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Low and High Temperature Limits to PSII[1]

A Survey Using *trans*-Parinaric Acid, Delayed Light Emission, and F_o Chlorophyll Fluorescence

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ABSTRACT

Many studies have shown that membrane lipids of chilling-sensitive plants begin lateral phase separation (*i.e.* a minor component begins freezing) at chilling temperatures and that chilling-sensitive plants are often of tropical origin. We tested the hypothesis that membranes of tropical plants begin lateral phase separation at chilling temperatures, and that plants lower the temperature of lateral phase separation as they invade cooler habitats. To do so we studied plant species in one family confined to the tropics (Piperaceae) and in three families with both tropical and temperate representatives (Fabaceae [Leguminosae], Malvaceae, and Solanaceae). We determined lateral phase separation temperatures by measuring the temperature dependence of fluorescence from *trans*-parinaric acid inserted into liposomes prepared from isolated membrane phospholipids. In all families we detected lateral phase separations at significantly higher temperatures, on average, in species of tropical origin. To test for associated physiological effects we measured the temperature dependence of delayed light emission (DLE) by discs cut from the same leaves used for lipid analysis. We found that the temperature of maximum DLE upon chilling was strongly correlated with lateral phase separation temperatures, but was on average approximately 4°C lower. We also tested the hypothesis that photosystem II (PSII) (the most thermolabile component of photosynthesis) of tropical plants tolerates higher temperatures than PSII of temperate plants, using DLE and F_o chlorophyll fluorescence upon heating to measure the temperature at which PSII thermally denatured. We found little difference between the two groups in PSII denaturation temperature. We also found that the temperature of maximum DLA upon heating was not significantly different from the critical temperature for F_o fluorescence. Our results indicate that plants lowered their membrane freezing temperatures as they radiated from their tropical origins. One interpretation is that the tendency for membranes to begin freezing at chilling temperatures is the primitive condition, which plants corrected as they invaded colder habitats. An alternative is that membranes which freeze at temperatures only slightly lower than the minimum growth temperature confer an advantage.

Thermotropic properties of plant membrane lipids have been proposed to influence plant responses to both low and high temperatures. It has been shown using several different techniques that membrane lipids of chilling-sensitive species begin undergoing lateral phase separations (*i.e.* a minor component begins freezing) at chilling temperatures, whereas this does not occur until lower temperatures in membranes of chilling-resistant species (2, 10, 12, 14). Lateral phase separation begins at about the temperature at which chilling injury develops, and it has been proposed that the presence of frozen lipid may trigger chilling injury in chilling-sensitive plants (2, 7, 10, 12, 14).

It has also been shown that PSII is the most thermolabile aspect of photosynthesis and that the temperature at which PSII denatures is influenced by the fluidity of the thylakoid membranes (2, 15). Thermal denaturation of PSII has been shown to be closely correlated with the temperature at which photosynthesis becomes thermally unstable (18) and the temperature at which leaf thermal injury develops (3). Thylakoid membrane fluidity may therefore influence the high temperature limit to plant growth and survivorship.

Thermal denaturation of PSII may be conveniently detected by an abrupt rise in F_o[3] Chl fluorescence at a critical temperature, T_c (2, 17, 18); this rise thus may provide information about the fluidity of the thylakoid membrane. It has also been proposed that thermal denaturation of PSII may be detected using DLE, which is thought to arise from charge recombination at the PSII reaction center (9). DLE provides information about the overall function of the photosynthetic apparatus, since it is affected by a number of membrane-linked reactions (6, 8, 9). In particular, it has been proposed that it may be used to detect lateral phase separation of thylakoid lipids upon chilling (8).

Chilling-sensitive plants are often of tropical origin (1, 2, 7). In this study we test the hypothesis that the tendency for membrane lipids to begin freezing at chilling temperatures is a common property of plants of tropical origin, which is lost as plants invade colder habitats. We also test whether there is a relationship between lateral phase separation temperatures and the temperature at which PSII thermally denatures. Our approach is a comparative survey of plant species in one family confined to the tropics (Piperaceae) and in three families with both tropical and temperate representatives (Faba-

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[3] Abbreviations: F_o, Chl fluorescence with all PSII traps oxidized. DLE, delayed light emission; NL, neutral lipids; PL, phospholipids; GL, glycolipids.

ceae [Leguminosae], Malvaceae, and Solanaceae). To minimize effects of acclimation to different habitats we test plants grown under identical conditions, wherever possible. We measure membrane lateral phase separation temperatures and relate this to physiological function by measuring the temperature dependences of DLE and F_o Chl fluorescence.

MATERIALS AND METHODS

Plant Materials

Plant materials were either grown in temperature-controlled greenhouses under natural lighting or were collected in the field, as indicated in Table I.

Lipid Extraction

Lipids were extracted by a modification of Radin (13). Five g (fresh weight) leaves were boiled 5 min in 40 mL isopropanol and ground in a tissue grinder. Sixty mL hexanes were then added, and the crude extract was left for 1 h in the dark at room temperature. It was then washed with 50 mL 6.7% Na_2SO_4 (w/v) in water, the upper phase was recovered, and the lower phase was reextracted with an equal volume of hexanes:isopropanol (7:2, v/v). The pooled upper phases were dried under vacuum and fractionated by column chromatography on silica gel 60/200 (Baker) sequentially eluted with 20 column volumes each of chloroform (NL) and acetone (GL). PL were recovered with 2 column volumes of methanol, dried, then further purified by ascending column chromatography on silica gel 60/200 to remove residual pigments. Two glass tubes (i.d. 4 mm) joined with a Teflon connector were plugged at one end and filled with silica gel. PL were loaded, then the column was plugged with glass wool, inverted, and placed in a vial containing chloroform:methanol:water (65:25:4). The column was developed until the pigments entered the Teflon connector, then the tubes were separated, silica gel containing pigments was discarded, and PL (which run below the pigments in this system) were eluted with methanol.

Liposome Preparation and Fluorescence Depolarization

Liposomes were prepared from 0.5 mg PL mixed with 3 nmol *trans*-parinaric acid and dried under N_2. Three mL of 20 mM Tris-acetate, 2 mM EDTA (pH 7.2) in 20% ethylene glycol (v/v) heated to 50°C was added, and the tubes were vortexed. The liposomes were then transferred to a stirred cuvette placed in a Perkin Elmer model MPF-3L spectrofluorimeter. Temperature was monitored with a copper-constantan thermocouple and regulated by peltier-effect modules in contact with the sample holder. The excitation beam (320 nm) was passed through a polarizing prism (Karl Lambrecht, Chicago). Fluorescence was passed through a film polarizer (Edmund Scientific, Camden, NJ) oriented either parallel ($I_{||}$) or perpendicular ($I_{\perp}$) to the excitation polarizer, and its intensity at 420 nm (20 nm half-bandwidth) was measured as the temperature was increased from −6°C to 45°C in 0.1°C increments. At each increment, the position of the polarizer was changed, and the ratio $I_{||}/I_{\perp}$ was calculated for every cycle (*i.e.* at 0.2°C intervals). A 21× data logger (Campbell Scientific, Logan, UT) was used to control temperature and polarizer orientation, and for data acquisition.

Delayed Light Emission

DLE as a function of temperature was measured as described (6). Briefly, the actinic beam provided by either a HeNe laser (632.8 nm) or a HeCd laser (442 nm) was conducted by a fiber optic guide to a temperature-controlled cuvette containing the sample. The beam was interrupted by a rotating sector producing 0.9 ms light and 5.9 ms dark cycles. Delayed light was conducted from the sample by a fiber optic through a Schott RG 665 and a Balzers Calflex C filter to the photomultiplier (Hamamatsu Photonics R662). This combination of filters allowed all delayed light of more than 660 nm wavelength to be measured with a Becquerel-type phosphoroscope.

Samples were illuminated by the actinic light for about 20 min at their growth temperature. Once a stable signal was obtained they were cooled to nearly 0°C at about $1°C \cdot min^{-1}$. They were then heated at about $1°C \cdot min^{-1}$ until the DLE signal disappeared (usually near 60°C). Temperature was measured with a copper-constantan thermocouple, using an ice bath reference. DLE and temperature were recorded continuously on an X-Y recorder. The signal from the thermocouple was amplified (Hewlett Packard 2470A) and connected to the X input, while the DLE signal was amplified with a lock-in amplifier (model 128A, EG and G- Princeton Applied Research) and connected to the Y input.

F_o Chl Fluorescence

F_o Chl fluorescence as a function of temperature was measured essentially as previously described (18). Discs (0.18 cm^2) punched from the leaf to be assayed were placed on a nylon mesh support inserted into a suitably machined indentation in a brass block (about 500 g). A brass cover containing a plexiglass light-guide was then fastened over the sample, and a spring under the support pressed the sample against the light guide. The chamber under the sample was ventilated by two holes, and was partially filled with water at the start of each run to maintain humidity. Aeration with moistened air forced through these holes did not affect the results (W Terzaghi, unpublished data). Actinic light (about 0.03 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from a very stable source was passed through an interference filter (λ_{max} 480 nm, 7.1 nm half-bandwidth; Ditric Optics, Hudson MA) then led to the sample by a fiber optic. Fluorescence was conducted by a fiber optic to an interference filter (λ_{max} 695 nm, 12 nm half-bandwidth; Ditric Optics, Hudson MA), then measured with a photomultiplier tube. Temperature was monitored with a copper-constantan thermocouple inserted into the sample. Fluorescence and temperature signals were collected at 1 s intervals by a 21x data logger (Campbell Scientific, Logan, UT). Data files were then transferred to a Macintosh computer for analysis.

RESULTS

An example of *trans*-parinaric acid fluorescence intensity and polarization ratio as a function of temperature (plotted

Table I. *Lateral Phase Separation Temperatures, Low and High Temperature DLE Maxima, and F_oT_c of Various Plant Species*

DLE and Fo Chl fluorescence as a function of temperature were measured as described in "Materials and Methods" on leaf discs cut from individuals of the indicated plant species. Lipids were then isolated from the sampled leaves and their lateral phase separation temperatures were determined as described in "Materials and Methods." F_o is mean of three replicates; phase separation temperature is mean of two replicates; DLE is from a single sample. TLPS, temperature of lateral phase separation; F_o T_c, critical temperature for F_o Chl fluorescence; Growth T, growth temperature; Ref, reference for origin; Trop, tropical; Temp, temperate.

Plant Species		TLPS	DLE Maxima		F_oT_c	Growth T (day/night)	Origin	Ref
			Low	High				
		°C						
Piperaceae								
Piper aduncum L.		20	11	46, 49	45	28/23	Trop (C America)	4
P. aequale Vahl.		20	14	46, 51	44	28/23	Trop (C America)	4
P. auritum H.B.K.		15	12	46, 50	45	28/23	Trop (C America)	4
P. hispidum Sw.		18	13	45, 49	46	28/23	Trop (C America)	4
P. lapathifolium Steud.		23	10	48, 52	45	28/23	Trop (C America)	4
P. sanctum Miq.		19	11	48, 51	46	28/23	Trop (C America)	4
P. umbellatum L.		19	10	46, 50	45	28/23	Trop (C America)	4
Leguminosae								
Arachis hypogaea L	(peanut)	8	9	48	43	25/23	Temp (S Andes)[a]	21
Erythrina folkersii Krukoff & Mold	(coral tree)	18			46	28/23	Trop (C America)	1
Glycine max Mill.	(soybean)							
cv Corsoy		14	13	44	45	25/23	Temp (NE China)	21
cv Minsoy		15	9	46	45	25/23	Temp (NE China)	21
cv Noir I		15	10	45	45	25/23	Temp (NE China)	21
Lens esculenta L.	(lentil)	9	10	45, 50	46	25/23	Temp (E. Med)[b]	21
Medicago hispida L.	(bur clover)	<0	3	38	43	Field (Feb)	Temp (S Europe)	1
Medicago sativa L.	(alfalfa)	4	2	45, 49	47	25/23	Temp (Europe)	1
Phaseolus vulgaris L.	(bean)	18	3	46, 49	47	25/23	Trop (C Am & Peru)	21
Pisum sativum L.	(pea)	9	8	43	43	15/15	Temp (Asia/Med)	21
Vicia faba L.	(fava bean)	9	8	45, 49	46	25/23	Temp (E Med/Turkey)	21
Vigna radiata L.	(mung bean)	18	9	47, 50	47	25/23	Trop (India)	21
Vigna sinensis Endl.	(cowpea)	24	5	47	47	25/23	Trop (C Africa)	1
Malvaceae								
Althaea rosea Cav.	(hollyhock)	5	5	44	46	25/23	Temp (China)	1
Gossypium hirsutum L.	(cotton)	11	8	49	47	25/23	Trop (C America)	16
Hibiscus syriacus L.	(hibiscus)	<0	<0	43	31	Field (Feb)	Temp (China)	1
Hibiscus esculentus L.	(okra)	9	10	47	46	25/23	Trop (Tropical Asia)	1
Malva parviflora L.	(cheese-weed)	<0	<0	43	39	25/23	Temp (Eur/N Af/Asia)[c]	1
Solanaceae								
Capsicum annuum L.	(bell pepper)	8	8	46	45	25/23	Trop (C America)	16
Datura innoxia Mill.	(Jimson weed)	7	6	41	45	25/23	Temp (US, Mexico)	22
Lycopersicon esculentum Mill.	(tomato)	8	7	45	45	25/23	Trop (WS America)	1
Nicotiana sanderae Hort.	(flowering tobacco)[d]	6	6	45	41	25/23	Trop	1
Nicotiana tabacum L.	(tobacco)	7	5	46	46	25/23	Trop (C America)	1
Petunia hybrida Hort.	(petunia)[e]	6	5	46	43	25/23	Temp	1
Physalis ixocarpa Brot.	(tomatillo)	4	5	46	41	25/23	Temp (Mexico)	23
Solanum melongena L.	(egg plant)	16	10	42	43	25/23	Trop (SW Asia)	1
Solanum tuberosum L.	(potato, white rose)	4	4	40	44	Field (May)	Temp (C Andes)	16

[a] S Bolivia/N Argentina. [b] E Mediteranean/W Iran. [c] Europe/N Africa/Asia. [d] *N. sanderae* is a modern hybrid constructed from *N. alata* and *N. forgetiana* in 1903. Both parents are native to Brazil (1). [e] *P. hybrida* is probably a result of a cross between *P. axillaris* and *P. violacea*, both from Argentina (1).

as 1/K) is presented in Fig. 1, using PL prepared from soybean (cv Corsoy) leaves. Sharp changes in the temperature dependence of both fluorescence intensity and polarization ratio occur at about 15°C. These changes reflect lateral phase separations, that is, lipids begin freezing and aggregate to form frozen domains. The probe preferentially partitions into these domains, whereupon its fluorescence properties change (19). Other techniques, such as differential scanning calorimetry and electron spin resonance spectroscopy, detect lateral phase separations at the same temperatures (14). We therefore call this the lateral phase separation temperature.

Representative plots of F_o Chl fluorescence and DLE as a function of temperature are presented in Figures 2 and 3, respectively. F_o Chl fluorescence shows little change with temperature until a critical leaf temperature, T_c, is reached, at which it increases sharply. T_c has been shown to be closely

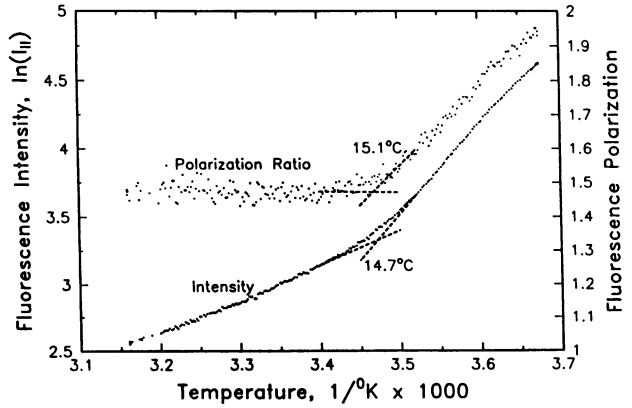

Figure 1. Lateral phase separation temperature of phospholipids prepared from soybean (cv Corsoy) leaves. Leaf lipids were extracted and liposomes containing the fluorescent probe *trans*-parinaric acid were prepared from the phospholipids as described in "Materials and Methods." Fluorescence intensity and depolarization of the probe were then measured as a function of temperature as described in "Materials and Methods." Note that data are presented as an Arrhenius-type plot. Also note the differing scales for fluorescence intensity and depolarization.

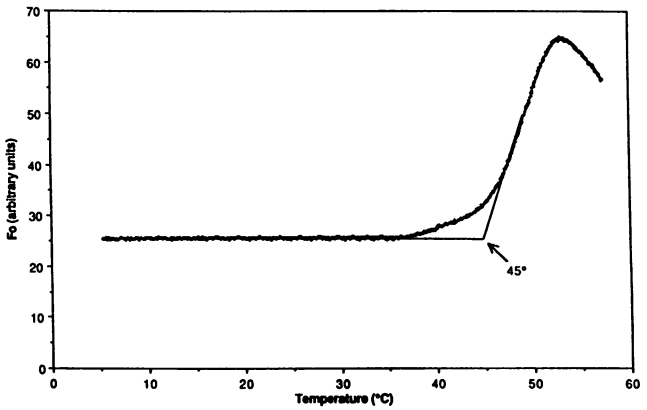

Figure 2. F_o Chl fluorescence of soybean (cv Corsoy) leaves as a function of temperature. F_o Chl fluorescence as a function of temperature was measured as described in "Materials and Methods," on a disc cut from the leaf used for Figure 1.

correlated with the temperature at which PSII begins thermal denaturation (18). DLE increases as the disc is chilled until a maximum is reached at a characteristic temperature (the low temperature DLE maximum), then decreases upon further chilling. Upon heating, DLE also increases to a maximum at a characteristic temperature (the high temperature DLE maximum) and then declines. We frequently observed a second maximum at higher temperature, whose magnitude varied greatly between samples, as shown in Figure 3. For plants showing a pronounced second peak, such as the bottom panel of Figure 3, we have included the temperature at which this occurred in Table I.

Lateral phase separation temperatures, low and high temperature DLE maxima, and F_o T_c temperatures for representative members of the Fabaceae, Malvaceae, Piperaceae, and Solanaceae are presented in Table I. All Piperaceae (an exclu-

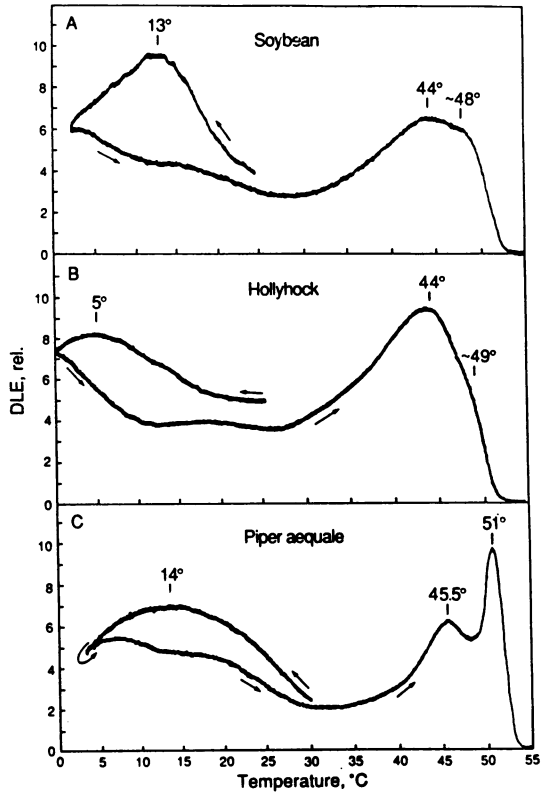

Figure 3. DLE of soybean (cv Corsoy), hollyhock (*A. rosea*), and *P. aequale* leaves as a function of temperature. DLE as a function of temperature was measured as described in "Materials and Methods." The soybean disc was cut from the leaf assayed in Figures 1 and 2.

sively tropical family) had lateral phase separations and low temperature DLE maxima at or above 10°C, whereas all other families had representatives both above and below 10°C. Phospholipids from species of tropical origin began lateral phase separation at a mean temperature of 15.4°C, while the mean separation temperature for species of temperate origin was 6.8°C (Table II). Species of tropical and temperate origin also had different average low temperature DLE maxima, although the difference was smaller, 8.9 and 6.0°C, respectively, for tropical and temperate species. However, although the means for both parameters differed significantly between the two groups, individual tropical and temperate species overlapped in both phase separation temperature and low temperature DLE maximum.

Results from the low temperature DLE measurements are closely correlated with those of the *trans*-parinaric acid studies (Table III). The mean temperature for lateral phase separation measured with *trans*-parinaric acid was 11.2°C while the mean low temperature DLE maximum was 7.5°C. The difference was even greater in the species of tropical origin, where the results were 15.4 and 8.9°C for *trans*-parinaric acid and low temperature DLE (Table III). In contrast, the mean results from *trans*-parinaric acid and low temperature DLE for species of temperate origin were not significantly different (Table III).

The temperature at which PSII thermally denatured was generally less variable (Table I). More than 85% of 68 readings

Table II. *Comparison of Lateral Phase Separation Temperatures, Low and High Temperature DLE Maxima, and F_o T_c between Species of Temperate and Tropical Origin*

Species origin based on the assignments in Table I. The significance of differences was tested with a Student's *t* test on unpaired values.

	Temperate Species	Tropical Species	
	°C		
Trans-parinaric acid			
Mean	6.8	15.4	
SD	5.0	5.7	
n	16	18	
t			4.64
P <			0.001
Low temperature DLE			
Mean	6.0	8.9	
SD	3.7	3.0	
n	16	17	
t			2.51
P <			0.017
High temperature DLE			
Mean	43.7	46.0	
SD	2.5	1.5	
n	16	17	
t			3.23
P <			0.003
Fo T_c			
Mean	43.25	45.33	
SD	3.9	1.5	
n	16	18	
t			2.1
P <			0.042

for both F_o T_c and high temperature DLE maxima were between 43 and 49°C, although the range for all plants was 31 to 49°C. The mean DLE high temperature maximum was not significantly different from the F_o T_c, and the two parameters were significantly correlated (Table III).

Species of temperate and tropical origin were less different at high than at low temperature extremes. The difference in the temperature of the high temperature DLE maximum was 2.3°C, whereas the difference in the F_o T_c was 2.0°C (Table II).

DISCUSSION

It has long been known that plants of tropical origin tend to be chilling sensitive, whereas plants of temperate origin are not, and that membrane lipids of chilling-sensitive plants begin freezing at chilling temperatures, whereas those of chilling-resistant plants do not (2, 4, 19). In this study we tested the hypothesis that the tendency for membrane lipids to freeze at chilling temperatures is a characteristic of tropical plants which is lost as plants invade colder habitats. Our results support this hypothesis. We found that membrane lipids of tropical members began lateral phase separation at temperatures at or above 10°C, whereas those of most temperate members began lateral phase separation at lower temperatures (Table I). Our survey of low temperature DLE maxima showed that these thermotropic changes in the physical state of the membrane lipids were correlated with physiological changes. By contrast, we found little difference between tropical and temperate plant species in the temperature at which PSII thermally denatured, as detected with DLE or F_o Chl fluorescence.

The magnitude of the DLE signal is influenced by many aspects of photosynthesis; DLE thus provides information about the overall functioning of the photosynthetic apparatus (9). The temperature of maximum DLE upon chilling has been proposed to signal the onset of lateral phase separation by thylakoid membrane lipids (8). One possible reason for the decline in DLE upon chilling below the low temperature maximum is dissipation of the proton gradient through leaks generated by frozen lipid, although it could be due to a decline in the activity of any component of photosynthetic electron transport (9).

We found that the low temperature DLE maximum was significantly correlated with lateral phase separation temperatures (Table III), although the DLE maximum was usually lower, especially in tropical plants (Tables I and II). DLE can thus be used to predict whether a plant's membrane lipids will begin lateral phase separation at chilling temperatures. One possible explanation for the difference between the lateral phase separation temperature in isolated lipids and the low temperature DLE maximum is that many plants use other mechanisms (which are discarded during the isolation of membrane lipids, such as nonpolar lipids or proteins) to lower

Table III. *Statistical Comparison of Two Methods for Detecting Lateral Phase Separations at Low Temperature (trans-Parinaric Acid and Low Temperature DLE Maximum) and Two Methods for Detecting Damage at High Temperature (F_o T_c) and High Temperature DLE Maximum*

All samples of <0°C were calculated as if they were 0°C. Data are for all plants.

Comparison	Mean	SD	*n*	*t*	P <
A. Students *t* tests on paired samples					
1. *trans*-parinaric acid	11.21	6.79			
Low temperature DLE_{max}	7.49	3.63	33	4.22	0.001
2. F_o T_c	44.96	2.29			
High temperature DLE_{max}	44.30	3.04	33	1.22	0.23 (NS)
Comparison			*r*	*n*	P <
B. Correlaton analysis					
1. *trans*-parinaric acid *versus* low temperature DLE_{max}			0.68	33	0.001
2. F_o T_c *versus* high temperature DLE_{max}			0.37	33	0.035

the *in vivo* phase separation temperature. Quinones are especially attractive candidates for membrane plasticizers.

Many studies have correlated the F_o T_c with various aspects of high temperature damage to leaves, including the thermal denaturation of PSII and the development of tissue necrosis (2, 3, 17, 18). In this study we found that the high temperature DLE maximum was correlated with the F_0 T_c, and usually occurred at about the same temperature. This was predictable, since DLE is affected by the inputs from many different processes related to photosynthesis. The slightly higher mean DLE maximum may reflect mechanistic differences between the two parameters. Immature or senescent cells in some of the samples may also account for some of the difference, as these give a relatively thermolabile F_0 signal (3, 18) but have less effect on DLE (W Terzaghi, unpublished data). The second DLE maximum at high temperature may also reflect the presence of different populations of cells, especially if some developed under different thermal regimes. Other possible sources include PSI or other types of PSII reaction centers (9).

We observed little variability in F_0 T_c and high temperature DLE maxima. These parameters were slightly higher in tropical species (Tables I and II); however, we did not observe the great variability described in other studies (2, 3, 5, 6, 15, 18). This probably occurred because we sampled plants grown under similar conditions in most cases, whereas the other studies sampled plants grown under differing thermal regimes (in fact, our greatest deviations were in field-grown material; Table I). Many plants can acclimate their F_0 T_c when grown in different habitats or when shifted to different growth temperatures (3, 5, 15, 18). A striking result of our survey is that all these plants chose roughly the same F_o T_c when grown under similar conditions.

Angiosperm plant families are generally thought to have arisen in the tropics and then invaded colder habitats (20). Also, global temperatures during the Late Cretaceous and Early Tertiary, the period in which Angiosperms first appeared, were warm relative to the present, probably 5 to 10°C warmer at low latitude and about 30°C warmer at high latitude (11). The observation that membrane lipids of tropical plants began phase separation at chilling temperatures might therefore be interpreted as indicating that this is a primitive condition which plants need to modify to invade colder habitats, perhaps because it is associated with chilling injury. An alternative hypothesis is that there is an advantage to constructing membranes which begin freezing at temperatures near the lowest expected temperature (although the two are not mutually exclusive). The former hypothesis is simplest. An argument favoring the latter hypothesis is that genetic exchange within families with both tropical and temperate representatives should diminish the frequency of tropical representatives with membranes which begin phase separation at chilling temperatures, if such membranes do not offer some advantage over membranes which do not begin freezing until lower temperatures. We did not observe such a trend.

Although the general trend was for lower lateral phase separation temperatures in plants adapted to colder habitats, there were some exceptions. For example, soybeans and peas are temperate plants; yet they began lateral phase separation at 15 and 9°C and low temperature DLE maxima at 9 and 8°C, indicating changes in both the physical properties of their membranes and in their physiological function at this temperature (Table I). Moreover, we have modified lipid compositions and lateral phase separation temperatures of photosynthetic soybean cell cultures without affecting their temperature dependence of photosynthesis (W Terzaghi, unpublished data). Thus, the presence of frozen lipid domains may not be sufficient to trigger chilling injury. Plants have undoubtedly invaded colder habitats on many independent occasions. Different plants appear to have solved problems created by frozen lipid domains in different ways, some of which may not involve lowering the temperature at which lipds freeze. Conversely, the most cold-labile component may limit range extensions into colder habitats; in some cases, this may be frozen lipid domains, while in others it may be cold-sensitive proteins.

We therefore propose that the tendency to undergo lateral phase separations at chilling temperatures is a symptom, but not necessarily a cause, of chilling sensitivity. As such, it is useful for predicting whether a plant is likely to be chilling sensitive, and DLE provides a convenient means of testing this, and the high temperature limit to PSII at the same time.

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LITERATURE CITED

1. **Bailey LH** (1928) The Standard Cyclopedia of Horticulture. MacMillan, New York
2. **Berry JA, Björkman O** (1980) Photosynthetic response and adaptation to temperature in higher plants. Annu Rev Plant Physiol **31:** 491–543
3. **Bilger H-W, Schreiber U, Lange OL** (1984) Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. Oecologia **63:** 256–262
4. **Burger WC** (1972) Evolutionary trends in the Central American species of *Piper* (Piperaceae). Brittonia **24:** 356–362
5. **Downton WJS, Berry JA, Seemann JR** (1984) Tolerance of photosynthesis to high temperature in desert plants. Plant Physiol **74:** 786–790
6. **Fork DC, Mohanty P, Hoshina S** (1985) The detection of early events in heat disruption of thylakoid membranes by delayed light emission. Physiol Veg **23:** 511–521
7. **Graham D, Patterson BD** (1982) Responses of plants to low, nonfreezing temperatures: proteins, metabolism and acclimation. Annu Rev Plant Physiol **33:** 347–373
8. **Havaux M, Lannoye R** (1983) Temperature dependence of delayed chlorophyll fluorescence in intact leaves of higher plants. A rapid method for detecting the phase transition of thylakoid membrane lipds. Photosynth Res **4:** 257–263
9. **Jursinic PA** (1986) Delayed fluorescence: current concepts and status. *In* Govindjee, J Amesz, DC Fork, eds, Light Emission by Plants and Bacteria. Academic Press, New York, pp 291–328
10. **Lyons JM, Raison JK** (1970) Oxidation activity of mitochondria isolated from plant tissue sensitive and insensitive to chilling injury. Plant Physiol **45:** 386–389
11. **Parrish JT** (1987) Global paleogeography and paleoclimate of the Late Cretaceous and Early Tertiary. *In* EM Friis, WGC Chaloner, PR Crane, eds, The Origin of Angiosperms and their Biological Consequences. Cambridge University Press, Cambridge, pp 51–73

12. **Pike CS, Berry JA** (1980) Membrane phospholipid phase separations in plants adapted or acclimated to different thermal regimes. Plant Physiol **66:** 238–241
13. **Radin NS** (1981) Extraction of tissue lipids with a solvent of low toxicity. Methods Enzymol **72:** 5–7
14. **Raison JK, Orr GR** (1986) Phase transitions in thylakoid polar lipids of chilling-sensitive plants. A comparison of detection methods. Plant Physiol **80:** 638–645
15. **Raison JK, Roberts JKM, Berry JA** (1982) Correlations between the thermal stability of chloroplast (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of the higher plant, *Nerium oleander*, to growth temperature. Biochim Biophys Acta **688:** 218–228
16. **Roberts EH, Summerfield RJ** (1987) Measurement and prediction of flowering in annual crops. *In* JG Atherton, ed, Manipulation of Flowering. Butterworths, London, pp 17–50
17. **Schreiber U, Armond PA** (1978) Heat induced changes of chlorophyll fluorescence in isolated chloroplasts and related heat damage at the pigment level. Biochim Biophys Acta **502:** 138–151
18. **Seeman JR, Berry JA, Downton WJS** (1984) Photosynthetic response and adaptation to high temperature in desert plants. A comparison of gas exchange and fluorescent methods for studies of thermal tolerance. Plant Physiol **75:** 364–368
19. **Sklar LA, Miljanich GP, Dratz EA** (1979) Phospholipid lateral phase separation and the partition of *cis*-parinaric acid and *trans*-parinaric acid among aqueous, solid lipid, and fluid lipid phases. Biochemistry **18:** 1707–1716
20. **Stebbins GL** (1972) Ecological distribution of centers of major adaptive radiation in angiosperms. *In* DH Valentine, ed, Taxonomy, Phytogeography and Evolution. Academic Press, London, pp 7–34
21. **Summerfield RJ, Roberts EH** (1987) Effects of illuminance on flowering in long- and short-day grain legumes: A reappraisal and unifying model. *In* JG Atherton, ed, Manipulation of Flowering. Butterworths, London, pp 203–233
22. **Thomas JH** (1961) Flora of the Santa Cruz Mountains of California. Stanford University Press, Stanford
23. **Zeven AC, Zhukovsky PM** (1975) Dictionary of Cultivated Plants and their Centres of Diversity. Pudoc (Centre for Agricultural Publication and Documentation), Wageningen, The Netherlands