



Dual-Layer Adaptation Mechanisms of the Karst Obligate-CAM Epiphytic *Dendrobium Loddigesii*

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Abstract

Obligate crassulacean acid metabolism (CAM) plants survive karst drought through daytime stomatal closure, which limits water loss but suspends gas exchange, raising the question of how intracellular metabolism continues under closed stomata. Using a reintroduced population of the orchid *Dendrobium loddigesii* shaped by 15 years of natural selection, we combined a two-season (January and April) between-population comparison with concurrent within-plant comparisons of different-aged leaves, and quantified leaf electrophysiology using an extended Hodgkin-Huxley model. Intracellular water transport rate (WTR) was highly conserved across seasons and leaf ages (overall mean 51.25, within-group CV $\leq 2.93\%$), suggesting an evolutionarily conserved “metabolic chassis trait” that may safeguard basal metabolism. In spring, the effective thickness of overwintered leaves decreased by 31.2% (Welch’s *t*-test, $p = 0.004$), whereas intrinsic physiological resistance (IR)

declined non-significantly (11.5%; $p = 0.724$). Measured IR was 19.7% of the value extrapolated from a winter power-law thickness-IR model and lay below the lower bound of its 95% prediction interval, a tentative indication of structure-function decoupling. Young leaves varied among individuals in IR (CV 57.1%), some adopting a “low-resistance, high-flux” strategy, consistent with risk-spreading across heterogeneous microhabitats. We propose a two-tier adaptation model of “conserved chassis homeostasis + plastic functional regulation” for epiphytic CAM plants, extending the static structure-function view of the plant economics spectrum, and introduce an individual-level storage-water exchange coordination index (SWCI) and a group-level source-sink transition response index (SSTRI) as descriptive tools for orchid conservation and karst restoration.

Keywords: *Dendrobium loddigesii*, electrophysiological dynamics, dual-layer adaptation model, structure-function decoupling, metabolic chassis, source-sink relationship.



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1 Introduction

The karst terrain of southwestern China represents one of the most extensively developed karst landscapes globally. Prolonged rock desertification has resulted in habitat fragmentation and extreme hydrological fluctuations, imposing severe stress on plant

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survival [1]. This region harbors exceptionally high orchid (Orchidaceae) diversity, among which the epiphytic genus *Dendrobium* Sw. has emerged as an ideal model group for investigating adaptive evolutionary strategies of plants in extreme habitats, owing to its distinctive niche preferences and phenotypic plasticity [2]. *Dendrobium loddigesii* Rolfe, a rare medicinal orchid distributed from southern China to Indochina and frequently found in karst areas, has experienced severe population decline under habitat fragmentation stress. Carbon isotope ($\delta^{13}\text{C}$) analysis has confirmed it as an obligate crassulacean acid metabolism (CAM) plant, which minimizes water loss through transpiration under seasonal drought via daytime stomatal closure [3]; epiphytic orchids in such habitats also employ structural adaptations such as thickened cuticles and water-storage pseudobulbs to buffer against intermittent water deficit [4]. However, this strategy creates a physiological paradox: while complete stomatal closure effectively curtails water loss, it simultaneously abolishes gas exchange between the plant and the external atmosphere. The fundamental question of how intracellular basal metabolism is sustained within such a closed microsystem remains a critical scientific issue awaiting elucidation [5].

In recent years, studies on the mechanisms by which obligate CAM plants sustain core metabolic fluxes under stomatal closure have begun to reveal multi-tiered integrated regulatory networks involving antioxidant defense, transcription factor networks, epigenetic reprogramming, and metabolic pathway remodeling [3, 5]. However, most of these insights are derived indirectly from transcriptomic and metabolomic analyses, and direct physiological evidence from the perspective of membrane ion transport and transmembrane water transport dynamics remains lacking. Plant electrophysiology, rooted in the Hodgkin-Huxley membrane equivalent circuit model, has now been extended to the scale of intact leaf organs, enabling non-destructive impedance detection to precisely capture the dynamic remodeling of membrane systems induced by phenological transitions and environmental stress [6–8]. This provides a key technological approach to elucidating, at the micro-electrophysiological level, the intracellular maintenance mechanisms underlying CAM photosynthesis during stomatal closure.

Despite progress in multiple aspects regarding the physiological and molecular basis of *Dendrobium* adaptation to drought habitats, three key issues

remain to be further elucidated. (1) Regarding research subjects, most existing studies have employed greenhouse-acclimated seedlings or individuals collected from short-term field sampling, focusing primarily on immediate physiological responses under controlled conditions [9]. Consequently, continuous *in situ* observations of wild reintroduced populations under long-term natural selection pressure are relatively scarce, limiting a comprehensive understanding of the true evolutionary adaptive strategies of the species. (2) In terms of experimental design, cross-sectional comparisons across different environments or species have been extensively accumulated, but systematic longitudinal comparisons across different developmental stages under the same genetic background have not yet been established. This hampers a clear distinction between genetically fixed traits and environmentally induced developmental plasticity [10]. (3) With respect to mechanistic understanding, during the winter-spring phenological transition, the overwintering mature leaves serve as source organs for nutrient export. Whether the changes in their membrane systems represent passive degenerative damage or active functional remodeling remains unresolved due to insufficient evidence. Furthermore, whether the functional construction of newly emerging young leaves (sink organs) involves strategic differentiation among individuals within the population also awaits systematic verification [11]. The root cause of these limitations lies in the lack of methodological support for “synchronous comparison of leaves of different ages on the same individual” — a design gap that makes it difficult to effectively exclude the interference of genetic background differences and environmental heterogeneity.

To address the research gaps outlined above, this study employs a reintroduced population of *D. loddigesii* that has established naturally for 15 consecutive years in a karst rocky desertification habitat without anthropogenic intervention. Leveraging an experimental design of “synchronous comparison of leaves of different ages on the same individual” within each season, we investigate the core scientific question of how hierarchical regulation of membrane electrophysiology during the winter-spring phenological transition coordinately achieves both the export of assimilated nutrients from overwintering mature leaves (source) and the functional construction of newly emerging young leaves (sink). We propose three testable hypotheses: (1) the conservative basal evolution

hypothesis-intracellular water transport rate (WTR) is an evolutionarily conserved trait that maintains basal metabolic homeostasis and shows no detectable modulation by leaf age or seasonal environmental fluctuations; (2) the source-sink functional decoupling hypothesis-overwintering mature leaves undergo active functional remodeling of their membrane systems in spring, with morphological structure and membrane transport functions exhibiting decoupled changes; (3) the developmental heterogeneity strategy hypothesis-newly emerging young leaves differentiate into divergent electrophysiological regulatory strategies within the population (i.e., a low-resistance sprint type vs. a conservative construction type) to adapt to the spatial heterogeneity of karst microhabitats. By integrating multi-dimensional data from morphological anatomy, organ electrophysiology, and developmental plasticity, this study aims to construct a “dual-layer source-sink transition adaptation” theoretical model applicable to epiphytic CAM orchids, thereby providing novel theoretical perspectives and empirical support for an in-depth understanding of the adaptive evolutionary mechanisms of orchids in extreme karst habitats.

2 Materials and Methods

2.1 Study Area and Experimental Materials

The experimental base is located at the *D. loddigesii* wild reintroduction demonstration base in Ganca Village, Xingyi City, Guizhou Province, China (24.9080°N, 104.9955°E), which features a typical karst peak-cluster depression landform. The region has a humid subtropical monsoon climate, with an annual mean temperature of 15–18 °C and annual precipitation of 1300–1600 mm. A persistent dry season occurs from November to April of the following year, resulting in high seasonal drought stress. The bedrock is predominantly limestone, with shallow soil layers and a high proportion of gravel, leading to extremely poor water and nutrient retention capacities. The experimental population consisted of clonal clumps of *D. loddigesii* transplanted and established in 2011 on vertical limestone rock faces on karst slopes. By 2026, these plants had been continuously subjected to no artificial irrigation, fertilization, or pest and disease management for 15 years. Through natural vegetative propagation, they have formed a stable population of over 260 individuals, driven entirely by natural selection throughout the entire period, ensuring the ecological authenticity and evolutionary representativeness of the experimental materials.

2.2 Experimental Design and Sample Grouping

This study employed a two-season (winter and spring) between-population comparative design, complemented by a concurrent within-plant control of leaves of different ages, to reduce genetic background confounding within each season and to disentangle the effects of two core physiological factors: seasonal environment and leaf developmental age. It should be noted that the winter and spring measurements were obtained from different individuals within the same natural population, rather than from repeated measurements of the same individuals; all cross-season comparisons were independent between-group comparisons at the population level.

Winter steady-state group (15 January 2026): Overwintering mature leaves were randomly collected from seven healthy mature individuals (coded mhsh-1 to mhsh-7). At this stage, the plants were in a dormant steady state with basal metabolic maintenance, and these samples were used to establish a baseline power-function model of the species' winter physiological steady state.

Spring dynamic group (10 April 2026): Synchronous sampling was performed within the same natural population, which was divided into two subgroups: (1) the spring old-leaf group (mhsh old leaves 1–4): functional leaves on old stems that were differentiated and formed in 2025 and had fully overwintered until the following spring; and (2) the spring young-leaf group (mhsh young leaves 1–5): newly formed young leaves differentiated from new shoots after dormant buds germinated in spring 2026. The spring old leaves and young leaves were obtained from the same individual plants, enabling concurrent comparison of leaves of different ages within the same plant.

2.3 Electrophysiological Measurements and Indicator System

All measurements were conducted during a unified time window of 10:00–12:00 (local time) on clear days to avoid circadian rhythm interference with electrophysiological parameters. A plant electrophysiology analyzer (Jiangsu Zhongtian Zhigan Life Data Co., Ltd., China; model to be specified) was employed. The measurement theory is based on the Gibbs free energy equation, extending the classical Hodgkin-Huxley membrane equivalent circuit model to the scale of intact leaf organs. Five gradients of mechanical clamping force were applied to acquire electrical response curves, and the zero-clamping-force state was

obtained by extrapolation to eliminate mechanical stress interference, thereby accurately capturing intrinsic electrophysiological parameters under non-destructive conditions [6, 18].

The core observation indicators were categorized into three groups:

- (1) Morphological-structural indicator: leaf specific effective thickness (d);
- (2) Membrane transport functional indicators: intrinsic physiological resistance (IR), intrinsic physiological impedance (IZ), intrinsic physiological capacitive reactance (IXc), intrinsic physiological inductive reactance (IXl), intracellular water transport rate (WTR), intracellular nutrient transport rate (NTR), nutrient active transport capacity (NAC), and resistance to low nutrition (RLN);
- (3) Overall metabolic indicators: leaf metabolic flux (MF) and relative metabolic activity (MA).

Units for all parameters are provided in Table 1.

2.4 Statistical Analysis and Construction of Quantitative Indices

All statistical analyses were performed in R 4.3.1 on the raw data of all 16 samples, and every value reported in the text, Table 2, Table 3, Figure 1, and Figure 2 was generated in a single analysis run (analysis script provided as a Supplementary File). Normality within groups was assessed with the Shapiro-Wilk test. Differences among the three groups were tested with classical one-way analysis of variance (ANOVA; F and p values in Table 2); Tukey's HSD *post hoc* test was applied only when the omnibus test was significant ($p < 0.05$). Two planned pairwise contrasts were tested separately: (i) winter versus spring mature leaves, which were sampled from different individuals, with Welch's two-sample t -test; and (ii) spring mature versus spring young leaves, which were sampled from the same plants, with a paired t -test across the plants bearing both leaf types. Effect sizes are reported as Hedges' g (bias-corrected Cohen's d). Because variances were heterogeneous for several parameters, Welch's one-way ANOVA was run as a sensitivity analysis (Supplementary File).

The winter steady-state d -IR relationship was fitted as $IR = a d^b$ by nonlinear least squares, with starting values taken from a log-log linear fit; R^2 was calculated as $1 - SS_{res}/SS_{tot}$. The 95% prediction interval for a new observation at the spring mean d was obtained by

the delta method, combining the parameter covariance matrix with the residual variance (t distribution with $n - 2$ degrees of freedom). This model was used for counterfactual prediction of IR in spring mature leaves. We acknowledge that the sample sizes are modest ($n = 4$ -7 per group), and the results should be interpreted with appropriate caution.

Two descriptive quantitative indices were developed in this study.

Storage-Water Exchange Coordination Index (SWCI):

$$SWCI = \frac{d}{IR} \quad (1)$$

This index characterizes the coordination between structural investment in leaf water storage and transmembrane exchange efficiency. Leaf electrophysiological parameters sensitively reflect intracellular water status, with specific effective thickness (d) being related to leaf water-storage structures and intrinsic physiological resistance (IR) reflecting the resistance to transmembrane ion and material exchange [6, 7]. Leaf hydraulic models also confirm the existence of both coordination and trade-offs between water-storage structures and water transport efficiency [17].

Source-Sink Transition Response Index (SSTRI):

$$SSTRI_{group} = \left| \frac{\overline{IR}_{spring} - \overline{IR}_{winter}}{\overline{d}_{spring} - \overline{d}_{winter}} \right| \quad (2)$$

where bars denote the group means of mature (source) leaves in each season. Because winter and spring measurements were obtained from different individuals, no individual-level change exists; SSTRI is therefore a single population-level descriptive value of the change in transport resistance per unit change in effective thickness during the phenological transition. Its uncertainty was estimated with a percentile bootstrap (5000 resamples of individuals within each group). Source-sink transition during leaf senescence involves active nutrient remobilization and metabolic reprogramming [12], and changes in source-sink relationships systematically affect leaf physiological status and senescence progression. SSTRI describes a physiological response only; it is not a measure of fitness, because survival, growth, and reproductive output were not measured.

3 Results

3.1 Winter Physiological Steady-State Baseline: The “Low-Resistance, High-Flux” Electrophysiological Fingerprint of *D. loddigesii*

Under winter dormant steady-state conditions, leaves of *D. loddigesii* exhibited a species-specific electrophysiological fingerprint (Table 1). The population mean intrinsic physiological resistance (IR) was only 11.92, which appears lower than that reported for most other *Dendrobium* species, providing a structural-physical basis for an efficient “low-resistance, high-flux” transmembrane transport mode. The coefficient of variation (CV) for intracellular water transport rate (WTR) was merely 2.93%, the lowest among all measured parameters, indicating that this trait is highly homogeneous within the population and may represent an evolutionarily conserved core trait. In contrast, the CV for leaf metabolic flux (MF) reached 170.51%, with extreme values differing by approximately 130-fold among samples (mhsh-4: 64.48; mhsh-1: 8399.79), indicating strong inter-individual differentiation in metabolic activity and suggesting possible niche divergence within the population.

Nonlinear least-squares regression of the winter steady-state data revealed a negative power-law relationship between leaf specific effective thickness (d) and intrinsic physiological resistance (IR):

$$IR = 5.53 \times 10^8 d^{-4.87}, \quad R^2 = 0.94, \quad p = 0.002, \quad n = 7 \quad (3)$$

(Figure 1), where p refers to the exponent b . The calibration domain of the model is $d = 33.8$ – 55.2 .

3.2 Seasonal Dynamic Variation: Hierarchical Differentiation between Conservative Baselines and Plastic Regulation

Comparison of core electrophysiological parameters among the three groups—winter mature leaves, spring mature leaves, and spring young leaves—revealed that the electrophysiological traits of *D. loddigesii* follow a hierarchical regulatory logic: conservative baseline traits remain stable, while functionally regulatory traits exhibit plastic changes in response to phenology and developmental stage (Table 2).

3.2.1 Evolutionary Conservation of WTR: A Core Safeguard for Intracellular Metabolic Homeostasis in CAM Plants

The intracellular water transport rate (WTR) of the three sample groups was highly convergent,

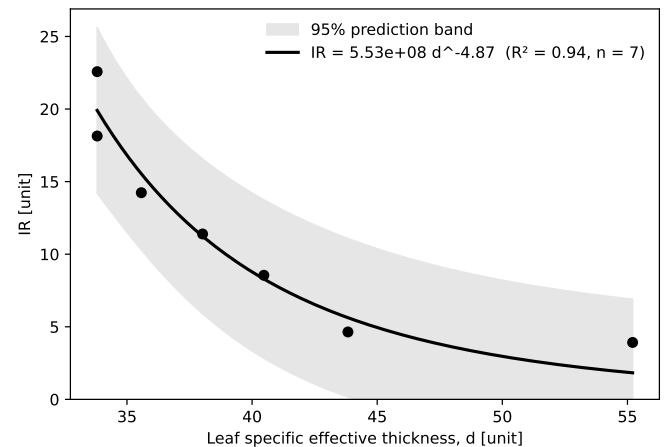


Figure 1. Power-law relationship between leaf specific effective thickness (d) and intrinsic physiological resistance (IR) of *Dendrobium loddigesii* in winter. Points, individual leaves ($n = 7$); line, nonlinear least-squares fit of Eq. (3); shaded band, 95% prediction band.

with an overall mean of 51.25 (all 16 samples) and no significant difference among groups (one-way ANOVA, $F = 0.92$, $p = 0.422$; Table 2). WTR is a characteristic parameter of retained water transport within leaves based on physiological impedance measurements and can effectively characterize intracellular water status [13]. Similar diurnal stability has also been observed in *Broussonetia papyrifera* in karst habitats, where photosynthetic efficiency increased with enhanced intracellular water use efficiency [14], suggesting that maintaining stable WTR may be an important strategy for karst plants to ensure sustained photosynthetic operation. The high conservation of WTR across seasons and leaf ages in this study supports the hypothesis that it is an evolutionarily conserved trait. It can be analogized to a “physiological heartbeat” at the cellular level. Under conditions in which CAM plants have completely closed stomata and an interrupted transpiration stream during the day, WTR continuously drives the basal cycling of intracellular water and solutes and may provide an irreplaceable underlying hydraulic safeguard for cell survival [15, 16].

3.2.2 Structural-Functional Decoupling in Spring Mature Leaves: Indirect Evidence for Active Membrane Remodeling in Source Organs

The mean d of spring old leaves was 27.58, 31.2% lower than that of winter old leaves (40.10; Welch's $t = 4.09$, $df = 7.5$, $p = 0.004$; Hedges' $g = 1.81$), consistent with the expectation that source organs undergo degradation of parenchymatous water-storage structures and morphological shrinkage

Table 1. Electrophysiological fingerprint statistics of overwintering mature leaves of *Dendrobium loddigesii* in winter ($n = 7$).

Parameter	Minimum	Maximum	Mean	SD	CV
Leaf specific effective thickness (d)	33.809	55.206	40.103	7.596	0.1894
Intrinsic physiological resistance (IR)	3.921	22.574	11.922	6.906	0.5793
Intrinsic physiological impedance (IZ)	0.731	1.652	1.235	0.322	0.2607
Intrinsic physiological capacitive reactance (IXc)	0.770	1.651	1.240	0.308	0.2484
Intrinsic physiological inductive reactance (IXI)	5.395	32.264	16.151	9.064	0.5612
Intracellular water transport rate (WTR)	49.491	53.461	51.756	1.518	0.0293
Intracellular nutrient transport rate (NTR)	3.431	11.353	6.297	2.895	0.4597
Nutrient active transport capacity (NAC)	22.201	112.710	49.822	29.400	0.5901
Resistance to low nutrition (RLN)	0.017	0.131	0.062	0.039	0.6320
Leaf metabolic flux (MF)	64.485	8399.788	1750.580	2984.944	1.7051
Relative metabolic activity (MA)	3.031	9.609	5.583	2.483	0.4447
Storage–water exchange coordination index (SWCI)	1.498	14.080	5.350	4.699	0.8783

Table 2. Comparative statistics of core electrophysiological parameters among three leaf groups in winter and spring (mean $\pm$ SD).

Parameter	Winter mature leaves ($n = 7$)	Spring mature leaves ($n = 4$)	Spring young leaves ($n = 5$)	F -value	p -value
d	40.10 $\pm$ 7.60a	27.58 $\pm$ 2.14b	31.16 $\pm$ 9.09ab	4.38	0.035
IR	11.92 $\pm$ 6.91a	10.55 $\pm$ 5.37a	20.22 $\pm$ 11.55a	1.94	0.183
IXc	1.24 $\pm$ 0.31a	1.21 $\pm$ 0.14a	1.32 $\pm$ 0.39a	0.16	0.853
IXI	16.15 $\pm$ 9.06a	16.19 $\pm$ 10.30a	42.61 $\pm$ 34.92a	2.75	0.101
WTR	51.76 $\pm$ 1.52a	50.72 $\pm$ 1.30a	50.96 $\pm$ 1.07a	0.92	0.422
NTR	6.30 $\pm$ 2.89a	5.85 $\pm$ 0.82a	5.81 $\pm$ 2.84a	0.07	0.936
MF	1750.58 $\pm$ 2984.94a	1029.99 $\pm$ 1151.56a	1551.77 $\pm$ 3140.71a	0.09	0.915
MA	5.58 $\pm$ 2.48a	4.97 $\pm$ 1.15a	4.22 $\pm$ 2.97a	0.46	0.640
SWCI	5.35 $\pm$ 4.70a	3.49 $\pm$ 2.51a	3.26 $\pm$ 4.59a	0.43	0.658

Note: F and p are from classical one-way ANOVA ($df = 2, 13$). Different lowercase letters within a row indicate significant differences among groups (Tukey's HSD, $p < 0.05$), shown only when the omnibus ANOVA was significant; otherwise all groups are marked "a". SWCI was calculated for each sample as d/IR before averaging.

after exporting assimilates. However, the mean IR was 10.55, showing a non-significant decrease relative to winter (11.92; -11.5% ; Welch's $t = 0.37$, $df = 7.8$, $p = 0.724$; Hedges' $g = 0.19$). This departed from the negative power-law coordination between d and IR observed during the steady-state phase and is at odds with the view that morphological structure passively determines membrane transport function, although the change in IR itself was not statistically significant.

Based on the winter steady-state power-law model, a counterfactual prediction was made (Table 3, Figure 2) by substituting the mean d of spring old leaves (27.58) into Eq. (3). Because this value lies outside the calibration domain ($d = 33.8$ – 55.2), the prediction is an extrapolation and should be interpreted with caution. The extrapolated prediction was $IR = 53.6$ (95% prediction interval: 25.9–81.4); the *in situ* measured IR (10.55) corresponded to 19.7% of the predicted value and lay below the lower bound of

the prediction interval. A measured value below the lower bound of the prediction interval indicates lower transport resistance than expected from the winter structure-function relationship, i.e., possible structure-function decoupling.

If confirmed by the re-fitted model, this deviation would suggest that, despite net loss of structural material, spring mature leaves may accomplish functional reprogramming of the membrane system. This interpretation is consistent with the hypothesis of active remodeling, although direct molecular evidence is not provided in this study.

3.2.3 Developmental Heterogeneity in Spring Young Leaves: Diversified Growth Strategies of Sink Organs

Although the mean IR of spring young leaves (20.22) was higher than that of overwintering old leaves (10.55) during the same period, this difference was not statistically significant (paired t -test across the 4 plants bearing both leaf types: $t = 0.88$, $df = 3$, $p = 0.442$),

Table 3. Comparison between measured IR of spring mature leaves and predicted values from the winter steady-state model.

Group	<i>d</i>	IR	Description
Winter mature leaves (modeling baseline)	40.10	11.92	Calibration data of Eq. (3) (<i>d</i> = 33.8-55.2)
Spring mature leaves - model prediction	27.58	53.6 (95% PI 25.9-81.4)	Extrapolated from the winter power-law model
Spring mature leaves - field measurement	27.58	10.55	Measured value is 19.7% of the prediction; below the lower bound of the 95% PI

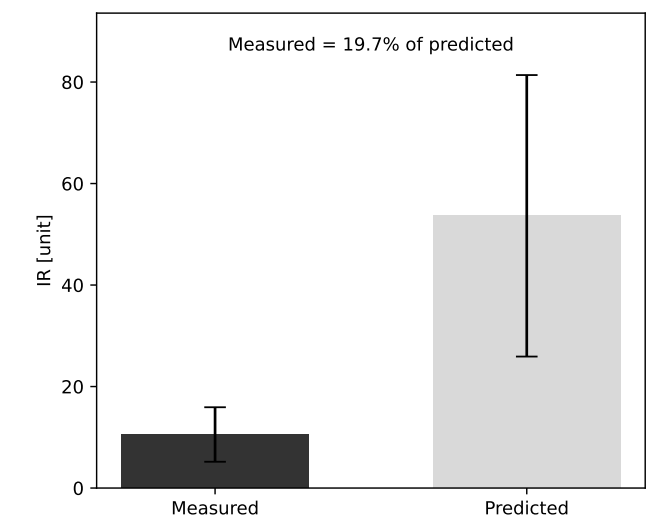


Figure 2. Measured IR of spring mature leaves compared with the IR predicted by the winter power-law model (Eq. 3) at the spring mean *d* = 27.58. Measured, mean $\pm$ SD (10.55 $\pm$ 5.37, *n* = 4); predicted, 53.6 with the 95% prediction interval (25.9-81.4; winter model *n* = 7).

which may reflect developmental-stage characteristics of incomplete membrane system differentiation and relatively high basal transport resistance in newly formed leaves. However, the within-population coefficient of variation in IR reached 57.1%, indicating extreme inter-individual differentiation in the construction of membrane transport function in young leaves. According to risk-spreading or bet-hedging theory, when environmental conditions fluctuate unpredictably across spatiotemporal scales, differentiated developmental strategies among individuals within a population can spread the mortality risk of a genet among ramets or individuals with different phenotypes, thereby reducing the annual reproductive variance of the population and maintaining long-term fitness [19]. Even among individuals with identical genotypes, stochastic fluctuations in gene expression can lead to pronounced phenotypic differentiation [20], and

such inter-individual variation may have important population-level adaptive value under stressful environments [21]; intraspecific trait variability (ITV) in plants has been demonstrated to be a key ecological factor determining individual and population performance, species interactions, and community assembly [22].

The two extreme differentiation types observed in this study may represent concrete manifestations of this theoretical framework in heterogeneous karst habitats. Individual mhsh young leaf 4 (IR as low as 3.945, *d* = 45.187) appears to adopt a “low-resistance sprint” strategy by rapidly assembling an efficient transmembrane transport system in parallel with morphological development to prioritize acquisition of limited resources during the dry season; individual mhsh young leaf 5 (IR up to 34.305) appears to follow a “conservative construction” strategy by preferentially accumulating cell wall and water-storage parenchyma structural materials while temporarily postponing optimization of membrane transport functions. The coexistence of these two developmental strategies may constitute an adaptive mechanism for diversifying environmental risks through phenotypic variation, which could be important for ensuring the overall stability and persistence of the population in the highly heterogeneous and unpredictable karst microhabitats.

3.3 Quantitative Indices: SWCI and SSTRI

For winter mature leaves, SWCI ranged from 1.498 to 14.080 (mean 5.350, CV 87.8%), reflecting substantial inter-individual variation in water storage-exchange coordination. Because winter and spring leaves were sampled from different individuals, SSTRI could be calculated only at the group level (Eq. 2): $SSTRI_{group} = 0.110$ (bootstrap 95% CI: 0.009-0.990). This is a single descriptive value for the population and carries no information on variation among individuals.

4 Discussion

4.1 Core Findings and Mechanistic Implications

Using a two-season between-population comparative framework, complemented by concurrent within-plant controls of leaves of different ages, this study systematically dissected the leaf electrophysiological dynamics of the obligate CAM epiphytic orchid *D. loddigesii* during the winter-spring phenological transition. The core findings can be distilled into three progressive tiers.

Tier 1 (baseline safeguard): The high conservation of WTR across seasons and leaf ages supports the hypothesis that it is an evolutionarily conserved “metabolic chassis trait.” Under conditions of complete stomatal closure and interrupted transpiration stream, this trait continuously drives the basal cycling of intracellular water and solutes, providing physiological clues at the membrane hydraulic level for understanding the core paradox of CAM plants, namely “stomatal closure with continued metabolic operation” [15, 16]. However, it should be noted that WTR is a passive parameter based on impedance measurements; its conservation alone does not directly establish causality, and future validation combining isotope tracing or metabolic flux analysis is required.

Tier 2 (structure-function decoupling trend): Against a background of a 31.2% decrease in the effective thickness of spring overwintering old leaves, IR showed a non-significant downward trend (−11.5%; Welch’s *t*-test, *p* = 0.724). The measured IR lay below the lower bound of the 95% prediction interval extrapolated from the winter power-law model, which, if below, suggests possible active functional reprogramming of the membrane system. However, given the lack of statistical significance and the extrapolative nature of the prediction, this conclusion should be regarded as suggestive rather than definitive evidence and requires further verification with an expanded sample size.

Tier 3 (diversified adaptation): The extreme inter-individual differentiation in IR of newly formed young leaves (CV 57.1%) is consistent with a bet-hedging strategy by which the population may spread environmental risk through phenotypic diversification. This developmental heterogeneity may itself be an adaptive product under the pressure of the highly heterogeneous karst habitat. Together, the three tiers of findings point to a unified regulatory logic: a two-tier architecture of “conserved chassis homeostasis + plastic functional regulation.”

4.2 Revision and Extension of the Plant Economics Spectrum Theory

The global spectrum of plant form and function and the “fast-slow” plant economics spectrum propose that plant morphological and physiological traits covary along a “fast resource acquisition-slow resource conservation” axis, with species exhibiting relatively fixed fast/slow survival strategies [23, 24]. The data from this study provide a possible revision and extension to this classical theory: fast and slow resource strategies may not be inherently fixed species attributes; rather, organs of different ages on the same individual and different phenological stages can undergo dynamic strategy switching. The overwintering mature leaves of *D. loddigesii* maintain a slow strategy (low metabolism, high storage) in winter and appear to switch toward a rapid resource-export strategy in spring, accompanied by decoupling between morphological and functional indicators—a phenomenon that would be difficult to explain under the traditional “structure-function coordination” framework. This finding suggests that the plant economics spectrum is not a fixed one-dimensional axis, but rather a highly plastic multi-dimensional trait regulatory space; the active physiological remodeling across organs and phenophases may substantially expand the degrees of freedom and strategic space for environmental adaptation.

4.3 Molecular Mechanism Hypothesis: Possible Pathways of Structure-Function Decoupling

Based on inferences from the literature, the phenomenon that membrane transport resistance did not increase synchronously in spring old leaves despite morphological shrinkage may involve the following synergistic mechanisms (which require further experimental validation): (1) adjustment of membrane lipid composition-during leaf senescence, membrane lipids undergo systematic remodeling, and phospholipid hydrolysis and fatty acid release provide the material basis for nutrient remobilization [25]. The maintenance or adjustment of the proportion of unsaturated fatty acids may ensure membrane fluidity and transport activity under conditions of structural shrinkage. (2) Phosphorylation of aquaporins-phosphorylation is a core mechanism by which plant cells finely regulate aquaporin gating and transport activity [26], and under drought stress, aquaporin phosphorylation status directly influences the efficiency of transmembrane water transport [27]. (3) Callose regulation of plasmodesmal permeability-plasmodesmal

permeability is dynamically regulated by callose deposition and degradation [28]; callose hydrolysis can enlarge the pore size of symplasmic channels and promote symplasmic unloading and partitioning of assimilates between source and sink. These hypotheses await direct verification by multi-omics approaches such as transcriptomics, proteomics, and phosphoproteomics.

4.4 Innovative Contributions and Methodological Advantages

Compared with previous studies, the core innovations of this study are reflected in three aspects. First, the ecological authenticity of the study material. Previous studies mostly used greenhouse-acclimated seedlings or short-term field sampling, making it difficult to capture the true adaptive characteristics of populations under long-term natural selection. This study relied on a wild reintroduced population that had been free of artificial management for 15 consecutive years; its trait differentiation is largely the result of natural selection rather than artificial intervention, and the conclusions therefore possess high ecological validity. Second, the design advantage of a two-season between-population comparison combined with concurrent within-plant controls using leaves of different ages. This design reduces genetic background confounding for within-season comparisons, allowing developmental stage effects to be resolved more clearly. Third, the application of a counterfactual prediction framework. Based on the winter steady-state model, counterfactual predictions were made for IR in spring old leaves; the deviation between measured values and the prediction interval provides a quantitative assessment of the “structure-function decoupling” hypothesis, although the extrapolative nature and statistical non-significance of the prediction require cautious interpretation.

In addition, the two quantitative indices constructed in this study provide descriptive tools for assessing stress adaptability in orchids. SWCI characterizes an individual's current coordination between water storage and membrane exchange, whereas SSTRI summarizes, at the population level, the change in transport resistance per unit change in effective thickness during the phenological transition. Because SSTRI is a group-level value, it cannot be used to rank individuals; using it for screening karst *Dendrobium* germplasm [29] would require repeated measurements of the same marked individuals across seasons.

4.5 Limitations, Application Value, and Future Perspectives

This study has the following limitations. First, the sample size was limited (winter: $n = 7$; spring old leaves: $n = 4$; spring young leaves: $n = 5$), which constrained statistical power; future studies should expand the sample size to enhance the robustness of the conclusions. Second, the seasonal change in IR did not reach statistical significance (Welch's t -test, $p = 0.724$), and the power-law prediction involved extrapolation; therefore, the evidence for structure-function decoupling should be regarded as suggestive rather than conclusive. Third, inferences regarding molecular mechanisms are mainly based on the literature and require direct validation using multi-omics approaches. Fourth, this study was based on *in situ* observations and did not include cross-validation experiments under controlled environments; subsequent studies should incorporate multifactorial interaction experiments. Fifth, winter and spring leaves were sampled from different individuals, so seasonal change could not be tracked within individuals; repeated measurements on marked plants are needed before any individual-level transition index can be defined.

In terms of application: (1) For the conservation of rare orchids, the successful maintenance of a naturally propagating population without artificial management for 15 years supports the feasibility of an *in situ* reintroduction conservation model. For field reintroduction and introduction of *Dendrobium*, individuals with high SWCI may be considered for preferential selection, while population physiological heterogeneity should be preserved. (2) For vegetation restoration in karst rocky desertification areas, *D. loddigesii* possesses both a low-resistance membrane transport system and physiological plasticity, making it suitable for limestone epiphytic habitats; it is therefore a potential species for characteristic economic-ecological composite restoration in rocky desertification areas. An *in situ* electrophysiological non-destructive detection system can enable rapid batch screening of genotypes adapted to different microhabitats [30]. (3) In terms of theoretical contribution, the two-tier adaptation model of “conserved chassis homeostasis + plastic functional regulation” proposed in this study provides a new framework for understanding the adaptive evolution of epiphytic CAM plants to extreme environments and opens a new direction for research on the plasticity dimension of the plant economics spectrum.

5 Conclusions

This study used a wild reintroduced population of the karst orchid *D. loddigesii* that had undergone 15 consecutive years of natural selection as experimental material. An *in situ* electrophysiological approach combining a two-season between-population comparison with concurrent within-plant controls of leaves of different ages was employed to systematically elucidate the regulatory mechanisms by which an obligate CAM epiphytic orchid adapts to the extremely arid karst habitat. Four core conclusions were reached:

(1) The intracellular water transport rate (WTR) was highly conserved across seasons and leaf ages (overall mean 51.25, within-group coefficient of variation $\leq 2.93\%$), suggesting that it is an evolutionarily conserved “metabolic chassis trait” and providing clues, from the perspective of membrane transport hydraulics, for understanding the continued operation of intracellular metabolism under daytime stomatal closure in CAM plants.

(2) The effective thickness of spring overwintering old leaves decreased by 31.2% (Welch’s *t*-test, $p = 0.004$), while IR showed a non-significant downward trend (-11.5% ; $p = 0.724$; Hedges’ $g = 0.19$). The measured IR was 19.7% of the value extrapolated from the winter power-law model ($IR = 5.53 \times 10^8 d^{-4.87}$, $R^2 = 0.94$) and lay below the lower bound of the 95% prediction interval, suggesting possible structure-function decoupling. However, this evidence is limited by statistical non-significance and the extrapolative nature of the prediction, and should therefore be interpreted cautiously as suggestive rather than conclusive.

(3) The population coefficient of variation in IR of newly formed young leaves was 57.1%, differentiating into two growth modes: a “low-resistance head-start type” and a “conservative construction type,” suggesting that the population may spread the risk of karst microhabitat stress through physiological heterogeneity.

(4) A two-tier adaptation theoretical model of “conserved chassis homeostasis + plastic functional regulation” was proposed for epiphytic CAM orchids, and two non-destructive descriptive indices were constructed—the individual-level SWCI and the group-level SSTRI—providing theoretical tools and technical references for the conservation of rare medicinal orchids and ecological restoration of karst rocky desertification.

Data Availability Statement

Data will be made available on request.

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Conflicts of Interest

The authors declare no conflicts of interest.

AI Use Statement

The authors declare that no generative AI was used in the preparation of this manuscript.

Ethical Approval and Consent to Participate

Not applicable.

References

- [1] Jiang, Z., Lian, Y., & Qin, X. (2014). Rocky desertification in Southwest China: Impacts, causes, and restoration. *Earth-Science Reviews*, 132, 1-12. [CrossRef]
- [2] Yang, M., Sun, D., Wang, X., Zhu, S., & Goodale, U. M. (2025). Different leaf strategies between lithophytic and terrestrial orchids in a subtropical karst forest. *Plants*, 14(8), 1161. [CrossRef]
- [3] Zhang, L., Chen, F., Zhang, G. Q., Zhang, Y. Q., Niu, S., Xiong, J. S., Lin, Z., Cheng, Z. M., & Liu, Z. J. (2016). Origin and mechanism of crassulacean acid metabolism in orchids as implied by comparative transcriptomics and genomics of the carbon fixation pathway. *The Plant Journal*, 86(2), 175-185. [CrossRef]
- [4] Yang, S. J., Sun, M., Yang, Q. Y., Ma, R. Y., Zhang, J. L., & Zhang, S. B. (2016). Two strategies by epiphytic orchids for maintaining water balance: thick cuticles in leaves and water storage in pseudobulbs. *Aob plants*, 8, plw046. [CrossRef]
- [5] Zou, L. H., Wan, X., Deng, H., Zheng, B. Q., Li, B. J., & Wang, Y. (2018). RNA-seq transcriptomic profiling of crassulacean acid metabolism pathway in *Dendrobium catenatum*. *Scientific Data*, 5(1), 180252. [CrossRef]
- [6] Zhang, C., Wu, Y., Su, Y., Xing, D., Dai, Y., Wu, Y., & Fang, L. (2020). A plant’s electrical parameters indicate its physiological state: A study of intracellular water metabolism. *Plants*, 9(10), 1256. [CrossRef]

- [7] Jócsák, I., Végvári, G., & Vozáry, E. (2019). Electrical impedance measurement on plants: a review with some insights to other fields. *Theoretical and Experimental Plant Physiology*, 31(3), 359-375. [CrossRef]
- [8] Kozlova, E., Yudina, L., Sukhova, E., & Sukhov, V. (2025). Analysis of electromyography as a tool for plant monitoring: progress and perspectives. *Plants*, 14(10), 1500. [CrossRef]
- [9] Qiu, S., Sultana, S., Liu, Z. D., Yin, L. Y., & Wang, C. Y. (2015). Identification of obligate C₃ photosynthesis in *Dendrobium*. *Photosynthetica*, 53(2), 168-176. [CrossRef]
- [10] Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., ... & van Kleunen, M. (2010). Plant phenotypic plasticity in a changing climate. *Trends in plant science*, 15(12), 684-692. [CrossRef]
- [11] Guo, Y., Ren, G., Zhang, K., Li, Z., Miao, Y., & Guo, H. (2021). Leaf senescence: progression, regulation, and application. *Molecular horticulture*, 1(1), 5. [CrossRef]
- [12] Clément, G., Moison, M., Soulay, F., Reisdorf-Cren, M., & Masclaux-Daubresse, C. (2018). Metabolomics of laminae and midvein during leaf senescence and source-sink metabolite management in *Brassica napus* L. leaves. *Journal of Experimental Botany*, 69(4), 891-903. [CrossRef]
- [13] Xing, D., Mao, R., Li, Z., Wu, Y., Qin, X., & Fu, W. (2022). Leaf intracellular water transport rate based on physiological impedance: A possible role of leaf internal retained water in photosynthesis and growth of tomatoes. *Frontiers in Plant Science*, 13, 845628. [CrossRef]
- [14] Qin, X. J., Xing, D. K., Wu, Y. Y., Wang, W. X., Li, M. Q., & Solangi, K. A. (2022). Diurnal variation in transport and use of intracellular leaf water and related photosynthesis in three karst plants. *Agronomy*, 12(11), 2758. [CrossRef]
- [15] Schulte, P. J., & Nobel, P. S. (1989). Responses of a CAM plant to drought and rainfall: capacitance and osmotic pressure influences on water movement. *Journal of Experimental Botany*, 61-70. [CrossRef]
- [16] Zotz, G., Andrade, J. L., & Einzmann, H. J. R. (2023). CAM plants: Their importance in epiphyte communities and prospects with global change. *Annals of Botany*, 132(4), 685-698. [CrossRef]
- [17] Luo, Y., Ho, C. L., Helliker, B. R., & Katifori, E. (2021). Leaf water storage and robustness to intermittent drought: A spatially explicit capacitive model for leaf hydraulics. *Frontiers in Plant Science*, 12, 725995. [CrossRef]
- [18] Zhang, C., Su, Y., Wu, Y., Li, H., Zhou, Y., & Xing, D. (2021). Comparison on the nutrient plunder capacity of *Orychophragmus violaceus* and *Brassica napus* L. based on electrophysiological information. *Horticulturae*, 7(8), 206. [CrossRef]
- [19] Gianella, M., Bradford, K. J., & Guzzon, F. (2021). Ecological, (epi)genetic and physiological aspects of bet-hedging in angiosperms. *Plant Reproduction*, 34(1), 21-36. [CrossRef]
- [20] Cortijo, S., Aydin, Z., Ahnert, S., & Locke, J. C. (2019). Widespread inter-individual gene expression variability in *Arabidopsis thaliana*. *Molecular systems biology*, 15(1), MSB188591. [CrossRef]
- [21] Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., ... & Vasseur, D. A. (2011). Why intraspecific trait variation matters in community ecology. *Trends in ecology & evolution*, 26(4), 183-192. [CrossRef]
- [22] Westerband, A. C., Funk, J. L., & Barton, K. E. (2021). Intraspecific trait variation in plants: a renewed focus on its role in ecological processes. *Annals of botany*, 127(4), 397-410. [CrossRef]
- [23] Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of ecology*, 102(2), 275-301. [CrossRef]
- [24] Díaz, S., Kattge, J., Cornelissen, J. H., Wright, I. J., Lavorel, S., Dray, S., ... & Gorné, L. D. (2016). The global spectrum of plant form and function. *Nature*, 529(7585), 167-171. [CrossRef]
- [25] Yang, B., Tan, Z., Yan, J., Zhang, K., Ouyang, Z., Fan, R., ... & Guo, L. (2024). Phospholipase-mediated phosphate recycling during plant leaf senescence. *Genome Biology*, 25(1), 199. [CrossRef]
- [26] Verdoucq, L., Rodrigues, O., Martinière, A., Luu, D. T., & Maurel, C. (2014). Plant aquaporins on the move: reversible phosphorylation, lateral motion and cycling. *Current Opinion in Plant Biology*, 22, 101-107. [CrossRef]
- [27] Shivaraj, S. M., Sharma, Y., Chaudhary, J., Rajora, N., Sharma, S., Thakral, V., ... & Deshmukh, R. (2021). Dynamic role of aquaporin transport system under drought stress in plants. *Environmental and Experimental Botany*, 184, 104367. [CrossRef]
- [28] Wu, S. W., Kumar, R., Iswanto, A. B. B., & Kim, J. Y. (2018). Callose balancing at plasmodesmata. *Journal of experimental botany*, 69(22), 5325-5339. [CrossRef]
- [29] Luo, M., Liu, X., Wu, R. J., Yang, P. F., Yang, L., Zhou, M., & Wu, M. K. (2024). Screening of new *Dendrobium officinale* strains adapted to karst forest environmental stress based on electrophysiological detection method. *Agronomy*, 14(7), 1530. [CrossRef]
- [30] Tran, D., Dutoit, F., Najdenovska, E., Wallbridge, N., Plummer, C., Mazza, M., ... & Camps, C. (2019). Electrophysiological assessment of plant status outside a Faraday cage using supervised machine learning. *Scientific reports*, 9(1), 17073. [CrossRef]

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