Ecophysiology: Migrations Between Different Levels of Scaling

Manfred Kluge

Contents

Abstract Causal explanation of the mechanisms behind ecological adaptation in plants requires research on different levels of scaling, ranging from molecules to plants in their natural environment. In the following article this is illustrated by a description of the author's scientific life-work mainly with respect to the crassulacean acid metabolism (CAM) and research on *Geosiphon*, an endosymbiotic consortium of a fungus and a cyanobacterium.

Introduction

The title chapters of *Progress in Botany* provide a forum where in the past celebrities of the plant sciences explored important fields of their scientific life-work. Thus, the invitation by the Editors of *Progress in Botany* to write the title chapter of

Manfred Kluge

Institute of Botany, Darmstadt University of Technology, Schnittspahnstrasse 10,
D-64287 Darmstadt, Germany
e-mail: klugemanfred@t-online.de

volume 69 honors me beyond measure. Simultaneously it confronts me with a dilemma because, with the title chapter of the preceding volume, Barry Osmond provided an excellent survey of the world of crassulacean acid metabolism (CAM), a topic which concerns also the major part of my scientific work. Therefore I cannot avoid that CAM plays an important role also in my treatise, but I want to consider this topic from a different point of view and to include also aspects other than CAM.

In his paper Barry explored CAM in all its diversity of recent and future aspects and the implications of CAM research for other disciplines of the plant sciences, and he expressed his "indebtedness to very many companions in CAM research". The latter holds very much true also for me and even concerns largely the same circle of personalities to whom I am indebted and whom I have to thank. Thus, in order to avoid repetition and hoping that it will not be misunderstood as immodesty, I will try to illustrate my personal adventure of nearly four decades of research on CAM. In particular I would like to show the logic which pushed me during my involvement in CAM to exciting migrations through the various levels of scaling, ranging from the plants in their environment down to the world of molecules.

From my beginning I considered CAM primarily as a matter of ecophysiology, irrespective of its implication also for other disciplines of plant sciences (compare Osmond 2007). Ulrich Lüttge (1997) clearly characterized the hierarchy of approaches required aiming to extend ecophysiology beyond the descriptive level. One has:

- "To start by depicting habitats and plants physiognomically,
- To deduce problems from such observations in the field as are suited for physiological, biochemical and biophysical and perhaps even molecular experimentation in the laboratory, and
- To return from the laboratory to the field with increasing sophisticated technologies for measurements and analyses applicable to field conditions."

My migrations included all these steps and thus are largely symptomatic for the path one has to follow when aiming to really understand even single facets of the breathtaking diversity of mechanisms evolved in plants to cope with the demands of their environments.

1 First Steps Towards Biology

My interest in biology emerged from two important experiences of my youth. When I was 12 years old, as a Christmas gift I got a little microscope. It opened to me a new exciting world of sometimes bizarre beauty; and to put anything under the microscope which looked somehow interesting became besides playing the piano a stern hobby.

The next, and this time a more serious impetus in the direction of biology, came from my school days. I graduated from a gymnasium practicing so-called "reformed senior classes" where I enjoyed excellent instruction in biology which not only comprised the prescribed topics of the gymnasial biology curriculum for the final two years, i.e. genetics and phylogeny, but also introduced us, based on the work of

L. v. Bertalanffy, to theoretical biology. Thus, I began to understand organisms as a steady state – maintaining dynamic open systems. Important for the direction of my later work was the introduction to classic ecology. It provided me first insights into the structure of ecosystems, food webs and matter cycling, and I began to suspect that, because of its complexity, ecology is a particularly demanding discipline within the biosciences. For me this seemed to be more attractive than deterrent.

For the development of the capability of scientific thinking and working it was very important that, in the "reformed senior class" system for the matriculation examination, a thesis based on own independent work had to be written. Within the frame of this thesis I conducted a limnological study on a pond where I investigated during the cycle of a whole year the changes in the biocenosis parallel to the alterations in water temperature, oxygen content, pH values and other environmental parameters. This study was a veritable introduction into research work. It provided me with a considerable knowledge of species, strengthened skills in handling microscopic and analytical techniques, developed the ability for independent scientific working and gave me the first feeling of satisfaction to find something that nobody had known before. Altogether, in particular the reformed senior classes of the gymnasium laid a sound fundament for my later studies, thus I have all reason to look gratefully back to my school days.

After the matriculation examination in spring 1957 it was clear to me to become teacher at a gymnasium, less because this was already tradition in my family, rather because I myself felt, strengthened by the example of my father, that teaching can be a passion and source of deep satisfaction. Briefly I was not sure whether I should choose music or biology as the main topic of my studies for the teacher's profession, but finally I decided myself in favor of science and kept music as a hobby. Thus, for the summer semester 1957 I enrolled in the Faculty of Natural Sciences of the University at Frankfurt.

2 Academic Studies and Entrance into Professional Activity

As topics for the academic studies for teacher's profession beside biology I chose chemistry and geography. In Frankfurt my studies were focused on zoology, but in 1959 I changed to the Darmstadt University of Technology where for the following three semesters I was engaged mainly in botany.

In Darmstadt the biology departments were quite small, and in particular the Institute of Botany was excelled by a close contact between the at this time sole professor, the scientific staff and the students, creating a nearly family-type and stimulating atmosphere. When I came to Darmstadt, Otto Stocker, one of the great pioneers and celebrities of experimental plant ecology, was just retired and Hubert Ziegler had become his successor. Although being still very young, H. Ziegler had already a great reputation as researcher on phloem transport, but his wide spectrum of interest included also physiological aspects of plant ecology, so that in Darmstadt the long tradition in this field was not only continued rather but, due to the introduction

of modern biochemical methodology, extended to the quest for the biochemical fundaments of ecological adaptation in plants.

H. Ziegler was an excellent, electrifying teacher, and due to him my main interest soon shifted to plant physiology and biochemistry, although on purpose I tried to avoid too early and too narrow a specialization and to retain curiosity towards the diversity of the biological world. Also in this respect H. Ziegler was a great mentor and exemplary for me.

In 1961 I won a scholarship of the Studienstiftung des Deutschen Volkes, and I began to work in H. Ziegler's group on the chemical composition of phloem exudates of trees. After I passed the teacher's examination in 1962, H. Ziegler accepted me as a candidate for a doctorate. He was a great mentor and really a friend to his pupils. Within the frame of my doctoral thesis I continued my investigation on phloem transport. In summer 1964 I finished my studies by taking the Doctor's degree. Moreover, that year became particularly important for me, because a new PhD student, Helga Müller, joined the group of Hubert Ziegler to conduct comparative studies on the carbohydrate composition of sieve tube saps. She not only appreciated my help in the time-consuming gathering of phloem exudates from the trees of the Botanical Garden but also found a partner who was happy to accompany her excellent playing of the flute on the harpsichord. It became a partnership for the whole life. Thank you, Helga.

3   Research on CAM

3.1   Initial Physiological Studies at the Level of the Entire Plant

After I took the Doctor's degree H. Ziegler encouraged me to enter an academic career and offered me the position of Scientific Assistant at his institute which I happily accepted. Looking for my own field of research, accidentally I read a paper by Nishida (1963) which trapped me so much that it determined the topic of my main future scientific work: the crassulacean acid metabolism (CAM). In 1965, when I began my investigations on CAM, at the level of science the phenomenon had already been known for 150 years, but it was still considered more or less as a physiological curiosity exhibited by certain specialists among plants. In the mentioned publication Nishida showed by viscous flow porometry that CAM plants perform a pattern of stomatal movement inverse to that of non-CAM plants, i.e. CAM plants open the stomata at night and close them during the day. This pattern coincides with typical CAM CO_2 exchange, namely net CO_2 uptake during the night (Phase I, denotation after Osmond 1978), a short period of enhanced net CO_2 uptake after the onset of the light period (Phase II), depression of net CO_2 uptake during several hours of the day (Phase III), and recommencement of net CO_2 uptake towards the end of the day (Phase IV). However, the question remained open whether the unusual stomatal behavior is the primary event determining the unique course of CAM CO_2 exchange, or whether this CO_2 exchange is otherwise controlled.

The attempt to answer this question was the beginning of my love story with CAM lasting now more than 40 years.

My start into CAM research was facilitated by several lucky circumstances. First, in 1965, because of its reputation as a physiological curiosity (Osmond 1978), CAM kept world-wide only few researchers busy. Thus, speaking in terms of ecology, for a young researcher CAM provided still a kind of niche without too much competitive pressure. Triggered by the discovery of C4-photosynthesis in 1966, this situation later changed considerably. Second, I had access to an efficient system of modern infrared gas analyzers already established by O. L. Lange when he worked from 1961 until 1963 in Darmstadt. This system allowed one to measure continuously net CO_2 exchange and transpiration by plants under controlled conditions. Finally, in the mid-1960s young scientists still at the beginning of their work like me had a good chance to get research funds from the Deutsche Forschungsgemeinschaft just by presenting a convincing research program, without the pressure that prior results, best published in the journals with highest impact factors, had to be shown.

Furnished with the mentioned equipment and a DFG grant of 5000 DM for three years, I started a study aiming to provide a causal explanation of the CAM gas exchange phenomena.

I began my investigations with a simple, now I would say brutal, experiment in one of the classic CAM plants, *Kalanchoë daigremontiana*, where fortunately it is possible to strip off the abaxial epidermis of the planar leaves remaining attached to entire plant, without substantial damage to the leaf mesophyll. Net CO_2 exchange and transpiration (the latter as an index for the stomatal behavior) were measured under constant water vapor pressure deficit of the ambient air before and after the leaf epidermis had been stripped off. The result was very clear (Fig. 1). As to be expected from the results by Nishida (1963), in the intact leaves both during day and night there was a perfect coincidence between net CO_2 exchange and the stomatal movements indicated by transpiration, whereas in the leaves without epidermis the typical CAM pattern of CO_2 exchange was preserved whilst expectedly transpiration stayed constant. My interpretation was "that not the stomatal behavior is the primary event determining the CO_2 exchange in CAM plants rather than that the CO_2 exchange of the mesophyll cells, which itself is the direct consequence of CAM, determines whether the stomata close or open at a given time" (Kluge and Ting 1978). However, the strict synchronization of CO_2 exchange and stomatal movement in the intact leaves implied that there must be a coupling between the mesophyll-inherent CO_2 exchange and the movements of the stomatal guard cells. I found a plausible explanation on the basis of the pioneering publication by K. Raschke (1966) showing in *Zea mays* that the stomata are links in a regulatory circuit which stabilizes the CO_2 concentration in the subepidermal intercellular spaces ($[CO_2]_{int}$). A consequence of such a circuit is that an increase in the internal CO_2 concentration leads to a closure of the stomata, and vice versa. On the basis of Raschke's regulatory circuit model I proposed that also in CAM plants $[CO_2]_{int}$ is the signal factor. For me it was just logic that CAM plants open their stomata at night because, due to dark CO_2 fixation, $[CO_2]_{int}$ in the mesophyll should be low, and they close them during the day because malic acid decarboxylation would lead
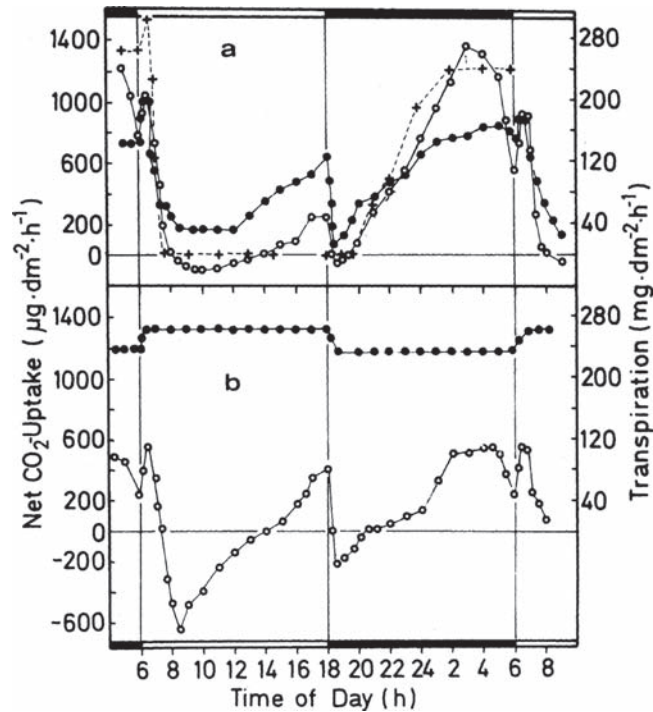
Fig. 1 Net CO_2 exchange and transpiration in *Kalanchoe daigremontiana*. **a** Coincidence of CO_2 exchange and transpiration in an intact plant. ○—○ net CO_2 exchange, ●—● transpiration, +—+ porometric values (relative units) obtained in the same species under the same conditions by Nishida (1963). **b** CO_2 exchange and transpiration in the same plant as **a**, however with the lower epidermis of all leaves having been stripped off (from Kluge and Fischer 1967)

to high $[CO_2]_{int}$. In the early years of my struggling with CAM I made a great effort to verify this hypothesis by following two strategies, namely (a) trying to measure $[CO_2]_{int}$ directly and to correlate it with the actual status of the stomatal aperture, and (b) trying to influence the duration of stomatal closure during the day (Phase III) by experimentally manipulating the source for a high internal CO_2 concentration, i.e. the malic acid pool built up during the previous night.

Already in our first CAM experiment (Fig. 1b) we observed a massive CO_2 outburst occurring upon removal of the epidermal diffusion barrier during Phase III. This outburst was drastically lower or even absent with intact leaves. Thus, for us this outburst was indicative of massive CO_2 enrichment built up behind the closed stomata. Actually, this observation was the first hint in favor of the now widely accepted view that CAM is also a CO_2 enrichment mechanism (see e.g. Osmond 2007). Later it could be demonstrated by direct measurements in air samples (Cockburn et al. 1979) taken along the day/night cycle from the succulent *Kalanchoë* leaves that, in the night, when the stomata were shown to be open,

$[CO_2]_{int}$ dropped close to zero, while during Phase III, when the stomata were closed, the leaf internal CO_2 was enriched by a factor of ten with respect to the ambient air (Kluge et al. 1981a).

The next step was to corroborate systematically the hypothesis that, optimal water status provided, the duration of stomatal closure in CAM plants during Phase III is mainly determined by the production of CO_2 from the previously synthesized and stored malic acid. I could directly prove this assumption by ^{14}C-pulse-chase experiments (Kluge 1968a).Subsequently a large series of gas exchange measurements followed which were paralleled by estimation of biochemical parameters in the plants under variation of the environmental conditions. These experiments not only uncovered the extraordinary phenotypic flexibility of CAM performance, but also showed that all experimental treatments which prevent filling of the malic acid storage pool during the night and all treatments which accelerate the consumption of the previously stored malic acid during the day shorten the duration of Phase III. After this we were quite sure that Phase III of CAM is a function of malate consumption (Kluge 1968a).

It was an interesting side-issue of the above-mentioned studies to find that application of CO_2-free air during the night inhibits not only the nocturnal synthesis of malate, but also the depletion of starch (Kluge 1969a). This showed that the breakdown of starch to generate phosphoenol pyruvate (PEP) as a CO_2 acceptor of the PEP carboxylase (PEPCase) reaction must be coupled rather directly to the availability of the substrate CO_2. Looking back I regret that neither we nor other researchers followed this finding further immediately, because presumably it could have guided us quite early into the investigation of carbohydrate metabolism in CAM which currently collects more attention (Osmond 2007).

We also observed that, at the beginning of the light period, a lag-phase in the onset of malic acid consumption occurred which coincided with Phase II. It was for me the first hint that, under the experimental condition applied, at the onset of light malate stored in the vacuole is not immediately disposable for decarboxylation. The duration of the lag phase turned out to depend on the actual vacuolar malate concentration and temperature. Thus it was reasonable to conclude that these two factors might be important for the determination of the malate release from the vacuole. Some years later I came back to these findings (Kluge et al. 1991d), and the investigation of temperature effects on the tonoplast became an important aspect in my research on CAM.

From the beginning I guessed that the pattern of the CAM gas exchange must be meaningful for the carbon and water balance of the concerned plants growing in the field (Kluge and Fischer 1967) so that CAM can reasonably be interpreted in terms of ecophysiological adaptation to environments where water is in short supply. I had the dream to get once the opportunity to verify this interpretation by studying the behavior of CAM plants in their natural habitats. A couple of years later this dream became reality. However, first we wanted to prove the ecophysiological relevance of CAM at least in the laboratory, and thus we conducted the (to my knowledge) first stress experiment on a CAM plant (*Kalanchoë daigremontiana*; Kluge and Fischer 1967). It turned out that just a few days cessation of watering

were sufficient to completely suppress opening of the stomata during the day (i.e. Phases II, IV), whereas nocturnal opening of the stomata with net CO_2 uptake continued, although on a somewhat lower level. All stress effects on CAM gas exchange disappeared within one hour after re-watering the plant. These findings suggested that CAM allows the plants to retain a positive carbon balance even if carbon acquisition during the day is prevented by water deficiency stress.

Because of compassion with our experimental plants we were not cruel enough to extent withholding of water for longer than five days. This was a pity because otherwise we would have discovered that, upon long-lasting water deficiency stress, CAM plants finally close their stomata also during the night but continue CAM behind the closed stomata by nocturnal recycling of respiratory CO_2 ("CAM idling"). Szarek and Ting (1975), who discovered CAM idling, observed that in the field it is ecophysiologically highly relevant for CAM plants which have to overcome long-lasting drought seasons.

Our stress experiments showed us also that the $[CO_2]_{int}$ regulatory circuit controlling the aperture of stomata operates undisturbed only if the plants do not suffer water deficiency stress. The conclusion was that drought factors which were still unknown to us at that time override the CO_2 regulatory mechanism. Today it is textbook knowledge that a drop in the plant water potential, with abscisic acid as molecular messenger, leads to hydroactive closure of the stomata dominating the other regulation circuits.

3.2 Investigations at the Metabolic Level: the Problem of CAM Regulation

Orderly functioning of CAM requires that during the day the malic acid derived free CO_2 is directed quantitatively to the Calvin cycle for final assimilation. From the beginning it was a striking question for me how it is prevented that during Phase III the malate derived intermediary CO_2 is trapped by PEPCase and thus futilely recycled into the malate pool instead of entering correctly the photosynthetic pathway. By means of $^{14}CO_2$ tracer experiments I could show that in the light during Phase III the PEPCase-mediated primary CO_2 fixation is switched off. In contrast, during the night even under illumination CO_2 is nearly exclusively fixed by PEPCase thus practically not available to the Calvin cycle (Kluge 1969b; Fig. 2). These in vivo changes in the activity of the primary CO_2 fixation turned out to be correlated with the diurnal malic acid rhythm of CAM. In other words: the PEPCase pathway is in an active state when the malate level in the CAM performing cells is low, and vice versa (Kluge 1971). This correlation was strictly retained when the course and the amplitude of the malic acid fluctuations were experimentally manipulated.

Looking for an explanation of the diel changes in the activity of the primary CO_2 fixation, the lucky circumstance came to my assistance that, at the same time when we discovered the in vivo down-regulation of PEPCase during Phase III, Queiroz (1966) found that in vitro this enzyme can be inhibited by L-malate. This led me to
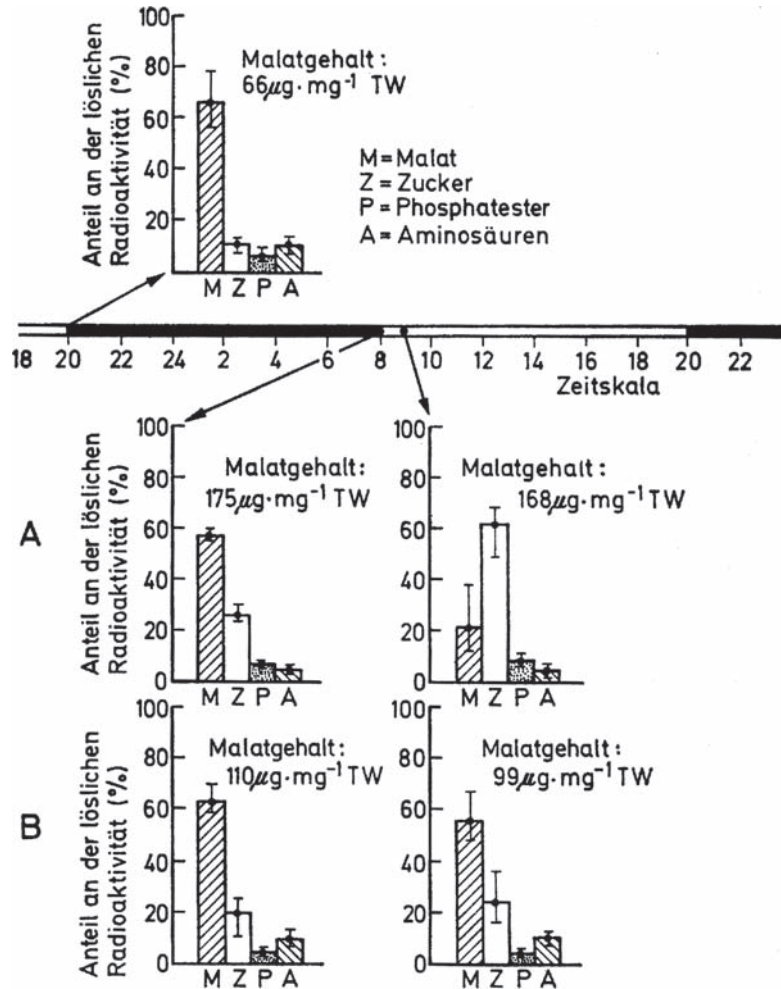
Fig. 2 *Kalanchoe tubiflora*: evidence for the inhibition in vivo of primary CO_2 fixation by PEPCase during Phase III of CAM. Indicative of this inhibition were changes in the labeling patterns in the soluble fraction after 15min $^{14}CO_2$ fixation in the light by phyllodia detached from the plant and labeled at the time points indicated by the *arrows*. The values indicate arithmetic means of three independent extractions. *Graph at top of diagram*: labelling pattern obtained at the beginning of the night. **A** Phyllodia with uninhibited nocturnal malic acid accumulation. In contrast to the labeling pattern obtained during the night (*closed horizontal bar*), one hour after the beginning of the light period (*open horizontal bar*) most of the tracer was trapped by sugars instead of malate, indicating that PEPCase was switched off. **B** Phyllodia with inhibited nocturnal malic acid accumulation. The labeling pattern in the light remained unchanged and indicative of PEPcase being still active. *TW* Dry weight, *M* malate, *Z* sugars (mainly sucrose), *P* phosphate esters, *A* amino acids (from Kluge 1971)

assume that feed-back inhibition of PEPCase by the nocturnally synthesized and stored malate might be important for suppressing CO_2 fixation by PEPCase during Phase III. However, it was also evident to me that an enzymatic feed-back inhibition alone cannot provide the complete explanation. Logically, there must be a factor which during the night keeps the malate level low in the cytoplasm, otherwise the PEPCase located there would be inhibited. In contrast, during Phase III the cytoplasmatic malate level has to be high to bring about feed-back inhibition of the enzyme. I proposed that diel changes in the source-sink behavior of the storage compartment for malate, notably the vacuole, could be the factor which determines the fluctuation of the cytoplasmatic malate level important for the feed-back control of PEPCase (Kluge 1969b). Without having already clear ideas about the mechanism behind this, I imagined that in CAM the vacuole acts with respect to malate in analogy to a siphon which is a highly attractive sink, thus keeping the cytoplasm free of malate, as long it is not filled, but which overflows thus enriching the cytoplasm with malate when it reaches a critical state of filling. In this model the sink situation corresponds to the CAM processes during Phase I and the overflow to the processes during Phase III (Kluge 1969a).

Although the model of co-operation between the vacuole and feed-back inhibition of PEPcase in CAM regulation appeared to be altogether conclusive, I was aware of the many questions remaining open and that the effort to answer them required to learn more: (a) on the structural, kinetic and regulatory properties of PEPCase, and (b) on the mechanisms controlling the reversible transport of malic acid across the tonoplast. Consequently mainly of these two complexes of questions determined my research on CAM during the following years.

3.3   Biographic Insert: Visiting Research Fellow at the ANU in Canberra, Associate Professor in Munich, Return to Darmstadt

The outlines in Sections 1.3.1 and 1.3.2 describe my struggle with CAM until summer 1969 when I finished my qualification for an academic career by Habilitation at the Darmstadt University of Technology. Thereafter I became a Lecturer in the Institute of Botany of this university, and I held this position until spring 1971. During this time I won a stipend from the Deutsche Forschungsgemeinschaft which allowed me to spend six months as Visiting Research Fellow in the group of Ralf Slatyer and Barry Osmond (Research School of Biological Sciences, ANU, Canberra). Barry introduced me to enzymology, and our work was mainly dedicated to the PEPCase of CAM plants. We estimated kinetic and regulatory properties of the semi-purified enzyme and found first indications that PEPCase is allosterically regulated, mainly by malate as negative effector (Kluge and Osmond 1972), We also demonstrated that pyruvate-P_i-dikinase, previously supposed to be a marker enzyme of C4-plants, is active also in CAM plants and permits in the light their incorporation of malate-derived pyruvate into carbohydrates (Kluge and Osmond 1971).

One of the highlights of my stay in Australia was the international meeting "Photosynthesis and Photorespiration" held in November 1970 in Canberra. Since this meeting the view has emerged and is now generally accepted that CAM represents an ecophysiologically relevant mode of photosynthetic carbon assimilation equivalent to C3- and C4-photosynthesis. Thus, by no means could CAM be any longer considered as a physiological curiosity featured by some specialists among higher plants, and in the following decades this new understanding initiated a boom in international CAM research. In this context it is worth mentioning that later we could demonstrate that even classic C3 plants such as spinach can be driven experimentally to a photosynthetic pathway analogous to CAM, with CO_2 fixed in darkness into malate entering the Calvin cycle during light (Böcher and Kluge 1978).

I owe to my stay in Australia also my first impressions of tropical rain forests, coral reefs and deserts, really unforgettable key experiences for me as an ecologically interested biologist.

After my stay in Canberra I went to the Technical University Munich and became Associate Professor at the Institute of Botany. The Director of this institute was Hubert Ziegler who, briefly before I departed to Australia, had moved from Darmstadt to Munich. There I stayed until 1974, and my work during this time strengthened my conviction that transport across the tonoplast is a key factor in the metabolic regulation of CAM. To get deeper insights into this matter I looked to contact experts in cellular transport. In this situation I was offered by the Darmstadt University of Technology the position of a Full Professor of Botany, freshly established parallel to the traditional chair held by Ulrich Lüttge as a follower of H. Ziegler. Ulrich, a leading transport physiologist, was already successfully active in CAM research. So, the call to Darmstadt opened to me the ideal possibility for co-operation and to learn more on vacuolar transport. I was the more pleased since I already knew Ulrich from our common time in Darmstadt as pupils of H. Ziegler and I was sure that we were scientifically and personally on the same wavelength.

Thus, my family and I decided to follow the call to Darmstadt, a decision which we never repented. Compared with other universities, in Darmstadt the Faculty of Biology was quite small, but it provided me such a beneficial environment for my work that in 1978 I refused to follow a call to the University of Essen. In particular the friendship and effective co-operation with Ulrich in research, teaching and administration belong to the most valuable experiences of my scientific career. This co-operation was the fundament that over the years could be founded first a DFG Researcher Group, later the DFG Center of Excellence 199 and finally the DFG Graduate School 340 at the Faculty of Biology in Darmstadt.

Towards the end of my stay in Munich the international boom in CAM research had increased the knowledge on CAM to such an extent that a comprehensive consideration of the many new results was highly desired. This was for me the impetus to publish the first monograph on CAM (Kluge and Ting 1978) which was then followed by the book by Winter and Smith (1996).

3.4   Investigations at the Level of Enzymes: the PEP Case

The central importance of PEPCase not only for CAM and C4-photosynthesis but also for many other metabolic processes in plants has triggered internationally a vast number of investigations on this enzyme, covering the whole range from its function and regulation to its molecular structure and genomic expression. Here I want to stress briefly our own involvement in the PEPCase story.

As already outlined, aiming to understand the role of PEPCase in the regulation of the complex CAM carbon flow, it is necessary to study the kinetic and regulatory properties of the enzyme. I began such studies during my sabbatical in Canberra. There we worked with plant extracts freed from low molecular substances by Sepadex filtration aiming to measure pH optimum curves of enzyme activity, K_m values and K_i values. Usually professional enzymologists refuse to take such kinetic data into account if they are not derived from measurements on the highly purified enzyme protein. Of course, such data are available also from PEPCase of CAM plants. However, it turned out that sometimes it can be useful not to obey the experts too much. Namely, it was discovered independently and nearly simultaneously by three laboratories (including ours), that PEPcase extracted from the plants at different times during the diurnal CAM cycle showed different K_i values for malate, provided the enzyme extracts were quickly desalted by passing through molecular sieves and measuring strictly within a few minutes after the plant material had been collected (v. Willert et al. 1979; Kluge et al. 1980; Winter 1980). Upon purification of the protein the differences vanished. In close co-operation with Jeanne Brulfert and Orlando Queiroz (CNRS, Gif-sur-Yvette, France) we then found that not only the sensitivity of PEPCase towards malate varies along the diurnal CAM cycle but also its sensitivity towards the activator glucose-6-phospahte (G-6-P) and the K_m for the substrate PEP. Thus, in the early 1980s it became clear that, during the diurnal CAM cycle, PEPCase switches reversibly from a physiologically active night form to a physiologically inactive day form. In vivo measurements of gas exchange, $^{14}CO_2$ labeling patterns and malic acid fluctuations parallel to the enzyme extraction supported the view that the changing properties of PEPCase are relevant for up- and down-regulation of the enzyme in vivo and with it for the regulation of the carbon flow in CAM (Kluge et al. 1981b).

It is worth mentioning that our work on the PEPCase of CAM plants founded a long-lasting co-operation and friendship with French colleagues around Orlando Queiroz in Gif-sur-Yvette, in particular with Jeanne Brulfert to whom I owe important stimulation and input for my work. Jeanne and I organized in 1983 a first international workshop on PEPCase which assembled world-wide most of the researches involved in this enzyme. During this workshop the idea was first discussed that the diurnal changes in the regulatory properties of CAM PEPCase might be due to post-translational modification of the enzyme protein. Indeed, Nimmo et al. (1986) then discovered that in the PEPCase of *Bryophyllum fedtschenkoi* the night-form is phosphorylated, not however the day-form. Soon after we found the same in other *Kalanchoë* species (Brulfert et al. 1986) and could demonstrate that the protein is

predominantly labeled with $^{32}PO_4$ in serine residues. The discovery of reversible PEPCase phosphorylation was also per se interesting because in the mid-1980s only few plant enzymes were known to be regulated by this way, and it was the only known example of a plant enzyme where protein phosphorylation leads to up- instead of down-regulation. Now it is generally accepted that not only in CAM is the reversible phosphorylation of PEPCase part of a complex regulatory network which includes also the involved PEPCase specific protein kinases and phosphatases (Nimmo 2000).

We have also conducted studies on PEPCase on the molecular level. Already by means of serological techniques we could show that, in the leaves of the facultaltive CAM-plant *Kalanchoë blossfeldiana* upon photoperiodic induction of CAM, a new isoform of PEPCase appeared additionally to that already present in the C3-state of the plant (Brulfert et al. 1982). This finding was then specified on the level of PEPCase gene sequences (Gehrig et al. 1995). We also compared in the CAM performing orchid *Vanilla planifolia* the amino acid sequences of the PEPCase isoforms (deduced from the c-DNA) present in the different organs of the plant (Gehrig et al. 1998a, b). We found that in all organs a constitutive PEPCase isoform (a "house-keeping" form) is expressed functioning most likely in context with anaplerotic reactions, whereas in the CAM-performing organs (leaves and stem) besides the housekeeping isoform an additional one occurs specifically responsible for the start reaction of CAM. Finally, a comparison of PEPCase nucleotide and aminoacid sequences in 50 plant species comprising the whole range from Bryophyta to Spermatophyta (Gehrig et al. 1998a, b, 2001a, b) convinced us that PEPCase sequences are valuable markers for the reconstitution of phylogenetic relations between organisms and metabolic pathways. In this context it was interesting to learn that in the spermatophytes the PEPCase isoforms supposed to be functionally related to CAM are widely dispersed over the different levels of taxa which support the view (Cushman and Bohnert 1999) that CAM is of polyphyletic origin.

3.5   Investigations at the Level of Cellular Compartmentation: the Role of the Vacuole and of Tonoplast Properties in CAM

As outlined in Section 1.3.2 already the results of my early studies on CAM forced me to the conclusion that diel oscillation in the PEPcase activity is the consequence of the rhythmic changes of the source/sink behavior of the vacuole for malate. This assumption pushed me into a long-lasting friendly controversy with Orlando Queiroz. Orlando was convinced that the diurnal CAM carbon flow is principally regulated by an endogenous circadian rhythm of the PEPC activity (Queiroz 1979), following a master oscillator intrinsic to the enzyme system. Finally he believed to have evidence "that an enzyme molecule behaves by itself as an oscillator shifting between different conformations" (Queiroz and Queiroz-Claret 1992).

This controversy forced me to look for more theoretical and experimental arguments in favor of my conviction that in CAM the vacuole is the basic oscillator.

Stimulated by discussions with the great cyberneticist W. Oppelt and his colleague H. Tolle, both professors of the Department of Regulation Techniques of the Technical University Darmstadt, and in co-operation with them we developed a dynamic computer model of the metabolic and regulatory processes in CAM (Nungesser et al. 1984). The model was based on a minimalistic metabolic scheme of CAM and allowed a quite realistic simulation of its typical phenomena such as diurnal acid fluctuation and gas exchange patterns. Later Freder Beck (Department of Physics, Darmstadt University of Technology) in co-operation with Ulrich Lüttge and his team developed a more sophisticated and thus more efficient theoretical model, which also identified the vacuole as master switch governing the circadian rhythmicity of CAM (Lüttge 2000).

Already our first computer model, and even more so the version developed by Freder Beck, clearly showed that proper simulation of the diurnal CAM behavior can be achieved by only inserting the changing flux behavior of the vacuole as an input variable into the model. Other approaches, where rhythmic changes of enzyme activities instead of the vacuole were supposed to represent the oscillator, failed nearly completely.

At the experimental level we could directly demonstrate by ^{14}C-tracer studies the changing source-sink behavior of the vacuole for malate (Fig. 3). Then the question arose for determining the factors controlling this changing behavior. The mechanisms behind the loading of the vacuole were studied by Ulrich Lüttge and his group (see e.g. Lüttge 2000, 2004; Osmond 2007). I was more interested in the mechanisms controlling the efflux of malate. It had been shown that this efflux is thermodynamically passive and it was assumed that at the end of the night, with vacuolar pH being low, malic acid efflux proceeds presumably by diffusion of the undissociated molecule through the lipid phase of the membrane (Lüttge and Smith 1984). Looking for the factors which might influence this diffusion I paid attention mainly to cell water relations and temperature.

1. *Water relations.* Still during my time in Munich we could show that the efflux of malate from the vacuole might be under the control of osmotic gradients (Kluge and Heininger 1973). In co-operation with Ulrich later in Darmstadt these investigations were continued and confirmed the view that osmotic gradients between the vacuole and its environment, thus finally the turgor, are important factors determining the acidification and deacidification of the vacuole (Lüttge et al. 1975).

2. *Temperature.* For a long time it was known that the gas exchange and carbon flow of CAM are phenotypically affected by temperature. As the literature shows, this phenomenon can be explained partially at the level of the enzymes involved in CAM. In this context we studied the temperature response of PEPCase in relation to its allosteric effectors (Buchanan-Bollig and Kluge 1984). However, there were also observations suggesting that the vacuole is the target of temperature effects (Kluge and Ting 1978). For instance, one of my PhD students, Detlev Ritz, found by ^{13}C mass-spectrometric analysis that a high temperature during the night prevents the storage of malate, while its synthesis is still possible (Ritz et al. 1987). Then we could show that temperature, via the
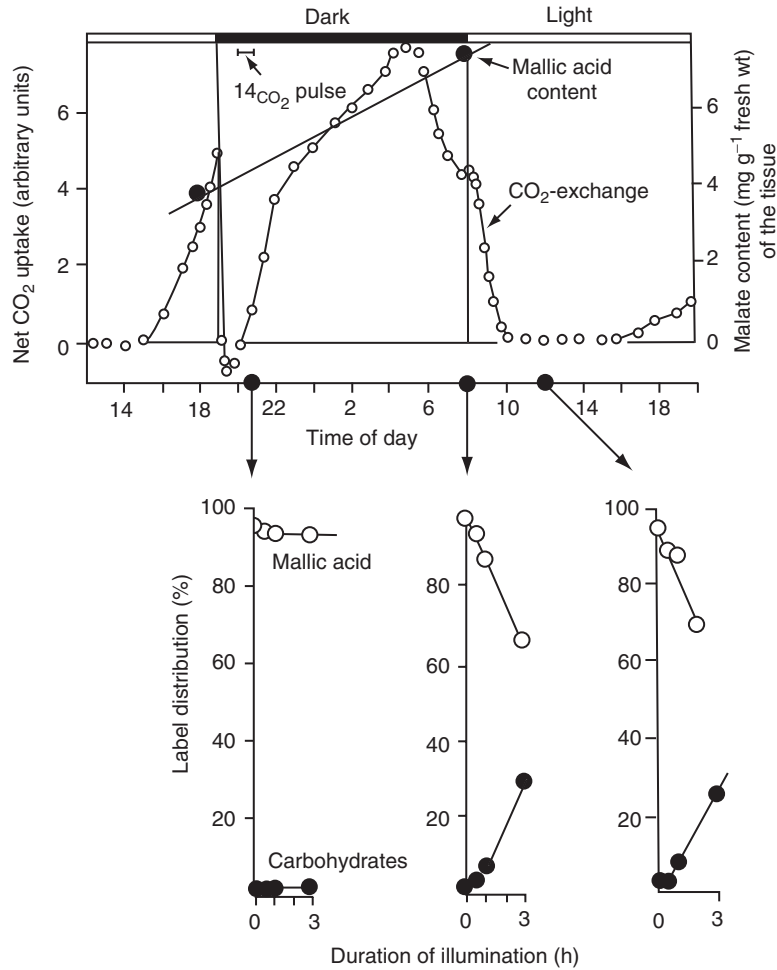
Fig. 3 ^{14}C pulse-chase experiments to show changes in the storage behavior of the vacuole during the night and the first hours of the following day. About 50 phyllodia of *Kalanchoe tubiflora* were detached from a single plant and enclosed in a glass cuvette to follow the CAM gas exchange and the nocturnal malic acid accumulation (*upper panel*). At the beginning of the night 30 phyllodia were exposed for 1 h in darkness to $^{14}CO_2$ as indicated in the upper panel ("$^{14}CO_2$ pulse"). After the pulse the labeled phyllodia were transferred back to normal air and remained in darkness. At the time points indicated by the *arrows* subsamples were taken from the labeled phyllodia and exposed to light for 3 h. During this time the phyllodia were continuously analyzed with respect to changes in the label distribution. In the *lower panel* it can be seen that with empty vacuoles at the beginning of the night even under illumination the labeled malate is retained in the vacuole, thus its label is not transferred to other metabolites. In contrast, at the end of the night, when the vacuole is filled up, upon illumination malate-derived label appears quickly in carbohydrates as photosynthetic end products. The upper panel shows the net CO_2 exchange during the CAM cycle and the nocturnal malic acid accumulation by the detached phyllodia. *Closed bar at top of the graph*: duration of the night (from Kluge 1977)

malic acid efflux from the vacuole, affects the carboxlation pathways of CAM (Friemert et al. 1988), and clear-cut evidence came also from studies on temperature effects on the onset of malic acid release from the vacuole at the beginning of the light period (Kluge et al. 1991d). This release showed at 10 °C a lag-phase of more than two hours, while at temperatures higher than 30 °C no lag-phase occurred, suggesting that at high temperatures malic acid easily leaks out from the vacuole and thus becomes instantaneously available for decarboxylation. The Arrhenius plot of the temperature effects on the duration of the lag-phase showed a break in the slope at about 15 °C. We interpreted this physiological effect in terms of phase separation of the tonoplast lipids beginning in the range of 15 °C. At temperatures higher than this critical value the tonoplast becomes increasingly fluid, thus leaky for malic acid. At temperatures lower than the phase separation range the opposite holds true (Kluge et al. 1991d).

The next step was to verify this hypothesis by measuring the fluidity of the tonoplast as a function of temperature directly. Thus, we had to shift our investigation to the level of the isolated vacuolar membrane. For this approach again co-operation with the group of Ulrich Lüttge was important because there we learned how to isolate, to purify and to characterize the tonoplasts from CAM plant cells. We learned in the laboratory of H.-J. Galla (Department of Organic Chemistry, Darmstadt University of Technology) the biophysical techniques required to measure the thermotropic phase behavior of membrane lipids (electron power magnetic resonance spectroscopy, measurement of fluorescence depolarization). The results of our studies were fully consistent with the predictions derived from physiological experiments (Kluge et al. 1991d). Namely, we found that in *K. daigremontiana*, grown at "standard conditions" (25 °C day, 17 °C night), below 10 °C the tonoplast is in a rigid state. With increasing temperature up to 52 °C tested so far, the membrane becomes steadily more fluid, with a break in the curve in the range of 14–18 °C, suggesting spontaneous phase separation (Fig. 4, upper panel; Kluge et al. 1991b). Moreover, we discovered that the fluidity of the tonoplast can adapt to the growth temperature (Kliemchen et al. 1989), a phenomenon called "homeoviscous adaptation" (HVA) and at that time not known to occur in plant biomembranes, except the thylakoids of *Nerium oleander*. Concerning the tonoplast of *K. daigremontiana*, the HVA to high temperatures means that in the acclimated plants much higher temperature than in the controls is required to reach a given degree of membrane fluidity, thus to elicit the phase transition in the membrane lipids. It was a great satisfaction for us to see that, indeed, in the plants acclimated to the higher temperature, thus having a more rigid tonoplast, the efflux from the vacuole at a given temperature was significantly slower with respect to the not-acclimated controls (Fig. 4). HVA of the tonoplast occurs also upon acclimation to low temperatures, but as to be expected in the other direction (Kliemchen et al. 1989). Finally, we could make clear that HVA of the tonoplast is due to changes in the membrane protein and phospholipid composition (Schomburg and Kluge 1994; Behzadipour et al. 1998).

Our data on the temperature response of tonoplast fluidity provided the experimental input needed by Ulrich Lüttge, Freder Beck and their groups for the development of
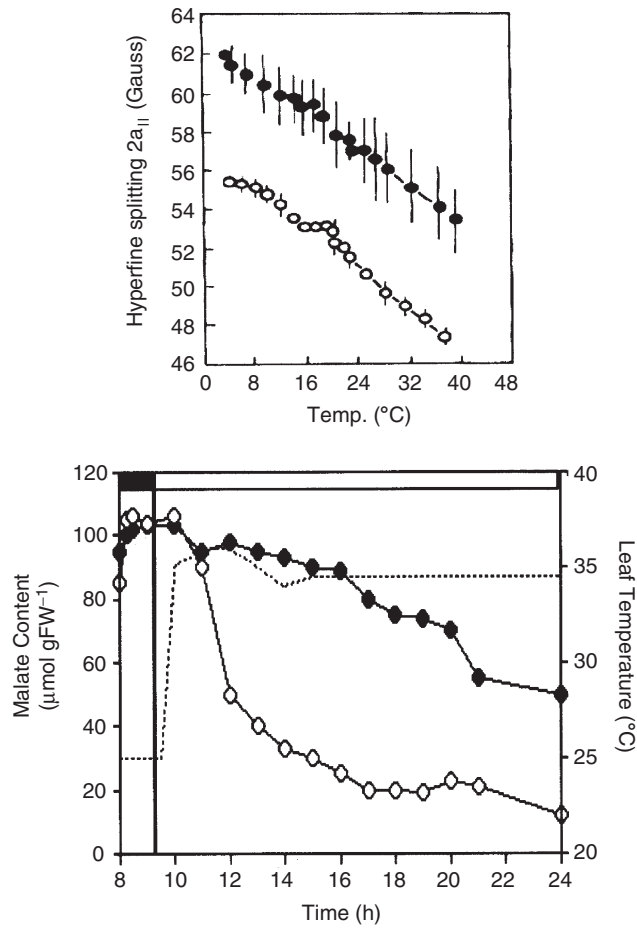
Fig. 4 Thermotropic changes of membrane fluidity and homeoviscous adaptation in the tonoplast in *Kalanchoe daigremontiana* upon acclimatization of the plants to higher growth temperature. *Upper panel* (from Kliemchen et al. 1993) shows the tonoplast fluidity, indicated by the electron power magnetic resonance (EPR) parameter "hyperfine splitting" ($2a_{\parallel}$) in spin-labeled tonoplast preparations, in relation to the incubation temperature during EPR spectroscopy. The lower the $2a_{\parallel}$ value, the more fluid is the tonoplast. *Open symbols*: tonoplasts from plants grown under standard conditions (25 °C day, 17 °C night), mean values of two independent repetitions of the experiment. *Closed symbols*: tonoplasts from plants grown for four weeks at "high temperature, HT" conditions (34 °C day, 25 °C night). Values represent arithmetic means with standard deviation of seven independent experiments. *Lower panel* (redrawn from Kliemchen et al. 1993): Course of malic acid depletion during the light period of CAM in *K. daigremontiana* under the HT conditions mentioned above. *Closed symbols*: plants grown already for four weeks under HT. *Open symbols*: plants grown under standard conditions and exposed without acclimation to HT. The figure shows that, in the plants having a more rigid tonoplast due to HT acclimation, depletion of the vacuolar malate in light is retarded compared with the plants grown at standard temperature thus having more fluid tonoplasts. *Dotted line*: leaf temperature. *Black bar on top of the graph*: Last hour of the dark period; *open bar*: light period

their theoretical model of the master switch determining the circadian rhythmicity CAM. For instance, we could show that in *Kalanchoë daigremontiana* HVA of the tonoplast prevents the endogenous CAM rhythms from being abolished by high temperatures, which is the case in not-acclimated plants (Grams et al. 1995). This supports the prediction from the theoretical model that temperature affects the biological clock controlling CAM by changing the state of order in the tonoplast.

Thus, particularly in the context with the investigation on the vacuolar malate transport, the great benefit of our close co-operation with Ulrich's group was evident and became a supporting pillar for the DFG Center of Excellence 199.

3.6 *Investigations at the Biotope Level: Performance of CAM in the Field*

From the beginning of my work on CAM I was certain that even the most plausible hypothesis on the ecophysiological relevance of CAM delineated from studies in the laboratory or climate chambers essentially requires verification by field studies, in the case of CAM necessarily sometimes at quite exotic places. Some dyed-in-the-grain biochemists and molecular biologists considered our plans for field studies as "ecotourism", which made the problem of fund raising no easier.

Field studies on CAM are still a challenging task, although today considerably facilitated by advanced experimental technology. Modestly we began our field work in the surroundings of Darmstadt where at dry sites the mini-succulents *Sedum acre* and *S. mite* are abundant. By means of continuous in situ gas exchange measurements on plants enclosed in a fully climate-conditioned cuvette paralleled by biochemical analyses, in situ $^{14}CO_2$ tracer experiments and estimation of the carbon isotope composition ($\delta^{13}C$ values) we could show that the above-mentioned species are C3-CAM intermediates which are permanently capable of CAM performance, but make use of this pathway only if water is in short supply (Schuber and Kluge 1981). Stimulated by P.N. Avadhani (National University of Singapore) we investigated CAM behavior in epiphytic ferns and orchids growing in tropical forests near Singapore (Ong et al. 1986; Goh and Kluge 1989; Kluge et al. 1989).

In 1989 I had the opportunity to join the team of Ulrich Lüttge for field work on St. John (US Virgin Islands) dealing with the tropical CAM-tree *Clusia rosea* (Ball et al. 1991).

For me a highlight of our field work was our studies on CAM in plants of the vegetation of Madagascar. In this context once again I have to mention gratefully Jeanne Brulfert. To her I owe not only my contacts at the University of Antanarivo where in 1989, 1991 and 1993 I had the opportunity to give lectures and courses in plant ecophysiology for graduate students. Without her scientific input, enthusiasm and practical support our research in Madagascar would have been less successful. Also the late Didier Ravelomanana (Botany Department at Antananarivo) and other Malagasy scientists and graduate students contributed substantially to our work.

Although we conducted in Madagascar also the usual in situ measurements of CAM performance by means of portable CO_2/H_2O porometers, a suitable screening method for large-scale analysis was required. Therefore we used mainly the estimation of the stable carbon isotope composition ($\delta^{13}C$ values) to get information on the abundance, mode of behaviour and ecophysiological relevance of CAM in the plants we thought were interesting. Moreover, $\delta^{13}C$ estimation addressing such ecological questions can be successfully applied to plant material obtained from herbaria. For instance, supplementary to the material collected by ourselves in the field, we analyzed among many other species nearly all Malagasy and Continental-African *Kalanchoë* samples deposed at the Musée National d'Histoire Naturelle de Paris. I still remember the absolute silence and the specific smell which surrounded us during our many days of work in the huge herbarium of this museum, an initially unexpected facet of field studies. Hubert Ziegler in Munich and Eliane Deleens in Orsay were essential partners in our work by carrying out the $\delta^{13}C$ analysis in the samples we sent to them. The predictions for CAM behavior of given species derived from their $\delta^{13}C$ values were in many cases verified by experiments on living plants in the phytotron.

Our $\delta^{13}C$ screening on plants of the Malagasy vegetation comprised all species of Didiereaceae, many Euphorbiaceae, Orchidaceae and other families (Kluge et al. 1995). For two reasons, however, our main interest was focused on the comparison of CAM modes in the genus *Kalanchoë* (Kluge et al. 1991a). First, historically CAM was discovered in a species of this genus (*K. pinnata*) and many of the fundamental insights into the biochemistry and physiology of CAM have derived from studies on *Kalanchoë*s, for instance *K. daigremontiana*. Second, most of the species are endemic to Madagascar, they show an extraordinary diversity of growth forms, and they are abundant in all of the very different climatic zones and in climatically very different microhabitats of the island. Thus, we were interested to find out whether the mode of CAM performance of the various *Kalanchoë* species could be related to the specific habitat preference and to the morphological characteristics of the plants.

First we realized that most likely all species of the genus *Kalanchoë* are capable of CAM, but that there are considerable differences among the species in the way CAM is conducted. Although potentially CAM performers, some species behave as C3 plants with CO_2 acquisition mainly during the day, thus low water-saving effectiveness. Many other species show stereotypically the water-conserving mode of "strong" CAM with opening of the stomata and CO_2 fixation taking place only during the night, irrespective of water availability. Finally, there are species performing highly flexible CAM with CO_2 fixation both during night and day, with the relative contribution of the two modes depending on the actual water availability.

We were excited by the finding that the differences in CAM behavior are mirrored at the biotope level (Fig. 5). In arid zones and dry microhabitats, the *Kalanchoë* species performing water-saving strong CAM mode are abundant. In contrast, species of the C3 type prefer the humid climate zones or humid, shady habitats in otherwise dry regions, and finally, the climatic zones and habitats with frequently changing water availability are the sites preferred by the species performing highly flexible CAM (Kluge et al. 1991a, 1993; Kluge and Brulfert 1996).
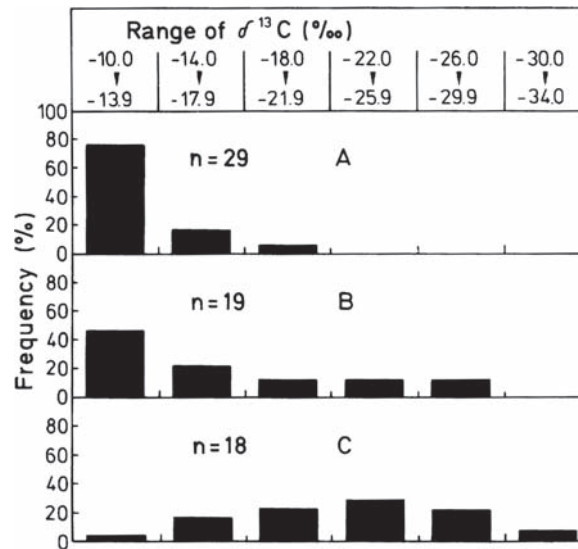
Fig. 5 Quantitative evaluation of $\delta^{13}C$ distribution in *Kalanchoë* species over the vegetation zones of Madagascar. The frequency is expressed as a percentage of the total number of species (*n*) analyzed for the given zone. **A** Xerophilous bush, **B** evergreen dry forest and savannah, **C** evergreen rain forest and montane forest. Where CAM is taking place, $\delta^{13}C$ values in the range of $-10‰$ to $-16‰$ are indicative of CO_2 uptake exclusively during the night, and those more negative than $-25‰$ indicate CO_2 uptake entirely during the day, i.e. a C3 pattern of CO_2 acquisition. $\delta^{13}C$ values between these extreme ranges indicate that the plants in question acquired external carbon in a changing proportion during both night and day (Kluge et al. 1991)

It was also fascinating for us to realize that the various modes of CAM behavior in the *Kalanchoë* species are clearly related to the intragenic taxonomic position of the species and their growth forms. This taxonomic approach was triggered by the lucky circumstance that Dr. Lucile-Allorge Boiteau (CNRS, Gif-sur-Yvette, France) generously made accessible to us a posthumous draft of a monograph on the Malagasy *Kalanchoë*s by her father, the late Pierre Boiteau, formerly Director of the Botanical Gardens Zimbazaza (Antananarivo) and one of the leading authorities on the Malagasy succulents. P. Boiteau divided the genus *Kalanchoë* into Section I (Kitchingia), Section II (Bryophyllum) and Section III (Eukalancho*ë*). It turned out that the C3 performers among the *Kalanchoë*s belong altogether to the ancestral Section I and the first two groups of Section II. They represent herbaceous, quite thin-leafed plants. The strong CAM performers belong mainly to the phylogenetically more derived groups of Section II and Section III. The species of these taxa exhibit pronounced leaf succulence. We also included the *Kalanchoë* species of continental Africa in our screening. They belong to the Section III, and $\delta^{13}C$ analysis predictably showed that CAM with predominantly nocturnal CO_2 uptake is the rule.

It is worth mentioning that, encouraged by our ecophysiological studies on the genus *Kalanchoë*, Lucile Allorge-Boiteau finished and finally published P. Boiteau's posthumous monograph (Boiteau and Allorge-Boiteau 1995).

With the results of our field work in mind we turned back to the laboratory to find out whether the grouping of the *Kalanchoë* species based on the ecophysiological and taxonomic criteria is mirrored also on the molecular level. Hans Gehrig, a post-doc in my group, investigated in about half of the known *Kalanchoë* species DNA polymorphism derived from random-amplified polymorphic DNA (RAPD)-PCR, and he compared the nucleotide sequences of internal transcribed spacer (ITS)-1 and ITS-2 regions (Gehrig et al. 1997, 2001a, b). He found out that within the Crassulaceae the genus *Kalanchoë* forms a monophyletic clade. Within this clade the species form three main clusters which coincide with the three intragenic sections of the genus *Kalanchoë* distinguishable by classic taxonomical and ecophysiological criteria, including the modes of CAM performance. ITS analysis showed that Section I is phylogenetically the most ancestral, Section III however the most derived one, thus confirming the propositions by P. Boiteau derived from classic taxonomic criteria. Another interesting finding of the molecular study was that the Section III species are divided into two sister groups, with one comprising only Malagasy species and the other all African species of the genus. The African sister group appears more derived with respect to the Malagasy Section III sister group. Thus, our molecular data provided strong support in favor of the view that the center of phylogenetic radiation of the genus *Kalanchoë* is located in Madagascar, from where the species spread into continental Africa (Boiteau and Allorge-Boiteau 1995).

Our field studies in Madagascar on the genus *Kalanchoë* and other Malagasy CAM plants verified very clearly the selective advantage of CAM. They permitted the conclusion that CAM, because of its high phenotypic plasticity and genotypic variability, allows those species furnished with this mode of photosynthesis to cope both with short-term fluctuations and long-lasting constellations of environmental demands. Thus, CAM enables these plants to conquer successfully highly diverse ecological niches along gradients in the physical environment and resource availability (Kluge et al. 2001; Kluge 2005). In this context a comparison with the CAM diversity in all 11 species of Didiereacea and in all eight Malagasy species of leafless orchids was very instructive to us and supported our interpretation of the results obtained with *Kalanchoë*s (Kluge et al. 2001). Both the Didiereacean species and the leafless orchids are specialists restricted to sites where water is long-lasting of short supply, namely in the case of the Didiereacea the thorn bush in the semi-arid South and South-West of Madagascar, and in the case of leafless orchids epiphytic habitats. As predictable from the uniform environmental conditions of these habitats, the $\delta^{13}C$ of these plants were in all cases indicative of inflexible CAM with CO_2 fixation taking place exclusively during the night.

4 Fascinated by a Curiosity: *Geosiphon pyriformis*

During my later years at the university besides the research on CAM I was trapped by *Geosiphon pyriformis*, an organism far away from the world of succulent higher plants. It was due to my teaching that I became involved in the organism.

For a better understanding of the following some introductory explanations might be helpful. *Geosiphon* is until now the only known example of a coenocytic soil fungus that lives in endosymbiotic (!) association with a cyanobacterium, i.e. *Nostoc punctiforme*. The fungal hyphae grow in the upper layers of soil together with free-living *Nostoc* cells. If the tip of such a hypha meets *Nostoc*, some cells are incorporated into the hypha by a kind of phagocytosis, and upon this act the fungus forms an unicellular pear-shaped "bladder" up to 2 mm long where the *Nostoc* cells are accommodated, multiply and become physiologically active (Fig. 6). *Geosiphon* is apparently very rare and, because of the unpretending appearance of the bladders, difficult to find in nature. That is the reason why relatively little was known on this organism when we began our work on it in 1990. It has to be mentioned that in the early 1960s Eberhard Schnepf had investigated already the ultrastructure of *Geosiphon* bladders (Schnepf 1964). This pioneering study led to a general theory of cell compartmentation and provided strong arguments in favor of the endosymbiosis theory of the eukaryotic cell. After E. Schnepf's studies the organism was forgotten for more than 20 years, until Dieter Mollenhauer rediscovered it on an arable site near Bibergemünd (Spessart Mountains; Germany; Mollenhauer 1988). This is at present the only known site world-wide where *Geosiphon* is naturally abundant.

Many years before I became directly involved in research on *Geosiphon*, within the frame of my teaching I had already covered it in lectures on "Symbiosis and Parasitism in Plants" as an especially fascinating example of endocytosis. Then, in 1986 I needed a photograph of *Geosiphon* for the textbook on botany I was going to write with Ulrich Lüttge (see Section 5). With Dieter Mollenhauer I made an excursion to the field where he had rediscovered the organism. We sampled soil carrying some dozens of bladders and went back to the laboratory to take the needed photographs. Dieter's wife Resi, gifted with admirable patience and subtle intuition for the in vitro cultivation of algae, refused to discard the precious sample and succeeded in establishing a culture of *Geosiphon* (Mollenhauer and Mollenhauer 1988), although previous attempts in this direction by other researchers always had failed. The possibility of growing *Geosiphon* in the laboratory was the breakthrough opening the way for experimental work on this organism. We used this chance and started a close co-operation between my group and Dieter Mollenhauer. Sharing the tasks, we began to investigate at first physiological and metabolic aspects, while Dieter and his wife studied the processes during the initiation and further development of the symbiotic consortium.

Looking for financial support for research on *Geosiphon*, still at the beginning of our work, we were immediately confronted with the question by referees: "And where is your molecular approach?" To me this type of question seems to be symptomatic for certain trends in modern plant science trying to put the cart before the horse. It was always my opinion, and I was reconfirmed by the further development of the *Geosiphon* project, that first careful work at higher levels of the scale is required to uncover clear starting points for meaningful molecular approaches. Nevertheless, finally our standpoint was accepted and the project could be anchored in the DFG Center of Excellence (SFB) 199 and the DFG
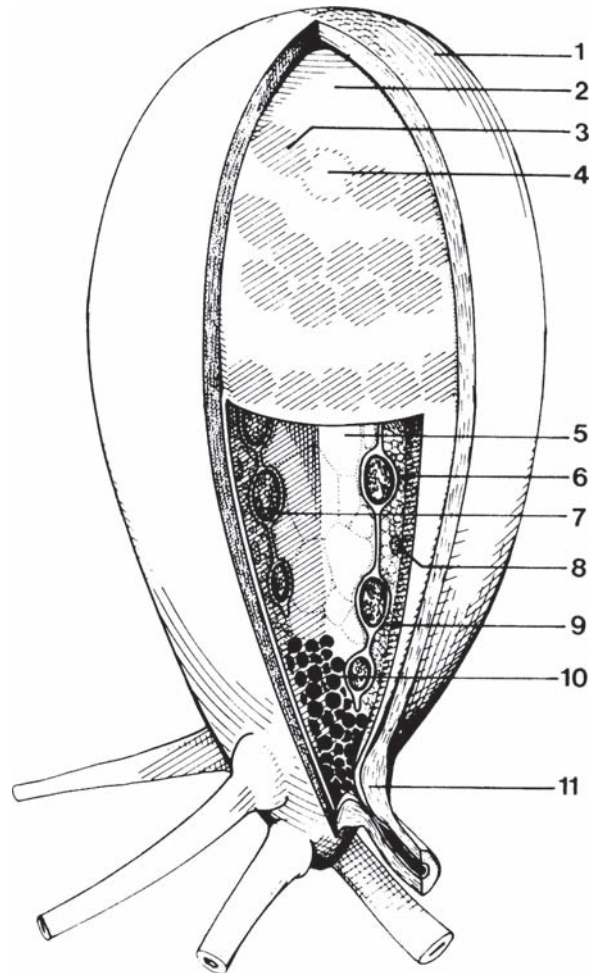
Fig. 6 Organization of a *Geosiphon* bladder (semi-schematic): *1* cell wall of the bladder, *2* plasma-lemma of the fungus (outside view), *3* endocytotic *Nostoc* seen through the plasmalemma and outer layer of the fungal cytoplasm, *4* heterocyte in a *Nostoc* filament, *5* central fungal cytoplasm with large vacuoles, *6* peripheral fungal cytoplasm with small vacuoles, *7* section of a vegetative *Nostoc* cell, *8* mitochondrion, *9* pericyanobacterial membrane, i.e. the membrane of the symbiosome, *10* glycogen granules, lipid droplets, microbodies and other inclusions, *11* hypha of the mature bladder with narrow lumen. The function of the constriction is unknown (from Kluge et al. 1994)

Graduate School 340 "Communication in biological systems: from the molecule to the organism in its environment". A graduate student, Arthur Schüssler, joined us to work under the supervision of E. Schnepf (University of Heidelberg) and myself on a doctoral thesis. Arthur became particularly important for the development of the *Geosiphon* research project and now, after the retirement of the initiators, he continues it.

An extended discussion of all results obtained over the years on *Geosiphon* is beyond the scope of this chapter (for reviews see Kluge et al. 1997, 2002; Schüßler and Kluge 2001). In the following just some highlights are mentioned.

We found that inside the *Geosiphon* bladders the *Nostoc* cells are photosynthetically active (Kluge et al. 1991c), with higher efficiency as compared with free-living *Nostoc* cells of the same strain (Bilger et al, 1994). Due to its endosymbionts *Geosiphon* is most likely capable of nitrogen fixation (Kluge et. al. 1992), and it enriches phosphate in the fungal cytoplasm, not however in *Nostoc* cells (Maetz et al. 1999).

A. Schüßler studied the wall of the symbiosome bordering endosymbiotic *Nostoc* cells inside bladders against the fungal cytoplasma. He showed that this interface is produced by the fungus and that its structure is strikingly similar to that bordering the fungus against the plant root cell in arbuscular mycorrhiza (AM). Thus, Arthur postulated that the internal structure of the *Geosiphon* bladder is comparable with the within-root situation of AM. This and other arguments make *Geosiphon* a promising model system for studies on AM (Schüßler 2002) and shifted the project unexpectedly in the direction of AM research.

D. and R. Mollenhauer uncovered the complete development of the *Geosiphon* consortium from the first contacts between the future symbiotic partners via incorporation of the *Nostoc* cells by the fungus in the formation of the bladders (Mollenhauer et al. 1996). In co-operation with the "Institut für den wissenschaftlichen Film" (Göttingen) they documented this process by an excellent video movie (reference: VHS C 1955) which is now commercially available for teaching and research. Tragically Resi Mollenhauer was not allowed during her life to see the publication of the video movie and the whole success of the *Geosiphon* project which owed so much to her, because in 1996 after a long-lasting illness she died.

It was also found out that only immobile *Nostoc* primordia, but not motile hormogonia are susceptible to recognition and incorporation by the fungus (Mollenhauer et al. 1996). Then A. Schüßler showed that the extracellular slime of the primordia contains mannose. This sugar is absent in the other stages of the *Nostoc* life cycle (Schüßler et al. 1997) so that it is tempting to speculate but remains to be shown that mannose has something to do with the specific partner recognition required to establish the *Geosiphon* consortium.

Finally, after a couple of years of patient basic research on *Geosiphon*, the time was ripe for approaches at the molecular level. A. Schüßler and his co-workers started gene expression studies. In this context Holger David succeeded in establishing a cDNA library derived from fungal mRNA of the *Geosiphon* bladders. This led to the first molecular characterization of a carbohydrate transporter from a symbiotic glomeromycotan fungus and resulted in a paper published in *Nature* (Schüßler et al. 2006). The gate is now open for expression studies on further *Geosiphon* genes.

The question about the taxonomic position of *Geosiphon* initiated a study of the small subunit (SSU) rRNA genes of *Geosiphon* (Gehrig et al. 1996), which then led to a comprehensive analysis of the molecular taxonomy of AM fungi (Schüßler et al.

2001). This triggered a revision of fungal taxonomy by the description of a fifth phylum, the Glomeromycota, which comprises all AM forming fungi. *Geosiphon* turned out to represent an ancestral lineage within the Glomeromycota. Finally, the molecular analysis of the taxonomic position of *Geosiphon* provided important arguments for the reconstruction of the early evolution of Fungi using a six-gene phylogeny. This study by an international consortium including A. Schüßler was also published in *Nature* (James et al. 2006).

The discovery leads back to ecology in that *Geosiphon* belongs taxonomically to the AM fungi, which are obligatory symbiotic. Because of its close relationship to the classic AM fungi, most likely (however remaining to be shown) the mycelium of *Geosiphon* interacts in situ not only with *Nostoc* to form the endosymbiotic consortium rather but also with the roots of higher plants. Moreover, in nature *Geosiphon* grows always together with the hornwort *Anthoceros punctatus* and the liverwort *Blasia* spp. It is known that *Anthoceros* at *Geosiphon* stands forms with a glomeromycotan fungus an AM-like symbiosis. In contrast, D. Mollenhauer showed that, at the natural stand, *Nostoc punctiforme* not only interacts with *Geosiphon* but is also a symbiotic partner of *Anthoceros* and *Blasia*. Thus, most likely within its habitat *Geosiphon* could be a link in an ecological network (Kluge et al. 2002) which integrates *Nostoc*, *Anthoceros*, *Blasia* and higher plants. This network opens interesting questions for future ecological research.

5 Teaching: Mainly Great Pleasure, Sometimes Frustration

After graduation I decided myself deliberately in favor of a university career because it promised the possibility to combine two passions: research and teaching. Looking back I think it was the right decision, although the politically motivated over-regulation of the German university system which began in the late 1960s became increasingly conter-productive for the genuine tasks of a university professor. How often I wished to have the time wasted in endless and ineffective debates in the institutions of the academic self-administration to be available for more intense work with the students. Also in this context I mention gratefully the close co-operation with Ulrich Lüttge which was very important to overcome the frictions born by the university system of today, thus facilitating the effort to maintain efficiency in the work of the Institute of Botany in Darmstadt at the highest level.

Teaching, including the basic lectures in General Biology and General Botany, always meant pleasure for me and were a permanent source of inspiration, never an inconvenient obligation. In particular teaching to undergraduates I experienced as a challenging and important task, because today the freshmen coming from the gymnasium begin their study of biology with very different functional prerequisites, sometimes even without clear motivation for the topic. Of course, occasionally there was also frustration when all efforts to raise real interest failed. With the lecture "General Botany" Ulrich Lüttge and I took turns from one year to the other and collected by this way a lot of common experience on the difficulties and

demands of teaching this topic under the allowance of the current curriculum. On the basis of this experience we wrote our textbook "Botanik" (Lüttge et al. 2005), dedicated in particular to undergraduate students. Writing such a book requires a permanent dialogue and reciprocal tuning between the authors, and the success of the opus tells us that here we were on the right path.

Retrospectively sometimes I ask myself if it would not have been better to focus the research onto a more narrow topic instead of migrating between the various levels of scaling; for instance, to stay at the level of biochemistry and to investigate the exciting problem of PEPCase and its regulation cascade in all its ramifications instead of having always the whole CAM syndrome in mind. Given my propensity, the holistic approach was more satisfying for me and corresponded more to my understanding of the essence of ecophysiology as outlined in the introduction. Probably I would try to follow anew the same line, but I am aware that under the challenges of modern biology and current research politics this would be very difficult, if not impossible. With regard to teaching at a botanical institute with a relatively small teaching staff like Darmstadt an utmost broad spectrum of scientific interest and experience is essential to cope with the requirements of the curriculum. Also in this context my holistic orientation was very helpful.

References

Ball E, Hann J, Kluge M, Lee H S J, Lüttge U, Orthen B, Popp M, Schmitt A and Ting IP (1991) Ecophysiological comportment of the tropical CAM-tree *Clusia* in the field. II. Modes of photosynthesis in trees and seedlings. New Phytol 117:483–491

Behzadipour M, Ratajczak R, Faist K, Pawlitschek P, Trémolières A, Kluge M (1998) Phenotypic adaptation of tonoplast fluidity to growth temperature in the CAM Plant *Kalanchoë daigremontiana* Ham. et Per. is accompanied by changes in the membrane phospholipid and protein composition. J Membr Biol 166:61–70

Bilger W, Büdel B, Mollenhauer R, Mollenhauer D (1994) Photosynthetic activity of two developmental stages of a *Nostoc* strain (Cyanobacteria) isolated from *Geosiphon pyriforme* (Mycota). J Phycol 30:225–230

Boiteau P, Allorge-Boiteau L (1995) *Kalanchoe* (Crassulaceés) de Madagascar. Karthala, Paris

Böcher M, Kluge M (1978) Der C_4-Weg der CO_2-Fixierung bei *Spinacea oleracea*. II. Pulse-chase Experimente mit suspendierten Blattstreifen. Z Pflanzenphysiol 86:405–421

Brulfert J, Müller D, Kluge M, Queiroz O (1982) Photoperiodism and crassulacean acid metabolism I: Immunological and kinetic evidences for different patterns of phosphoenolpyruvate carboxylase isoforms in photoperiodically inducible and non-inducible crassulacean acid metabolism plants. Planta 154:326–331

Brulfert J, Vidal J, Le Marechal P, Gadal P, Queiroz O, Kluge M, Krüger I (1986) Phosphorylation–dephosphorylation process as a probable mechanism for the diurnal regulatory changes of phosphoenolpyruvate carboxylase in CAM plants. Biochem Biophys Res Commun 136:151–159

Buchanan-Bollig IC, Kluge M, Müller D (1984) Kinetic changes with temperature of phosphoenolpyruvate carboxylase from a CAM plant. Plant Cell Environment 7:63–70

Cockburn W, Ting IP, Sternberg CO (1979) Relationship between stomatal behavior and internal carbon dioxide concentration in crassulacean acid metabolism plants. Plant Physiol 63:1029–1032

Cushman JG, Bohnert B (1999) Molecular genetics of crassulacean acid metabolism. Annu Rev Plant Physiol Plant Mol Biol 50:305–332

Friemert V, Heininger D, Kluge M, Ziegler H (1988) Temperature effects on malic-acid efflux from the vacuoles and on the carboxylation pathways in CAM Plants. Planta 174:453–461

Gehrig H, Taybi T, Kluge M, Brulfert J (1995) Identification of multiple PEPC iso-genes in leaves of the facultative crassulacean acid metabolism (CAM) plant *Kalanchoë blossfeldiana* Poelln. cv Tom Thumb. FEBS Lett 377:399–402

Gehrig H, Schüßler A, Kluge M (1996) *Geosiphon pyriforme*, a fungus forming endocytobiosis with *Nostoc* (Cyanobacteria), is an ancestral member of the Glomales: evidence by SSUrRNA analysis. J Mol Evol 43:71–78

Gehrig H, Rösicke H, Kluge M (1997) Detection of DNA polymorphism in the genus *Kalanchoë* by RAPD-PCR fingerprint and its relationships to infrageneric taxonomic position and ecophysiological photosynthetic behaviour of the species. Plant Sci 125:41–51

Gehrig H, Faist K, Kluge M (1998a) Identification of phosphoenolpyruvate carboxylase isoforms in leaf, stem and roots of the obligate CAM plant *Vanilla planifolia* Salib. (Orchidaceae): a physiological and molecular approach. Plant Mol Biol 38:1215–1223

Gehrig H, Heute V, Kluge M (1998b) Towards a better knowledge of the molecular evolution of phosphoenolpyruvate carboxylase by comparison of partial c-DNA sequences. J Mol Evol 46:107–114

Gehrig H, Gaußmann O, Marx H, Schwarzott D, Kluge M (2001a) Molecular phylogeny of the genus *Kalanchoe* (Crassulaceae) inferred from nucleotide sequences of the ITS 1 and ITS 2 region. Plant Sci 160:827–835

Gehrig H, Heute V, Kluge M (2001b) New partial sequences of phosphoenolpyruvatecarboxylase as molecular phylogenetic markers. Mol Phyl Evol 20:262–274

Goh JC, Kluge M (1989) Gas exchange and water relations in epiphytic orchids. Ecol Stud 76:139–166

Grams TEE, Kluge M, Lüttge U (1995) High temperature adapted plants *Kalanchoe daigremontiana* show changes in the temperature dependence of the endogenous CAM rhythm. J Exp Bot 46:1927–1929

James TY, et al (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443:818–822

Kliemchen A, Schomburg M, Galla H-J, Lüttge U, Kluge M (1989) Phenotypic changes in the fluidity of the tonoplast membrane of crassulacean acid metabolism plants in response to temperature and salinity stress. Planta 189:403–409

Kluge M (1968a) Untersuchungen über den Gaswechsel von *Bryophyllum* während der Lichtperiode. I. Zum Problem der CO_2-Abgabe. Planta 80:255–263

Kluge M (1968b) Untersuchungen über den Gaswechsel von *Bryophyllum* während der Lichtperiode. II. Beziehungen zwischen dem Malatgehalt des Gewebes und der CO_2-Aufnahme. Planta 80:359–377

Kluge M (1969a) Zur Analyse des CO_2-Austausches von Bryophyllum. II. Hemmung des nächtlichen Stärkeabbaus in CO_2-verarmter Atmosphäre. Planta 86:142–150

Kluge M (1969b) Veränderliche Markierungsmuster bei $^{14}CO_2$-Fütterung von *Bryophyllum tubiflorum* zu verschiedenen Zeitpunkten der Hell-Dunkel-Periode. I. Die Fütterung unter Belichtung. Planta 88:113–129

Kluge M (1971) Veränderliche Markierungsmuster bei $^{14}CO_2$-Fütterung von *Bryophyllum tubiflorum* zu verschiedenen Zeitpunkten der Hell-Dunkel-Periode. II. Beziehungen zwischen dem Malatgehalt des Gewebes und dem Markiermuster nach $^{14}CO_2$-Lichtfixierung. Planta 98:20–30

Kluge M (1977) Regulation of carbon dioxide fixation in plants. Proc Symp Soc Exp Biol 31:155–175

Kluge M (2005) Options of photosynthesis in the genus *Kalanchoe*: an approach integrating the levels of biochemistry, ecophysiology, molecular taxonomy and phytogeographic distribution of the species. Nova Acta Leopold 92:195–205

Kluge M, Brulfert J (1996) Crassulacean acid metabolism in the genus *Kalanchoë*: ecological, physiological and biological approaches. In: Winter K, Smith AP, Smith JAC (eds) Crassulacean acid metabolism. biochemistry, ecophysiology and evolution. Springer, Berlin Heidelberg New York, pp 324–335

Kluge M, Fischer K (1967) Über Zusammenhänge zwischen dem CO_2-Austausch und der Abgabe von Wasserdampf durch *Bryophyllum daigremontianum*. Planta 77:212–223

Kluge M, Heininger B (1973) Untersuchungen an *Bryophyllum* über den Efflux von Malat aus den Vakuolen der assimilierenden Zellen und mögliche Einflüsse dieses Vorgangs auf den CAM. Planta 113:333–343

Kluge M, Osmond CB (1971) Pyruvat-P_i-dikinase in crassulacean acid metabolism. Naturwissenschaften 58:414–415

Kluge M, Osmond CB (1972) Studies on PEP-carboxylase and other enzymes involved in crassulacean acid metabolism of *Bryophyllum tubiflorum* and *Sedum praealtum*. Z Pflanzenphysiol 66:97–105

Kluge M, Ting IP (1978) Crassulacean acid metabolism. Ecol Stud 30:3

Kluge M, Böcher M, Jungnickel G (1980) Metabolic control of crassulacean acid metabolism: evidence for diurnal changing sensitivity against inhibition by malate of PEP-carboxylase in *Kalanchoë tubiflora* Hamet. Z Pflanzenphysiol 97:197–204

Kluge M, Böhlke C, Queiroz O (1981a) Crassulacean acid metabolism in *Kalanchoë*: variations of the intercellular CO_2 concentration during a normal CAM cycle, and during cycles with either light or darkness only. Planta 152:87–92

Kluge M, Brulfert J, Queiroz O (1981b) Further evidence for diurnal changes in regulatory properties of PEP-carboxylase in crassulacean acid metabolism (CAM). Plant Cell Environment 4:251–256

Kluge M, Friemert V, Ong BL, Brulfert J, Goh JC (1989) In situ studies of crassulacean acid metabolism in *Drymoglossum piloselloides*, an epiphytic fern of the humid tropics. J Exp Bot 40:441–452

Kluge M, Brulfert J, Ravelomanana D, Lipp J, Ziegler H (1991a) Crassulacean acid metabolism in *Kalanchoë* species collected at various climatic zones of Madagascar: a survey by $\delta^{13}C$ analysis. Oecologia 88:407–414

Kluge M, Kliemchen A, Galla H-J (1991b) Temperature effects on crassulacean acid metabolism: EPR spectroscopic studies on the thermotropic phase behaviour of the tonoplast membranes *Kalanchoë daigremontiana*. Bot Acta 104:355–360

Kluge M, Mollenhauer D, Mollenhauer R (1991c) Photosynthetic carbon assimilation in *Geosiphon pyriforme* (Kützing) F. v. Wettstein, an endosymbiotic association of fungus with a cyanobacterium. Planta 185:311–315

Kluge M, Wolf H, Fischer A (1991d) Crassulacean acid metabolism: temperature effects on the lag-phase in the photosynthetic oxygen evolution occurring at the outset of the light period. Plant Physiol Biochem 29:83–90

Kluge M, Mollenhauer D, Mollenhauer R, Kape R (1992) *Geosiphon pyriforme,* an endosymbiotic consortium of a fungus and a cyanobacterium (*Nostoc*), fixes nitrogen. Bot Acta 105:343–344

Kluge M, Brulfert J, Lipp J, Ravelomanana D, Ziegler H (1993) A comparative study by $\delta^{13}C$-analysis of crassulacean acid metabolism (CAM) in *Kalanchoë daigremontiana* (Crassulaceae) species of Africa and Madagascar. Bot Acta 106:320–324

Kluge M, Mollenhauer D, Mollenhauer R (1994) *Geosiphon pyriforme* (Kützing) von Wettstein, a promising system for studying endocyanoses. Prog Bot 55:5

Kluge M, Brulfert J, Rauh W, Ravalomanana D, Ziegler H (1995) Ecophysiological studies on the vegetation of Madagascar: $\delta^{13}C$ and δD survey for incidence of crassulacean acid metabolism (CAM) among orchids from montane forests and succulents from the xerophytic thorn-bush. Isotopes Environ Health Stud 31:191–210

Kluge M, Gehrig H, Mollenhauer D, Mollenhauer R, Schnepf E, Schüßler A (1997) News on *Geosiphon pyriforme*, an endocytobiotic consortium of a fungus with a cyanobacterium. In: Schenk HEA, Herrmann R, Jeon KW, Müller NE, Schwemmler W (eds) Eukaryotism and symbiosis: intertaxonic combination versus symbiotic adaptation. Springer, Berlin Heidelberg New York, pp 469–476

Kluge M, Razanoelisoa B, Brulfert J (2001) Implications of genotypic diversity and phenotypic plasticity in the ecophysiological success of CAM plants, examined by studies on the vegetation of Madagascar. Plant Biol 3:214–222

Kluge M, Mollenhauer D, Wolf E, Schüßler A (2002) The *Geosiphon/Nostoc* endocytobiosis. In: Rai AN, Bergmann B, Rasmussen M (eds) Cyanobacterian symbiosis. Kluwer, Dordrecht, pp 19–30

Lüttge U (1997) Physiological ecology of tropical plants. Springer, Berlin Heidelberg New York

Lüttge U (2000) The tonoplast functioning as the master switch for circadian regulation of crassulacean acid metabolism. Planta 211:761–769

Lüttge U (2004) Ecophysiology of crassulacean acid metabolism (CAM). Ann Bot 93:629–652

Lüttge U, Smith JAC (1984) Mechanism of passive malic-acid efflux from vacuoles of the CAM plant *Kalanchoe daigremontiana*. J Membr Biol 81:149–158

Lüttge U, Kluge M, Ball E (1975) Effects of osmotic gradients on vacuolar malic storage. A basic principle in oscillatory behaviour of crassulacean acid metabolism. Plant Physiol 56:613–616

Lüttge U, Kluge M, Bauer G (2005) Botanik, 5th edn. Wiley–VCH, Weinheim

Maetz M, Schüßler A, Wallianos A, Traxel A (1999) Subcellular trace element distribution in *Geosiphon pyriforme*. Nucl Instrum Methods B 150:200–207

Mollenhauer D (1988) Weitere Untersuchungen an *Geosiphon pryriforme*- einer Lebensgemeins chaft von Pilz und Blaualge. Nat Mus 118:289–309

Mollenhauer D, Mollenhauer R (1988) *Geosiphon* cultures ahead. Endocyt Res 5:69–73

Mollenhauer D, Mollenhauer R, Kluge M (1996) Studies on initiation and development of the partner association in Geosiphon pyriforme (Kütz) v. Wettstein, a unique endocytobiotic system of a fungus (Glomales) and the cyanobacterium *Nostoc punctiforme*. (Kütz)Harriot. Protoplasma 193:3–9

Nimmo GA, Nimmo HG, Hamilton ID, Fewson CA, Wilkins MB (1986) Purification of the phosphorylated night form and dephosphorylated day form of phosphoenolpyruvate carboxylase from *Bryophyllum fedtschenkoi*. Biochem J 239:213–220

Nimmo HG (2000) The regulation of phosphoenolpyruvate carboxylase in CAM plants. Trends Plant Sci 5:75–80

Nishida K (1963) Studies on stomatal movement of crassulacean plants in relation to the acid metabolism: Physiol Plant 16:281–298

Nungesser D, Kluge M, Tolle H, Oppelt W (1984) A dynamic computer model of the metabolic and regulatory processes in CAM. Planta 162:204–214

Ong BL, Kluge M, Friemert V (1986) Crassulacean acid metabolism in the epiphytic ferns *Drymoglossum piloselloides* and *Pyrrosia longifolia*: studies on the response to environmental signals. Plant Cell Environ 9:547–557

Osmond CB (1978) Crassulaceen acid metabolism: a curiosity in context. Annu Rev Plant Physiol 29:379–414

Osmond CB (2007) Crassulaceae acid metabolism: now and then. Prog Bot 68:3–32

Queiroz O (1966) Recherche d'un modéle enzymatique pour le déterminisme de la désacidification diurne chez les Crassulacees. C R Acad Sci 265:1928–1931

Queiroz O (1979) CAM: rhythms of enzyme capacity and activity as adaptive mechanisms. In: Gibbs M, Latzko E (eds) Encyclopedia of plant physiology, photosynthesis II, vol 6. Springer, Berlin Heidelberg New York, pp 263–270

Queiroz O, Queiroz-Claret C (1992) Seasonal and daily control of enzyme synthesis and activity by circadian clocks. In: Tuitou Y, Hans E (eds) Biological in clinical and laboratory medicine. Springer, Berlin heidelberg New York, pp 78–89

Raschke K (1966) Die Reaktionen des CO_2-Regelsystems in den Schließzellen von *Zea mays* auf weißes Licht. Planta 68:111–140

Ritz D, Kluge M, Veith HJ (1987) Effect of temperature and CO_2-concentration on malate accumulation during CAM: evidence by ^{13}C-mass spectrometry. Plant Physiol Biochem 25:391–399

Schnepf E (1964) Zur Feinstruktur von *Geosiphon pyriforme*. Arch Mikrobiol 49:112–131

Schomburg M, Kluge M (1994) Phenotypic adaptation to elevated temperatures of tonoplast fluidity in the CAM plant *Kalanchoë daigremontiana* is caused by membrane proteine. Bot Acta 107:328–332

Schuber M, Kluge M (1981) In situ studies on crassulacean acid metabolism in *Sedum acre* L. and *Sedum mite* Gil. Oecologia 50:82–87

Schüßler A (2002) Molecular pylogeny, taxonomy and evolution of *Geosiphon pyriformis* and arbuscular myccorrhizal fungi. Plant Soil 244:75–83

Schüßler A, Kluge M (2001) *Geosiphon pyriforme*, an endocytosymbiosis between fungus and cyanobacteria, and its meaning as a model system for AM research. In: Hock B (ed) The Mycota IX. Springer, Berlin Heidelberg New York, pp 151–161

Schüßler A, Mollenhauer D, Schnepf E, Kluge M (1994) *Geosiphon pyriforme*, an endosymbiotic association of fungus and cyanobacteria: the spore structure resembles that of mycorrhizal (VAM) fungi. Bot Acta 107:36–45

Schüßler A, Meyer T, Gehrig H, Kluge M (1997) Variations of lectin binding sites in extracellular glycoconjugates during the life cycle of *Nostoc punctiforme*, a potentially endosymbiotic cyanobacterium. Eur J Phycol 32:233–239

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol Res 105:1413–1421

Schüßler A, Martin H, Cohen D, Fitz M, Wipf D (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. Nature 444:933–936

Szarek SR, Ting IP (1975) Physiological responses to rainfall in *Opuntia basilaris*. Am J Bot 62:602–609

Willert DJ von, Willert K von (1979) Light modulation of the activity of the PEP-carboxylase in CAM plants of the Mesembryanthemaceae. Z Pflanzenphysiol 95:42–49

Winter K (1980) Day/night changes in the sensitivity of phosphoenolpyruvate carboxylase to malate during crassulacean acid metabolism. Plant Physiol 65:792–796

Winter K, Smith JAC (1996) Crassulacean acid metabolism; biochemistry, ecophysiology and evolution. (Ecological Studies, vol 114). Springer, Berlin Heidelberg New York