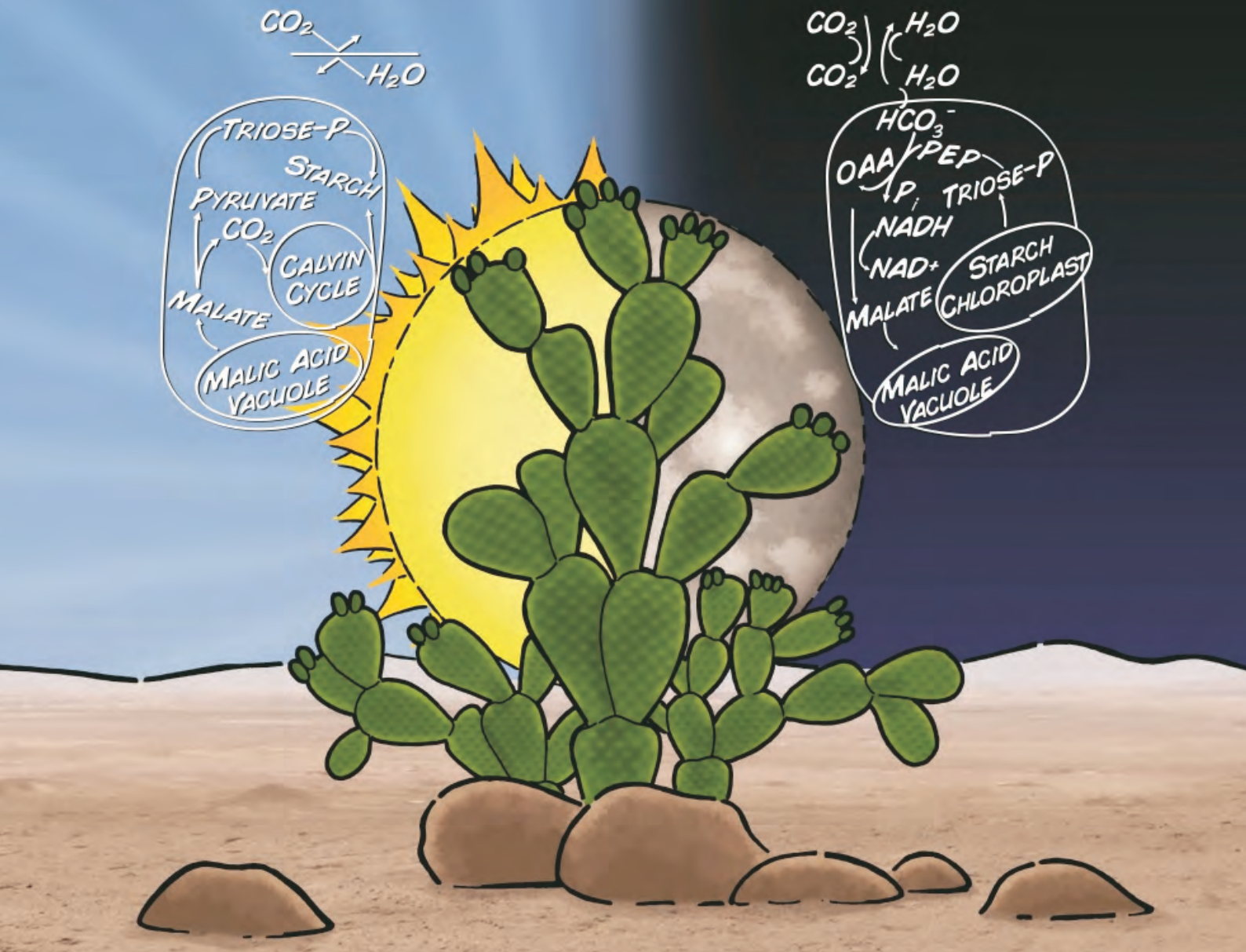
34th New Phytologist Symposium

Systems biology and ecology of CAM plants



15–18 July 2014
Granlibakken resort, Lake Tahoe,
Tahoe City, CA, USA

New Phytologist

Programme, abstracts and participants

34th New Phytologist Symposium

Systems biology and ecology of CAM plants

Granlibakken Conference Center and Lodge, Lake Tahoe, Tahoe City, CA, USA

15–18 July 2014

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Anne Borland *(Newcastle University, UK)*
John Cushman *(University of Nevada-Reno, USA)*
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Programme, abstracts and participant list compiled by Jill Brooke
'Systems biology and ecology of CAM plants' logo by A.P.P.S., Lancaster, UK

Contact email: *np-symposia@lancaster.ac.uk*

Table of contents

Information for delegates

Symposium location
The 34th New Phytologist Symposium will be held at Granlibakken Conference Center and Lodge, on the north shore of Lake Tahoe, near Tahoe City, CA, USA (postal address: Granlibakken Conference Center & Lodge 725 Granlibakken Road Tahoe City, CA 96145, USA). Further information about Granlibakken can be found on their website: http://www.granlibakken.com/

Map
A map showing the location of the main lodge and cabins can be found at the back of this book.

Catering
Breakfast will be served from 07:30–09:00 daily.
Coffee breaks will be served in the centrally located area outside the main meeting room.
Lunch and Dinner will be served outside on the Garden Deck.

Accommodation
If you have booked a registration option which includes accommodation then accommodation will be available for you for three nights: 15th, 16th and 17th July.
Check-in is from 16:00 on Tuesday 15th July. Please note you must check-out of your room by 11:00 on the morning of Friday 18th July. There will be space allocated where you can leave your baggage until departure.

Posters
Posters should be prepared so that they are no larger than A0 size, portrait orientation (118 cm high x 84 cm wide). Posters should be put up during registration (13:00–14:30 on 15th July) and will be displayed for the duration of the meeting. There will be two dedicated poster sessions at 20:00–21:00 on Tuesday, 15th July and 19:00–21:00 on Wednesday 16th July. Beer, wine and soft drinks will be served during the Wednesday poster session. Please stand by your poster at these sessions – there will be prizes for the best poster presentations.

Field trip
At the end of the meeting we have arranged an excursion to Emerald Bay with some scenic hiking. Buses will take us to Emerald Bay and will depart promptly at 13:15 on Friday 18th July. The buses will return us to Granlibakken by 18:00.

Internet access
Free wifi will be provided throughout the venue. Log in to 'Granlibakken main'. No password is required.

Social media
We encourage all attendees to join in discussions on social media sites. Follow @NewPhyt on Twitter and fb.com/NewPhytologist on Facebook for updates before, during and after the meeting. Please use #34NPS in all of your tweets.

Altitude
The altitude of Lake Tahoe is 6,225 feet (1897 metres). This elevation does not usually cause altitude sickness, but to avoid problems such as dry sinuses and shortness of breath, particularly if you plan on hiking any of the surrounding peaks, drink plenty of water and cut down on your exercise for the first few days of your stay. You should also note that alcohol consumption can exacerbate the effects of altitude.

Contact
For further information, and in case of any emergencies, please contact Michael Panagopulos. Email: m.panagopulos@lancaster.ac.uk, np-symposia@lancaster.ac.uk; tel: +44 7966 984 319.

Meeting programme

Tuesday 15th July

13:00–14:30	Registration	
14:30–14:45	Welcome from the organisers	
14:45–15:45	Andrew Smith	K1 Turgor, succulence, acidity and CAM: the central role of the vacuole in carbon capture and storage
15:45–16:35	Thomas Brutnell	K2 A systems approach to understanding photosynthetic differentiation in the grasses
16:35–17:00	*Break*	
Session 1	***Genomics***	**Chair: Anne Borland**
17:00–17:40	James Hartwell	S1.1 Genomic, transcriptomic and metabolomic adventures with CAM
17:40–18:20	Xiaohan Yang	S1.2 Comparative genomics of CAM, C_3 and C_4 plants
18:20–19:00	David Weston	S1.3 The transcriptomic and proteomic networks underlying *Agave americana* physiology
19:00–20:00	*Dinner*	
20:00–21:00	*Poster session*	

Wednesday 16th July

08:25–08:30	Announcements	
Session 1 (cont.)	***Genomics***	**Chair: Xiaohan Yang**
08:30–09:10	June Simpson	S1.4 Comparative transcriptome analysis in *Agave* species
09:10–09:50	Hengfu Yin	S1.5 The draft genome of *Agave tequilana*
09:50–10:30	Bernard Wone	S1.6 Transcriptional dynamics of CAM in the facultative CAM species, *Mesembryanthemum crystallinum*
10:30–11:00	*Break*	
11:00–11:40	Ray Ming	S1.7 The draft genome of pineapple (*Ananas comosus* L.) and the evolution of CAM photosynthesis
11:40–12:40	Discussion (CAM genomics)	Chair: James Hartwell
12:40–14:00	*Lunch*	

14:00–14:40	Sarah Davis	K3 Potential for converting light to liquid fuel using CAM crops in semi-arid regions
Selected poster talks		
14:40–14:55	Paul Abraham	**P1** Capturing the dynamics of CAM: transcriptome and proteome analysis of diel cycle gene expression in *Agave americana*
14:55–15:10	Zong-Ming (Max) Cheng	**P4** The comparative transcriptomic analysis of ten species in five subfamilies of Orchidaceae
15:10–15:25	Won Cheol Yim	**P35** Transcriptome sequencing and RNA-seq mRNA expression profiling in the facultative CAM model species *Mesembryanthemum crystallinum*
15:25–15:40	Nanako Isshiki	**P19** Promoter characteristics of CAM related genes
15:40–16:00	*Break*	
Session 2	***Metabolism, metabolomics & proteomics***	**Chair: James Hartwell**
16:00–16:40	John Cushman	S2.1 Novel insights into CAM from metabolomics studies
16:40–17:20	Anne Borland	S2.2 Orchestration of starch degradation in CAM plants
17:20–18:00	Luciano Freschi	S2.3 Developmental, environmental and hormonal regulation of CAM expression in bromeliads
18:00–19:00	*Dinner*	
19:00–21:00	*Poster session*	

Thursday 17th July

08:25–08:30	Announcements	
Session 2 (cont.)	***Metabolism, metabolomics & proteomics***	**Chair: John Cushman**
08:30–09:10	Johan Ceusters	S2.4 Malate metabolism and leaf physiology 'enlightened'
09:10–09:50	Susanna Boxall	S2.5 Ground-truthing the CAM genetic blueprint using transgenic approaches in the genus *Kalanchoë*
09:50–10:30	George Ratcliffe	S2.6 Analysis of CAM using constraints-based metabolic modelling
10:30–11:15	Discussion (Roadmap for CAM research)	Chair: Xiaohan Yang
11:15–11:30	*Break*	

11:30–12:20	Rowan Sage	K4 The functional anatomy of C_3, C_4 and CAM photosynthesis
Selected poster talks		
12:20–12:35	Karolina Heyduk	**P16** Lability of CAM expression in the homoploid hybrid *Yucca gloriosa*
12:35–12:50	Jacob Vogenberg	**P34** Adaptation to deep shade: evolution of tropical cacti
12:50–13:05	Lonnie Guralnick	**P13** Variability in the contribution of CAM in populations of *Portulacaria afra* (L.) Jacq. in the Eastern Cape
13:05–13:20	Paulo Mioto	**P25** Abscisic acid production in leaves of different age in *Guzmania monostachia* with CAM up-regulated by drought
13:20–14:20	*Lunch*	
Session 3	***Evolution, ecology & ecophysiology***	**Chair: Klaus Winter**
14:20–15:00	Erika Edwards	S3.1 Rethinking the CAM evolutionary trajectory
15:00–15:40	Katia Silvera	S3.2 Functional diversification of CAM in tropical orchids
15:40–16:20	Joe Holtum	S3.3 Towards an Australian CAM flora: beginning with *Calandrinia*
16:20–16:50	*Break*	
16:50–17:30	Howard Griffiths	S3.4 Shifty work on CAM: has working the late shift led to a phase shift in CAM ecology and productivity modelling?
17:30–18:10	Casandra Reyes Garcia	S3.5 Ecophysiological traits that influence colonization and survival of epiphytic bromeliads in a changing climate
18:10–18:50	David Williams	S3.6 Isotopic ecology and physiology of columnar cacti
18:50–20:30	*Dinner*	

Friday 18th July

08:25–08:30	Announcements	
Session 3 (cont.)	***Evolution, ecology & ecophysiology***	**Chair: Joe Holtum**
08:30–09:10	Klaus Winter	S3.7 Observations on phase-III-CO_2 leakage, CAM idling, and *Jatropha*

09:10–09:50	Erick de la Barrera	S3.8 Effects of climate change on CAM crops
09:50–10:10	*Break*	
10:10–10:50	Jose Luis Andrade	S3.9 CAM plants of the Yucatán Peninsula: what have we learned? What else do we need to see?
10:50–11:50	Discussion (Evolution & ecophysiology)	Chair: Howard Griffiths
11:50–12:00	Closing remarks	
12:00–13:15	*Lunch*	
13:15–18:00	Trip to Emerald Bay	

Speaker abstracts

**K=keynote speaker abstract; S=speaker abstract; P=poster abstract; bold=presenting author*

Speaker abstracts

Turgor, succulence, acidity and CAM: the central role of the vacuole in carbon capture and storage

K1

J. ANDREW C. SMITH

14:45–15:45

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The assimilatory cells of crassulacean acid metabolism (CAM) plants that engage in photosynthetic carbon fixation are typically dominated by a huge lytic vacuole, which can occupy more than 95% of cell volume. In this sense, the vacuole is central to the CAM plant's entire carbon and water economy: it acts as the temporary storage site for malic acid synthesized at the night, and as the reservoir for most of the water acquired from the environment during periods of episodic water availability. The outward manifestation of these features is the succulence of the photosynthetic tissues and organs so characteristic of most CAM plants. At the same time, the vacuole must also play a dynamic role in fulfilling other essential functions such as maintenance of cell turgor, ion balance and cytoplasmic homeostasis. In this presentation, I shall first explore the relationship between the osmotic characteristics of CAM plants at the cellular level and the defining ecological features of their habitats. I shall then assess our current understanding of the role of the vacuole in the process of malic-acid accumulation and remobilization that underpins the diel rhythm of the CAM cycle. This will serve to highlight some outstanding gaps in our knowledge and questions that may soon be resolved with the aid of new developments in CAM-plant genomics and systems biology.

A systems approach to understanding photosynthetic differentiation in the grasses

K2

TOM BRUTNELL[1,2], KEVIN AHERN[3], TIM ANDERSON[1], PINGHUA LI[4], RACHEL MERTZ[5], TONY STUDER[1], LIN WANG[6], SARIT WEISSMANN[1]

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15:45–16:35

C_4 photosynthesis has evolved over 65 times in the history of angiosperms. In the grasses alone C_4 has evolved over 22 times independently and is dependent on the metabolic cooperation of two specialized cell types, the bundle sheath and the mesophyll. In maize, carbon is fixed in the mesophyll as a C_4 acid, either as malate or aspartate, that then diffuses to the bundle sheath where it is decarboxylated releasing CO_2 in the vicinity of Rubisco. This CO_2 pump results in high local CO_2 concentrations in the BS plastids that effectively eliminates photorespiration. As photorespiration can reduce photosynthetic capacity of C_3 plants by as much as 30%, C_4 plants are at a competitive advantage under hot dry conditions when photorespiration is prevalent in C_3 plants. In the sister taxon *S. viridis*, malate appears to be the sole source of carbon that is shuttled between the two cell types and additional members of the Panicoid family utilize alternative decarboxylases to release CO2 in the BS. Thus, a deeper understanding of the genes and networks underlying C_4 photosynthetic differentiation in the grasses will provide new opportunities for breeding improved varieties of C_4 crops and for the engineering of C_4 traits into C_3 grasses.

We have begun the functional dissection of C_4 photosynthesis by exploiting the excellent genetic and genomic resources available in maize including transposon collections and RNAseq expression profiling. I will present the findings of our recent genetic studies to define the components of the C_4 carbon concentrating mechanism and present preliminary studies into the use of a new model system, *Setaria viridis*, to accelerate gene discovery in the grasses and begin the engineering of C_4 pathways.

Session 1: Genomics
Chair: Anne Borland

Genomic, transcriptomic and metabolomic adventures with CAM

S1.1

JAMES HARTWELL[1], SUSANNA F. BOXALL[1], LOUISA V. DEVER[1], JANA KNEROVA[1]*, NIRJA KADU[1], JADE WALLER[1], JACK DAVIES[1], PHAITUN BUPPHADA[1], RACHEL BRENCHLEY[2], RICHARD GREGORY[2], YONGXIANG FANG[2], ROY CHAUDHURY[2], GARETH WEEDALL[2], ALISTAIR DARBY[2], STEVEN SALZBERG[3], DANIELA PUIU[3], TANJA MAGOC[3], ILIA LEITCH[4], NEIL HALL[2]

17:00–17:40

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Our core research goal is to define all of the genes and proteins required for crassulacean acid metabolism (CAM) to develop and operate efficiently – these can be referred to collectively as the 'CAM genetic blueprint', 'CAM parts-list' or even the 'CAMome'. To facilitate this, we have decoded the genome and transcriptome of *Kalanchoë fedtschenkoi,* and the transcriptome of *Agave sisalana,* as representative dicot and monocot CAM species. Using quantitative transcriptome sequencing (RNA-seq), we have been able to identify hundreds of known and novel genes as candidates for CAM-specific functions; based both on their increased transcript abundance in CAM leaves relative to C_3, and their regulation over the light/ dark cycle. A combination of global quantitative data for both gene transcripts and metabolites has allowed us to model the metabolic steps of CAM in unprecedented detail, including *in silico* prediction of subcellular compartmentation of key metabolic steps. This sets the stage for testing the predicted CAM pathway with transgenic approaches in order to further refine our CAM parts-list. In addition to requiring the core biochemistry of CAM in leaf mesophyll cells, CAM also requires reverse stomatal opening, allowing atmospheric CO_2 into the leaf in the dark, and preventing the escape of CO_2 from decarboxylation in the light. To understand the gene signalling networks associated with reverse stomatal control, we have undertaken RNA-seq to compare gene regulation in separated epidermal peels and mesophyll tissue. This data can be used to predict a model for stomatal control during CAM.

This work is supported in part by the Department of Energy (DOE), Office of Science, Genomic Science Program under Award Number DE-SC0008834. The contents of this abstract and presentation are solely the responsibility of the authors and do not necessarily represent the official views of the DOE. This work was also supported by the Biotechnology and Biological Sciences Research Council, U.K. (grant BB/F009313/1 awarded to JH and NH).

Comparative genomics of CAM, C_3 and C_4 plants

S1.2

17:40–18:20

XIAOHAN YANG[1], JAMES HARTWELL[2], JOHN C. CUSHMAN[3], HENGFU YIN[1], JERRY JENKINS[4], JEREMY SCHMUTZ[4], ANNE M. BORLAND[1,5], DAVID WESTON[1], GERALD A. TUSKAN[1,4], BERNARD WONE[3], WON CHEOL YIM[3], JUNGMIN HA[3], KAREN A. SCHLAUCH[3], SUSANNA F. BOXALL[2], RACHEL BRENCHLEY[2], RICHARD GREGORY[2], NEIL HALL[2], TANJA MAGOC[6], STEVEN L SALZBERG[7], STEPHEN M. GROSS[4], ZHONG WANG[4], AXEL VISEL[4], JUNE SIMPSON[8]

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Photosynthesis in higher plants provides the basic source for food, fiber, forage and renewable fuels through solar energy-driven CO_2 fixation in three different pathways: C_3, C_4 and crassulacean acid metabolism (CAM). In CAM plants, net CO_2 uptake is shifted predominantly to the night, resulting in a diel separation of primary and secondary carboxylation processes which can produce comparable amounts of biomass to that of C_4 and C_3 plants but with significantly lower (20 to 80%, respectively) inputs of water. CAM is thought to have evolved from the C_3 pathway through reprogramming of metabolic processes already present in C_3 plants. However, the molecular basis of this reprogramming is largely unknown. To understand the genomic changes underpinning the evolution of CAM, we performed comparative genomics analysis of diverse plant species including CAM, C_3, C_4 and non-vascular plant species. Our comparative analysis of protein sequences identified genes conserved among C_3, C_4, and CAM plants as well as CAM/C_4-specific genes. Also, our comparison between CAM and C_3 plants revealed changes in diel expression patterns of some genes relevant to CAM. Lastly, we compared the syntenic regions among CAM, C_3, and C_4 genomes to gain new insights into the genomic context of CAM evolution. This research builds a foundation for studying evolutionary genomics of photosynthesis.

The transcriptomic and proteomic networks underlying *Agave americana* physiology

S1.3

DAVID J. WESTON[1], XIAOHAN YANG[1], ANNE M. BORLAND[1,2], GERALD A. TUSKAN[1,3], PAUL ABRAHAM[4], ROBERT L. HETTICH[4]

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18:20–19:00

Increasing demand for food, fiber and bioenergy resources have heightened the need for sustainable plant production systems. Within arid regions, crassulacean acid metabolism (CAM) is a promising photosynthetic adaptation as it enhances plant water-use efficiency through an inverse day/night pattern of stomatal closure/opening that facilitates nocturnal CO_2 uptake and improves photosynthetic efficiency. The genetic basis of the diel reprogramming of metabolism that distinguishes CAM from other modes of photosynthesis is largely unknown, yet the understanding of which is critical for the development of biodesign strategies to transfer CAM into other commodity crops. In this presentation, we will introduce analytical methods for the genetics, gene expression, and proteomics used to infer CAM metabolic reprogramming. Central to this approach is the correlation of omics' networks to physiological measures. A usecase example with data from *Agave americana* and the open-source and open-development cyber infrastructure used to model those data will be demonstrated.

Session 1 (continued): Genomics
Chair: Xiaohan Yang

Comparative transcriptome analysis in *Agave* species

S1.4

E. AVILA DE DÍOS, L. DELAYE ARREDONDO, JUNE SIMPSON

08:30–09:10

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The genus *Agave* is ubiquitous in Mexico but despite economic, cultural and social importance, few studies have been carried out on this genus at the molecular level. We describe transcriptome analysis for *Agave tequilana, Agave victoriae-reginae* and *Agave striata* by RNA-seq of 8 different tissues by reconstruction of the transcriptome of the 3 species and analysis of the similarity of the transcripts at the level of amino acid sequences reported for other plant species. Prediction of non-coding sequences based on covariance models, the functional annotation of the sequences based on homology at the amino acid level, relative quantification *in silico* and differential expression analysis both interspecific and intraspecific were also carried out. The three reconstructed transcriptomes are similar in terms of average length and number of transcripts that could be classified. However, despite these similarities, there are significant differences in the expression patterns of a certain group of transcripts in different tissues within the same species and also in the same tissue in different species. These differences, mostly at the intraspecific level could be responsible for significant differences in enriched biological terms, indicating differences in the molecular processes controlling the distinctive morphology of each species.

The draft genome of *Agave tequilana*

S1.5

HENGFU YIN[1], GERALD A. TUSKAN[1,2], XIAOHAN YANG[1]

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09:10–09:50

Agaves are economically important plants primarily featuring constitutive crassulacean acid metabolism (CAM). They hold a great potential for biofuel and fiber production in water-limited areas due to high water use-efficiency, low lignin and high cellulose content. *Agave tequilana* is widely cultivated for commercial alcohol production. It has a diploid genome of *c*. 4.0 Gb. To generate genomic resources for systems biology research and genetic improvement in Agaves, we performed whole-genome sequencing of *A. tequilana* using next-generation sequencing technologies. Specifically, we generated over 50X paired-end reads and mate-pair reads with using Illumina MiSeq platform. A draft genome assembly was obtained and the quality of the genome assembly was assessed by comparing with core eukaryotic genes as well as the transcriptome sequencing reads for three *Agave* species (*A. americana*, *A. deserti*, *A. tequilana*). More than 90 percent of the Agave transcripts were found in the assembly. Furthermore, we sequenced and assembled the chloroplast genome of *A. americana* (~157 kb). Genes encoding key photosynthesis components from both nuclear and chloroplast genomes were analyzed to characterize the diversification of photosynthetic regulation in CAM. Ongoing work towards a reference genome of *Agave* includes improving genome assembly using long sequencing reads and genome annotation based on transcriptome and proteome sequencing data.

Transcriptional dynamics of CAM in the facultative CAM species, *Mesembryanthemum crystallinum*

S1.6

BERNARD W. M. WONE, **BAHAY G. BILGI, REBECCA L. ALBION, WON CHEOL YIM, RICHARD L. TILLETT, KAREN A. SCHLAUCH, JOHN C. CUSHMAN**

09:50–10:30

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Crassulacean acid metabolism (CAM) is an elaboration of C_3 photosynthesis wherein carbon assimilation occurs at night to reduce daytime water losses through a temporal separation of primary C_4 and secondary C_3 carboxylation reactions. The circadian clock controls the temporal separation of these potentially competing reactions. However, the underlying orchestration of transcriptional network modules of CAM is poorly understood. Comparative RNA-seq analysis was performed in well-watered (C_3 performing) and water-deficit-stressed (CAM performing) common ice plants (*Mesembryanthemum crystallinum* L.), a facultative CAM model species. Leaves were collected every 4 h over a 72 h time course under both 24 h light/dark and 48 h light/light conditions to characterize the circadian clock-controlled transcriptome in both the C_3 photosynthesis and CAM states. Under water-deficit conditions that induce CAM, greater numbers of transcripts become rhythmic indicating that the stress-adaptive and CAM transcriptional machinery is directly under circadian clock control. Weighted co-expression network analyses of differentially expressed genes upon CAM induction ($\log_2$ fold-change in CAM performing compared to C_3 photosynthesis performing) revealed both persistent and induced circadian clock-controlled transcriptional network modules. Together these results tentatively identified CAM-specific network modules and provide insights into the circadian clock-controlled transcriptional expression of CAM.

The draft genome of pineapple (*Ananas comosus* L.) and the evolution of CAM photosynthesis

S1.7

ROBERT VANBUREN[1], CHING MAN WAI[1], MICHAEL C. SCHATZ[2], HAIBAO TANG[3], JISEN ZHANG[4], ROBERT E. PAULL[5], QINGYI YU[6], RAY MING[1,4]

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11:00–11:40

Pineapple is the third most cultivated tropical fruit in the world behind banana and citrus. The available genetic and genomic resources and a large collection of pineapple germplasm make pineapple an excellent model for studying obligate crassulacean acid metabolism (CAM) photosynthesis and it serves as an important outgroup for comparative genomics in the well-studied panicoid grasses. The pineapple genome was sequenced using an integrative whole genome shotgun and BAC based approach with next-generation Illumina, Moleculo, PacBio and 454 techology. The draft genome has a contig N50 of 9.5kb and a scaffold N50 of 408kb, and covers about 375Mb of the estimated 525Mb genome. The pineapple draft genome contains 43% repetitive sequences, and was annotated to have 25,862 genes. We identified genes in the CAM pathway based on homology to C_4/C_3 orthologs in maize, sorghum, and rice, and discovered that the pineapple genome contains fewer CAM/C_4 pathway and photosynthesis genes than other monocot genomes. Extensive transcriptome data coupled with physiology measurements were used to identify the genes and regulatory sequences involved in the expression and circadian regulation of the CAM pathway. RNAseq expression data was collected at 2 hour intervals in photosynthetic (green) and non-photosynthetic (white) leaf tissue as well as along the leaf development gradient at diurnal and nocturnal time points. Gene expression in the leaf is dynamic both spatially and temporally. The pineapple genome serves as a framework for dissecting the gene network and regulation of the CAM pathway.

Potential for converting light to liquid fuel using CAM crops in semi-arid regions

K3

SARAH C. DAVIS[1], EMILY KUZMICK[1], DAVID LEBAUER[2], STEPHEN P. LONG[2]

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14:00–14:40

Drought is expected to become a growing problem in many regions as climate change progresses. Plants that use crassulacean acid metabolism (CAM) for photosynthesis have exceptional water use efficiency and some CAM species have greater potential yields than many commodity crops that use C_3 and C_4 photosynthesis, including those that are considered high-yielding bioenergy crops. Using *Agave* spp. as a model, theoretical yields of biofuel from CAM crops meet or exceed that of typical bioenergy cropping systems while maintaining far lower water requirements. Preliminary data from the first field trial of *Agave* spp. in the United States suggest that *Agave americana* is more productive in the semiarid southwest than *Agave tequilana* and *Agave fourcroydes,* two species that are grown commercially in Mexico. Water use efficiency of an *Agave americana* crop in the arid southwestern US is up to six times greater than the WUE of cotton, a major commodity crop in the region. Pesticide requirements may however be substantial. Environmentally sustainable production of *Agave* ultimately depends on prior land use and the amount of inputs required to achieve commercially viable biomass yields.

Session 2: Metabolism, metabolomics & proteomics
Chair: James Hartwell

Novel insights into CAM from metabolomics studies

S2.1

16:00–16:40

JOHN C. CUSHMAN[1], BAHAY G. BILGI[1], BERNARD W. M. WONE[1], JESSE A. MAYER[1], REBECCA L. ALBION[1], JULI PETEREIT[2], KAREN A. SCHLAUCH[1], DANNY C. ALEXANDER[3], LINING GUO[3], JOHN A. RYALS[3]

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Metabolic phenotypes are particularly informative as metabolites represent the end products of cellular regulatory processes. To probe the metabolic phenotypes of crassulacean acid metabolism (CAM) plants, metabolomic profiling was used to characterize the diel and circadian changes in metabolite abundance patterns over a 72 h time course in wild type (WT) and a CAM-deficient mutant ice plant (*Mesembryanthemum crystallinum* L.) genotypes performing either C_3 photosynthesis or CAM induced by water-deficit stress. A total of 469 (256 named and 227 unnamed) metabolites were identified. Most C_4 and tricarboxylic acid (TCA) cycle metabolites exhibited circadian clock-controlled increases in WT CAM-performing plants, whereas the CAM-deficient mutant accumulated far less of these compounds. The CAM-deficient mutant failed to accumulate starch breakdown products, such as maltose and maltotriose, but showed increased accumulation of soluble sugars, sugar alcohols, nitrogen-rich compounds, and lipids in the CAM-deficient mutant likely reflecting a redirection of carbon into alternative pathways and storage pools to compensate for reduced starch accumulation. A second metabolomics study was performed on prickly pear cactus (*Opuntia ficus-indica*) to compare metabolites within mesophyll and epidermis tissues over a 24 h diel time course under well-watered and water-deficit stress conditions. A total of 382 (210 named and 172 unnamed) metabolites were identified.

Orchestration of starch degradation in CAM plants

S2.2

ANNE M BORLAND[1,2], JOHAN CEUSTERS[1,3], ERIN CASEY[1], TAHAR TAYBI[1], SUSIE BOXALL[4], LOUISA DEVER[4], JAMES HARTWELL[4]

16:40–17:20

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In plants with crassulacean acid metabolism (CAM), carbohydrate availability is a major limiting factor for nocturnal CO_2 uptake. In several model CAM species, starch is degraded at night to provide phosphoenolpyruvate as substrate for PEPC. The nocturnal requirement for PEP represents an additional sink for carbohydrate that must be reconciled alongside the carbohydrate demands imposed by growth and maintenance processes. This presentation considers whether or not the specialized requirement for carbohydrate in CAM plants is reflected by a're-engineering' of carbohydrate processing compared to that in C_3 plants, both in terms of regulation and the engagement of alternative metabolic pathways for carbohydrate breakdown. Data will be presented which indicates that leaf starch is degraded via a different route in *Kalanchoë fedschenkoi* compared to that in *Arabidopsis.* Findings will be discussed in line with the specialized metabolic requirements of CAM and the implications for carbon and water balance will be considered. Future areas of research allied to this topic will be highlighted.

Developmental, environmental and hormonal regulation of CAM expression in bromeliads

S2.3

LUCIANO FRESCHI, M. A. RODRIGUES, P. T. MIOTO, A. MATIZ, B. N. K. GOBARA, F. C. PIKART, P. N. PEREIRA, L. HAMACHI, H. MERCIER
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17:20–18:00

About half of the 3200 Bromeliaceae species perform crassulacean acid metabolism (CAM) photosynthesis at some point of their lifespan. To provide some insight into the developmental and environmental regulation of CAM expression in bromeliads, and to identify signaling molecules associated with the control of CAM in these plants, we have analyzed key physiological parameters in young and adult bromeliad individuals exposed to contrasting environmental conditions. The impacts of water and/or nutrient availability on specific biochemical components of the CAM pathway in both CAM constitutive and facultative bromeliad species will be presented and compared with alternative responses triggered by these same environmental cues in C_3 bromeliads. In addition, the impacts of CAM up- and down-regulation in other metabolic pathways, such as nitrogen acquisition and assimilation, will be also discussed. Evidence of water and nutrient mobilization between distinct leaf regions and data establishing a clear functional gradient along the leaves of these rosette plants have also been obtained, thereby indicating further complexity in the control of CAM and other metabolic pathways. Morphological and biochemical leaf attributes will be related to this peculiar spatial pattern of CAM expression and, whenever possible, the potential ecological significance as well as the signaling mechanisms underlying this compartmentalization will be discussed.
(Financial support: FAPESP, CAPES and CNPq)

Session 2 (continued): Metabolism, metabolomics & proteomics
Chair: John Cushman

Malate metabolism and leaf physiology 'enlightened'

S2.4

JOHAN CEUSTERS[1], ANNE M BORLAND[2], J ANDREW C SMITH[3]

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08:30–09:10

The diel, intracellular fluxes of malate across the tonoplast are a crucial feature of crassulacean acid metabolism. Nocturnally accumulated malate exits the vacuole during daytime and decarboxylation liberates CO_2 for subsequent processing into organic carbohydrates *via* Rubisco. It is of vital importance that the decarboxylation reactions keep up with malate efflux from the vacuole to prevent over-acidification of the cytoplasm, which has recently been showed to induce chlorenchyma cell death and leaf necrosis. Although the photosynthetic photon flux density (PPFD) has been found an important determinant of organic acid remobilization out of the vacuole, the precise mechanisms and their interaction with environmental parameters, responsible for this export process are still matter of conjecture. Therefore the influences of light on malate processing were investigated in more detail in the obligate crassulacean acid metabolism (CAM) bromeliad *Aechmea* 'Maya'. Besides continuous light and dark treatments plants were provided with different low fluence monochromatic light treatments (10 µmol m^{-2} s^{-1}) such as blue (470 nm), green (530 nm), red (630 and 660 nm) and far red (735 nm). Determinations of malic and citric acid, titratable acidities, cell sap pH and cell sap osmolalities were performed to shed more light on the intracellular movements of malate.

Ground-truthing the CAM genetic blueprint using transgenic approaches in the genus *Kalanchoë*

S2.5

SUSANNA F. BOXALL, LOUISA V. DEVER, JANA KNEROVA*, JACK DAVIES, JADE WALLER, NIRJA KADU AND JAMES HARTWELL

09:10–09:50

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Current genomic and transcriptomic approaches in diverse crassulacean acid metabolism (CAM) species are permitting the rapid identification of candidate CAM genes. Similarly, earlier pioneering biochemical work defined many of the enzymes and transporters that are present at high specific activities in CAM leaves. This wealth of background data has perhaps encouraged the community to believe that we have already defined the 'parts-list' for CAM. However, aside from one phosphoglucomutase mutant isolated for ice plant, we have not had access to CAM mutants in order to test which members of CAM gene families perform each step of the pathway. Using a draft genome sequence in concert with extensive quantitative RNA-seq data, we have been able to identify many candidate CAM genes in both *Kalanchoë fedtschenkoi* and *K. laxiflora*. Due to the ease of *Agrobacterium*-mediated stable transformation in *Kalanchoë*, we are able to switch candidate CAM genes either off, with RNAi constructs, or on, with constitutive over-expression constructs, in order to test gene function *in planta*. Here we will describe progress in defining the minimal CAM parts-list in *Kalanchoë* using loss-of-function and gain-of-function transgenic approaches. Phenotypic analysis of transgenic lines lacking key enzymes and/ or transporters in both the carboxylation (dark) and decarboxylation (light) phases of CAM will be presented.

This work is supported in part by the Department of Energy (DOE), Office of Science, Genomic Science Program under Award Number DE-SC0008834. The contents of this abstract and presentation are solely the responsibility of the authors and do not necessarily represent the official views of the DOE. This work was also supported by the Biotechnology and Biological Sciences Research Council, U.K. (grant BB/F009313/1 awarded to JH).

Analysis of CAM using constraints-based metabolic modelling

S2.6

C. Y. MAURICE CHEUNG, MARK G. POOLMAN, DAVID A. FELL, R. GEORGE RATCLIFFE, LEE J. SWEETLOVE

09:50–10:30

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Constraints-based metabolic modelling provides a method for predicting metabolic fluxes in large-scale networks. The subcellular compartmentation of plant metabolism complicates the approach, but robust protocols for flux-balance analysis (FBA) are emerging that allow realistic exploration of such networks. Most FBA applications to photosynthesis have modelled a system with continuous light, but a more representative model of leaf metabolism can be obtained using an FBA framework in which the two phases of the diel cycle are solved as a single optimisation problem. In this approach the requirement to support continued export of sugar and amino acids from the leaf during the night, as well as to meet night-time cellular maintenance costs, forces the model to set aside stores of carbon and nitrogen during the day. The diel model captures many of the known features of C_3 and crassulacean acid metabolism (CAM) photosynthesis with only minimal constraints. It also predicts that there is no overall energetic advantage to CAM, despite the potential for suppression of photorespiration. Moreover, any savings in enzyme machinery costs through suppression of photorespiration are likely to be offset by the higher flux demand of the CAM cycle. Thus energetic or nitrogen-use considerations are unlikely to be evolutionary drivers for CAM photosynthesis.

The functional anatomy of C_3, C_4 and CAM photosynthesis

K4

ROWAN F. SAGE, TAMMY L. SAGE

11:30–12:20

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In recent years, there has been renewed interest in leaf anatomy as improved understanding of photosynthetic physiology has drawn attention to anatomical constraints on performance. In the C_4 and crassulacean acid metabolism (CAM) pathways, similar structural designs have repeatedly appeared to enable CO_2 concentration. The best known are the Kranz syndrome of C_4 photosynthesis, and the succulent anatomy of CAM. There are however, additional structural modifications that are not widely recognized. For example, in CAM plants, the cells where CAM is active are tightly packed with low intercellular air space, forming an internal structure that reduces diffusive efflux of CO_2 during malate decarboxylation. Recently, we demonstrated dramatic reductions in the number of mesophyll cell chloroplasts during C_4 evolution, probably to optimize diffusive access to the site of PEP carboxylation in the cytosol. C_3 plants have high numbers of chloroplasts that cover most of the mesophyll cell perimeter, which maximizes diffusive transfer of CO_2 into the chloroplast. In CAM species, chloroplast investment patterns appear similar to those observed in C_4 plants. An intriguing possibility is that certain CAM species employ a dual strategy of a C_3-active outer layer of M cells, and an inner layer of enlarged CAM-active cells. If so, their chloroplast investment patterns may vary, with the C_3-type cells having many chloroplasts while the CAM-active cells have few chloroplasts.

Session 3: Evolution, ecology & ecophysiology
Chair: Klaus Winter

Rethinking the CAM evolutionary trajectory

S3.1

ERIKA EDWARDS
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14:20–15:00

Crassulacean acid metabolism (CAM) photosynthesis is best described not as a trait, but as a trait syndrome- many anatomical, biochemical, and physiological characters interact to produce CAM behavior. CAM shares many qualities with C_4 photosynthesis, another complex trait syndrome. Both CAM and C_4 syndromes have evolved numerous times across the plant tree of life, and researchers have been extremely successful in using the phylogeny of C_4-evolving groups to piece together a rough sketch of a C_4 'evolutionary trajectory': which pieces of the syndrome evolved first, and in what order. C_4 appears to proceed in a roughly linear fashion, and there is a remarkable consistency to the relative order of trait evolution. We have made less headway with CAM evolution, which is partly due to the lack of phylogenetic resolution in CAM-evolving clades, partly due to a lack of clearly identified key modules that act as the building blocks of CAM- and might also be due to, in reality, a lack of a linear trajectory. I'll review how the CAM evolutionary trajectory has been typically portrayed and what we have learned (and can learn) from a phylogenetic perspective, and suggest that we might revisit some fundamental assumptions about the evolutionary 'progression' of CAM.

Functional diversification of CAM in tropical orchids

S3.2

KATIA SILVERA[1], JOHN C. CUSHMAN[2], KLAUS WINTER[1]

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[1]Smithsonian Tropical Research Institute, Panama; [2]Biochemistry and Molecular Biology, University of Nevada Reno, USA

15:00–15:40

Crassulacean acid metabolism (CAM) is a water-conserving mode of photosynthesis present in 7% of vascular plant species. In tropical epiphytic orchids, CAM is abundant and many species use a combination of CAM and C_3 pathways. To investigate patterns of functional diversification related to the expression of CAM, multiple isoforms of phosphoenolpyruvate carboxylase (PEPC) were sequenced from related orchid species with distinct photosynthetic types based on cDNA clone sampling and mRNA relative abundance. Phylogenetic analysis revealed the existence of two main PEPC lineages in flowering plants, two PEPC lineages within eudicots and three PEPC lineages within the Orchidaceae. Our results indicate CAM-associated isoforms originated from gene duplications and adaptive sequence divergence. Measurements of 24h gas-exchange confirmed photosynthetic pathways and showed that some orchid species with weakly expressed CAM can significantly increase their CAM activity when water stressed and, in some cases, revert to weak CAM upon re-watering, suggesting the presence of a facultative CAM component. When CAM is overlain upon a phylogeny of orchids, C_3 photosynthesis is the ancestral state, and CAM has evolved more than once. A large CAM radiation event was prominent within the Epidendroideae, which provided the majority of extant CAM species today.

Towards an Australian CAM flora: beginning with *Calandrinia*

S3.3

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15:40–16:20

There is no check-list of Australian plant species that express crassulacean acid metabolism (CAM). Here we present continuous whole-plant or leaf gas-exchange surveying for CAM in five *Calandrinia* species (*C. creethae*, *C. polyandr*a, *C. reticulata*, *C. stagnensis* and *C. volubilis*), all five Australian rubiaceous ant-house plants (*Myrmecodia beccarii*, *M. tuberosa*, *M. platytyrea*, *Hydnophytum ferrugineum* and *H. moseleyanum*), and in *Lecanopteris sinuosa*, an ant-house fern. Carbon isotope surveys of components of Australian Apocynaceae (*Hoya* and *Dischidia*), Portulacaceae, Montiaceae, Rubiaceae, Orchidaceae and ferns will be presented. Comparison of isotopic values from herbarium specimens with gas-exchange indicates that any CAM check-list based upon a survey of isotopes will underestimate the prevalence of CAM in the Australia flora. Strong-CAM isotopic signals evident in vines and epiphytes are uncommon in terrestrial succulents. Gas-exchange indicates that, at least in the *Calandrinia* (which number perhaps 70 species) and in ant-plants, the levels of CAM may be low and expression is frequently facultative. Australian members of many genera in which CAM has been detected outside Australia, e.g. *Euphorbia* and *Bulbine*, have yet to be surveyed. Speculation will be entertained as to why strong-CAM appears to be less common in Australian terrestrial succulents than in succulents from the New World and Africa.

Shifty work on CAM: has working the late shift led to a phase shift in CAM ecology and productivity modelling?

S3.4

HOWARD GRIFFITHS, JAMIE MALES, NICK OWEN

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16:50–17:30

Crassulacean acid metabolism (CAM) continues to engender interest because of the need to integrate molecular, biochemical, physiological and ecological contexts. The challenge has been to develop modeling approaches which are predictive, ecologically robust and translational. The presentation will summarize some of the physiological and metabolic insights from laboratory and field measurements associated with the work of this group, and by colleagues at Newcastle upon Tyne. Some of the outputs have already been integrated into a systems dynamics model, which manages to capture the contrasting phases of gas exchange and CAM activity typically associated with leaf succulent and 'stem' succulent morphologies (Owen & Griffiths, 2013). We now need to address tissue succulence in relation to water supply and demand, so as to enhance model predictions, as well as setting phylogenetic relationships in terms of habitat preference and climate space. Recent developments have focused on characterizing water use by contrasting members of the Bromeliaceae and fieldwork on the niche separation of *Aechmea aquilega* and *A. fendleri*. Other efforts in refining the more traditional Nobel environmental productivity index (EPI) will help to improve predictions of CAM biomass production potential and responsiveness to likely climate-change scenarios

Owen NA, Griffiths H. 2013. A system dynamics model integrating physiology and biochemical regulation predicts extent of crassulacean acid metabolism (CAM) phases. *New Phytologist* **200**: 1116−1131.

Ecophysiological traits that influence colonization and survival of epiphytic bromeliads in a changing climate

S3.5

CASANDRA REYES GARCÍA, NAHLLELI CHILPA-GALVÁN, MANUEL CACH-PÉREZ, CELENE ESPADAS-MANRIQUE, MANUELA TAMAYO-CHIM, JOSÉ LUIS ANDRADE, ROGER ORELLANA

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17:30–18:10

We investigated both abiotic and biotic constrain to epiphytic bromeliad species distribution and the ecophysiological traits that enable species specific responses in seasonally dry environments. The study took place in Yucatán, Mexico, using species showing crassulacean acid metabolism (CAM) photosynthesis of known distribution and abundance along a climatic gradient from the drier mangrove forest to the wetter sub-perennial forest. Species showed marked differences in traits such as succulence and carbon uptake, related to their geographic distribution. Under controlled conditions, species from drier environments showed increased stress tolerance in seedlings, compared to species from wetter environments that had increased sensitivity in smaller plants. Seeds may also be subjected to species specific pressures that affect the morphology. Chemical extracts from tree trunks of non-hosts inhibited germination; survival rate increased in seeds germinated in hosts compared to non-hosts. In general, the epiphytes were found to inhabit the lower canopy strata in these stressful environments, where light is attenuated and shade decreases leaf temperature. The findings stress that epiphytic distribution is affected by the identity of hosts, state of conservation of the forest and annual climatic variations. Establishment was the most vulnerable stage in the life cycle. Recent variations in weather patterns, along with habitat destruction, are already threatening the local epiphyte community.

Isotopic ecology and physiology of columnar cacti

S3.6

DAVID WILLIAMS
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Botany, University of Wyoming, Laramie, Wyoming 82071, USA

18:10–18:50

Predicting responses of crassulacean acid metabolism (CAM) plants to globally changing environmental conditions demands an understanding of integrated physiological and metabolic reactions over timescales ranging from seconds to centuries. Our work has focused on isotopic records of physiological change in long-lived columnar cacti. These very long-lived CAM plants record the history of plant responses to climate variability in isotopic patterns of spine tissue built over decades and centuries. The carbon and oxygen isotopic records in spines of the giant saguaro (*Carnegiea gigantea*) of the Sonoran Desert reveal systematic and correlated adjustments in plant water balance and CAM photosynthetic behavior to seasonal and inter-annual change in humidity and precipitation. This talk will explore some of the details of carbon isotope discrimination in saguaro and two other important columnar cacti: *Stenocereus thurberi*, the organ pipe cactus of the Sonoran Desert; and *Echinopsis atacamensis*, the Pasacana cactus of Bolivia. We measured instantaneous carbon isotope discrimination in these species using a customized cuvette attached to a tunable diode laser and gas exchange system. Extremely low internal conductance values were necessary to account for the difference between observed and modeled ^{13}C discrimination in these species. Importantly, variation in nighttime CO_2 uptake rate was strongly correlated with photosynthetic ^{13}C discrimination, suggesting that carbon isotope ratio values in organic matter, such as spine tissue, may record variation in CAM photosynthetic capacity over time. Long-term patterns of ^{13}C discrimination in columnar cacti therefore may provide useful validation for CAM photosynthetic models in complex changing environments.

Session 3 (continued): Evolution, ecology & ecophysiology
Chair: Joe Holtum

Observations on phase-III-CO_2 leakage, CAM idling, and *Jatropha* **S3.7**

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08:30–09:10

Since the C_4-CAM meeting at the University of Illinois at Urbana-Champaign in August last year, new research on three different topics was initiated: (1) Preliminary data show the extent to which net CO_2 loss occurs in illuminated crassulacean acid metabolism (CAM) tissues during phase-III gas exchange; (2) results are discussed from experiments attempting to demonstrate and quantify CAM idling, i.e. the degree of residual CAM activity based upon the recycling of respiratory CO_2 in severely droughted CAM tissues exhibiting close-to-zero net CO_2 exchange during the 24 h cycle; (3) day/night patterns of net CO_2 exchange and tissue acidity are presented for *Jatropha curcas* (Euphorbiaceae), a biofuel species.

Effects of climate change on CAM crops

S3.8

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Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Morelia, Mich., Mexico

09:10–09:50

Agriculture is the productive sector that is most vulnerable to climate change. Indeed, the increase in air temperature and shifts in precipitation regimes projected to occur during the present century will add on to the inherently high uncertainties of an activity that relies on weather. The risk of substantial economic loss and even food insecurity is even greater for developing countries for which agriculture is predominantly rainfed. In particular, for tropical regions a reduction of annual precipitation may be more damaging to conventional agriculture than atmospheric warming. An alternative is to utilize crassulacean acid metabolism (CAM) crops such as *Ananas comosus* and various species of *Agave*, *Opuntia* or other cacti, some of which are drought-adapted. This talk will discuss the potential productivity of selected CAM crops under scenarios of climate change and ponder their viability as sustainable crops.

CAM plants of the Yucatán Peninsula: what have we learned? What else do we need to see? **S3.9**

JOSÉ LUIS ANDRADE, EDILIA DE LA ROSA-MANZANO, EDUARDO CHÁVEZ-SAHAGÚN, CASANDRA REYES-GARCÍA **10:10–10:50**
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The Yucatán Peninsula shows an array of plant communities in a gradient of precipitation, where the drier and short-statured communities are located on the north and the wetter and taller forests are on the south. Accordingly, crassulacean acid metabolism (CAM) plants are all terrestrial in the coastal dunes and become mainly epiphytical and hemiepiphytical in the forests on the south of the peninsula. Within each plant community there is a mosaic of microenvironments, where we have observed that CAM plants perform differently mainly because of differences in water and light availability. For epiphytic orchids in dry forests, environmental and physiological variations are greater among seasons than among microenvironments within the canopy. Although all orchid species show different strategies to cope with drought, small stomata and reduced leaf area are apparently related to abundance. Also, in the driest forest, plant size may be important for epiphytic bromeliads to establish on the soil: individuals of *Aechmea bracteata* can complete their life cycle on the forest floor despite the environmental differences among seasons, especially during the dry season. Environmental regulation and plant size should be taken into consideration to study survival and growth in CAM plants of the dry tropics.

Poster abstracts

** S=speaker abstract; P=poster abstract; bold=presenting author*

Poster abstracts

Poster abstracts are ordered alphabetically by presenting author (underlined).

P1 Capturing the dynamics of CAM: transcriptome and proteome analysis of diel cycle gene expression in *Agave americana*

P. ABRAHAM, H. YIN, G. TUSKAN, X. YANG, R. L. HETTICH

Oak Ridge National Laboratory, Oak Ridge, TN, USA

Of the fresh water used by humans, 70 percent of the total is consumed by agriculture each year and, according to the International Water Management Institute, the amount of water consumed in agriculture is expected to increase by 70–90% by 2050. Therefore, improvements are needed in crop water-use efficiency (WUE). The inherently high WUE of plants with crassulacean acid metabolism (CAM) highlights their potential as a model for improving WUE and sustainable production of biomass in warmer and drier environments. In this study, we define the transcriptome and proteome patterns of CAM gene expression over a 24-hour period in *Agave americana*. By sampling the transcriptome and proteome across the 24-hour period, we have identified and quantified temporal expression of genes that define CAM. For example, the data set describes the temporal-dependent expression of the genes related to carboxylation and decarboxylation processes as well as stomatal regulation across. Interestingly, when correlating the abundance values for the transcripts and proteins across these diurnal expression patterns, there were 4 major types of correlations: strong, delayed, weak, and no correlation. Encompassed within the delayed correlations are genes likely under post-transcriptional and/or post-translational regulation processes, which may underpin the metabolic basis for plasticity.

P2 Evolutionary origins and ecophysiology of CAM photosynthesis in the montane genus *Puya* (Bromeliaceae)

J.D. BELTRAN, J.A.C. SMITH

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK

It is estimated that nearly 50% of all Bromeliaceae (~3000 species) show CAM photosynthesis, a trait that has arisen at least five times independently within this Neotropical family. Most bromeliad genera contain either wholly C_3 or CAM species, reflecting a pattern of ecological niche conservatism at this taxonomic level, but the Andean genus *Puya* contains significant numbers of both CAM and C_3 taxa. To determine which evolutionary and ecological factors have shaped the occurrence of CAM photosynthesis in *Puya*, phylogenetic reconstruction and ecological niche modelling have been performed to reconcile the geographical distribution of the identified CAM and C_3 species. CAM photosynthesis is present in some of the earliest-diverging lineages of *Puya* with a southerly distribution in Chile, but no CAM species are found amongst the more derived taxa occurring north of the Western Andean Portal (WAP) in northern Peru/southern Ecuador. We assess whether the lack of CAM species of *Puya* in the northern Andes is due to absence of suitable habitats, or rather to a biogeographical barrier such as the WAP preventing northerly migration of CAM species. This work provides new insights into the historical and ecological factors determining the occurrence of CAM photosynthesis in these distinctive high-elevation species.

P3 Functional genomics of CAM in the monocot biofuels feedstock crop *Agave sisalana*

P. BUPPHADA, S. F. BOXALL, J. HARTWELL

Institute of Integrative Biology, Biosciences Building, University of Liverpool, Crown Street, Liverpool L69 7ZB, UK

Certain crassulacean acid metabolism (CAM) crops have been recognised as having great potential for the production of renewable biomass from seasonally dry lands. In this project, we are studying the functional genomics of CAM development and light/dark regulation in *Agave sisalana*. The *A. sisalana* transcriptome was sequenced *de novo* using 454 sequencing and assembled and annotated. Semi-quantitative RT-PCR analysis was employed to study the regulation of CAM and circadian clock-related genes in leaf tissues. The transcript level of CAM genes was highest in the mature leaf tip, lower in the young, expanding leaf base and very low to undetectable in the most basal white leaf tissue. Several CAM and clock genes were found to display robust light dark oscillations in transcript abundance. Quantitative Illumina RNA-seq has recently been completed using these different parts of the *A. sisalana* leaf sampled in the light and dark. Comprehensive analysis of the obtained RNA-seq data is now underway to identify novel CAM regulatory genes. This research is funded mainly by Agricultural Research Development Agency (ARDA), Thailand and partly by Biological Sciences Research Council (BBSRC), UK.

P4 The role of the chloroplastic glucose transporter in the operation of CAM in *Kalanchoë fedtschenkoi*

E. CASEY[1], A. BORLAND[1,2], T. TAYBI[1], J. CEUSTERS[1,3], S BOXALL[4], L. DEVER[4], J. HARTWELL[4]

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Starch degradation in the leaves of crassulacean acid metabolism (CAM) plants is thought to primarily occur via the phosphorylytic pathway, and the hydrolytic pathway is believed to serve as a secondary route for starch breakdown. Here it is hypothesized that a chloroplastic glucose transporter, involved in the hydrolytic pathway, is fundamental for the process of starch breakdown, and as such is needed for optimal CAM activity. A knock-down mutant of the chloroplastic glucose transporter (GlcT 12) was generated for the CAM plant *Kalanchoë fedtschenkoi.* The GlcT12 mutant had significantly impaired growth compared to that of wild type. Data for leaf succulence, chlorophyll content, 24 h and circadian patterns of leaf gas-exchange also showed differences between the mutant and wild type. In the GlcT12 mutant, gas exchange data showed an impact upon the daytime phases of CAM, with enhanced release of CO_2 from the mutant compared to wild type, indicating incomplete closure of stomata during the day. Increased maltose and sucrose concentrations in leaves of the mutant additionally infer the use of the hydrolytic pathway to generate maltose for export from the chloroplast when glucose export is curtailed.

P5 The comparative transcriptomic analysis of ten species in five subfamilies of Orchidaceae

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Orchids, belong to family of Orchidaceae, are a group of highly diverse monocotyledonous plants that are globally distributed in tropical, subtropical to temperate climates. The Orchidaceae species display a spectacular array of adaptations and rapid speciation that are linked to several innovative features, including the presence of crassulacean acid metabolism (CAM) and colonization of epiphytic habitats. To better understand the role of CAM of Orchidaceae, we generated high quality transcriptome sequence of ten representative species in five subfamilies of Orchidaceae that cover diverse habitat from terrestrial to epiphyte. In this paper, we report the comparative transcriptomics among these 10 species and with those in other CAM plants like agaves. The evolution and expression of the enzyme phosphoenolpyruvate carboxylase kinase (PPCK), phosphoenolpyruvate carboxylase (PEPC), malate dehydrogenase (MDH), NADP-malic enzyme (NADP-ME), and pyruvate phosphate dikinase (PPDK) are particularly investigated in these transcriptome data. Our work presents a comprehensive transcriptome resource for ten orchid species and provides insight into their biology and evolution. These resources also serve as a foundation for further elucidation of orchid development, developmental morphology, molecular developmental genetics, and molecular evolution.

P6 Leaf anatomy and CAM cycling in the understory plant *Sedum ternatum*: unusual adaptations of a forest floor succulent

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Sedum ternatum, a prostrate succulent native to Appalachian deciduous forests, belongs to the minority of plants that exhibit some degree of crassulacean acid metabolism (CAM) photosynthesis yet grow in shaded environments. We hypothesize that in the understory, the high water use efficiency and facultative CAM cycling of this species permit adaptation to intermittently dry microhabitats that many shade plants would not tolerate, including shallow soil atop mossy boulders and rapidly draining slopes. Because the leaf succulence underlying this high WUE may hamper CO_2 conductance in a CAM intermediate relying primarily on C_3 photosynthesis, we hypothesize that *S. ternatum* circumvents mesophyll conductance limitations through features including amphistomaticity, and by exploiting high CO_2 concentrations arising from root respiration near the forest floor. We are currently measuring leaf thickness, intercellular air spaces, and stomatal density, and designing experiments to test (1) the extent to which CAM cycling enhances survival during prolonged drought; (2) whether CAM idling can be induced; (3) whether *S. ternatum* has an exceptionally efficient response to sunflecks; and (4) how it responds to naturally and artificially enhanced CO_2. Our results will clarify how this unusual species balances tradeoffs between its primary dependence on C_3 photosynthesis and leaf anatomical features typically associated with CAM.

P7 Tonoplast function in CAM in *Kalanchöe fedtschenkoi*

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Coordinated import and export of malate from the vacuole is key to the temporal separation of the fixation and re-release of carbon dioxide in crassulacean acid metabolism. Malate import during phase I in the dark is mediated by an inward-rectifying malate/H+ antiporter, posited as an aluminium-activated malate transporter (ALMT). Malate export beginning in phase II at dawn is mediated by a distinct malate transporter thought to be the tonoplast dicarboxylate transporter (TDT). RNAi lines of the crassulacean acid metabolism (CAM) model species *Kalanchöe fedtschenkoi* in which *almt4*, the most abundant ALMT transcript in this species, is knocked down display decreased malate accumulation at the end of the dark period. Furthermore, extracted malate levels at the end of the light period remain elevated suggesting that export of malate from the vacuole is dependent upon the rate of import in the preceding phase I. Lines overexpressing full-length *tdt* display decreased accumulation of malate, suggesting that aberrant expression of *tdt* promotes premature export of malate from the vacuole.

P8 An innovative cloning platform for pathway engineering in living organisms

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There has been increasing interest in transferring crassulacean acid metabolism (CAM) to C_3 crops using synthetic biology for genetic improvement in water-use efficiency. Here we describe a novel synthetic biology method, called TNT-cloning, that *in vitro* assembles DNA parts in a simple, fast, efficient, flexible and, if desired, automated manner. Our system combines all cloning elements into one single universal library allowing a pre-determined assembly without the need for linkers/adaptors, which ultimately creates a scar-free product. Also, there are no sequence homology requirements and up to 3 fragments can be cloned at once in as little as one hour using our optimized TNT-buffer. The binary plasmids support secondary and tertiary assembling, which require minimal re-cloning of fragments and makes the final construct reusable as well as ready for plant transformation. We demonstrated the system is wholly functional by cloning, assembling and testing several fragments from 30bp to >4kb. Because this technique is compatible with isothermal (Gibson) assembly, virtually any fragment can be used as an element in the library and circularized without the need to carry the binary-backbone, expanding the technology use to other systems. This novel cloning platform will greatly facilitate the engineering of different pathways, like CAM into C3.

P9 Characterization of the transcriptional regulation of CAM in *Kalanchoe laxiflora* mesophyll tissue

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Increasing bioenergy crop production is vital to meet future energy demand. Much of the world, however, is too arid for biomass production. Engineering biofuel crops for growth in these environments would greatly increase biomass yields. Introducing crassulacean acid metabolism (CAM) into these crops would confer the improved water-use efficiency required for growth in arid lands. CAM is strongly reliant on rhythmic circadian clock-dependent mRNA regulation. Several putative CAM-related transcription factors have been identified whose mRNA expression fluctuates with circadian phase in a manner distinct from that observed in C_3 photosynthesis. To elucidate the temporal regulation of CAM in mesophyll tissue as an initial step in engineering CAM into C_3 crops, target genes of these transcription factors will be identified. To accomplish this, INTACT (isolation of nuclei tagged in specific cell types) will be employed to purify mesophyll nuclei from transformed lines of the obligate CAM species *Kalanchoe laxiflora* which express epitope-tagged transcription factors of interest. INTACT utilizes mesophyll-specific nuclear envelope biotin labeling followed by affinity isolation of biotinylated nuclei. Chromatin bearing tagged transcription factors will be immunoprecipitated from purified mesophyll nuclei. Lastly, ChIP-seq will be conducted to identify transcription factor target genes.

P10 Differential competence for CAM expression along leaf blades at different ontogenetic phases in *Vriesea gigantea* under drought

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Vriesea gigantea is a tank-epiphytic bromeliad with a high uptake capacity of water. Besides, isolated leaves of this species display a certain degree of crassulacean acid metabolism (CAM) when treated with hyperosmotic solutions. This study investigated the competence for CAM expression in leaves at different ontogenetic stages along the *V. gigantea* rosette when exposed to several watering regimes. Measurements of water potential, relative water content (RWC), and key components of CAM machinery were comparatively studied in three leaf groups (younger, intermediate, and mature leaves) of 5-year-old plants subjected to daily watering or water withdrawal for different periods. All leaves were also analyzed as regards the blade portions (apex, middle and base). Water potential decreased sharply in intermediate leaves after 14 days of drought while only mature leaves showed reduced RWC, indicating a possible water remobilization from older to younger leaves. At 21th day of water withdrawal the water potential remained stable whereas all leaf tissues displayed significant RWC decrease. This longer drought period also induced the up regulation of PEPC/MDH activities and nocturnal accumulation of organic acids in the middle portion of younger and intermediate leaves. Thus, water availability modulates CAM expression in a tissue-compartmented manner along the *V. gigantea* rosette. Support: FAPESP

P11 Baseline phenotyping of *Populus* candidates for CAM bioengineering

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The primary goal for 'Engineering CAM Photosynthetic Machinery into Bioenergy Crops for Biofuels Production in Marginal Environments'[1] is to produce *Populus* transformants with a crassulacean acid metabolism (CAM)-like phenotype to potentially increase water use efficiency. To facilitate bioengineering of CAM machinery into a C_3 species, baseline measurements are needed to select the most appropriate genotype to target as host for CAM: that is, to identify a poplar species with a more 'CAM-like' phenotype that is also amenable to genetic transformation. We are looking at leaf anatomical traits relevant to the operation of CAM (e.g. leaf thickness, cell size, % intercellular air space, stomatal size and density) in different *Populus* genotypes to establish a baseline expectation on the genetic limits of modification. We present preliminary data on specific leaf area, stomatal density, cell size and stomatal conductance in several *Populus* species/genotypes that fall within the range of values established for two weak CAM-inducible species of *Clusia*. We will discuss observed genotypic differences in these traits which will allow us to predict theoretical potential for leaf water loss in an engineered CAM-inducible poplar.

[1]Cushman *et al*. 2012. DE-FOA-0000640 - Genomic Science: Biosystems Design to Enable Next-Generation Biofuels. GRANT 11099732.

P12 Structure-based functional and phylogenetic analysis of nuclear-encoded photosynthetic proteins in *Agave americana*

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Protein structural information is very important for understanding the biological function of a protein and evolutionary relationship among homologous genes (i.e. paralogs within a species, orthologs among species). Transcriptome sequencing and proteomics studies of the crassulacean acid metabolism (CAM) plant *Agave americana* revealed that many nuclear-encoded protein subunits, which belong to or are associated with the photosynthetic super-complexes photosystem I and II (PSI/II), cytochrome b_6/f ($Cytb_6f$) as well as the ATP synthase, exhibit peak expression during the night. To understand the roles of these proteins in *Agave*, we performed 3D structure modeling and structure-based phylogenetic analysis. Specifically, 3D modeling was used to construct the structures of the photosynthetic proteins with peak expression during the night, and identify their localization in the photosynthetic chain assembly. Our structure-based gene ontology analysis predicted the role of each protein subunit in CAM photosynthesis. Furthermore, a structure-based phylogenetic tree was constructed to study evolutionary relationship among orthologs in cyanobacteria (*Thermosynechococcus elongatus*), C_3 (*Arabidopsis thaliana*) and C_4 (*Zea mays*) plants. The findings from this study may shed lights on the molecular basis of the CAM pathway in *Agave*.

P13 Variability in the contribution of CAM in populations of *Portulacaria afra* (L.) Jacq. in the Eastern Cape

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Portulacaria afra is endemic to semi-arid areas of the Spekboom Thicket in the southeastern Cape of South Africa. As a facultative CAM species which can utilize both day and nighttime CO_2 uptake, *P. afra* is well adapted to the region where rainfall is limited and sporadic. This photosynthetic flexibility of both day and nighttime CO_2 uptake for carbon gain and increased water use efficiency allows *P. afra* to be a dominant species in some regions. *P. afra* communities are able to sequester carbon at a rate typical of more mesic environments even under water limited conditions. Little research has been performed on the contribution CAM in different populations. Carbon isotope composition of leaf samples were analyzed from various populations ranging from the coast to elevations of 1400 m. Carbon isotope values ranged from -16.1 ‰ in Plutosvale to -21.1 ‰ to -23.2 ‰ in Port Alfred and Grahamstown populations, respectively. The carbon isotope composition values indicate variable contributions of the CAM pathway from approximately 22% to 50% to the overall growth in the various populations. *P. afra* illustrates a large phenotypic plasticity in the various populations and may indicate genotypic differences which may be valuable in reestablishment and carbon sequestration.

P14 Genome assembly of the common ice plant (*Mesembryanthemum crystallinum* L.) a facultative CAM and halophytic plant model

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The common or crystalline ice plant (*Mesembryanthemum crystallinum* L.) is a facultative crassulacean acid metabolism (CAM) and halophytic model species that can switch from C_3 photosynthesis to CAM following high salinity or water-deficit stress treatments to reduce transpiration during the day and improve water-use efficiency. To reveal the molecular basis of halophytism and CAM, the ice plant genome is being sequenced and analyzed through hybrid assembly approaches. Results from three different sequencing platforms, Illumina Mate Pair (MP), Illumina Paired End (PE) and PacBio (PB) long reads, are being used to sequence the genome.. Two strategies will be applied for hybrid genome assembly: 1) contigs from PB reads will be error-corrected by PE data followed by scaffolding using MP reads, 2) contigs build from PE reads will be combined with scaffold assembly using MP reads, followed by gap filling with PB reads. The completed ice plant genome sequence will be the first genome sequence of a facultative CAM species and will facilitate our understanding of the genomic basis of salt tolerance and water-use efficiency that allows CAM species to inhabit semi-arid and arid environments.

P15 A new look at an old method for isotopically characterizing low-level CAM activity

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Constitutive crassulacean acid metabolism (CAM) plants are closely related to and phylogenetically interspersed with known CAM-'intermediates'. Hypotheses posit that these CAM-like phenotypes (CAM-cycling and facultative CAM) may be transitional states or represent intermediate phenotypes between C_3 and full CAM photosynthesis. To understand the evolutionary significance of these CAM-intermediates, we need to characterize the distribution of C_3, CAM-like, and CAM photosynthesis across lineages that include the full spectrum of C_3 - CAM phenotypes. The $^{13}C/^{12}C$ ratio is commonly used to distinguish between C_3, facultative CAM and constitutive CAM, however, this measure cannot be used to differentiate CAM-cycling. The hydrogen isotopic value of cellulose nitrate (dD), which is generated from plant cellulose, has been shown to detect CAM-cycling. This method has not been widely adopted because generating cellulose nitrate is laborious, dangerous, and due to differences in the D/H ratio of source water, difficult to evaluate. In this study we replaced cellulose nitration with water-vapor equilibration thus advancing the resurrection of this method as a potentially useful tool to understand the phylogenetic distribution of CAM-cycling behavior across many species. Combining this method with ^{13}C analyses, we present a preliminary attempt to characterize photosynthesis type across taxa known to use C_3, CAM-like and CAM photosynthesis.

P16 Lability of CAM expression in the homoploid hybrid *Yucca gloriosa*

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Yucca gloriosa is a homoploid hybrid cross between *Y. aloifolia* and *Y. filamentosa*, a crassulacean acid metabolism (CAM) and C_3 species respectively. All three species are sympatric in the southeastern United States, overlapping particularly along the coast. Earlier work indicated that *Y. gloriosa* utilizes an intermediate form of CAM photosynthesis, with nighttime acid accumulation but without concurrent nighttime CO_2 uptake. The intermediacy of CAM expression in these hybrids paralleled molecular work, which showed *Y. gloriosa* segregates for parental alleles in the wild. While CAM has been in the spotlight recently for its evolutionary novelty and potential importance for improving drought tolerance of food and energy crops, little is still known about how a plant transitions to CAM. Using the system found in *Y. gloriosa*, we explored variation in the ability of genotypes from across the range to perform CAM under drought stress. We find that while the two parental species are fixed in their photosynthetic pathway, the hybrid shows segregation for the ability to utilize CAM. Here we present preliminary physiological and expression data, as well as future plans for studies using the intermediate *Yucca* species.

P17 Facultative CAM: does water availability influence photosynthetic mode shifting in *Agave utahensis*?

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Several succulent species exhibit considerable plasticity in crassulacean acid metabolism (CAM) expression, shifting from CAM to C_3 photosynthesis under changing environmental conditions. Such mode shifting could enable plants native to harsh desert environments, such as agaves, to take advantage of seasonally abundant water for growth and development. Three-year-old *Agave utahensis* plants were subjected to constant soil moisture levels ranging from drought to well-watered conditions. Whole plant photosynthetic rates were measured over 24-hour periods for several days. We observed differences in the rate of CO_2 uptake across moisture treatments. *Agave utahensis* exhibits the potential to shift photosynthetic rates to adjust to variations in soil water availability, which may have implications for its survival and productivity in its native environment.

P18 Stable isotope physiology of stem succulents detect functional tradeoffs across a broad range of volume to surface area ratio

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Volume-to-surface area ratio across stem succulent taxa varies by almost two orders of magnitude. We examined the intrinsic physiological tradeoffs across diverse stem morphologies in three divergent evolutionary groups where stem succulence is common: Cactoideae and Opuntioideae (Cactaceae) and Euphorbiaceae. We predicted that variation in photosynthetic gas exchange, growth and response to stress would be (1) highly constrained by stem volume-to-surface area ratio (V:S), and (2) detectable in the stable isotope ratios of plant tissues. Stable isotope ratios were measured in the spines and prickles of over 60 succulent species occurring in a well-watered common garden setting at the Desert Botanical Garden in Phoenix, AZ. Across all major groups, tissue $\delta^{18}O$ increased with stem V:S indicating that the turnover time of internally stored water increased with V:S. Both $\delta^{13}C$ and $\delta^{2}H$ also increased with V:S but patterns were specific to the major taxa reflecting various levels of crassulacean acid metabolism (CAM) strength; Cactoideae stems had the strongest CAM function, followed by Opuntioideae and Euphorbiaceae, respectively. Taken together, these data suggest that functional tradeoffs associated with stem V:S are similar across broad evolutionary groups, despite differences in CAM strength and photosynthetic physiology.

P19 Promoter characteristics of CAM related genes

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To study the molecular basis of circadian regulation in CO_2 fixation of crassulacean acid metabolism (CAM) plants, the structure of the 5'-flanking sequences for CAM-related genes were analyzed. We have determined the nucleotide sequences of about 3 kbp from the respective genes of phosphoenolpyruvate carboxylase (PEPC), PEPC kinase (PEPCK) and NADP-malic enzyme (NADP-ME). These genes contained the core site required for binding of Dof proteins in the region. The region of PEPC from *Kalanchoe pinnata* (*KpPEPC*) included two types of sequence motif which are related to the transcriptional regulation of circadian clock genes. It also contained the sequence motif related to mesophyll-specific expression of the gene for PEPC in C_4 plants. The promoter region of PEPCK from *K. fedtschenkoi* (*Kfppck*) and NADP-ME from *Mesembryanthemum crystallinum* (*Mod1*) contained the sequence motif related to the transcriptional regulation of circadian clock genes and salt-, drought- and ABA-inducible genes. The promoter region of *Ppc1*, a CAM specific PEPC isogene from *M. crystallinum* included sequence motif of the promoters for circadian-regulated genes and ABA-inducible genes, but not *Ppc2*, the other isogene of PEPC that may have a nonphotosynthetic anapleurotic function. We will discuss the factors contributing to the differences in gene expression of CAM-related genes in C_3 and CAM plants at transcriptional level.

P20 Investigating the viability of *Agave americana* as a potential bioenergy feedstock

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Agave americana is an example of a succulent that exhibits high water use efficiency (WUE), enabling increased water conservation and the ability to thrive in arid and semi-arid lands, which avoids of the issue of further land degradation and ecosystem displacement. We aimed to assess the potential of *Agave americana* as a bioenergy crop and determine whether irrigation is required for a commercially viable yield. A field experiment established contains replicated plots receiving four irrigation treatments: control (rainfall only), 200, 400, and 800 mm annually. Two years after establishment, biomass measurements were obtained and gas exchange rates assessed between dusk and dawn. The control treatment resulted in 46% lower mean biomass than the 800 mm y^{-1} treatment. Plots receiving 800 mm y^{-1} irrigation showed a maximum CO_2 uptake of 27.2 µmol $m^{-2}s^{-1}$, while plants in the control plots exhibited 19.9 µmol CO_2 $m^{-2}s^{-1}$. WUE ranged from 7.93 kg $ha^{-1}mm^{-1}$ in 800 mm y^{-1} irrigation to 10.96 kg $ha^{-1}mm^{-1}$ in the control, exceeding that of conventional cropping systems in the region (e.g. cotton crops require 1,674 mm y^{-1} irrigation with 1.04 kg $ha^{-1}mm^{-1}$ WUE). These results indicate that water might be conserved with the adoption of *Agave* as a cropping system.

P21 Identification and testing of circadian clock-controlled and drought-inducible promoters from *Arabidopsis* and *Mesembryanthemum* for CAM biodesign

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The common or crystalline ice plant (*Mesembryanthemum crystallinum* L) performs C_3 photosynthesis, but when subjected to water or salt stress conditions switches into crassulacean acid metabolism (CAM). CAM is an adaptation to reduce water loss through stomata by reversing the diurnal rhythm of CO_2 fixation from day to night, which results in improved water-use efficiency (WUE). Introducing the CAM pathway into C_3 plants is expected to confer improved WUE in order to expand crop production into semi-arid or arid regions. The introduction of the CAM pathway into C_3 plants such as *Arabidopsis thaliana* will require the proper temporal and mesophyll expression patterns of multiple transgenes in order to reconstitute CAM. However, repeated use of the same promoters within multi-gene constructs should be avoided to minimize the chance of repeat-induced silencing or recombination events. In this study, we have retrieved various kinds of promoters from *Arabidopsis* and ice plant with leaf-specific-, drought inducible-, and circadian expression patterns, which are expected to provide optimal expression of multi-gene CAM modules in the *Arabidopsis*. The expression patterns of candidate promoters from *Arabidopsis* and *Mesembryanthemum* will be confirmed under control and water-deficit stress conditions using the *GUS*/*LUC* dual reporter in transgenic *Arabidopsis* plants.

P22 Understanding the role of hydrophysiology in adaptive radiations of the Bromeliaceae

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The Bromeliaceae is a Neotropical monocot family that displays remarkable diversity in terms of morphology, physiology, and ecology. Several lineages have undergone dramatic adaptive radiations since 10-15 Mya following colonisation of niche spaces created by orogeny in the Andean cordillera. The evolution of a suite of key innovations has been linked to increased diversification rates, including the tank-forming habit, specialised absorptive trichomes, epiphytism, and crassulacean acid metabolism (CAM). Relatively little attention has been paid to the contribution of hydrophysiological traits to adaptations of bromeliads to specific environments. We have developed a phylogenetic framework for the investigation of trends in hydrophysiological character evolution and their correlation with ecological characters. Taxa sampled from across the phylogeny of the family and representative of the diversity of ecological types exemplified by Pittendrigh's seminal classification have been characterised using pressure-volume analysis, xylem vulnerability curves, and a sophisticated version of the evaporative flux method for measuring hydraulic conductivity. Significant differences in quantified hydraulic properties were found between taxa that we related to their distributions and habitat preferences. Evidence of convergent evolution of hydraulic properties in different ecological types suggests that hydrophysiology is an important factor that must be optimised in the exploitation of novel niches.

P23 Urea as a possible source of CO_2 for malate synthesis in detached leaves of *Vriesea gigantea* under water shortage

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Under water shortage condition, stomata are usually closed and the influx of atmospheric CO_2 into the plant is limited. Since urea is hydrolyzed by urease into ammonium and CO_2, it could be an important CO_2 source for plants during water limitation. Previous studies have shown the preference of *Vriesea gigantea* (Bromeliaceae) for urea as nitrogen source. Nevertheless, it is not yet known whether under water shortage this bromeliad would display an even more expressive preference for urea (reflected by an increased activity of urease). Therefore, after an 8 day-period of water deficit imposed by using a 30% PEG solution, detached leaves of *V. gigantea* were treated with urea, followed by measurements of urease activity and CO_2 accumulation by cytolocalization. Besides, for characterizing the photosynthetic state of the leaf, the relative water content, nocturnal organic acid storage, gas exchange, and the phosphoenolpyruvate carboxylase activity were measured. The results indicated that apical portions of detached leaves of *V. gigantea* can express CAM under water shortage. Moreover, the apical portion of leaves kept in PEG and urea showed higher urease activity and CO_2 accumulation at night, suggesting that the CO_2 buildup from urea hydrolysis might represent an important carbon source for malate synthesis. Supported by FAPESP.

P24 Metabolic engineering of *Opuntia ficus-indica* for use as a low water use biofuel feedstock

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Global climate change will require drought tolerant crop production platforms. Increased biofuels production will demand crops that possess reduced water and nutrient requirements for utilization of semi-arid lands. High productivity and water-use efficiency makes prickly pear cactus (*Opuntia ficus-indica*) a promising biofuel production platform. The overall goal of this program is to reengineer *Opuntia* to produce and store lipids in its cladodes and fruit instead of storage carbohydrates. The major tasks of the project include (1) using next-generation and single-molecule sequencing to sequence the *O. ficus-indica* genome and transcriptome, and (2) developing innovative strategies to increase lipid production and storage in *Opuntia* cladodes using candidate gene modifications. Gene constructs have been designed with constitutive, mesophyll-specific and drought-inducible promoters to trigger lipid production under post-harvest conditions. Sequencing has been completed for Illumina-based RNA-seq analysis of plants grown under well-watered and water-deficit stress conditions and results of the analysis will be presented. Genomic DNA samples for Illumina- and PacBio-based sequencing have been isolated and submitted for sequencing. For oleogenic reprogramming of *Opuntia*, multiple candidates modeled after *Arabidopsis* genes have been selected. Overexpression of diglycerol acyltransferase (DGAT), structurally-stabilized oleosin, and WRINKLED1 (WRI1) were chosen and combinatorial strategies for their use will be outlined.

P25 Abscisic acid production in leaves of different age in *Guzmania monostachia* with CAM up-regulated by drought

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Guzmania monostachia is a tank bromeliad capable of up-regulating crassulacean acid metabolism (CAM) in response to water deficit. Moreover, there are differences in CAM expression along the length of the leaves and between leaves of different ages, with younger leaves performing stronger CAM. When this plant is droughted, there also seems to be a remobilization of water from the oldest to the youngest leaves. Therefore, we believe that the different groups of leaves may perceive the water shortage differently. ABA is plant hormone known to act in environmental stresses and may be a signal worthy of investigation. In order to achieve this, ABA content, PEPC transcript accumulation and tissue water were measured in droughted versus well watered plants. After the treatments, the leaves were divided in three groups, according to their position in the rosette. Our results showed that the production of ABA in response to drought was higher in older leaves, while in the younger ones ABA contents showed much less variation. Younger leaves, however, also expressed CAM conspicuously when water was withheld. Our results indicate that younger leaves may not perceive the water shortage directly, but respond to signals originated in the older leaves. Supported by FAPESP.

P26 Examination of photosynthetic genes across the Portullugo using NGS targeted sequence capture

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Within the Portullugo clade in the Caryophyllales, which includes Cactaceae, Portulacaceae and related families, and Molluginaceae, both CAM and C_4 photosynthesis have evolved multiple times. These plants provide an opportunity to investigate the evolution of photosynthetic physiology in relation to adaptation to the desert and dryland habitats where they occur. To this end, we are reconstructing the phylogeny of the group using targeted sequence capture. This technique uses next-generation sequencing to obtain the sequences of hundreds of loci for phylogeny inference while simultaneously offering opportunity to determine divergence among genes associated with CAM and C_4 photosynthesis across lineages. We will thus be able to determine the changes in coding regions associated with one or the other photosynthetic pathway and establish whether patterns of selection changed concurrently with a shift in photosynthetic physiology.

P27 A system dynamics model integrating physiology and biochemical regulation predicts extent of CAM phases

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Crassulacean acid metabolism (CAM) was modelled by taking a system dynamics (SD) approach. SD considers that the interplay between elements of a complex system have the capacity to explain counterintuitive non-linearities arising from ostensibly simple inputs. This allowed measured physiological constants to be quantitatively related to CAM expression. Simulated CAM-expression for Kalanchoë daigremontiana displayed strong correlation with measured gas-exchange and malic-acid accumulation (R^2=0.931, 0.852 respectively). The four-phases of CAM-expression were further resolved into parameters that rate-limit carbon-uptake over the diel-cycle. A series of sensitivity analyses identified parameter-manipulations that could simulate stem-succulent CAM-expression (phase I bias, near-elimination of phases II, III, and IV gas exchange signatures). Simulated carbon-uptake for A. tequilana displayed strong correlation with measured data (R^2=0.950).

P28 Establishment of transformation capabilities for CAM engineering

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Crassulacean acid metabolism (CAM), a photosynthetic adaptation found in close to 6% of higher plants, maximizes water use efficiency (WUE) relative to C_3 and C_4 photosynthesis. Evidence that CAM has emerged from C_3 photosynthesis in diverse plant lineages through convergent evolution indicates that engineering CAM in C_3 plants is feasible. Engineering CAM photosynthetic machinery into potential C_3 bioenergy crops like *Populus* can significantly enhance WUE, thus allowing for productive cropping of marginal lands with minimal inputs. To facilitate both the functional characterization of CAM genes and the transfer of CAM genes into C_3 species, genetic transformation systems for multiple species are being explored. In vitro shoot cultures of *Agave tequilana*, *Clusia minor*, *Kalanchoë laxiflora*, and *Populus* spp. were established as representative species of obligate, facultative, and non-CAM source material. Using both established and novel in vitro shoot regeneration protocols, biolistic and *Agrobacterium*-mediated transformation methods are being tested with marker genes as platforms for engineering CAM traits and validating gene modules with down-regulation and over-expression strategies. These initial successes with in vitro culture and transformation create the foundation for implementing a multi-gene engineering system to induce CAM from C_3 photosynthesis in *Populus* under drought conditions.

P29 CAM induction in leaves of the bromeliad *Guzmania monostachia* by nutrient deficiency and water deficits

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Leaves of *Guzmania monostachia*, an epiphytic facultative C_3–CAM (crassulacean acid metabolism) bromeliad, when subject to water deficit have been shown to perform stronger CAM photosynthesis in the apical compared with the basal portion of the leaf blade. To investigate the possible interaction of nutrient deficiency and water deficits in CAM induction, detached leaves of *G. monostachia* from adult plants were subjected to treatment for seven days with 30% PEG 6000 plus modified Knudson solution deficient in specific nutrients (N, P, K, or Ca). A further experiment was conducted to test the influence of alternative nitrogen sources on CAM expression by supplying detached leaves with either 5 mM NO_3^- or NH_4^+ in modified Knudson solution in the presence of 30% PEG 6000. To assay CAM activity, nocturnal malate and citrate contents were measured together with extractable PEPC, MDH and PEPCK activities in both apical and basal leaf portions, in combination with determinations of relative water content and chlorophyll and carotenoid contents. We observed that nitrogen deficiency and water deficits were the most important signals for CAM induction in the apical portion of leaves of *G. monostachia*, and furthermore that treatment with NH_4^+ induced higher CAM expression than treatment with NO_3^-.

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P30 Constructing approximate scale-free and small-world networks

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Co-expression networks have become standard tools in the systems biology toolbox to examine possible functional relationships in whole-organism studies. A co-expression network is a simple node-edge graph in which nodes represent elements such as genes or proteins, and are connected by an edge if the two nodes are deemed similar by their expression values across all measured experiments. These networks provide simple yet effective models of putative gene or protein interactions and potential functional groupings. Current methods often use parametric metrics, a misleading default because most high-throughput data is not normally distributed. To provide statistically sound network construction, our tool includes parametric and non-parametric similarity measures to compensate for various types of data distributions. Biological networks are known to have approximately scale-free and small-world structure, thus we believe it is imperative that any co-expression network model follow these two properties, a feature often not provided or supported by current available methods. By allowing the network construction to be governed by these properties, our networks are guaranteed to be both scale-free and small-world. The algorithm is being implemented into R, a popular Open Source high-level statistical programming language, with minimal effort required from the user. Input requirements consist only of a text file of quantified expression measures, and the choice of similarity metric to be used for measuring similarity between expression profiles. The output is a simple text file indicating connections between all nodes in the expression data file.

P31 Spatial patterns of drought-induced CAM expression along *Guzmania monostachia* leaves under different nutrient availability

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Plasticity in crassulacean acid metabolism (CAM) expression is mainly under environmental control, especially nutrient and water availability. Epiphytic-tank bromeliads usually show functional compartmentalization along their leaves with apical and basal portions being specialized in photosynthesis and water/nutrient absorption, respectively. Guzmania monostachia is an epiphytic-tank bromeliad that up-regulate the CAM pathway under water shortage and the drought-induced responses can vary along the leaves. We investigated the diel fluctuation of CAM expression in different leaf portions of *G. monostachia* subjected to presence or absence of a weekly-supplied nutrient solution during one month, following by treatments with either daily watering or water withdraw for seven days. Water loss was more conspicuous in the base of the drought-treated leaves (with or without nutrition). Besides, the apical and middle portion of all drought-treated leaves showed an increasing trend of water loss, nocturnal accumulation of malate, and higher levels of proteins and PEPC activity. However, the apical portion of non-nourished leaves lost relatively less water than other leaf portions and displayed the drought-induced CAM up regulation. Curiously, the equivalent responses of the fertilized, drought-treated leaves were observed in the middle portion, indicating that nutrient availability modulated CAM expression in a tissue-compartmented pattern along the drought-treated leaves. Supported by FAPESP.

P32 Seasonal variation in microclimate, plant phenology, and leaf physiology for *Carpobrotus edulis* and *Dudleya edulis* in the San Clemente coastal bluff vegetation

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Carpobrotus edulis (Aizoaceae) and *Dudleya edulis* (Crassulaceae) are co-dominant succulent perennials in the coastal bluff vegetation of Southern California. Previous studies indicate that *Carpobrotus edulis* exhibits inducible crassulacean acid metabolism (CAM) when exposed to controlled drought treatments. However, this plastic drought response is poorly documented for this species under natural field conditions. Previous studies for various species in the genus *Dudleya* indicate reliance on either constitutive CAM or inducible CAM. But photosynthetic pathway utilization has not been documented for *Dudleya edulis*. To further understand the ecophysiology of each species, monthly measures of microclimatic conditions (soil moisture, near-ground air temperature and solar radiation), plant phenological state (dormant, vegetative, reproductive), and leaf physiological state (water content, nocturnal acidification, and carbon isotopic composition) for selected, established plants inhabiting the near-shore bluffs at the San Clemente State Beach were assessed monthly for two full years. Microclimate data indicated the plants experienced typical Mediterranean climate conditions with long, warm, bright, dry summers. Both species flower during the spring. *Carpobrotus edulis* plants were evergreen and grew throughout the year, whereas *Dudleya edulis* plants were semi-dormant during the late summer months when drought was most extreme. Carbon isotope analyses and leaf nocturnal acidification assays indicated that *Carpobrotus edulis* used C_3 photosynthesis year-round, whereas *Dudleya edulis* relied exclusively on CAM. Both of these co-dominants species employ different combinations of growth phenology and photosynthetic pathway use under common sets of dynamic environmental conditions. Neither species supported simple theories regarding the ecological significance of C_3, CAM, and inducible CAM for seasonal drought tolerance.

P33 The CAM plant lineages of planet Earth

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Crassulacean acid metabolism (CAM) represents one of the most striking examples of a biochemical adaptation known in the plant kingdom. Extensive ecological, physiological and taxonomic work over several decades has resulted in identification of thousands of CAM species amongst the terrestrial succulents typical of semi-arid environments and tropical epiphytes occupying microclimatically exposed habitats, together with a number of submerged aquatic macrophytes that exhibit CAM-like biochemistry. Altogether, it has been estimated that CAM species may comprise over 6 % of all vascular plants, but this figure is due for reappraisal in the light of recent research. Significant advances in phylogenetic research mean that it is now possible to assess with greater precision the number of independent lineages in which CAM photosynthesis has arisen. This is relevant to attempts to understand the evolutionary processes by which complex physiological traits arise, and also prompts comparison with analyses of the multiple origins of C_4 photosynthesis. We thus present an updated inventory of the genera of vascular plants currently known to show CAM photosynthesis, together with a breakdown of the number of independent lineages in which this trait has evolved. We invite participants in the symposium to highlight hitherto understudied lineages in which further research may reveal the existence of novel CAM species.

P34 Adaptation to deep shade: evolution of tropical cacti

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The obligate crassulacean acid metabolism (CAM) family, Cactatceae, has a myriad number of species representing almost all known growth forms over a wide geographic range. A shift from high light tolerant, deep desert species of cacti to high light inhibited species of epiphytic tropical cacti suggests an evolutionary spectrum of light adaptations correlatory with an increase in an outside structural support requirement. A greenhouse study examined photosynthetic parameters of over 40 species of tropical and desert cacti of varying growth forms using fluorescence techniques. Relevant parameters derived from the measures include maximum electron transport rate (ETR), saturating photosynthetically active radiation (PAR) and non-photochemical quenching (NPQ). Initial results show a negative correlation between maximum ETR and the amount of outside growth support. Suggesting an adaptation in the cacti in low light, the data is currently being placed in a phylogenetic context to examine the effects of phylogenetic placement, extant geographic range and effects of PAR and rainfall; thus allowing us to predict the evolution of growth forms in the Cactaceae.

P35 Transcriptome sequencing and RNA-seq mRNA expression profiling in the facultative CAM model species *Mesembryanthemum crystallinum*

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The common ice plant (*Mesembryanthemum crystallinum*) is an important facultative crassulacean acid metabolism (CAM) model that can switch from C_3 photosynthesis to CAM following high salinity or water-deficit stress treatments. In order to explore the circadian clock-regulated mechanisms that control the expression of CAM, transcriptome sequencing was performed on well-watered and water-deficit stress treated leaf tissue collected in parallel every 4 h over a 72 h time course under both 24 h light/dark (diel entrainment) and 48 h light/light (zeitgeber or free running) conditions in order to capture the full repertoire of circadian clock-controlled transcriptional outputs. cDNA libraries were constructed for 114 ice plant samples and Illumina HiSeq2000 was performed using a total of 19 flow-cell channels, with 6 samples in each. Approximately 227.87 Gbp of raw data were generated yielding a total of 254 Gbp trimmed sequence reads. Roche 454 pyrosequencing of RNA from salt- and drought-stressed leaves, roots, mature flowers, seed pods, and seeds produced 1.25 Gbp of sequence. A total of 16 Mbp EST data was downloaded from NCBI. All trimmed, cleansed reads and ESTs were assembled *de novo* using the SOAPdenovo-Trans program. The complete read dataset assembled into 35,681 contigs, which ranged in size from 200 bp to 15,229 bp with a N50 contig length of 1,341 bp. RSEM and Bowtie were then used to assign reads to multiple genes and count relative transcript abundances. Read counts were mapped, normalized and differentially expressed genes were detected using the DESeq package. Circadian clock output differences between the C_3 photosynthesis and CAM states will be discussed in detail. These results will facilitate the identification of the molecular genetic machinery required for CAM using comparative genomics methods as well as aid in future ice plant genome annotation efforts.

P36 RLSB, an *rbc*L mRNA binding protein specific to bundle sheath chloroplasts of maize that regulates Rubisco expression and photosynthetic development in C_4 plants, is present and conserved in monocot and dicot CAM plants

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The RBCL RNA S1-BINDING DOMAIN protein (RLSB) was isolated from chloroplasts of C_4 plants by affinity-purification based on its ability to bind rbcL mRNA in vitro. This protein, encoded by the nuclear *RLSB* gene, contains a conserved S1 nucleic acid binding domain and is highly conserved among a wide variety of plant species. It contains a plastid transit sequence, and co-localizes with LSU to chloroplasts. Comparative functional analysis in both C_4 and C_3 plants indicates that RLSB activates/enhances post-transcriptional *rbcL* gene expression in both plants. RIP-qPCR analysis demonstrated that RLSB selectively binds *rbcL* mRNA, but not other plastid-encoded mRNAs, in vivo. Evolutionary modification of *RLSB* expression, from a C_3 'default' state to BS cell-specificity, may represent one mechanism by which rbcL expression has become restricted to this one cell type in C_4 plants. Two copies of this gene are found in the crassulacean acid metabolism (CAM) plants *Mesembryanthemum crystallinum* and *Erycina*. These genes have no circadian regulation, but further analysis might shed some light on the evolution of different types of photosynthetic processes in green plants. RLSB co-localizes with LSU in C_3, C_4 and C_3–C_4 intermediate species of genus *Flaveria*, further establishing the role of RLSB.

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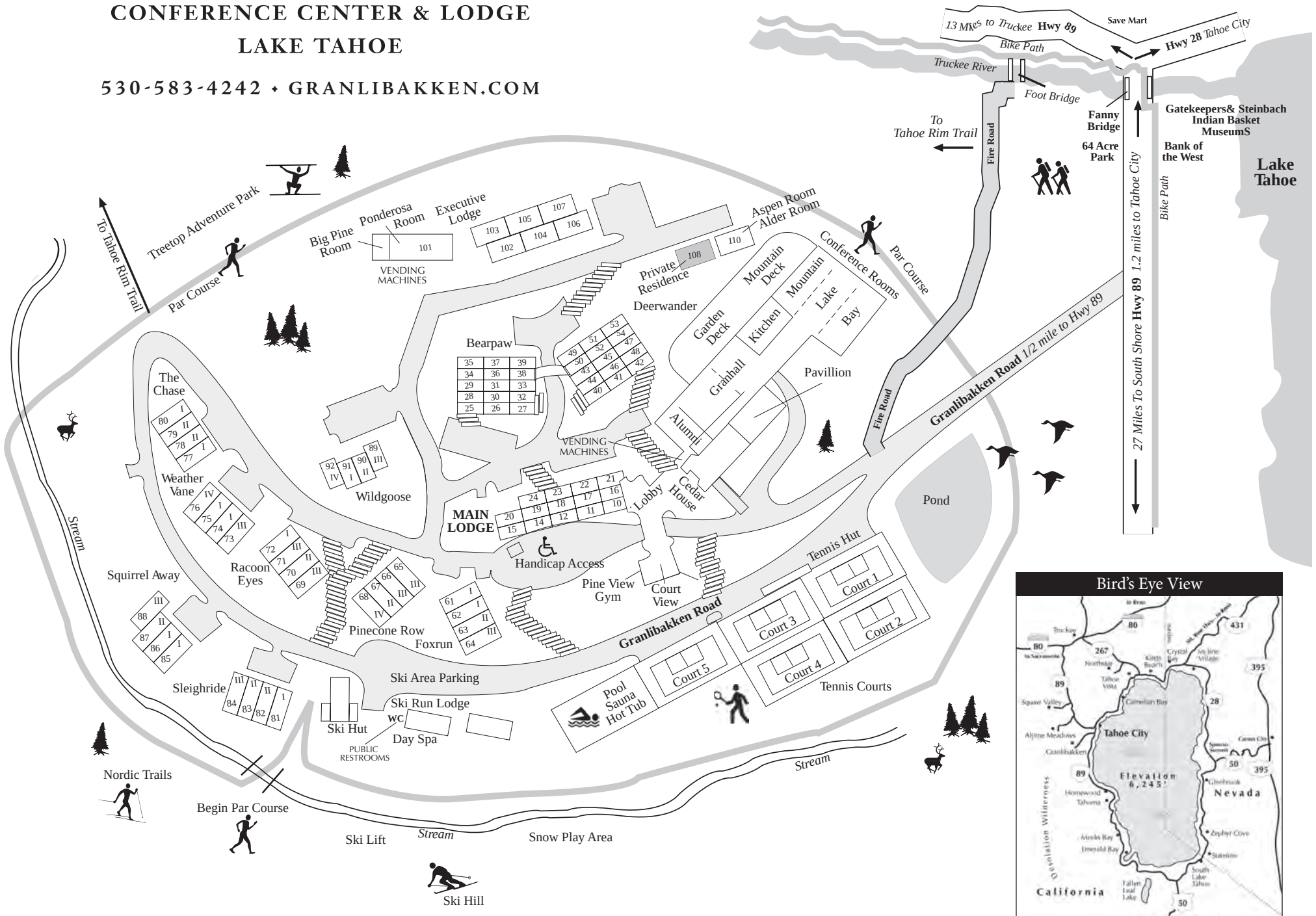

Bird's Eye View