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### YOUNG SCIENTIST AWARD SESSION

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#### TIP1 encodes the first S-acyl transferase to be identified in plants

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S-acylation (palmitoylation) targets and recruits proteins, such as ion channels and signalling molecules, to defined membrane domains and directs vesicle trafficking. S-acylation is hence a prime determinant of polar and non-polar growth, and protein S-acylation defects are responsible for some cancers and are implicated in Huntington's disease and schizophrenia. Polar growth occurs in many cells including animal nerves and plant root hairs. Root hairs are of agronomic importance as they acquire up to 80% of a plants nutrients and water and are essential to nitrogen fixing bacteria–legume symbioses. This symbiosis is of international importance as without it soil nitrogen levels would decline ultimately limiting all plant and animal life.

During my PhD I have identified *TIP1*, the first S-acyl transferase to be found in any plant. Arabidopsis plants lacking the *TIP1* gene are dwarfed, hypersensitive to the plant hormone ABA and defective in root hair development. Inhibition of S-acylation in wild type roots mimics the *tip1* mutant morphology implicating S-acylation involvement in root hair polar growth. *TIP1* functionally replaces a yeast S-acyl transferase in-vivo and binds acyl chains demonstrating that TIP1 is an S-acyl transferase. Increasing endogenous TIP1 levels results in longer root hairs. This indicates a possible link with the same mechanisms underlying human cancers due to increased S-acyl transferase activity. Tests are underway to determine if oncogenic S-acyl transferases can substitute for TIP1 in plants. These studies of TIP1 are leading towards discoveries of importance to cell biology, agronomy and human diseases, such as cancer and Huntington's disease.

#### Structure, stability and strength of leading edge vortices in insect flight

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Leading Edge Vortices (LEVs) form the secret of insect flight as they suck insect wings with an extraordinary force through the

air, affording insects a remarkable agility and performance in flight. This study focuses on the importance of rotational accelerations (e.g. the centrifugal and Coriolis acceleration) on the structure, stability and strength of LEVs. The significance of these rotational accelerations is expressed by the dimensionless Rosby number ( $Ro = U/\omega L$ ) of the flapping wing. The maximum Rosby number in hovering flight reduces to the aspect ratio of the wing semi-span ( $r/2L$ ), linking it directly to the wing geometry. Force measurements and flow visualizations were obtained with a hovering robotic flapper in a tank filled with mineral oil and water. The wing shape was based on a fruit fly wing (*Drosophila melanogaster*). The experiments were carried out over a Reynolds number range of 110 to 14000 using three types of kinematics; propeller kinematics, synthetic flapping kinematics and fruit fly kinematics. The Rosby number at the base of the wing was varied from zero (true flapping) to infinite (translational flapping) by changing the distance between the axis of rotation and the base of the wing. The maximum angle of attack was varied between 0° and 90°. We found that the characteristics of LEVs in insect flight strongly depend on the Rosby number, which measures the strength of rotational accelerations. The results are also relevant for other animals that flap their wings, fins or paddles in nature and in technology for propellers and windmills.

Keywords: Insect, Aerodynamics, LEV, Centrifugal, Coriolis

#### The beat goes on: The heart of anoxic crucian carp

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Prolonged anoxic survival requires balancing energy supply and demand and coping with anaerobic end products. In ectothermic vertebrates, this is usually accomplished through a reduction in metabolic rate that is mirrored by decreased cardiovascular function. For example, anoxic freshwater turtles reduce metabolism by 90%, enter a comatose-like state and buffer lactate with their bone and shell<sup>1</sup>. Correspondingly, cardiac power output is reduced by 95%, precluding an up-regulation of glycolysis<sup>2</sup>, and

autonomic cardiovascular control is blunted<sup>3,4</sup>. However, we recently found that the extremely anoxia-tolerant crucian carp, which remains active during anoxia, up-regulates glycolysis to maintain ATP supply, and avoids acidosis by converting lactate into ethanol, maintains cardiovascular function at normoxic levels during 5 days of anoxia with sustained autonomic cardiovascular control<sup>5</sup>. Further research suggests that this high level of cardiac activity may be needed to maintain ethanol excretion across the gills. We tested the idea that ethanol loss is related to gill perfusion by measuring venous blood ethanol concentration ( $V_{[Eth]}$ ) of anoxic (72 h at  $11 \pm 1$  °C) carp prior to and following manipulation of cardiac output ( $Q$ ), and therefore gill perfusion, with serial intra-arterial injections of the  $\alpha$ -adrenergic agonist isoproterenol ( $50 \mu\text{g kg}^{-1}$ ) and antagonist propranolol ( $1 \text{ mg kg}^{-1}$ ). Following isoproterenol,  $Q$  increased from anoxic control levels by 12%, while ( $V_{[Eth]}$ ) decreased by 19%. Conversely, propranolol decreased  $Q$  by 10% from anoxic control. Subsequently,  $V_{[Eth]}$  increased by 20%. These findings are consistent with gill ethanol excretion being perfusion-limited. Thus, a maintained  $Q$  during prolonged anoxia may prevent ethanol accumulation and even intoxication in tissues.

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### Low flow, low pressure, low cost: the cuttlefish (*Sepia officinalis*) ventilator system is highly efficient

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Laboratory raised *Sepia officinalis* (Cephalopoda) were capable of extracting 70–90% of available oxygen from the ventilatory current (as measured with oxygen microoptodes in the funnel) within the natural thermal window of the population (11–17 °C, English Channel), which represents the highest rates of extraction recorded for a cephalopod so far. Low ventilation volumes of less than 40% body weight  $\text{min}^{-1}$  result. In combination with low ventilation pressures of <0.025 kPa, ventilatory power output and ventilatory costs are minute. Less than 2% of cuttlefish total power is consumed by ventilatory mechanics in the mentioned temperature range. Both the mode of venous return and of blood oxygen binding characteristics sustain an efficient ventilatory system: Cephalic vein blood flow towards the gills (measured with a miniature doppler probe) is tightly coupled to ventilation pressure oscillations in the mantle cavity, with vein flow pulses being elicited consistently at the maximum mantle pressure increase of each ventilatory pressure cycle, regardless of ventilation pressure amplitude or frequency. Additionally, in vivo venous blood oxygen partial pressures are lower than previously reported, ranging from 1 to 1.5 kPa. Highest pH sensitivity of the cuttlefish hemocyanin consequently was found at much lower oxygen partial pressures (1–2 kPa) than in squid species (*Loligo*, *Illex*), which display low oxygen extraction rates from the ventilatory current (of less than 15%), and can thus afford to maintain higher venous oxygen partial

pressures (which, in turn can sustain higher rates of oxygen flow to tissues). The merits of the cuttlefish ventilatory system clearly lie in minimizing water flow and energy demand, thereby sustaining the energy conserving mode of life of a sit-and-wait predator and supporting camouflage strategies (low ventilation volumes imply less movement) in their highly uniform muddy bottom habitat.

### Regulation of UDP-galactose flux into cell wall polymers of Arabidopsis

J. Rösti, K. Roberts, G.J. Seifert, (Dept. Cell and Dev. Biol., John Innes Centre, Norwich, UK)

For the assembly of its complex and diverse cell wall polymers, the plant requires a sophisticated biosynthetic machinery comprising nucleotide sugar interconversion enzymes, nucleotide sugar transporters, and glycosyltransferases. Surprisingly plants have multiple isoforms for most of the nucleotide sugar interconversion enzymes providing activated monosaccharides for incorporation into the cell wall but also into glycolipids, glycoproteins and low molecular glycoconjugates. Although in most cases the biochemical function of these enzymes has been established the regulation and specific function of the different isoforms is yet unclear. We study UDP-D-glucose 4-epimerase in *Arabidopsis thaliana*, which converts UDP-D-glucose to UDP-D-galactose. UGE4, one of five isoforms of this enzyme, is specifically required for the galactosylation of xyloglucan (XG) and type II arabinogalactan. This specific requirement led to the hypothesis, that UGE4 is part of a substrate channel with Golgi localized XG-specific galactosyltransferases (1). Such a hypothesis predicts localization of UGE4 at the Golgi membrane and involvement of UGE4 in a metabolic complex. We therefore investigate the subcellular localization of UGE4 and try to isolate interacting proteins.

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### Thermal sensitivity of cellular energy budgets in Antarctic fish hepatocytes

F.C. Mark, T. Hirse and H.-O. Pörtner

Oxygen demand elicited by the main cellular energy consumers was examined in isolated hepatocytes of sub-Antarctic and high-Antarctic notothenioid and zoarcid (*Pachycara brachycephalum*) fish with respect to the role of cellular metabolism in co-defining thermal tolerance. The relative proportions of energy allocated to protein and RNA/DNA synthesis, ion regulation and ATP synthesis were quantified between 0 and 15 °C by analysis of inhibitor sensitive cellular respiration. In all investigated species, protein synthesis constituted 25–37%, RNA synthesis 24–35%,  $\text{Na}^+/\text{K}^+$ -ATPase 40–45% and mitochondrial ATP synthesis 57–65% of total respiration. The sub-Antarctic nototheniid *Lepidonotothen larseni* displayed lower cellular protein synthesis rates but somewhat higher active ion regulation activities than its high-Antarctic confamilials, as is typical for more eurythermal species. Assumed thermal optima were mirrored in minimized overall cellular energy demand. Onset of thermal stress indicated by elevated energy turnover became visible between 3 and 0 °C as well as beyond 6 °C in the sub-Antarctic *L. larseni* and *P.*

*brachycephalum*; whereas the high-Antarctic species displayed progressively rising respiration rates during warming with a cellular energetic minimum at 0 °C.

Sub-Antarctic fish showed signs of cold-eurythermy and appear to live close to their lower limit of thermal tolerance, while high-Antarctic notothenioids show high degrees of energetic efficiency at 0 °C. All cellular preparations maintained energy budgets over a wide thermal range, supporting the recent concept that thermal limits are set by oxygen and associated energy limitations at the whole organism level.

### **Nectar xylose metabolism in a rodent pollinator: defining the role of gastrointestinal microflora using <sup>14</sup>C-labelled xylose**

S.A. Johnson, S.W. Nicolson and S. Jackson

The Namaqua rock mouse *Aethomys namaquensis*, a rodent pollinator of certain geoflorous *Protea* species, consumes nectar containing xylose. Xylose is not known to be efficiently utilized by mammals. It is however utilized by certain bacteria, yeasts and fungi, particularly gastrointestinal bacteria, which yield end products that are utilized by the host in oxidative metabolism. We investigated the contribution of intestinal bacteria of *A. namaquensis* to xylose metabolism. Mice were caught during *Protea humiflora* flowering and non-flowering seasons and given an oral dose of <sup>14</sup>C-labelled xylose. Exhaled CO<sub>2</sub> and excreted urine and faeces were continuously collected for 30 hours after dosing and radioactivity of the samples determined. Each mouse was then treated with antibiotics to eliminate gut microflora and the experiment was repeated. With their natural gut flora population intact, mice caught during the flowering season exhaled significantly more <sup>14</sup>CO<sub>2</sub> than mice caught during the non-flowering season. Also, during both the flowering and non-flowering seasons, mice exhaled significantly more <sup>14</sup>CO<sub>2</sub> before antibiotic treatment than after. After antibiotic treatment significantly more <sup>14</sup>C-labelled xylose was excreted in the urine. These results provide further evidence that diet influences the composition of the gastrointestinal community, and that *A. namaquensis* relies on its gut microflora to ferment xylose and produce end products that are used by the mice for metabolism. Following these experiments caecal bacteria were isolated from mice, cultured anaerobically and assayed for xylose utilization using <sup>14</sup>C-labelled xylose. Positive xylose utilizers were identified using 16S rRNA-based taxonomy.

### **Aerodynamics of Vortex Lift in Insect Flight**

G.R. Nolan

Rotating wing models were used to study the aerodynamics of vortex lift in insect flight. Like a flapping wing, a spanwise pressure gradient is formed on the upper surface of a rotating wing because the wingtip moves faster than the wing base. Vorticity shed from the leading edge rolls into a spiral leading edge vortex (LEV), which was previously identified as an important high-lift mechanism in insect flight.

Complementary techniques were used to elucidate the structure of the LEV and overall flow patterns around wing models. Dye-flow visualisation revealed a spiral vortex at angles of attack ( $\alpha$ ) greater than 20°, which intensified with increasing Reynolds number ( $Re$ ) and  $\alpha$ . Spanwise flow was observed along the leading edge from

$Re=140$  to 6,500. Dye also revealed boundary layer flow patterns on the upper and lower wing surfaces, indicating the location of fluid reattachment on the upper surface, as well as reverse (tip-to-base) flow on the lower, inboard wing surface.

Stereo particle image velocimetry (PIV) was used to quantify the 3D fluid velocities around wing models. Vorticity within the LEV was used to calculate the circulation, and hence lift, attributed to the vortex. LEV circulation was consistently a high percentage of total circulation around the wing, highlighting the importance of vortex lift at  $Re$  appropriate to insect flight. PIV also revealed an intense spanwise flow at the centre of the vortex, and simple physical arguments suggest that the spanwise pressure gradient drives this flow. Overall, the results presented in this study suggest that the spiral LEV is stabilised by strong spanwise flow over a wider range of  $Re$  (appropriate to insect flight) than previously thought.

### **Physiological ageing in marine bivalves is modulated by environmental temperature and lifestyle**

E. Philipp, H.-O. Pörtner, D. Abele, (Alfred-Wegener Institute for Polar and Marine Research)

A tendency for higher maximum life span (MLSP) of cold adapted marine ectotherms was found, when comparing related species with similar lifestyle from permanently cold and temperate waters. We are interested in the physiological principles underlying differences of MLSP in marine ectotherms, especially with respect to the effect of temperature and lifestyle on metabolic rates and reactive oxygen species (ROS) formation. We investigated basic metabolic, antioxidative defence and oxidative damage parameters in different aged individuals of two burrowing mud clams, the Antarctic *Laternula elliptica* (MLSP ~36years) and the temperate *Mya arenaria* (MLSP ~ 13years), and two swimming scallops, the Antarctic *Adamussium colbecki* (MLSP~45years) and the temperate *Aequipecten opercularis* (MLSP ~ 8–10 years). Of the mud clams, the longer-lived Antarctic species had lower standard metabolic rates and mitochondrial H<sub>2</sub>O<sub>2</sub> generation, resulting in slower decrement of mitochondrial functions and conservation of tissue redox state with age. In the scallop group, a less pronounced decrease in mitochondrial and antioxidant enzyme activities in the Antarctic scallop might account for the higher MLSP. Despite a lower MLSP and the active lifestyle, mitochondria of the temperate scallop exhibit slower senescence than temperate mud clam mitochondria, indicating lifetime energy allocation adjusted to conserve optimal physiological functioning until a threshold is reached where physiological maintenance requires more energy than justified by the reproductive gain.

### **STUDENT AWARD SESSION**

#### **Root-ABA1: A QTL influencing L-ABA concentration and root traits in maize**

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A previous study conducted on a maize mapping population derived from Os420 × IABO78 identified a quantitative trait locus

(QTL) for leaf-abscisic acid concentration (L-ABA) on chromosome 2 (bin2.04) (Tuberosa *et al.*, 1998, T.A.G. 97: 744–755). To characterize more accurately the direct and associated effects of this QTL, sets of near isogenic lines (BDLs), were developed for both parental lines. The isogenization of a QTL in more than one genetic background provides the opportunity for evaluating the QTL in an otherwise hybrid background, an important prerequisite for appropriately testing QTL effects for traits, such as yield, affected by inbreeding depression. These materials were field tested under water-stressed and well-watered condition for two years. The QTL confirmed its effect on L-ABA and interestingly, the high L-ABA hybrids showed a significantly lower root lodging

(44.6%) compared to the low L-ABA hybrids (66.1%). To further elucidate the effects of the QTL on root architecture, root traits of two pairs of BDLs were measured in plants grown in controlled environment at three water regimes. Root differences among BDLs were not affected by water regimes. Across water regimes, the QTL confirmed its effect on L-ABA and showed a concurrent effect on root angle, branching, number, diameter and dry weight. Based on these results, we speculate that the QTL effects on root traits and L-ABA are likely due to pleiotropy rather than linkage and propose a model in which the primary effect of the QTL is on root architecture and not L-ABA.

Keywords: Maize, ABA, Drought stress, Roots