater (p=0.024) than that observed across all C3 species (123±16%, n=15), as predicted. Individually, not every C3 species exhibited sunfleck-use values lower than *Aechmea magdalenae* indicating that sunfleck use varies among species independent of effects strictly attributable to differences in photosynthetic pathway. Results from comparative studies of the 24-hr C gain QY for several tropical understory species will also be discussed.

1.8
Epiphytic CAM plants as biorecorders of atmospheric water vapor $\delta^{18}O$: a case study on *Tillandsia usneoides*

B. Helliker (University of Philidelphia)

Globally, there is a paucity of data-based information on mean values of the oxygen isotope ratio ($\delta^{18}O$) of atmospheric water vapor. Through models and observations, we show that $\delta^{18}O$ of leaf water in the tropical Crassulacean acid metabolism (CAM) epiphyte *Tillandsia usneoides* is controlled by and reflects the $\delta^{18}O$ of atmospheric water vapor in a predictable manner, irrespective of precipitation inputs. By assuming that this leaf-water-atmospheric signature is recorded in plant organic material, we reconstructed the atmospheric water vapor $\delta^{18}O$ signature for Miami, Florida (USA) for several years between 1878 and 2005 using contemporary and herbarium specimens. *T. usneoides* ranges from Virginia, USA southwards through the tropics to Argentina, and the CAM epiphytic lifeform is not unique to this species. Therefore, there is significant potential for reconstructing the isotope ratio of atmospheric water (both $\delta^{18}O$ and δD) for spatial scales that span over 60° of latitude and temporal scales that cover the last century of global temperature increase.

1.9
Features of single-cell C_4 photosynthesis in terrestrial plants

G Edwards (Washington State University), E Voznesenskaya (V.L. Komarov Botanical Institute of Russian Academy of Sciences)

In some terrestrial plants, a carbon concentrating mechanism occurs through the process of C_4 photosynthesis. Until recently, a definitive characteristic of these plants has been the presence of Kranz leaf anatomy, with two structurally and biochemically specialized photosynthetic cell types functioning coordinately in carbon assimilation. C_4 photosynthesis has evolved independently many times, with great diversity. Dramatic variants of C_4 terrestrial plants were discovered recently in three species in family Chenopodiaceae. These species have novel compartmentation, which allows them to perform C_4 photosynthesis in individual chlorenchyma cells which have dimorphic chloroplasts, as shown by anatomical, histochemical and biochemical studies. One cytoplasmic domain is specialized for supporting fixation of atmospheric CO_2 in the C_4 cycle, and the other for accepting CO_2 from decarboxylation of C_4 acids and assimilating it by Rubisco in the C_3 cycle. Two means have evolved to accomplish this spatial separation of functions. Suaeda aralocaspica has the equivalent of a Kranz mesophyll compartment at the distal end, and a Kranz bundle sheath compartment at the proximal end, of chlorenchyma cells. Bienertia cycloptera and B. sinuspersici have a peripheral cytoplasmic compartment, which is proposed to function like Kranz mesophyll cells, and an unusual central cytoplasmic compartment, which is proposed to function like Kranz bundle sheath cells. The two compartments are interconnected by cytoplasmic channels through the vacuole. Emerging information on the biochemical and structural strategies for accomplishing C_4 has promise for improving productivity of crops like rice, which lack a CO_2 concentrating mechanism.

1.10
Curiosity and context revisited: CAM in the Anthropocene

Barry Osmond (Duke University), Tom Neales (University of Melbourne) and Gert Stange (RSBS,Australian National University)

Having gained some understanding of the consequences of the CO_2 concentrating mechanisms in CAM that internalise the photosynthetic environment of the Cretaceous on a daily basis, it may be time to consider potential long-term effects of the planetary CO_2 concentrating mechanism on growth and ecology of these plants in the Anthropocene. This paper emphasises our limited understanding of the carbohydrate economy of CAM in relation to growth processes in these plants and briefly reviews the work of Park Nobel and others on the response of large CAM plants to elevated atmospheric [CO_2]. It revisits an inadvertent long-term experiment from the past in which an Opuntia monoculture grew to occupy 50 million hectares, expanding at 10-100 hectare per hour on a regional scale. We examine whether expert plant biologists of the time were aware of the role of CAM in this "eco-catastrophe". Fortunately, a rural economy was restored and remains sustained by the voracious appetite of larvae whose mothers' are able to distinguish CAM. Their CO_2 sensing apparatus is temperature compensated to pre-industrial atmospheric [CO_2] and we review FACE experiments that show this exquisite system of biological control may be compromised by rising global [CO_2] in the Anthropocene.

1 - Genetic, Molecular and Biochemical Correlates for C4 and CAM (2)

1.1
The merits of adopting *Kalanchoe fedtschenkoi* as a genomic and transgenic model for CAM research

J Hartwell (University of Liverpool), S Boxall (University of Liverpool), J Foster (University of York), C Dall'omo (University of Liverpool), R Meszter (University of Liverpool)

There is a pressing need for a CAM model system that is highly amenable to transformation. I will discuss the merits of using *Kalanchoe fedtschenkoi* as a transformable model for CAM. *K. fedtschenkoi* is a Madagascan obligate CAM plant in the Crassulaceae (order Saxifragales) with a ~790 Mb genome. There is a wealth of whole plant physiology, biochemistry and molecular biology data concerning the regulation of CAM in this species. In particular, the study of the circadian rhythm of nocturnal CO_2 fixation was pioneered in this species, and we understand most about CAM circadian control from studies on *K. fedtschenkoi*. *K. fedtschenkoi* is also an excellent model system for CAM because there is a clear ontogenetic progression from C3 to CAM during leaf development. We have greatly streamlined and optimised the transformation protocol for *K. fedtschenkoi*, such that it is possible to produce rooted transgenic plants in 3-4 months. Studies of transgenic K. fedtschenkoi lines over-expressing the central circadian clock genes CCA1/LHY and TOC1 have revealed perturbations in the period of the rhythm of CAM CO_2 fixation and in the regulation of clock-controlled, CAM associated genes. This data supports the hypothesis that CAM is regulated by the CCA1/LHY-TOC1 autoregulatory gene-loop oscillator in the nucleus, rather than the tonoplast-as-oscillator hypothesis. The discoveries that these plants have facilitated also demonstrate the incredible value of taking a transgenic approach to the study of CAM, and emphasise the urgent need for the CAM community to join forces to work on a transformable CAM species.

1.2
The integrated circadian control over carbohydrate and crassulacean acid metabolism

T Taybi (Newcastle University), A Borland (Newcastle University)

The circadian clock controls a number of metabolic reactions associated with crassulacean acid metabolism. To determine if the ecological and taxonomic diversity of CAM plants is manifest at the level of the clock, we compared Mesembryanthemum crystallinum, a dicotyledonous CAM-inducible species and variegated Ananas comosus, a monocot with constitutive CAM. Within the variegated *A. comosus* we compared leaf tissues of different functions, the photosynthetic green tissue and the achlorophyllous white tissue. Specifically, we sought to determine if the species show differences in the extent of clock control over CAM expression and carbohydrate metabolism and if this is influenced by metabolic status. Acidity levels showed similar rhythms in M. crystallinum and the green leaf tissue of A. comosus green tissue both under day/night (L/D) and continuous light conditions (L/L). However, a significant delay in nocturnal acidification took place in the white A. comosus tissue under L/L conditions indicating differences between source and sink tissues in terms of circadian control. Starch and soluble sugar contents showed similar rhythms in the two species but under L/L conditions a second peak of soluble sugars was apparent during the virtual night. The expression of two clock-associated genes (CCA1 and TOC1) peaked at similar times in the green and white tissues of *A. comosus* under L/D but shifted to different phases under L/L. This suggests that autonomous circadian clocks operate in the two tissue types. This result was further confirmed by the independent shift in the rhythm of starch phosphorylase activities in the two tissue types.

1.3
Zm*hcf136* is a mesophyll defective mutant that lacks a photosystem II complex

S Covshoff (Boyce Thompson Institute for Plant Research), W Majeran (Cornell University), P Liu (Iowa State University), J Kolkman (Cornell University), K van Wijk (Cornell University), T Brutnell (Boyce Thompson Institute for Plant Research)

Chloroplast development and maintenance depends on the coordination of nuclear and plastid gene expression. One mechanism used to coordinate the activities of these organelles is redox sensing. In C4 photosynthetic plants such as maize, redox regulation is more complex because two morphologically and biochemically distinct cell types, mesophyll (M) and bundle sheath (BS), perform different photochemical reactions. Photosystem II (PSII) and linear electron transport occur in M cells, and cyclic electron transport takes place in BS cells. Thus, M and BS cells establish unique photosynthesis-derived redox states and may also utilize distinct plastid-nuclear communication networks. Mutants that are disrupted in either M or BS cell development provide an opportunity to explore these pathways. Here, we identify the maize homologue of *Hcf136*, previously identified in *Arabidopsis* as a PSII stability or assembly factor. In maize, *hcf136* is a M defective mutant that lacks PSII supercomplexes and granal stacking in M plastids. To gain further insights into the coordination of C4 photosynthetic metabolism, we compared the global transcript profiles of separated M and BS cells in *hcf136* and wild-type siblings. Microarray analysis reveals that mutant M and BS cell transcript profiles are minimally impacted by the *hcf136* mutation and the loss of PSII. Interestingly, the *hcf136* mutant is also defective in processing the full *psbB-psbT-psbH-petB-petD* polycistron specifically in the M cell. These results suggest that an oxidized redox state associated with a severe disruption in PSII activity does not significantly alter nuclear or plastid transcriptional profiles.

1.4
Phosphorylation of phosphoenolpyruvate carboxylase is not essential for high photosynthetic rates in the C_4 species *Flaveria bidentis*

S von Caemmerer (Australian National University), T Furumoto (Hiroshima University), K Izui (Kinki University), V Quinn (Australian National University), R Furbank (CSIRO)

Phosphoenolpyruvate carboxylase (EC4.1.1.31; PEPC) plays a key role during C_4 photosynthesis. The enzyme is activated by metabolites such as glucose 6-phosphate and inhibited by malate. This metabolite sensitivity is modulated by the reversible phosphorylation of a conserved serine residue near the N-terminus in response to light. The phosphorylation of PEPC is modulated by a protein kinase specific to PEPC (PEPC-PK). To explore the role PEPC-PK plays in the regulation of C_4 photosynthetic CO_2 fixation we have transformed *Flaveria bidentis* (L.) Kuntze (a C_4 dicot) with antisense or RNAi constructs targeted at the mRNA of this PEPC-PK. We generated several independent transgenic lines where PEPC is not phosphorylated in the light, demonstrating that this PEPC-PK is essential for the phosophorylation of PEPC in vivo. Malate sensitivity of PEPC extracted from these transgenic lines in the light was similar to the malate sensitivity of PEPC extracted from darkened wild type leaves but greater than the malate sensitivity observed in PEPC extracted from wild type leaves in the light, confirming the link between PEPC phosphorylation and the degree of malate inhibition. There were, however, no differences in the CO_2 and light response of CO_2 assimilation rates between wild type plants and transgenic plants with low PEPC phosphorylation showing that phosphorylation of PEPC in the light is not essential for efficient C_4 photosynthesis for plant grown under standard glass house conditions. This raises the intriguing question of what role this complexly regulated reversible phosphorylation of PEPC plays in C_4 photosynthesis.

1.5
The development and evolution of C4-type cellular differentiation patterns

J Langdale (University of Oxford)

Abstract not submitted

1.6
The regulation of genes in C_3 species that encode proteins important for C_4 photosynthesis

J Hibberd (University of Cambridge)

The activities of four-carbon decarboxylases and pyruvate orthophosphate dikinase are high in tissue around veins of C_3 plants. We are interested in determining the extent to which the regulation of genes encoding these enzymes is different in C_3 and C_4 species. Using Arabidopsis thaliana we have investigated the role of elements 5' and 3' to each of these genes in controlling expression. In each case, promoters determine expression in specific cells around the veins, and 3' regions can modify the amount of expression. To provide insight into the likely alterations to these genes associated with the evolution of C_4 photosynthesis, we initiated work on Cleome, the most closely related genus to Arabidopsis known to contain C_4 species. We have shown that Cleome contains species spanning a developmental progression from C_3 to C_4 photosynthesis, and that it belongs to the NAD-ME subtype. We have cloned genes important for the C_4 pathway from the C_4 species C. gynandra to allow direct comparison with their orthologues in Arabidopsis.

1.7
Post-transcriptional control of bundle sheath cell-specific Rubisco gene expression

James O. Berry and Minesh Patel (State University of New York at Buffalo)

4 plants, ribulose 1,5 bisphosphate carboxylase (Rubisco) accumulates only in bundle sheath (bs) cells that surround the vascular centers, and not in mesophyll (mp) cells. We have shown previously that control of mRNA translation and stability mediate the C_4 expression patterns of genes encoding the large and small Rubisco subunits (chloroplast rbcL and nuclear RbcS, respectively). The current focus of our research is the identification and characterization of mRNA/protein interactions associated with Rubisco gene expression in C_4 plants. We hypothesize that many regulatory systems occur in both C_3 and C_4 plants, some of which must have diverged between these two groups, through modification of pre-existing factors, or by acquisition of novel processes not present in C_3 species. In support of this hypothesis we have recently identified plastid-localized mRNA binding activities specific to the 5' UTR of rbcL mRNA, with properties that correlate with C_4 Rubisco gene expression. Binding to rbcL mRNA occurs only in light, when rbcL is expressed, and at least one rbcL mRNA binding protein, p44, is present primarily in bs cell chloroplasts, where Rubisco is specifically localized in C_4 leaves. In addition, analysis of Arabidopsis insertion mutants indicates that p44 is essential for the synthesis/accumulation of both Rubisco subunits in this C_3 plant. The occurrence and conservation of orthologs of this protein in C_4 dicots (Flaveria, Amaranth), a C_4 monocot (maize), and a C_3 dicot (Arabidopsis) suggests an important regulatory role in many plants, with possibly modified function in C_4 species.

1.8
Functional analysis of cloned C3 and C4 PPDK regulatory protein

C Chastain (Minnesota State University-Moorhead), Wenxin Xu (Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE), Kate Parsley (Department of Plant Sciences, University of Cambridge, UK), Julian M. Hibberd (Department of Plant Sciences, University of Cambridge, UK)

As a pivotal regulator of the C4-cycle, the recent cloning1 of the maize C4 pyruvate,orthophosphate dikinase regulatory protein (RP) represented a significant breakthrough for the field of C4 photosynthesis. This pivotal role is to adjust the level of PPDK activity, a rate-limiting C4-cycle enzyme to the available light energy incident on the leaf. It does so by catalyzing the rapid, reversible phosphorylation of the PPDK active-site Thr-residue. In addition to its well known role in C4-plants, RP is also present in C3-plants where it confers a similar light/dark regulation of chloroplast localized PPDK, but unlike C4-PPDK, C3-PPDK plays no direct role in photosynthesis, although it is a key antecedent to C4-evolution. To further understand the C3 basis of C4-pathway evolution, we recently isolated two unique C3-RP homologues from Arabidopsis, AtRP1 and AtRP2, for comparison of their functional properties with C4-RP. We show that AtRP1 encodes a typical chloroplast targeted, bifunctional C4-like RP while AtRP2 encodes a cytosolic localized polypeptide that possesses invitro RP-like protein kinase activity but lacks protein phosphatase activity. Although both C3-RP enzymes are functionally Ser-Thr protein kinases, deduced primary structure for these polypeptides are devoid of any known eukaryotic or prokaryotic Ser-Thr protein kinase subdomain structure. Instead, the C3-RPs and the related C4-plant homologues, encode a centrally positioned, ~260 aa sequence currently described as the domain of unknown function 299. We propose that RP represents the first functionally identified member of a novel protein kinase gene family and that this unorthodox structure likely facilitated its recruitment into the C4-pathway.

1 Burnell JN, Chastain CJ (2006) Biochem and Biophys Res Commun 345:675–680

1 - C4 into Rice

1.1
Introduction of C_4-like photosynthetic pathway into rice: possibilities and limitations

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We have produced transgenic rice plants that overproduced four enzymes, namely, the maize C_4 PEPC, the maize C_4 PPDK, the rice C_3 NADP-ME, and the sorghum NADP-MDH. The photosynthetic CO_2 assimilation rate, which was reduced by overproduction PEPC alone, was restored in these quadruple transformants to reach levels comparable to or even higher than that of non-transformants. Although preliminarily, the d^{13}C value once reduced by the PEPC overproduction was recovered as well. These transformants, however, showed slight stunting. Comparison of transformants overproducing different sets of enzymes indicated that the stunting was caused by co-overproduction of PEPC and ME, and that overproduction of MDH acted to restore photosynthesis and mitigate stunting in the quadruple transformants.

In general, PPDK and MDH are active only in the daytime while PEPC and ME are active day and night. It is likely that the co-overproduction of PEPC and ME results in consumption of once fixed carbon at night and under weak light illumination and thereby leads to stunting. There remain possibilities for mitigating the stunting simply by reducing the PEPC protein level. On the other hand, it seems likely that the stunting is unique to rice. In rice leaves, both endogenous and foreign PEPCs are phosphorylated only at night, that is, more active at night than in the daytime: among 30 plant species tested, leaf PEPC was phosphorylated at night only in three species. The stunting might be less pronounced in other C_3 plants than in rice.

1.2
Identification of a plastid Na+ dependent pyruvate transporter in plants

Tsuyoshi Furumoto (University of Hiroshima) Teppei Yamaguchi, Yumiko Ichie, Yosuke Takahashi, Katsura Izui

Pyruvate is an essential metabolite in plastids as an initial substrate of lipid-, isoprenoid-, branched-chain amino acid synthesis and a major metabolite of C4 cycle. The cross-talk between cytosol and plastid has been speculated, however, a molecule of specific transporter is not identified.
By transcriptome analysis between C3 *Flaveria pringlei* and C4 *F. trinervia,* we isolated a novel type plastid transporter gene as a candidate of the pyruvate transporter functioning in C4 cycle. This gene product was localized at chloroplast envelop in mesophyll cells and was commonly abundant in several Na+-dependent pyruvate transporter type-C4 species. The expression of a single corresponding gene in *Arabidopsis thaliana* was strictly restricted at young stages, cotyledons and the first- and the second true leaves. Although the chloroplasts isolated from the T-DNA insertional mutants lost, those of wild type plants showed significant Na+-dependent pyruvate transport activity. By pharmacological analysis, T-DNA insertional mutants were sensitive to Mevastatin, cytosolic isoprenoid synthesis inhibitor, indicating that at least the plastid Na+-pyruvate transporter contribute the plastid isoprenoid synthetic pathway. These results indicate that these genes encode plastid Na+-dependent pyruvate transporters, functioning in C4 carbon cycle and in isoprenoid synthesis in plants.

1 - Genetic, Molecular and Biochemical Correlates for C4 and CAM (3)

1.1
Evolutionary origins of CAM photosynthesis

J Andrew C Smith (University of Oxford)

Crassulacean acid metabolism is widely recognized as a photosynthetic adaptation to plant growth in water-limited environments in tropical and subtropical regions of the world. Many CAM-plant taxa are found in families with leaf- or stem-succulent terrestrial life-forms that characterize semi-arid habitats with seasonal rainfall. But an increasing number of CAM species have now been identified amongst tropical vascular epiphytes, which can be exposed to microclimatically arid environments and intermittent was availability. In total, there are estimated to be more than 30 families of vascular plants containing CAM species, suggesting that this mode of photosynthetic carbon fixation has evolved independently on many occasions. Indeed, more detailed surveys of carbon-isotope ratios combined with phylogenetic analysis suggests that there have been multiple origins of CAM photosynthesis even within individual families, such as Bromeliaceae, Orchidaceae, Crassulaceae and Portulacaceae. Reversion from CAM to C_3 photosynthesis may have occurred in certain lineages, but has yet to be formally demonstrated by phylogenetic analysis. The lack of a fossil record for the typical families of CAM plant means that the evolutionary origins of this mode of photosynthesis cannot be dated with certainty. However, with the increasing number of molecular–systematic studies, application of the relaxed molecular clock and other techniques is providing constraints on the possible timing of these major evolutionary transitions. These, in turn, can be related to detailed models of palaeoclimate reconstruction to provide further broad tests of the adaptive significance of the CAM mode of photosynthesis.

1.2
The extent of C_4 and CAM photosynthesis in three *Grahmia* species of the Portulacaceae.

L Guralnick (Western Oregon University), A Cline (Western Oregon University)

CAM has evolved independently in over 30 different plant families. The Portulacaceae is plant family whose members show photosynthetic diversity in that some are C_3 plants; others are C_4 plants which show some CAM characteristics and some members are facultative CAM plants (switching between C_3 and CAM photosynthesis). Previous research has looked at the evolution and distribution of CAM in the Portulacaceae. The only genus known to have C_4 photosynthetic members is the genus Portulaca. The phylogeny indicated that Portulaca evolved the C_4 pathway after evolving the CAM pathway. Grahmia coahuilensis, G. frutescens, G. bracteleata are members of the Portulacaceae. Previous reports have indicated that these species and the Anancampseros genera may also contain C_4 photosynthetic members. Based on the phylogeny, this would indicate multiple origins of C_4 photosynthesis within the Portulacaceae. However, the placement of Grahmia as a C_4 plant is not well supported in the literature. This study will study the true photosynthetic characteristics of C_4 and CAM photosynthesis of the Grahmia spp. Preliminary data indicate that Grahmia may be a facultative CAM species. The carbon isotope composition of the Grahmia spp. was -24.5 o/oo which places it toward the C_3 range. The titratable acidity levels are high and PEP carboxylase enzyme activity is low under well watered conditions which may mean that CAM may be induced by water stress. Leaf samples have been obtained for anatomical studies. Further studies will be needed to determine the actual photosynthetic characters of this genus.

1.3
Characterization of photosynthesis in C_3 and C_4 species of the genus *Halosarcia* (Salicornioideae)

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Family Chenopodiaceae has the largest number of C_4 species and greatest diversity of C_4 photosynthesis and anatomical types among dicotyledons. In subfamily Salicornioideae, C_4 photosynthesis has only been found in the genus *Halosarcia*. Comparative anatomical and cytochemical studies were made on C_3 species *H. pergranulata*, and two C_4 subspecies of *H. indica* which have near-aphyllous photosynthetic stems. Molecular studies show their phylogenetic placement in the subfamily. In the C_3 species *H. pergranulata*, the stems have two Rubisco-containing layers of chlorenchyma cells surrounding the centrally located water storage tissue. In *H. indica*, the stems have Kranz anatomy with an outer layer of mesophyll cells (MC) and inner layer of bundle sheath cells (BSC); but in the MC, there is an unusual occurrence of large colorless cells (CC) between groups of MC. Phosphoenolpyruvate carboxylase and pyruvate, Pi dikinase are localized preferentially in the palisade MC, while Rubisco is predominantly localized in BSC. *Halosarcia indica* is an NAD-malic enzyme type C_4 species, with MC chloroplasts having reduced grana and BSC chloroplasts having well-developed grana; BSC are atypical in having chloroplasts in a centrifugal position and rather small mitochondria. The unique CC cells have some plastids which have significant labeling for Rubisco and high starch content. The MC have very thin cell walls, compared to epidermal, BS, CC and water storage cells. Cell walls in fresh sections have a bright blue fluorescence corresponding to wall thickness, which changes to green with NH_4OH treatment, indicating the presence of ferulic acid, a cell wall cross-linker.

1.4
Phylogenetic analysis of natural selection in Rubiscos of C_3, C_4 and CAM plants

M Kapralov (University of Birmingham), D Filatov (University of Birmingham)

The large subunit of the key photosynthetic enzyme, Rubisco, is encoded by the chloroplast gene rbcL. Being one of the most conservative genes, *rbcL* is often chosen by botanists for phylogenetic reconstructions and it has been sequenced in thousands of plant species. Surprisingly, despite abundance of sequence data, Rubisco's great physiological importance and well studied protein structure the question whether this ancient enzyme still evolves to improve its function has received little attention. We conducted the phylogeny-based and protein structure-based analyses of natural selection in rbcL sequences from over 3000 species representing C_3, C_4 and CAM plants. Our analysis revealed that while most of the rbcL sites are evolving under purifying selection there are several hot spots where positive selection is common in most analyzed plant lineages. The mapping of the positively selected residues on the Rubisco tertiary structure revealed that they are located in regions important for dimer-dimer, intradimer, large subunit-small subunit and Rubisco-Rubisco activase interactions. The signal of positive selection is weaker in aquatic C_3 and terrestrial C_4 and CAM plants compared to terrestrial C_3 ones possibly reflecting different subcellular conditions of Rubisco performance in these groups. Our results demonstrate that after billions of years of evolution Darwinian selection still fine-tunes performance of Rubisco, although strength of positive selection varies in different functional groups.

1.5
Single-cell C_4 photosynthesis in marine diatoms?

R Leegood (University of Sheffield), K Roberts (University of Dundee), E Granum (University of Sheffield), J Raven (University of Dundee)

Marine planktonic diatoms are responsible for 15% of primary productivity on Earth, fixing more than 10 billion tonnes of inorganic carbon (C_i) each year. Diatoms achieve this by using CO_2-concentrating mechanisms (CCMs) which increase the CO_2 concentration around Rubisco, diminishing photorespiration. It was generally held that diatoms have biophysical CCMs, based on direct acquisition of C_i, until evidence emerged of single-cell C_4 photosynthesis, a biochemical CCM, in the marine diatom Thalassiosira weissflogii. Recent whole genome sequencing of marine phytoplankton, including the diatoms T.

pseudonana and Phaeodactylum tricornutum, suggests that these organisms possess the enzymic apparatus (including phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase) to operate C_4 photosynthesis. We studied short-term photosynthetic carbon fixation by ^{14}C labelling in two diatoms, and found that T. weissflogii produced both C3 and C4 (mainly malate) compounds, while T. pseudonana produced only C3 compounds. Labelling patterns in neither diatom were altered by growth at different C_i concentrations. This lack of environmental modulation was supported by measurements (in T. pseudonana only) of metabolic gene transcripts and proteins. Growth C_i concentration had little effect on the expression of any of the genes studied except glycine decarboxylase P-protein, which was highly upregulated by low C_i, indicating induction of photorespiratory carbon oxidation. Moderately nitrogen-starved cells had reduced carbon metabolic transcript levels, but C_3 and C_4 carboxylase/decarboxylase gene transcripts increased rapidly when nitrate was added. The evidence from this study suggests C_3-C_4 intermediate photosynthesis in T. weissflogii and exclusively C_3 photosynthesis in T. pseudonana, in which C_4 carboxylases play key anaplerotic roles.

1.6
A Cyanobacterial CO_2 Concentrating Mechanism Enhances Rice Photosynthesis and Productivity

M Ku (National Chiayi University), S Yang (National Chiayi University), C Chang (National Chiayi University), M Yanagisawa (National Chiayi University)

Under low CO_2 or alkaline water conditions, cyanobacteria use bicarbonate transporters to pump in bicarbonate as a major carbon source. This adaptive CO_2 concentrating mechanism allows cyanobacteria to survive unfavorable growth conditions. In this study, we have constitutively expressed the high affinity bicarbonate transporter gene, *ictB,* from cyanobacterium in rice. All four transgenic rice lines expressing the transporter exhibited enhanced photosynthetic capacity, growth and grain yield. Relative to untransformed wildtype plants, the transgenic plants had 10-30% higher photosynthesis rates, 15-20% higher carboxylation efficiencies, and lower photosynthetic CO_2 compensation points. Activities of ribulose 1,5-bisphosphate carboxylase and PEP carboxylase were also 25-30% and 10-20% higher in the transgenic lines, respectively. Consistently, the transgenic plants produced 10-120% more tillers or panicles per plant and 10-70% more grains, relative to the wildtype. The enhancements in growth and grain yield are closely related with the increased photosynthetic capacity among the transgenic lines. This study demonstrates that the simple CO_2 concentrating mechanism from cyanobacterium can largely improve the photosynthetic efficiency, growth and productivity of C_3 crops.

1.7
Stable isotopic determinants of water exchange by bromeliads along climatic gradients

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Epiphytic bromeliads are subject to intermittent precipitation inputs, such as rainfall and fog, which can be exploited over varying timescales, depending on seasonality, growth form (use of tank and/or trichome), and exposure within the forest canopy. By comparing leaf-water and organic material 18O signals, as well as d13C composition, along an altitudinal and latitudinal gradient between Panama and Mexico, we relate the habitat preference of C3 and CAM bromeliads to exposure, and seasonality of precipitation inputs. We examine the 18O isotopic relationship between precipitation inputs, leaf water and variation along the monocot leaves, and the analysis of water vapour to distinguish isotopic enrichment due to water vapour exchange and transpiration. We will show how epiphytes act as sensitive markers of changing environmental conditions and could be used throughout the neotropics to characterise environmental gradients and changing climatic conditions.

1.8
Diel leaf growth cycles in *Clusia spp.* are related to changes between C_3 and Crassulacean acid metabolism during development and during water stress

A Walter (Research Center Juelich), M Christ (Research Center Juelich), U Rascher (Research Center Juelich), B Osmond (Australian National University, Canberra), D Randewig (Research Center Juelich), S Jahnke (Research Center Juelich)

Leaves grow with diel fluctuations that are plant-specific. Here we report the first combined time series of leaf growth and gas exchange in developing leaves of two Clusia species under C_3 and crassulacean acid metabolism (CAM) mode of photosynthesis. Strongest growth rates were observed during the day in the obligate CAM species C. alata and facultative C. minor during drought induced CAM photosynthesis. In contrast, when C. minor was well watered and performing C_3 photosynthesis, leaf growth was maximal at night. Expanding leaves of C. alata were shown to switch from C_3 to CAM-mode during development. The results demonstrate the close interaction of diel growth patterns with the timing of carbohydrate metabolism. We hypothesise that, when C_3 photosynthesis switches to CAM, the observed shift in leaf growth cycle is mainly caused by the primary demand of CAM for substrates for nocturnal CO_2 fixation and acid synthesis thus reducing the availability of carbohydrates for leaf growth.

1.9
Windows of opportunity … or portholes of pain? The light environment *inside* leaves of CAM succulents with adaxial epidermal windows

C E Martin (University of Kansas), K J Egbert (University of Kansas), T C Vogelmann (University of Vermont)

The leaves of a number of CAM succulents have adaxial epidermal “windows,” areas of the leaf which are transparent or translucent. Some, but not all, of these plants grow underground with only the leaf windows exposed, and it has been surmised that the windows allow light to penetrate through the clear hydrenchyma of the leaf interior to the underlying chlorenchyma tissue below. In aboveground species, such windows presumably enhance photosynthetic rates by illuminating the internal chlorenchyma from two sides, outside and inside the leaf. Although logical and attractive, these hypotheses have never been tested. Early results from this laboratory indicate, unexpectedly, that covering these leaf windows with reflective tape results in a stimulation of photosynthetic activity in these plants. No data were available, however, about the effectiveness of the tape in reducing light levels inside the leaves of these succulents. In this study, light quantity and quality were measured, for the first time in any plant, with an omni-directional light microprobe in six CAM succulents. Also, for three of the species, the effects of applying reflective tape to the epidermal windows were quantified by direct measurements of the light environment of the leaves before and after the application of the tape. The light quantity and quality inside leaves of CAM succulents are discussed as a reflection of the growth habit of the plant (underground vs aboveground), the size of the window (relative to the size of the leaf), and the presence or absence of reflective tape on the leaf window.

1 - C4 CAM Futures

1.1
C_3-C_4 Intermediate Species: Lessons in the Evolution of Biochemical and Anatomical Novelty

R Monson (University of Colorado)

Various approaches have been used in the past to infer the driving forces and selective advantages of C4 photosynthesis. Many of these approaches are based on analysis of the phenotypes of fully-expressed C3 and C4 species, which provide clear evidence of differences in resource-use efficiency, growth, and performance in various climatic and environmental regimes. It is tempting to interpret these differences within the context of the evolutionary process and progressive phases of adaptation as species have evolved from the C3 to C4 phenotype. Over the past three decades, the discovery and analysis of numerous species at intermediate stages of C3-to-C4 evolution have provided an alternative to understanding how evolution works to produce the novel anatomical and biochemical forms of C4 photosynthesis. The picture that has emerged from such analyses is one of changing adaptive advantage, periodic maladaptive modification, and evolutionary reversal. I will use examples from research over the past three decades, including a focus on more recent breakthroughs in phylogenetic analysis, to describe the lessons we can derive concerning the C4 evolutionary process.

1.2
Ecological Distribution and Diversity of C4 Plants in Iran

H Akhani (University of Tehran)

The C_4 plants are known that successfully grow under high temperatures and xeric climatic conditions and tolerate hypersaline soils. Iran with 1.6 m km^2 and 15° latitudinal range and extremely diverse environmental conditions is one of the rich countries for evolving many C_4 plants and C4 dominated ecosystems. The C4 plants of Iran occur from see level to ca. 4000 m. So far 303 C4 species are known from Iran belonging to 91 genera and 17 families of flowering plants, out of 19 known families containing C_4 species in the world. The Chenopodiaceae with 123 species and 25 genera ranks first which follow by Poaceae with 115 species and 43 genera, Cyperaceae with 22 species and 4 genera, Polygonaceae with one genus and 13 species and Amaranthaceae with 2 genera and 11 species. The Chenopodiaceae and Polygonaceae are rich in Irano-Turanian elements, but most of gramineous C_4 species show tropical and subtropical origin with wide distribution range. Five major C_4 dominated ecosystems in Iran include the C_4 grass dominated communities in steep rocky outcrops of the northern Iran, the C_4 dominated communities in the disturbed habitats and weedy communities, C_4 aquatics, C_4 communities on the desert dunes with domination of grasses, chenopods and *Calligonum* shrubs and finally the most extensive ones are the C_4 chenopod dominated ecosystems in diverse halophytic and xerophytic communities. The C_4 chenopodiaceae in Iran show great morpho-anatomical variation among them is the recent discovery of novel single-cell system of photosynthesis in *Bienertia cycloptera* and *B. sinuspersici.*

1.3
A C_4-dominated savanna responds to elevated concentrations of atmospheric CO_2: the OzFACE experiment

J Holtum (James Cook University), C Stokes (CSIRO Sustainable Ecosystems), A Ash (CSIRO Sustainable Ecosystems), I Woodrow (University of Melbourne)

C_4 plants account for almost a quarter of global terrestrial primary production, largely in tropical grasslands and savannas. In a five-year CO_2 enrichment experiment on a natural savanna, the first such study in the tropics, a C_4 grass community responded strongly to elevated CO_2 concentration. Above-ground net primary production increased 36% and community composition shifted towards an important pan-tropical species, *Themeda triandra*.
A 170 ppm increase in atmospheric CO_2 concentration correlated with an approximately 6% increase of soil water content, a response ameliorated by defoliation and nutrient addition. The soil water content was still increasing at the end of the experiment. Moisture accumulation was not uniform throughout the soil profile, the strongest response occurred at 35 cm, with about half the increase occurring below this depth. Elevated CO_2 reduced short-term rates of moisture depletion following rainfall such that CO_2-induced temporal lags in soil drying increased with depth, prolonging moisture availability into dry periods. Such interactions between CO_2 and moisture are significant since savanna ecosystem structure and function is strongly influenced by highly seasonal and interannually variable rainfall. The changing patterns of water availability under elevated CO_2 are likely to favour deeper-rooted species, thereby influencing plant community composition and productivity.
If our observations apply to other savanna systems it would indicate that exposure of tropical C_4 grass communities to elevated CO_2 may affect vegetation composition, nitrogen cycling, carbon sequestration, and that there are likely to be flow-on effects to other trophic levels and in responses to fire.

1 - C4 and CAM Poster Session

1.1
Molecular evolution of four-carbon decarboxylase genes recruited into C_4 photosynthesis

N Brown (University of Cambridge), J Hibberd (University of Cambridge)

Evolution of efficient C_4 photosynthesis required compartmentalisation of biochemistry into specific cell-types and alterations to leaf morphology, most likely through changes in gene expression. Despite this apparent complexity, it has evolved independently 45 times among the angiosperms. Although Arabidopsis (At) uses C_3 photosynthesis, its vascular cells show characteristics of the C_4 pathway; for example, activities of the decarboxylase enzymes NAD-ME, NADP-ME and PEPCK are highly enriched in these cells. We are interested in how the regulation of genes encoding these decarboxylases has altered in association with the evolution of C_4 photosynthesis and have adopted a comparative approach using C_3 and C_4 plants. Promoter regions for two AtNADP-ME genes and both AtNAD-ME genes direct expression of uidA in xylem parenchyma cells and the NADP-ME 3' regions greatly increase expression. We have identified Cleome as being a genus containing C_4 species that is within the Brassicaceae and therefore closely related to Arabidopsis (TIPS, 10, 215-221). Two NAD-ME genes have been isolated from Cleome gynandra (Cg), an NAD-ME sub-type C_4 species, and in situ hybridization indicates that the transcripts for both genes are localised in the bundle-sheath. We are using transgenic Arabidopsis containing CgNAD-ME2 –uidA constructs to determine whether Arabidopsis contains the trans factors required for bundle-sheath expression. We have demonstrated that 850 bp of promoter region is not sufficient for bundle-sheath expression in transgenic Arabidopsis, and the 5' and 3' UTRs do not mediate bundle-sheath expression by a post-transcriptional mechanism.

1.2
Effects of drought on water relations and nonstructural carbohydrates in the starch utilizing CAM-bromeliad *Aechmea* 'Maya'

J Ceusters (KULeuven), E Londers (KULeuven), V Verdoodt (KULeuven), M De Proft (KULeuven)

One of the main advantages of CAM plants is their improved water use efficiency due to the unique separation between nocturnal CO_2 uptake and daytime re-assimilation behind closed stomata. At night CO_2 is fixed on phospho-enolpyruvate (PEP) resulting in malic acid which releases its CO_2 again the next day. The supplier to deliver the PEP building blocks can be soluble sugars or starch.
In this research a starch-utilizing CAM-bromeliad, Aechmea 'Maya', is used to investigate long term effects of severe water stress. Water was withheld from plants for a period of 195 days compared to a well watered control group. At regular time points Relative Water Content (RWC) and water potentials of the plants and the soil were recorded in following up the drying process. Remarkably the stressed plants seem to maintain their RWC comparable to that of the controls for 60 days. However inside the leaves dramatic changes take place already after 15 days. A sudden drop in the content of starch is followed by a more steady decrease in malic acid content. Soluble sugars share the decreasing trend during water stress but to a lesser extent. In order to be able to interpret these results on the plant level, growth measurements were executed during the sampling period.
By investigating all these parameters the authors gain more insight into the extreme drought resistance of these CAM-bromeliads. Moreover results are interpreted in the scope of using starch as PEP-precursor in the CAM-cycle.

1.3
Postharvest metabolism of leaves on the crown of pineapple fruit

E Londers (KULeuven), J Ceusters (KULeuven), V Verdoodt (KULeuven), M De Proft (KULeuven)

Pineapple is the most important crop species exhibiting CAM (Crassulacean Acid Metabolism). The stomata in mature leaves are open at night and closed during the day, associated with organic acid build-up during the night. The pineapple crown is a continuation of the vegetative stem leaves, and the spirally arranged leaflets have a similar morphology. However, the stomata of young leaves such as on the crown, may not shown this same diurnal function, as water-loss rate appears to be constant during storage.
In practice, not only the pineapple fruit takes part of the grading standard, but also the crown quality is an aesthetic character of economic concern. However, little is known on the postharvest metabolism of he leaves on the crown of the pineapple fruit, and even less is known on the impact of the environmental storage conditions on this metabolism.
In this poster, the results of a metabolic activity analysis (based on porometer measurements and leaf organic acid analysis) are presented. To assess the impact of the environmental storage conditions on the crown leaf metabolism, metabolic activity analysis was executed under different storage conditions. The impact of the environmental temperature was studied comparing measurements at both 10°C (general storage condition) and room temperature. In addition, measurements were executed under dark and daylight conditions.
Finally, in this poster, results are placed in the broader context of pineapple crown leaf quality.

1.4
pH-dependent regulation revealed by X-ray structure of phosphoenolpyruvate carboxylase from maize

H Matsumura (Osaka University, CREST-JST, Osaka, Japan)

Phosphoenolpyruvate carboxylase (PEPC) catalyzes the irreversible carboxylation of phosphoenolpyruvate and plays an important role in fixing atmospheric CO_2 in C_4 and CAM plants. PEPC is an allosteric enzyme, which is activated by glucose-6-phosphate and is inhibited by L-malate. The sensitivity to L-malate inhibition of maize PEPC seems to be pH-dependent, which might be involved in regulation *in vivo*. Our biochemical study indicates that the activity is sensitive to inhibition at pH 7 but is insensitive at pH 8. The crystal structure of PEPC purified from maize leaves has been reported; however, the N-terminal amino residues of crystalline enzyme were cleaved during purification or crystallization, and the enzymatic properties of the truncated enzyme are very different from a full-length PEPC.
Here, we describe the crystal structures of recombinant maize PEPC. N-terminal sequencing identified the crystalline enzyme as a full-length PEPC. The crystals were prepared by soaking in solution containing L-malate at pH 7 and pH 8, respectively. The obtained two structures show L-malate bound to the enzyme at pH 7, but not at pH 8. The structural comparison shows a conformational change of a loop comprising 124-140, which is well-defined in electron density at pH 7, but is disordered at pH 8. The carboxyl groups of Asp and Glu on this loop interacts with the side chain of His on the adjacent helix at pH 7, which induces a conformational change and allows L-malate to bind to the inhibitory pocket. We also compare the structure function with E. coli enzyme.

1.5
Cold Tolerant Crassulacean Acid Metabolism in Mongolia's Extreme Continental Climate

S Oyungerel (National University of Mongolia), P Dondog (NUM), D Sodov (NUM), C C Black (Georgia University)

Mongolia is in the center of the Asian continent, which leads to extreme continental climate temperatures plus a very low precipitation. The temperatures vary from -50°C in long winters to 40°C in summers. We found that Crassulacean Acid Metabolism (CAM) occurred in succulent plant genus Orostachys in Mongolia, where it is used as food for pastoral livestock and herbal in medicine. How do CAM plants survive the extreme winter colds as in Mongolia? In the winter visually, apparently healthy, bright green specimens of O. spinosa are readily observed living both in exposed areas and under snow covers. Therefore, O. spinosa seems to be amongst the most cold adapted CAM plants known; however its special cold environment adaptation mechanisms remain to be elucidated.

We determined the content of aldo-sugar, starch, protein and activity of µ-amylase every month (in 2005-2006) in leaves of O. spinosa grown in laboratory and natural conditions. The curves of seasonal fluctuation of starch and sugar were gradually opposite. When the minus and plus temperature were increased and there was a water deficit in plant grown condition, then the content of of sugar is increased and it is proved that the sugar is a main protecting material from freezing, hotting and drying.
Content of protein and activity of amylase decrease and establish gradually in the warm season. O. spinosa is decreased the water and increased protein and solid substance in winter time for survive into cold condition. We think that, this increasing protein is a second protecting material from freezing like as a sugar.
The general regularity of change for a-amylase enzyme and starch were similar during the year. There is a related pattern that, which the content of starch increases, the activity of a-amylase enzyme increases. Also, the activity of a-amylase enzyme is decreased gradually for falling the little starch. For remaining green and living O. spinosa in winter time. The activity of a-amylase enzyme is increased gradually in this time. It is proved that O. spinosa live healthy both exposed areas and under the snow covers, and it is the most cold adapted succulent and CAM plant. The activity of a-amylase enzyme was a concrete level with a little fluctuation on O. spinosa during the winter time in Mongolian extreme climate condition.
Water content of O. spinosa, growing in the wild, was relatively constant from July to the middle of August (93±0.95%) and in other times (autumn and winter) it was low (82,34±3,17). But the water content of O. spinosa, cultivated in the laboratory, was relatively constant (94.2±0.94%). O. spinosa is a succulent plant, but it's the water content decreased in winter time for not freezing, however it has a concrete water content (79.6-83%) and it can endure to cold condition and goes on metabolism. Then it is proved that O. spinosa is the most cold adapted CAM and succulent plant.

1.6
Evolution of Crassulacean Acid Metabolism in Neotropical Orchids

K Silvera (University of Nevada Reno), L Santiago (University of California, Riverside), M Whitten (Florida Museum of Natural History, Gainesville, Fl), N Whitten (Florida Museum of Natural History, Gainesville, Fl), K Neubig (Florida Museum of Natural History, Gainesville, Fl), K Winter (Smithsonian Tropical Research Institute, Republic of Panama), J Cushman (University of Nevada, Reno)

Crassulacean Acid Metabolism (CAM) is a water-conserving mode of photosynthesis present in 7% of vascular plant species. The molecular mechanisms responsible for the evolution of this important photosynthetic adaptation to water-limitation are completely uncharacterized. We used stable carbon isotopic composition from leaf samples to determine if photosynthetic carbon assimilation occurs predominantly by C_3 photosynthesis or CAM pathway. Carbon isotopic composition of leaf material from 1,000 orchid species showed a bimodal distribution with most species exhibiting peak values near -27, suggesting a C_3 photosynthetic pathway, or around -15, suggesting a CAM pathway. Titratable acidity measurements of species within the C_3 photosynthesis peak revealed species with a weak CAM capacity. When overlain onto a molecular phylogeny of orchids, the distribution of photosynthetic pathways showed that C_3 photosynthesis is the ancestral state and that CAM has evolved more than once within Orchidaceae. High quality leaf RNA samples has been obtained from closely related orchid species from the Subtribe Oncidiinae with a range of photosynthetic pathways from C_3 photosynthesis to weak to strong CAM, and the mRNA abundance of molecular markers diagnostic for CAM has been analyzed. An analysis of PEPC gene family structure and mRNA relative abundance showed that at least five isoforms are present in orchids, with one putative CAM-specific PEPC isogene identified in CAM and weak CAM species based on cDNA clone sampling. This interdisciplinary project integrates ecophysiological, biochemical and molecular genetic approaches to understand the evolutionary origins of CAM within the context of a highly resolved orchid phylogeny.

1.7
Structural, biochemical and physiological characterization of C_4 photosynthesis in species having two vastly different types of Kranz anatomy in genus

N Koteyeva (Komarov Botanical Institute RAS), E Voznesenskaya (Komarov Botanical Institute RAS), S Chuong (Washington State University), V Franceschi (Washington State University), H Freitag (Institute of Biology, Kassel University), G Edwards (Washington State University)

Family Chenopodiaceae has the largest number of C_4 species and greatest diversity of C_4 photosynthesis among dicotyledons. In genus Suaeda, two types of Kranz anatomy are represented by S. taxifolia, Salsina type with chlorenchyma cells, mesophyll (M) and bundle sheath (BS) distributed around the leaf periphery, and S. eltonica, Schoberia type with M and BS surrounding vascular bundles in the central plane. The chloroplasts in BS cells are located centripetally in *Salsina* and centrifugally in *Schoberia* type. Western blots on decarboxylases showed that both forms are NAD-malic enzyme (ME) type C_4 species. Immunolocalization studies with both species showed Rubisco was preferentially expressed in precursor BS cells very early. This was followed by positioning of organelles in BS centrifugally in S. eltonica and centripetally in S. taxifolia, which occurred before structural differentiation of chloroplasts and full expression of C_4 enzymes. Expression of C_4 enzymes became apparent in mature leaves, including distinct localization of NAD-ME, along with Rubisco, in BS cells and phosphoenolpyruvate carboxylase and pyruvate,Pi dikinase in M cells. Starch distribution correlated with Rubisco expression. During leaf development, $\delta^{13}C$ values changed from C_4-like to C_4. In mature leaves M cells have chloroplasts with reduced grana while BS cells have chloroplasts with well-developed grana and large, specialized mitochondria, characteristic of NAD-ME type C4 chenopods. Their photosynthetic response to varying CO_2 and light was similar, and typical of C_4 plants. The results indicate that two structural forms of Kranz anatomy evolved in parallel in species of subfamily Suaedoideae having NAD-ME type C_4 photosynthesis.

1.8
Structural and biochemical characterization of the photosynthetic apparatus in chlorophyllous organs of representative species of *Cleome* (Cleomaceae)

A Ivanova (Komarov Botanical Institute RAS), N Koteyeva (Komarov Botanical Institute RAS), S Chuong (Washington State University), E Voznesenskaya (Komarov Botanical Institute RAS), G Edwards (Washington State University)

C_4 photosynthesis evolved many times in 18 different families of land plants with great variation in leaf anatomy, ranging from various forms of Kranz anatomy to occurrence of C_4 within a single type of chlorenchyma cell. Recent surveys showed that genus Cleome contains species with leaves having C_4, C_3 and C_3-C_4 intermediate photosynthesis. Since many Cleome species have petioles, young stems and pods which are green, a comparative study of anatomy and cytochemistry was made on these organs in representative photosynthetic types. C_4 species Cleome gynandra has green pods with Kranz anatomy, but non-Kranz petioles and stems. Immunolocalization in pod walls shows characteristic C_4 plant cell-specific labeling of Rubisco and phosphoenolpyruvate carboxylase, and glycine decarboxylase labeling in bundle sheath mitochondria. Kranz anatomy is well-developed towards the outer, but not the inner, pod wall, suggesting fixation of atmospheric CO_2 by C_4 photosynthesis and refixation of respired CO_2 from the embryo by C_3 photosynthesis. Cleome paradoxa leaves have C_3-C_4 intermediate type photosynthesis. Its petioles and young stems are green, with chlorenchyma tissue having intermediate features similar to those of leaves. The anatomical and ultrastructural features of these organs, and the selective localization of glycine decarboxylase in mitochondria of bundle sheath cells, are indicative of C_3-C_4 photosynthesis. In C_3 Cleome species studied, all the chlorophyllous organs (leaves, stems, petioles, and pod wall) have chlorenchyma tissue characteristic of C_3 type photosynthesis. The results indicate that during evolution of C_4 photosynthesis, new forms of carbon assimilation can be expressed in various photosynthetic organs.

1.9
The role of the *CCA1/LHY-TOC1* oscillator in the circadian control of CAM

C Dall'omo (University of Liverpool), S Boxall (University of Liverpool), J Foster (University of York), A Hall (University of Liverpool), J Hartwell (University of Liverpool)

CAM is co-ordinated and optimised by a circadian oscillator, with primary CO_2 fixation phased to the dark period and malate decarboxylation and secondary

CO_2 fixation phased to the light period. This prevents a futile cycle of simultaneous malate synthesis and decarboxylation, which would waste ATP and NAD(P)H. Although we are approaching the 50th anniversary of the first report of a circadian rhythm of nocturnal CO_2 fixation in the CAM plant *Kalanchoe fedtschenkoi*, the identity of the underlying circadian oscillator has remained an enigma. We show that a molecular oscillator in the nucleus, consisting of the single MYB-repeat transcription factor *CCA1/LHY,* and the pseudo-response regulator *TOC1,* is most likely responsible for the temporal co-ordination of CAM. An high temperature-induced phase advance of the *CCA1/LHY-TOC1* oscillator was accompanied by a concomitant phase advance in the CAM circadian rhythm. This provides correlative evidence that supports the hypothesis that the *CCA1/LHY-TOC1* oscillator controls CAM. Furthermore, transgenic *K. fedtschenkoi* lines that constitutively express either the *TOC1* or *CCA1/LHY* genes show changes in the period of the CAM CO_2 fixation rhythm under constant light and temperature (LL) free-running conditions. For example, high-level constitutive expression of *TOC1* leads to a very short period rhythm of CO_2 fixation that approaches arrhythmia, whilst an intermediate level of *TOC1* over-expression results in period lengthening to 24 h compared to the 21 h wild type period. This demonstrates that this autoregulatory positive-negative feedback loop of interacting genes in the nucleus is responsible for the temporal optimisation of CAM.

1.10
Molecular dissection of the circadian clock output pathway that mediates the temporal coordination of CAM

R Meszter (University of Liverpool), S Boxall (University of Liverpool), M Jones (University of Liverpool), J Hartwell (University of Liverpool)

Strict temporal control of CAM is essential in order to avoid a futile cycle of simultaneous malate synthesis and decarboxylation. This is achieved through circadian control, such that rhythms of nocturnal CO_2 fixation persist in constant conditions. Whilst we now know that the underlying CAM circadian oscillator consists of genes such as *CCA1/LHY* and *TOC1* working as part of an autoregulatory loop that sets the time in the nucleus, we have yet to identify any of the components of the output signal transduction cascade that links the central oscillator to circadian-regulated, CAM-associated genes. We have screened over 60 previously uncharacterised transcription factors (TFs) to identify novel TFs that could potentially function in the output pathway that controls CAM. Our screen focussed on TF genes that are induced and subject to circadian control in CAM leaves of *Mesembryanthemum crystallinum*. Members of the MYB, bHLH, bZIP, AP2/EREBP, C2C2 CONSTANS-like, DOF-like and NAM/NAC-like families of TFs were found to have circadian-regulated transcript abundance profiles. One particular TF, named *CAM-Induced bZIP* (*CIB*), has been chosen for more detailed analysis. This TF is strongly induced in salt-stressed, CAM-induced leaves of *M. crystallinum*, and its transcript abundance oscillates in constant light and temperature conditions with peak transcripts levels in the subjective morning. Interestingly, the orthologue of this bZIP was identified amongst 5000 *Kalanchoe fedtschenkoi* expressed sequence tags that were randomly sequenced from a cDNA library representing transcripts expressed in the dark period. Preliminary analysis of the regulation of this bZIP in *K. fedtschenkoi* will be presented.

1.11
Crassulacean acid metabolism (CAM) in Australian ant-plants (Rubiaceae)

J Holtum (James Cook University), J Hepworth (University of Oxford), A Smith (University of Oxford), A Field (James Cook University), H Field (James Cook University)

Five species of rubiaceous ant-house plants are currently known from tropical north-eastern Australia: *Myrmecodia platytyrea* ssp. *antoinii* (Becc.) C.R.Huxley & Jebb, *M. tuberosa* Jack, *M. beccarii* Hook.f., *Hydnophytum moseleyanum* Becc. and *H. ferrugineum* P.I.Forst. Like other ant-house plants in the Rubiaceae, the Australian taxa are characterised by the formation of chambered ‘tubers' at the base of the stem (hypocotyls) that are often inhabited by ants. All of these species are also epiphytes of tropical forests, but the forests range from humid tropical forests (e.g. simple notophyll vine forests) to more seasonally dry complex mesophyll vine forests and open eucalypt/*Melaleuca* woodlands. The ant-house plants exhibit varying degrees of adaptation to water stress, including differences in leaf succulence, leaf size and leaf angle. In the only report of CAM photosynthesis in the Rubiaceae to date, Winter *et al.* (1983) recorded carbon-isotope ratios consistent with CAM for *M. beccarii* and two other ant-house species of uncertain lineage collected in the McIlwraith Range (far-north Queensland) as part of a large survey of the vascular epiphytes of eastern Australia. We have collected Australian ant-house plants from their natural habitats, have authenticated their taxonomic identities and, using gas-exchange techniques, have investigated further the extent to which these species exhibit CAM photosynthesis under well-watered and water-limited conditions. The contribution of net CO_2 uptake in the dark to daily 24-hour carbon gain was quantified and will be discussed in relation to the ecology of these unusual myrmecophytes

1.12
The effects of overproduction of C_4 enzymes on photosynthesis and growth of rice plants.

T Fukuda (University of Tsukuba), C Masumoto (Natl. Inst. Agrobiol. Sci.), Y Taniguchi (Natl. Inst. Agrobiol. Sci.), H Ohkawa (Hirosaki Univ.), H Fukayama (Kobe Univ.), M Miyao (Natl. Inst. Agrobiol. Sci.)

To introduce the C_4 pathway into C_3 plants, at least three C_4 enzymes (PEPC, PPDK, and NADP-ME) have to be overproduced. The maize C_4 PEPC and the maize C_4 PPDK were overproduced in the mesophyll cell of rice leaves by introducing the respective intact genes, and the maize C_4 NADP-ME and the sorghum NADP-MDH by introducing the rice *Cab* promoter::cDNA constructs. Overproduction of the maize PEPC did not affect growth of rice plants but slightly suppressed photosynthesis. Overproduction of any of the maize PPDK and the sorghum MDH did not at all affect photosynthesis and growth. By contrast, overproduction of the maize ME led to serious stunting and leaf photobleaching, which resulted from enhanced photoinhibition of photosynthesis due to an increase in the level of NADPH inside the chloroplast. These phenotypes were not relieved by co-overproduction of any of MDH alone, PEPC+PPDK, and PEPC+PPDK+MDH, although they were mitigated slightly by co-overproduction of these enzymes. Therefore, the rice C_3 NADP-ME that did not show any detrimental effects was used instead of the maize enzyme.

Double transformants overproducing PEPC and PPDK were produced by crossing, and triple (PEPC+PPDK+ME) and quadruple (PEPC+PPDK+MDH+ME) transformants by gene introduction into the PEPC+PPDK hybrids. Contrary to the single transformants overproducing the rice ME, the triple transformants showed stunting, which was more marked with increasing ME activities. Such stunting was largely mitigated by overproduction of MDH in the quadruple transformants. These results suggest that overproduction of MDH would be prerequisite for introduction of the C_4 pathway into rice.

1.13
Comparison of transgenic rice plants overproducing different sets of C4 enzymes

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Five transgenic rice plants that overproduced different sets of four C4-related enzymes (maize C4 PEPC, maize C4 PPDK, rice C3 NADP-ME, and sorghum NADP-MDH) in leaf mesophyll cells were produced. Overproduction of PEPC alone slightly reduced the photosynthetic rate. The photosynthetic rate reduced by the PEPC overproduction remained unaffected by co-overproduction of any of PPDK alone, ME alone, and PPDK + ME, while it was restored by co-overproduction of PPDK + ME + MDH to reach levels comparable to or even higher than that of non-transformants. These results raise the possibility of improving photosynthetic performance of C3 plants by co-overproduction of all the four C4 enzymes. Comparison of growth of five different transformants indicated that co-overproduction of PEPC and ME led to stunting. The stunting was more marked in the PEPC + ME and PEPC + PPDK + ME transformants than in the transformants overproducing the four enzymes, an indication that overproduction of MDH mitigated the stunting caused by the PEPC + ME co-overproduction. In general, PPDK and MDH are active only in the daytime while PEPC and ME are active day and night. It is likely that the co-overproduction of PEPC and ME consumes once fixed carbon at night and under weak light illumination and thereby leads to stunting.

1.14
Put up or shut up: can comparisons of transcript abundance under drought stress in two *Clusia* species allow the molecular distinction between drought tolerance and CAM-associated drought postponement strategies

K Shorrock (Newcastle University), T Taybi (Newcastle University), A Kohli (Newcastle University), A Borland (Newcastle University)

Microarray experiments using the model plant *Arabidopsis thaliana* and crop plants such as barley have allowed us to study global changes in gene expression in plants subjected to a variety of environmental stresses. However, the problem remains that plants for which this array data is available are typically species that are not well adapted for surviving water shortages, and thus cannot be expected to effectively demonstrate the full range of strategies for coping with drought. Furthermore, studies involving a single species are limited in their ability to disentangle concurrent but distinct processes occurring within plant cells. Here, two closely related and morphologically similar neotropical tree species from the genus *Clusia* are compared. One of the species, *C. minor* is a C_3/CAM intermediate, where the capacity for CAM is hypothesised to postpone tissue desiccation under water-limiting conditions. The other, *C. multiflora* is a constitutive C_3 plant but appears to be more tolerant of leaf dehydration than *C. minor*. In order to investigate the molecular bases of these divergent strategies of drought acclimation we have used suppressive subtractive hybridisation to identify genes that are differentially expressed in the leaves of the two species in response to water limitation. The longer term aims of the project are to establish a greater understanding of what it takes to be a CAM plant and to offer insight into the metabolic constraints for the evolution of CAM in water-limited environments.

1.15
Have stress-induced changes in starch metabolism in C_3 plants been recruited to satisfy the nocturnal requirements for substrate supply in CAM plants?

R French (Newcastle University), A Borland (Newcastle University)

The high number of independent origins of Crassulacean acid metabolism (CAM) in over 30 taxonomically diverse plant families is an example of convergent evolution of a complex metabolic trait. Convergent evolution assumes that there are a finite number of effective solutions to certain environmental challenges and some of them emerge independently again and again, implying that the new adaptive trait exploits existing biochemistry. Since the expression of CAM is extremely responsive to environmental conditions (particularly those that influence water availability), it might be hypothesised that CAM exploits many of the metabolic changes that accompany acclimation to drought and salinity. The nocturnal mobilisation of starch for the production of PEP is a key limiting factor for the expression of CAM in the facultative species *Mesembryanthemum crystallinum.* It has been suggested that starch mobilisation also serves a more general role in acclimation to salinity by providing substrates for respiratory repair, for the synthesis of compounds responsible for osmo-regulation and for curtailing oxidative stress. We wished to test the hypothesis that the enhanced enzymatic capacity for starch degradation for the production of PEP in CAM-performing *M. crystallinum* exploits a general increase in starch-degrading enzyme activity elicited by salinity. The poster presents a comparison of the impact of salinity on starch content and the activity of selected starch degrading enzymes in *M. crystallinum* (which belongs to the higher order core Caryophyllales) with that in two other halophytes: *Beta vulgaris* (lower order core Caryophyllales) and *Thellungiella halophila* (Brassicales).

1.16
Where is CAM? Distribution of epiphytic bromeliads in a dry forest of Brazil

F Reinert (UFRJ), T Fontoura (UESC)

Epiphytes from dry forests are particularly vulnerable to water stress and are limited to the most specialised families, such as the Orchidaceae and Bromeliaceae. In this study we investigated abundance and distribution of epiphytic bromeliads from the dry forest of Jacarepiá and determined their photosynthetic mode. The forest receives less than 1000mm rainfall annually and this aspect is similar to the dry forests of Ecuador (800 mm y^{-1}). 800 trees were investigated, 82 hosted nine bromeliad species: (*Aechmea fasciata; A.floribunda; A.sphaerocephala; Billbergia pyramidalis; B.amoena; Neoregelia eltoniana; Tillandsia stricta; T.usneoides; Vriesea procera*). Eight species were CAM and *V.procera* was C_3. Phorophyte height varied from three to 16m and bromeliad vertical distribution varied from seven (*B.pyramidalis*) to 13m (*T.stricta*). Degree of exposure was assessed for the two most abundant species (*A.fasciata* and *V.procera*). *V.procera* was significantly more exposed than *A.fasciata*, it was higher and occurred more often on the ramifications than on the main trunk. If the abundance of CAM among bromeliads is related to water economy, the fact that C_3 species found was subjected to more exposed conditions is striking. The importance of CAM over C_3 is challenged among the epiphytic bromeliads in dry forests when comparing species abundance instead of richness 90% (eight in nine species) to 70% (79 specimens) of bromeliads. Our findings emphasise the continuing need to broaden our knowledge of bromeliad physiology to better understand their ability to inhabit dry forests.

1.17
Genetic engineering of "C_4 rice" Expression of maize PCK in rice mesophyll chloroplast to raise CO_2 concentration and photosynthesis

M Ku (National Chiayi University), K Izui (Kini University), Y Huang (National Chiyi University), Y Wu (National Chiyi University)

Phospho*enol*pyruvate carboxykinase (PCK) is a C_4-acid decarboxylation enzyme located in the cytosol of bundle sheath cells of PCK subtype C_4 plants. In this study, we have introduced maize PCK gene into rice genome and targeted the enzyme to mesophyll chloroplast as a first step in an attempt to raise CO_2 concentration in the chloroplast of rice through a synthetic C_4 pathway. Molecular analyses confirmed the integration of the maize gene into transgenic rice genome and its expression at transcript level. Expression of maize PCK was driven by tomato *rbcS3C* promoter in an organ-specific manner: high in leaf, stem and floret, but not in root. The protein amounts in the leaf were several folds higher in transgenic than in wild type plants. Although transgenic rice exhibited enhanced growth than did wild type, no significant differences in photosynthetic rate were detected between the two genotypes. This is expected since PCK is only one of the biochemical steps in the entire C_4 pathway; other affiliated mechanisms, such as effective CO_2 acquisition and rapid carboxylation by PEP carboxylase, will have to be in place in order to have a functional "C_4 rice". In order to raise CO_2 concentration in C_3 mesophyll chloroplast, an artificial C_4 pathway, which involves bicarbonate transport, PEP carboxylation and OAA decarboxylation via PCK, will be discussed.

1.18
Characterization of thiol-disulfide interchange of maize recombinant C4 and non-C4 NADP-malic enzyme

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C4 photosynthetic NADP-malic enzyme has evolved from non-C4 isoforms during evolution. In maize, the plastidic non-photosynthetic NADP-ME represents the more recent and direct ancestor of the C4–isoform. Although the high sequence similarity (85%) between these two NADP-MEs, they display well distinct kinetic and structural properties. In order to identify possible differences in response to -SH oxidation, recombinant C4- and non-C4 NADP-ME were subjected to oxidation and further reduction. Incubation of maize C4 NADP-ME with the oxidant *o*-iodosobenzoate (IBZ) leads to the complete inactivation of the enzyme. The reversal of the inactivation by reductive agents suggests that the modification of the enzyme by IBZ occurs concomitant with the oxidation of one or more pairs of -SH groups to the disulfide state. On the other hand, maize non-C4 NADP-ME isoform was not inactivated at all by -SH oxidation. In order to identify Cys residues involved in C4 NADP-ME oxidation, site directed mutagenesis of four different Cys residues was performed. The four mutants obtained, C192A, C231A, C246A and C270A, showed significant lower catalytic efficiency than the wild-type C4 NADP-ME. Moreover, the four mutants exhibit different responses to oxidation and further reduction, which may allow to infer the possible disulfide bond/s that may take place during maize C4 NADP-ME oxidation. The results obtained suggest that the redox state of maize C4 NADP-ME is important for the expression of the maximal catalytic activity of this enzyme, although the non-photosynthetic counterpart of the enzyme seems to be not so sensitive to -SH oxidation.

1.19
Elevation effects on foliar carbon gain attributes in the alpine C4 grass *Muhlenbergia richardsonis* and co-occurring C3 grasses in California's White Mountain range

A Meyer (California State University), R Sage (University of Toronto), J Skillman (California State University)

Muhlenbergia richardsonis, a widely distributed C4 grass, holds the high elevation record for any C4 species in North America at nearly 4000 meters in California's White Mountains and may be moving higher with climate change. A comparative leaf-level study was done along a 3000-3800 meter elevation gradient with *M. richardsonis* and three co-occurring C3 graminoid species. Elevation-dependent variation in stomatal density (SD), leaf nitrogen content (%N), and leaf d^{13}C was examined. These traits often vary with elevation in C3 species but have not been examined across elevation in C4 species. Leaf SD and d^{13}C increased with elevation as expected in the C3 plants but were unchanging with elevation in *M. richardsonis*. %N increased with elevation in all species. We interpret the C3 vs. C4 contrasts in SD and d^{13}C responses to mean that C4 photosynthesis is not limited by the low partial pressures of atmospheric CO_2 (pCO_2) present at high elevations. C3 plants are presumably photosynthetically limited by low pCO_2 at high elevation and shifts in SD and d^{13}C reflect responses to the thinning atmosphere. The similar trend in %N for C3 and C4 tissues suggests this is a response to altitudinal variables other than pCO_2 that affect C3 and C4 plants similarly. C4 plants are rare at high elevations for reasons that are not well understood. This study indicates C4 plants are not excluded from high elevations by low pCO_2. Rather, in this respect, C4 plants appear to be pre-adapted to the thin atmospheres of alpine habitats.

1.20
A geographically constrained phylogeny of Panamanian *Aechmea* (Bromeliaceae, sub-family Bromeliodeae)

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Aechmea is a large (>200 described species) and biologically diverse genus in the Bromeliaceae, a neotropical plant family with a Brazilian origin. Bromeliads, including *Aechmea*, occupy a broad array of ecological habitats, include terrestrial and epiphytic life forms, and include both C3 and CAM species. Extant *Aechmea* are widely distributed throughout the New World tropics and subtropics. The isthmus of Panama (formed 3 MYA) is a critical biogeographical location because it allowed the recent interchange of species (including *Aechmea*) between the South and North American landmasses. We used chloroplast DNA markers (*matK* and *rps16*) to reconstruct the evolutionary relationships among the 18 Panamanian *Aechmea* species, along with reference species in the genus from regions outside of Panama. Our interpretation of the phylogeny suggests the last common ancestor of the extant Panamanian *Aechmea* was CAM and facultatively epiphytic. Among the sampled *Aechmea* species, it appears that the capacity for epiphytic growth has been independently lost on at least two occasions, and that there was a single reversion to C3 photosynthesis. Little genetic variance and an estimated maximum divergence time of 5.2 MYA among these ecologically diverse species suggest a recent rapid adaptive radiation. Our data also indicate that there were multiple colonizations of the Panamanian land bridge by this ecologically diverse and evolutionarily young group of plants.

1.21
Involvement of PI-Phospholipase C, Phospholipase D and extracellular calcium in the light up-regulation of sorghum PEPC-kinase

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The C_4 phosphoenolpyruvate carboxylase (PEPC) is regulated post-translationally by light-induced reversible phosphorylation. This phosphorylation event is controlled primarily by phosphoenolpyruvate carboxylase kinase (PEPC-k). The transduction cascade initiated by light in mesophyll protoplasts of *Digitaria sanguinalis* involves PI-PLC, the phosphoinositide pathway and a Ca^{2+} dependent step. The present study has been intended to investigate whether the same components are involved in PEPC-k synthesis in *Sorghum* leaf disks. PEPC-k synthesis was dependent on protein synthesis and photosynthetic electron transport, as previously reported in protoplasts. On the contrary, the inhibitor of calcium release from intracellular stores, TMB-8, which reduced up regulation of PEPC-k in protoplasts, was ineffective in leaf disks. Maximum inhibition was reached with the combination of EGTA and TMB-8, thus suggesting the involvement of both extracellular and intracellular calcium in the response. The PI-PLC inhibitor U 73122 partially blocked the increase in PEPC-k activity in leaf disks. Treatment with the PLD inhibitor *n*-butanol and TLC analysis of ^{32}P labelled phospholipids indicated PLD activation in response to light and the participation of PLD signalling in the regulation of PEPC-k synthesis. Analysis of *SbPPCK1* and *2* expressions suggested that either PLC or PLD activation is enough to trigger PEPC-k synthesis in response to light. The CDPK activity analyzed was activated by calcium and by PA; moreover, it was up-regulated by light and lithium, two factors which increase PEPC-k activity. The CDPK inhibitor W7 was able to block the light up-regulation of PEPC-k, pointing to a situation of this signalling element afterwards of the confluence of PLC and PLD signalling.

1.22
Characterisation of the interaction between the Arabidopsis PPDK and its regulatory protein (RP)

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Pyruvate orthophosphate dikinase (PPDK) catalyses the reversible interconversion of pyruvate and phospho*enol*pyruvate (PEP). This process is regulated by phosphorylation of a threonine residue in the PPDK catalytic domain, inactivating PPDK in the dark. The PPDK regulatory protein, RP, acts as both a kinase and a phosphatase to inactivate and reactivate PPDK in C_3 and C_4 plants. This unusual bifunctional protein has recently been identified after having proved elusive for over 20 years.

The Arabidopsis genome has two genes for the regulatory protein, AtRP1 and AtRP2. We have shown *via* transient assays that the AtRP1 protein is targeted to the chloroplast with a transit peptide of approximately 86 amino acids, and that the AtRP2 protein is located in the cytosol.

Yeast-2-Hybrid analysis has been used to show that both Arabidopsis RP proteins interact with PPDK. Further analysis with PPDK deletion constructs has shown that AtRP1 specifically binds the catalytic region of PPDK. Deletion constructs of both RP proteins show that removal of only 80-85 amino acids from either the C or N terminus of the protein abolishes the interaction with PPDK.

Mutation of the regulatory threonine residue of PPDK alters the strength of interaction between RP and PPDK. Mutation to an aspartate residue increases the strength of binding, presumably by increasing the ‘dwell time’ between the two proteins when the target residue is not phosphorylatable.

We are currently attempting to isolate large quantities of both AtRP1 and AtRP2 soluble protein in order to determine the mature protein sequence of AtRP1 and the crystal structure of this highly unusual regulatory protein.

1.23
Photosynthesis in veinal cells of C_3 plants controls leaf morphology

S Janacek (University of Cambridge), B Palmer (University of Sheffield), P Quick (University of Sheffield), J Hibberd (University of Cambridge)

Photosynthetic cells surrounding the vascular bundle have been well characterised in C_4 plants, due to the important function of bundle sheath cells in the C_4 cycle. However, the role of photosynthesis in equivalent cells of C_3 plants is less well understood. To investigate this, we have generated *Arabidopsi*s lines in which photosynthesis has been removed specifically from cells adjacent to the vascular bundle. This has been achieved by combining an RNAi approach with the use of enhancer traps. Several lines with varying degrees of silencing have been generated. In all lines, removing photosynthesis from the veins leads to a change in leaf development such that a layer of palisade mesophyll cells is lost. In addition to this, the rate of plant growth is reduced and seed production is compromised. These data reveal that there is a critical role for photosynthesis in vascular cells of C_3 as well as C_4 species.

1.24
Epigenetic regulation of the C_4 syndrome in maize

S Offermann (RWTH Aachen)

The anatomy and biochemistry of C_4 photosynthesis in maize have been well defined, but the processes regulating the establishment of the C_4 syndrome are not understood. The activity of C_4-specific genes is controlled by multiple environmental stimuli and the corresponding promoters are mostly either active in the mesophyll or the bundle sheath. The importance of chromatin modifications in gene regulation has been recently underlined in genome-wide studies by the correlation of specific covalent histone modifications with transcription. We analysed the methylation of histone h3 lysine 4, one of the key marks of active genes, by chromatin immunoprecipitation. Our results reveal high levels of methylation of C_4-PEPC exclusively in the mesophyll and C_4-ME in the bundle sheath. Interestingly, this pattern is not dependent on gene activity, but already established in etiolated plants. Our data suggest that cell-type specific chromatin modifications potentiate subsequent light activation of transcription during differentiation of photosynthetic tissues in C_4 plants.

1.25
Cleome- A new model for C_4 photosynthesis

D Marshall (University of Cambridge), H Griffiths (University of Cambridge), J Hibberd (University of Cambridge)

C_4 plants have evolved from those using C_3 photosynthesis at least 45 times in the angiosperms and involves biochemical, anatomical, and ultrastructural changes. *Cleome gynandra* is the C_4 species phylogenetically most closely related to the C_3 species *Arabidopsis thaliana*. The phylogenetic proximity between these two species provides the potential to translate our understanding of processes, such as the regulation of gene expression and leaf development, from *Arabidopsis* to a C_4 plant. We have determined that *C. gynandra* belongs to the NAD-ME subtype, and in addition identified species within the genus that possess characteristics of C_4 photosynthesis ranging from increased venation density to centripetal localisation of chloroplasts in the bundle sheath. This demonstrates important developmental flexibility within the genus and provides insights into the evolution of C_4 photosynthesis. To identify whether *Arabidopsis* possesses trans-factors that recognise *C. gynandra* genes important for the C_4 pathway we have generated a series of reporter constructs and placed them in *Arabidopsis*. The extent to which these constructs are recognised in *Arabidopsis* will be summarised.

1.26
Apical and basal leaf portions of the tank bromeliad *Guzmania monostachia* play differential roles during CAM induction by drought

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Leaves of tank bromeliads are subjected to strong environmental gradients. While the leaf base is in contact with water in the tank and receives low light irradiance, the middle and upper leaf parts have no direct access to the tank water and are the leaf portions most exposed to light. This work attempted to investigate the existence of morphological and functional variations along the leaves of the tank bromeliad *Guzmania monostachia* during CAM induction by drought. Anatomical features and diurnal patterns of gas exchange, chlorophyll fluorescence, titratable acidity and activities of PEPCase and MDH were analyzed in the basal, middle and apical leaf portions of well-watered and drought-subjected plants. The results showed that, irrespective of the water conditions, the maximum values of CO_2 exchange, ETR, chlorophyll content and stomatal density were always detected in the apical portion of the leaf. Interestingly, under drought conditions, increases in the nocturnal acid accumulation and activities of PEPC and MDH were also restricted to the leaf tip, indicating the establishment of the CAM pathway exclusively in this region. Conversely, the highest trichome density and hydrenchyma thickness were observed in the leaf base, supporting its role in water uptake and storage. Evidence of water translocation from the leaf base to the leaf tip during drought was also obtained. Altogether, the data suggested that the drought-induced C_3-to-CAM transition in *G. monostachia* occurs preferentially in the upper leaf portion, while the basal region is mainly involved in maintaining a stable water status in more photosynthetically active tissues.

1.27
Kranz anatomy and development of NAD-ME type C4 photosynthesis in *Blepharis ciliaris* (L.) B. L. Burtt (Acanthaceae)

H Akhan (University of Tehran), M Ghasemkhani (University of Tehran), S Choung (University of Waterloo), G Edwards (Washington State University)

Genus *Blepharis* (Acanthaceae) is an Afroasiatic C_4 genus comprising 126 species in arid and semi-arid habitats. *B. ciliaris* (L.) B. L. Burtt. is a semi-desert species distributed in Oman, Iran and Pakistan. The anatomy of cotyledons, bracts and young stems, and the occurrence, development and ultrastructure of Kranz tissue, was investigated. C_4 type carbon isotope composition, the occurrence of Kranz anatomy, and concentration of starch in bundle sheath (BS) chloroplasts indicate that both cotyledons and leaves perform C_4 photosynthesis. Leaves and cotyledons are characterized by Atriplicoid type anatomy in which a continuous BS with centripetal chloroplasts encircles the accessory interrupted vascular bundles. By developing sclerenchymatous tissue in the midrib during leaf development the Kranz tissue becomes interrupted on the abaxial side, and then becomes completely absent in the mature leaf base. The young leaf tips have well developed Kranz tissue with a high density of BS chloroplasts which are rich in starch indicative of high photosynthetic capacity in the younger tissue. As the plant develops, bract lamina which have an interrupted Kranz anatomy, and spines which have a reduced Kranz anatomy, have an increased role as assimilating organs. However, the stems lack Kranz anatomy, and apparently are not photosynthetically active. Analyses of C_4 acid decarboxylases (by western blot) and chloroplast structure, which shows chloroplasts of BS cells are rich in grana while those in mesophyll cells are deficient in grana, and the occurrence of abundant mitochondria in BS cells, collectively show *B. ciliaris* is an NAD-ME type C_4 species.

1.28
Organic acid transport across the vacuolar membrane of the tropical crop plant *Ananas comosus*

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Ananas comosus (pineapple) is the most commercially important CAM plant and the world's second most valuable tropical fruit in terms of export market. As part of the CAM cycle, the *Ananas* vacuole plays a pivotal role in storing soluble sugars during the light phase and C4 organic acids during the dark phase. While sugar transport has been previously investigated in *Ananas*, little is known about the tonoplast transport of organic acids. In this study, organic anion transport was measured in tonoplast vesicles using two methods: indirectly as the anion-dependence of H^+ transport driven by the V-ATPase, and directly as whole-vacuole recordings of organic anion currents using the patch-clamp technique. ATP-driven H^+-transport was greatly dependent on the nature of the anion present, Cl^- supporting the highest rate, followed by organic anions in the order fumarate^{2-} ~ succinate^{2-} > malate^{2-} >> citrate^{3-}. Stimulation of ATP-dependent H^+ transport by chloride showed Michaelis-Menten kinetics with an apparent K_M for Cl^- of 5.0 mM. Differences in $malate^{2-}$ and $fumarate^{2-}$ stimulation of H^+ transport were observed in the presence or absence of low concentrations of Cl^-, which may reflect a requirement for allosteric activation of the V-ATPase by Cl^-. Vacuolar $malate^{2-}$ currents were recorded in both the whole-vacuole configuration and in isolated outside-out patches. Relatively large currents, consistent with $malate^{2-}$ movement into the vacuole, were observed at physiological (cytoplasmic-side negative) membrane potentials, whereas the reverse currents at corresponding positive potentials were smaller. The characteristic inward rectification of these currents, together with the distinctive anion-dependence of ATP-dependent H^+ pumping, suggests that *Ananas* vacuoles possess an effective system for malate transport into the vacuole during the nocturnal phase of the CAM cycle

1.29
CAM photosynthesis in tropical epiphytes: ecological and evolutionary trends in Bromeliaceae

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Epiphytic niches in tropical environments can be water-limited and microclimatically arid, even in regions with relatively high annual rainfall. The Bromeliaceae represent a large (*ca.* 3000 spp.) and ecologically diverse family of Neotropical plants that contains approximately equal numbers of terrestrial and epiphytic species. These can occupy a very wide range of habitats from arid, almost rainless coastal deserts to the shaded understory of tropical rain forests. From a survey of carbon-isotopes ratios covering nearly two-thirds of the family, we estimate that about 44 % of all bromeliad species utilize the CAM pathway as their principal mode of carbon assimilation. Phylogenetic analysis suggests that CAM photosynthesis has evolved independently a minimum of three times within the family. Ecologically, there is a tight correlation between habitat aridity and the frequency of CAM photosynthesis in these independent lineages, arguing strongly for the adaptive significance of this highly water-use-efficient mode of photosynthesis. Even within individual taxa, such as the large epiphytic genus *Tillandsia*, there is a close relationship between the occurrence of CAM photosynthesis, the degree of morphological reduction in plant life-form, and more xeric habitats. Nevertheless, a number of CAM taxa can persist in less typical habitats, such as high-rainfall montane forest and at high-altitude sites in the Andes. These observations suggest that interpretation of the adaptive value of CAM photosynthesis must take into account both prevailing environmental conditions and the selective pressures that may have acted upon distinct lineages during their evolutionary history.

1.30
Activity and Regulation of Rubisco and PEPC in Mesembryanthemum crystallinum

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Abstract not submitted

1.31
Metabolic and Circadian Regulation of Rubisco activity in Mesembryanthemum crystallinum

B N. Davies (University of Cambridge), H Griffiths (University of Cambridge)

Abstract not submitted

1.32
Stable isotopes as indicators of water use and habitat preference in epiphytic bromeliads

M Mejia-Chang (University of Cambridge), B Helliker (University of Cambridge), H Griffiths (University of Cambridge)

Abstract not submitted

1.33
Ecophysiology of Tillandsia epiphytes from a dry seasonal forest: photosynthesis and water use

C Reyes-Garcia (University of Cambridge), H Griffiths (University of Cambridge), P Huante (UNAM, Mexico)

Abstract not submitted

1.34
Carbon isotope discrimination during the dark phase of CAM in *Kalanchoe diagremontiana*

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Abstract not submitted

1.35
Response of wild C_4 crop progenitors to subambient CO_2 highlights a possible role in the origin of agriculture

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The synchronous origin of agriculture in at least four independent climatic regions at the end of the glacial period (10,000 yrs BP) points to a global limitation for crop domestication. One hypothesis proposes that a rapid CO_2 increase from 180ppm to near 280ppm during deglaciation caused significant increase in growth rates of wild crop progenitors, thereby removing a productivity barrier to their successful domestication. However, early C_4 crops present a challenge to this hypothesis, because they were among the first domesticates, but have a carbon-concentrating mechanism that theoretically makes photosynthesis insensitive to CO_2. We investigated the CO_2-limitation hypothesis using a set of five C_4 and one C_3 crop progenitors from four independent centres of domestication. Plants were grown in controlled environment chambers at glacial (180ppm), postglacial (280ppm) and current ambient (380ppm) CO_2 levels, and phenology, photosynthesis, transpiration, biomass and leaf area were measured. An increase in CO_2 from glacial to postglacial levels caused a significant gain in vegetative biomass of up to 40% in C_4 progenitors. Investigation into the underlying mechanisms showed C_4 photosynthesis to be limited by low CO_2 levels. More significantly, the increase in CO_2 caused a reduced transpiration rate via a decreased stomatal conductance of approximately 30%. In combination, these physiological changes confer a large improvement in water use efficiency at the postglacial CO_2 concentration compared with the glacial level. Our data provide experimental support for the CO_2-limitation hypothesis, suggesting that these key physiological changes could have greatly enhanced the productivity of wild crop progenitors after deglaciation.

1.36
A comparison of two *Asclepiadaceae* species in terms of their water relations

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Leaf hydraulic conductivity (K_L) of *Hoya carnosa* (a CAM plant) and *Stephanotis floribunda* (a C_3 plant) was measured using a vacuum chamber method. The leaf hydraulic conductivity usually correlates positively with stomatal conductivity as it determines the capability of plant to maintain sufficiently high water potential of leaves at given transpiration rate. However in this case, K_L of *H. cornosa* was more than twice higher than K_L of *S. floribunda*, although *H. carnosa* has substantially lower transpiration rate than *S. floribunda*. We looked for possible reasons and consequences of that finding in leave morphology, anatomy, water relations and leaf gas exchange of these plants.

1.37
Sunflecks Increase Bundle Sheath Leakiness in C_4 Plants

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C_4 plants inhabit forest understorey very rarely. They are considered to be sunny plants demanding additional energy for CO_2 concentration prior its final fixation in PCR cycle. Recently, misbalances between C_4 and PCR cycles during transient state of photosynthesis were reported. Here we hypothesise, that intermittent light (sunflecks) increase BSC leakiness in C_4 plants. We tested the effect of sunflecks on non-forest C_4 plants (*Setaria macrostachya* and *Amaranthus caudatus*). Plants were cultivated in a growth box under two regimes: 1/ steady light (375 mmol s^{-1} m^{-2}) and 2/ dynamic light (altering 95 and 950 mmol s^{-1} m^{-2} with the high light duration between 1 -10 min), both with 16h photoperiod and time integral of PPFD the same for both regimes. Temperature was 26/16 °C (day/night). ^{13}C discrimination ($\Delta^{13}C$) in leaf dry mass was analysed by IRMS (Delta^{Plus}XL ThermoFinnigan).
$\Delta^{13}C$ values (mean ± StD, n = 5) at dynamic *vs*. steady light were: *Setaria* 6.74 ± 0.06 ‰ *vs*. 5.54 ± 0.1 ‰; *Amaranthus* 7.54 ± 0.15 ‰ *vs*. 5.32 ± 0.13 ‰. The sunflecks significantly increased discrimination. Ratio of the leaf internal to ambient CO_2 (C_i/C_a) averaged over the growth period were 0.33 for *Setaria* and 0.4 for *Amaranthus*.
Sunflecks increased the leakiness (percentage of CO_2 escaping from the bundle sheath cells) in both C_4 plants approximately by a factor of 1.8. It could be an ecologically significant constraint, disadvantaging C_4 plants in a dense canopy relatively to their C_3counterparts.

1.38
Relationships between leaf anatomy and CAM function in eight CAM species representing distinct evolutionary lineages.

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Increased cell size and leaf succulence, reduced intercellular air space (IAS), and reduced surface of mesophyll exposed to IAS (Lmes) are traits associated with the Crassulacean acid metabolism (CAM) photosynthetic pathway. In this study, we tested whether these anatomical traits are related to the strength of CAM function in eight CAM species representing independent evolutionary lineages of CAM. The degree of CAM function was assessed as the amount of overnight acid accumulation, night-time CO_2 assimilation, and the length of the CAM phases. Increased cell size and leaf succulence were positively related to CAM function. Reduced IAS and Lmes were also positively related to CAM function but were negatively related to C_3 function. These results support the hypothesis that lower IAS and Lmes increase CAM function via the reduction of CO_2 efflux and the improvement of the carbon economy of leaves. However, reduced IAS and Lmes limit C_3 photosynthesis, generating a bimodal distribution of weak and strong CAM species with high and low IAS and Lmes values, respectively.

1.39
The Identification of C_3-C_4 intermediates in the genus *Heliotropium* section *Orthostachys* (Bo

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Both C_3 and C_4 photosynthesis occur in Heliotropium section Orthostachys. Here, we identify four C_3-C_4 intermediate species and two C_3 species with Kranz-like characteristics in Heliotropium section Orthostachys. Ten species were investigated for C_3-C_4 intermediacy in *Heliotropium* using anatomical studies, activities of PEP carboxylase (PEPC) and C_4-acid decarboxylases, and the intercellular distribution of Rubisco and the P-subunit of glycine decarboxylase (GDC). All species are in section Orthostachys, except H. europaeum of section Heliotropium. *Heliotropium europaeum*, H. calcicola, *H. tenellum*, and H. karwinskyi are C_3 plants, while *H. texanum* and *H. polyphyllum* are fully-developed C_4 species. The rest are C_3-C_4 intermediates, with weakly-developed Kranz anatomy in *H. procumbens*, and Kranz anatomy in *H. convolvulaceum, H. racemosum* and *H. greggii*. A quantitative anatomical study showed an increase in BS area and decrease in the M to BS area ratio in the four intermediate species, and the two C_3 species (H. tenellum, H. karwinskyi) that are most closely related to the C_4 species. In the C_3-C_4 *H. convolvulaceum* and *H. greggii*, labeling for Rubisco occurred in both M and BS cells, but was more pronounced in BS cells. Specific labeling for the P-subunit of GDC occurred in all photosynthetic cells of the C_3 species, but exclusively in BS cells of the C_3-C_4 and C_4 species. The C_3-C_4 intermediates had elevated PEPC activities compared to C_3 species, but PEPC activities were much lower in the intermediates than in C_4 species. These results indicate that photosynthetic efficiency is enhanced in the *Heliotropium* intermediates by the concentration of photorespired CO_2 into the BS cells via a glycine shuttle. The presence of enlarged bundle sheath cells in close C_3 relatives of the intermediates supports a hypothesis that bundle sheath enlargement is the critical initial phase in the evolution of C_4 photosynthesis in *Heliotropium*.

1.40
Rubisco in Flaveria

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C_4 plants have been reported to have Rubiscos with higher kcat and Km_{CO2} than the enzyme from C_3 species, but variation in other kinetic parameters between the two photosynthetic pathways has not been examined. We determined the CO_2/O_2 specificity ($S_{c/o}$) and the Michaelis-Menten constants for CO_2 (Kc), O_2 (Ko), and RuBP (Kr) in Rubisco purified from 16 species of Flaveria (Asteraceae). Our analysis included 2 of the 3 recognised C_3 species, 3 of the 4 recognised C_4 plants, and 11 species of differing C_3-C_4 intermediacy. We also examined the enzyme from two additional C_4 species (Zea mays and Amaranthus edulis) and C_3 plants (Spinacea oleracea and Chenopodium alba). The C_4 species of Flaveria had $S_{c/o}$ values of about 77 mol mol^{-1}, about 5% lower than the corresponding value in the C_3 species. In Rubisco from the C_4 species Kc was more than 40% higher than in the C_3 plants. Importantly, we found that Ko in the C_4 species F. bidentis and F. trinervia was similar to that of the C_3 Flaverias, but that in F. australasica, Z. mays, and A. edulis Ko was less than half of the value determined in the C_3 species. We detected no pathway-related differences in Kr. Previous work in Flaveria suggested that C_3-C_4 intermediates possessed Rubiscos with C_3 kinetic characteristics. By contrast, in our study the photosynthetic intermediates had a range of Rubisco kinetic types, with some species have enzymes that more closely resembling the enzyme from recognised C_4 species.

1.41
Malate metabolism in *Hoya carnosa* mitochondria and its role in photosynthesis during CAM phase III

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This study investigated the respiratory properties and the role of the mitochondria in malate metabolism during CAM phase III of a phospho*enol*pyruvate carboxykinase (PCK)-CAM plant, *H. carnosa.* The mitochondria showed rather high mitochondrial malate dehydrogenase (mMDH) and aspartate amino transferase (mAST), and with a significant amount of malic enzyme (mME). *H. carnosa* readily oxidized malate via mME or mMDH in the presence of some cofactors such as thiamine pyrophosphate (TPP), coenzyme A (CoA) or NAD^+. A high respiration rate was observed at pH 7.2 with NAD^+ and glutamate (Glu). Addition of AST on state 3 in the presence of Glu significantly stimulated malate oxidation and this oxidation was gradually inhibited by an inhibitor of a-ketoglutarate (a-KG) carrier, pyridoxal-5'-phosphate (PLP). The mitochondria also oxidized aspartate (Aps) and a-KG as the single substrates with low rates. However, the simultaneous oxidation of Aps or a-KG showed higher rates than those of the single substrate oxidation, and this simultaneous oxidation was also inhibited by PLP. By directly measuring the capacity of mitochondrial shuttle, we found that the OAA produced in *H. carnosa* mitochondria seemed to limit for transporting out but mAST could interconvert OAA and Glu to Asp and a-KG via a malate-aspartate shuttle. The results suggest that although *H. carnosa* is classified to PCK-CAM plants, their mitochondrial malate metabolism is different from that of PCK-CAM, *Ananas comosus* but rather similar to that in ME-CAM *Kalanchoë daigremontiana*, therefore it is conceivable that *H. carnosa* is an intermediate of PCK-CAM and ME-CAM species.