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Short Communication

Membrane Lipid Physical Properties in Annuals Grown under Contrasting Thermal Regimes[1]

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CARL S. PIKE
Department of Biology, Franklin and Marshall College, Lancaster, Pennsylvania 17604

ABSTRACT

Trans-parinaric acid was used to determine the order/disorder transition temperatures of phospholipids extracted from leaves of warm- and cool-season annuals grown under contrasting thermal regimes. All species were capable of adjusting this property, although there was considerable variation in the extent of the adjustment.

Many organisms can alter the physical properties of their membrane lipids in response to a change in environmental temperature. Sinensky (14) postulated that in bacteria the temperature-induced alteration in lipid composition leads to the maintenance of a constant membrane fluidity at the various growth temperatures, hence the term homeoviscous adaptation. Membrane fluidity is also regulated in response to temperature changes in *Neurospora* (6) and *Tetrahymena* (5). In the case of perennial plants, we obtained evidence for a change in the membrane lipid order/disorder transition temperature, as detected by *trans*-parinaric acid, in response to a shift in the environmental temperature (8, 12). The acclimation was only partial and varied in extent in different species.

In addition to this environmental influence on plant lipids, there is also evidence for a genetic influence, observed in earlier experiments (8) with various wild and cultivated annuals (both monocots and dicots) grown at the same temperature. In nearly all instances, the temperature of the order/disorder transition was considerably higher in phospholipids from warm-season annuals than in phospholipids from cool-season annuals.

In the present work, I investigated the ability of annuals to adjust their phospholipid order/disorder transition temperature in response to growth at widely different temperatures. This work represented a test of the hypothesis that the restriction of annuals to a certain season might be, in part, a consequence of a limited ability to adjust the lipid properties.

MATERIALS AND METHODS

All plants were grown from seed at a warm or a cool temperature in growth chambers with a 16-h photoperiod and 60% RH. The warm condition was generally 42°C day/30°C night, although for a few cool-season plants the day temperature had to be lowered by a few degrees. The cool condition was 18°C/13°C. Procedures for lipid extraction and fluorometry with *trans*-parinaric acid were as described previously (8). The present experiments were conducted with an Aminco-Bowman spectrophotofluorometer.

RESULTS AND DISCUSSION

The major focus was on southwestern desert species, many of which had been studied previously (8). The order/disorder transition temperatures for desert dicots are shown in Table I. For the four warm-season species, the value for the lipids from the warm-grown plants is remarkably high. For the cool-season desert species, the transition temperatures for plants from either growth regime are generally several degrees lower than those for the warm-season plants.

The ability of these desert dicot species to acclimate is substantial but only partial; for a shift in day temperature of 25°C, the greatest shift in transition temperature was 15°C; the smallest, 7°C (Table I). The various field-grown perennials studied previously (8, 12) also exhibited only partial adjustment, and again there was a range of magnitudes of shifts (from 6 to 17°C for a 21°C shift in day temperature). Therefore, both annuals and perennials are able to adjust the order/disorder transition temperature, and it cannot be concluded that annuals, as a group, are any less capable than perennials of making this adjustment.

We suggested that our previous results (8) were compatible with the notion, developed in studies on the growth and lipid properties of mung beans (11), that the order/disorder transition temperature may set a rough lower limit for normal plant function. Although no quantitative studies of growth were performed here, I noted

Table I. *Phospholipid Order/Disorder Transition Temperatures for Desert Dicots*

Plants were grown from seed under warm (typically 42°C/30°C day/night, 16-h photoperiod) or cool (18°C/13°C) conditions. The transition temperatures were determined as the average value from *trans*-parinaric acid fluorescence intensity and polarization ratio plots of data from two samples.

Species	Transition Temperature	
	Warm-grown	Cool-grown
	°C	
Cool climate		
Perityle emoryi	16	4
Gerea canescens	14	4
Palafoxia linearis	12	1
Warm climate		
Boerhaavia coccinea	20	13
Amaranthus palmeri	23	16
Mollugo verticillata	22	15
Portulaca oleracea	22	7

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that *Boerhaavia*, *Amaranthus*, and *Mollugo* grew extremely slowly at 18°C/13°C; *Amaranthus* in particular looked quite unhealthy. Therefore, although these species can acclimate to some extent, they are clearly restricted to relatively warm conditions and would probably be unable to grow at temperatures much lower than those tested. The perennials can shift over a range of temperatures that is no broader than that of these warm-season annuals, but the latter cannot shift down into the low-temperature range that a desert evergreen perennial would experience in winter. *Portulaca*, which exhibited the greatest shift of transition temperature, is an extremely widespread species, occurring from the southwestern deserts into eastern Canada.

The cool-season desert species exhibited transition temperatures slightly above 0°C when grown at 18°C/13°C. These seeds were all collected in the Death Valley area, where the temperature range in the coldest month is 18°C/3°C and temperatures below 0°C are rare (8). Therefore, the observed transition temperatures approximate the lowest temperature the plants might likely experience. These species continue growth into the warm spring months, and the transition temperature is substantially higher for the warm-grown than for the cool-grown plants. However, the values for the warm-grown cool-season plants were several degrees below those for the warm-season species, and this observation may be related to the inability of the cool-season species to continue growth in the summer.

The magnitudes of the shifts in transition temperatures for the grasses, ranging from 2 to 6°C (Table II), were smaller than for the dicots. As an example of a cold-tolerant grass, *Bromus rigidus* showed transition temperatures well below 0°C, with some ability to acclimate (Table II). This plant grew extremely rapidly at the cool temperature and could not be grown above about 38°C day. In contrast, *Zea mays* and the four *Panicum* species have transition temperatures typical of warm-season plants. The *Panicum* species tested include C_4 (*P. maximum*, *P. miliaceum*), C_3 (*P. bisulcatum*), and 'intermediate' (*P. milioides*) types (7), but there is no dramatic difference related to photosynthetic pathway.

It appears that a shift in transition temperature is part of a plant's mechanism of acclimation to temperature. A given set of phospholipids is apparently not appropriate to normal function over a wide temperature range, yet it is not evident why some species shift the transition temperature more than others. As noted below, studies of plant lipid chemical properties have also demonstrated temperature-dependent changes.

Several chemical properties of lipids can influence their physical properties; these include the acyl chain length and degree of unsaturation, the nature of the polar head group, and the position of the fatty acids on the glycerol (4). Although in simple model systems it is possible to predict the effect of a given change in chemical properties on the physical properties, such predictions are not necessarily possible in a complex system of plant lipids; direct measurements of physical properties need to be made. For example, in comparing membrane lipids from tender and cold-hardened artichoke tubers, Hannon and Raison observed that changes in membrane fluidity and in transition temperature were not accompanied by changes in fatty acid unsaturation (2). In other experiments in which only phospholipid chemical properties were measured, a decrease in growth temperature was often associated with an increase in unsaturation (4, 12, 13, 17) or a change in polar head group composition (3).

Table II. *Phospholipid Order/Disorder Transition Temperatures for Grasses*

Experimental details are given in Table I.

Species	Transition Temperature	
	Warm-grown	Cool-grown
	°C	
Zea mays	15	10
Panicum miliaceum	18	15
Panicum maximum	15	13
Panicum bisulcatum	13	7
Panicum milioides	12	8
Bromus rigidus	−6	−10

Our studies to date have not provided information on the nature of the phospholipids whose state is probed by *trans*-parinaric acid. This molecule is sensitive to the appearance of a few per cent solid in a sample (16). Recently, Wright and Raison (unpublished) have shown that in mung bean phospholipids there is a small fraction (<5%) of high-melting point lipids with two saturated fatty acids, although the bulk of the fatty acids are typically polyunsaturated. As a result, both electron spin resonance and differential scanning calorimetry detect an order/disorder transition at about 12°C, typical of chilling-sensitive species. No such fraction was detected in the polar lipids of the chilling-resistant wheat, and no physical changes were seen above 0°C. Therefore, *trans*-parinaric acid could be detecting the solidification of some such minor high-melting point fraction or domain. Although the domain of gel phase may be relatively small, the fact that the transition occurs at a temperature close to the minimum temperature for growth suggests that this domain has physiological significance (11; Wright and Raison, unpublished).

Related to this idea, an experiment was conducted with *cis*-parinaric acid, which shows little discrimination between ordered and disordered lipids (16). Several plant phospholipid samples were analyzed, and essentially straight-line fluorescence intensity plots were obtained (Fig. 1). Therefore, I conclude that there is no bulk phase change occurring; rather, only a small fraction of lipid is undergoing a change in order. That fraction is too small to be probed by *cis*-parinaric acid because most of the probe molecules will still remain in the unchanged disordered regions, but the fraction is probed by the *trans* isomer, because of its strong preference for ordered domains in a mixed system. A related observation is that the *trans*-parinaric acid plots of phospholipids from plants with very low transition temperatures (*e.g.* barley

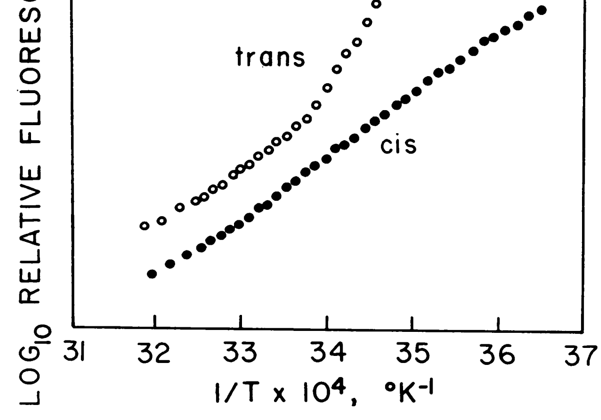

FIG. 1. *cis*- and *trans*-Parinaric acid fluorescence intensity of *A. palmeri* phospholipid vesicles.

[Figs. 1 and 2 in Ref. 8]) show much less dramatic deviations from linearity than those of plants such as corn with a higher transition temperature. This observation suggests that, as concluded by Wright and Raison, there is a smaller fraction of high-melting point lipids (whose change in order is detected by *trans*-parinaric acid) in the cold-season plants.

Raison and Berry (9, 10) have suggested that the viscosity of the membrane lipids is important in determining the high temperature limit to normal function. The temperature of an abrupt increase in Chl fluorescence (F_o) in intact leaves is an indicator of the point of lamellar membrane damage. This break point is higher in warm-season that in cool-season plants grown at the same temperature. Plants can adjust this point in response to shifts in environmental temperature (Downton *et al.*, unpublished; 1, 15). Of course, the Chl fluorescence technique probes the lamellar membranes, while our studies probe a mixture of phospholipids from various membranes (although lamellae contain very little phospholipid). However, acclimation to temperature may well involve many different parts of the cell.

By way of comparison to the present results, Downton *et al.* (unpublished) reported, for a 15°C shift in growth temperature, the following shifts in F_o break points: *Zea mays*, 2.6°C; *Amaranthus palmeri*, 4.0°C; *Portulaca oleracea*, 2.7°C; *Gerea canescens*, 6.8°C. Downton *et al.* suggested that the warm-season plants have a narrower range of thermal stability than the cool-season desert dicots, particularly because of the latter's greater ability to shift downward in cool conditions. With the exception of the wide-ranging *Portulaca*, a similar trend is evident in the present data (Table I), although the number of species studied here and by Downton *et al.* is small. These techniques, taken together, may be useful in studies of the ability of species to acclimate to temperature.

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