Adaptive Evolution of Seed Oils in Plants: Accounting for the Biogeographic Distribution of Saturated and Unsaturated Fatty Acids in Seed Oils

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abstract: Structural, energetic, biochemical, and ecological information suggests that germination temperature is an important selective agent causing seed oils of higher-latitude plants to have proportionately more unsaturated fatty acids than lower-latitude plants. Germination temperature is predicted to select relative proportions of saturated and unsaturated fatty acids in seed oils that optimize the total energy stores in a seed and the rate of energy production during germination. Saturated fatty acids store more energy per carbon than unsaturated fatty acids; however, unsaturated fatty acids have much lower melting points than saturated fatty acids. Thus, seeds with lower proportions of saturated fatty acids in their oils should be able to germinate earlier and grow more rapidly at low temperatures even though they store less total energy than seeds with a higher proportion of saturated fatty acids. Seeds that germinate earlier and grow more rapidly should have a competitive advantage. At higher germination temperatures, seeds with higher proportions of saturated fatty acids will be selectively favored because their oils will provide more energy, without a penalty in the rate of energy acquisition. Macroevolutionary biogeographical evidence from a broad spectrum of seed plants and the genus *Helianthus* support the theory, as do microevolutionary biogeography and seed germination performance within species of *Helianthus*.

Keywords: adaptive evolution, seed oil composition, latitudinal temperature gradient, germination, competition.

During germination, prior to initiation of photosynthesis, nearly all nonparasitic angiosperms and gymnosperms rely exclusively on reserves stored in seeds for both energy and carbon. The quantity and quality of these reserves are critically important because of their role in germination, a period in the life history of plants, especially annuals, that often plays a crucial role in determining plant fitness (Rees and Long 1992; Westoby et al. 1992). Annuals must successfully establish and reproduce during the year they germinate. Their competitive rank, and therefore their fitness, are often established by the timing of germination and their growth rate during establishment (Weiner 1985; Weiner and Thomas 1986; Silvertown 1988; Benjamin 1990; Schmitt and Ehrhardt 1990; Weiner and Thomas 1992). For perennial plants or shade-tolerant plants that emerge and grow for a time beneath a canopy, seed stores may also be important to attain a minimum size threshold before becoming completely dependent on photosynthesis for energy and carbon (Westoby et al. 1992). Insofar as the quantity and quality of stored reserves play a role in determining germination characteristics, they also play a role in plant fitness and are therefore under selection.

Energy and carbon reserves in seeds are either starches or triacylglycerols (TAGs)—more commonly called fats and oils. The majority (>80%) of plant species rely almost exclusively on TAGs in their seeds (Harwood 1980). Some attention has been paid to the evolutionary pressures acting on seed oil content (Levin 1974)—the proportion of total seed weight constituted of TAGs—but no work, empirical or theoretical, has attempted to account for the evolution and adaptation of seed oil composition—the types of fatty acids (FAs) in TAGs and their relative proportions. This theoretical and experimental gap is puzzling because the FAs that make up the TAGs in seeds are extremely varied, much more so than the FAs in phospholipids (Harwood 1980; Browse and Somerville 1991; Ohlrogge and Browse 1995; Harwood 1996), the other major sink for FA biosynthesis in plants. In plants, the FAs in phospholipids are almost completely restricted to 16 and 18 carbons and are either saturated or mono- and diunsaturated. In contrast, depending on the plant species, seed oil chain lengths may vary from as few as eight carbons to as many as 24 (Eckey 1954; Hilditch and Williams 1964; Gurr 1980), and the degrees of unsaturation range from

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none to as many as 4, as measured by the number of carbon-carbon double bonds in a chain. Here, I present a theory to account for the relative proportions of saturated and unsaturated FAs in seed TAGs and macro- and microevolutionary evidence in support of the theory.

Theory to Predict the Relative Proportions of Saturated and Unsaturated TAGs

TAGs are found almost exclusively in seeds, although a small proportion of plant species have oily fruits (Harwood 1980). From an ecological and selective perspective, this means that, for most plants, the production and direct effects of TAGs are confined to the seed stage of the life cycle. Therefore, TAGs are freed from direct selective pressures in other stages of the plant's life history. In addition, for species that have oily fruits, the oil composition of the fruit is usually quite different from the oil composition of the seed (Gurr 1980), indicating that the oil compositions of seeds and fruits are at least partially genetically independent of one another.

Energetics of Seed Oil Synthesis and Oxidation

Since one of the primary functions of TAGs is to store energy for the seed, we might expect selection on oil composition to maximize the energy stored. On a per carbon basis, unsaturated FAs cost more to produce and yield less energy when oxidized than saturated FAs (Lehninger 1993). In de novo FA synthesis, which occurs in plastids, saturated FAs are always the precursors of unsaturated FAs. The same FA synthase complex is used to synthesize all saturated FAs between four and 18 carbons (Ohlrogge et al. 1993; Harwood 1996), whereas a different desaturase is responsible for each degree of unsaturation of a FA having a specific number of carbons. Production of desaturase enzymes is the first incremental cost of producing unsaturated FAs. In addition, an energy penalty is exacted during formation of a double bond. The higher the degree of unsaturation, the greater the energy penalty.

During catabolization of TAGs (β-oxidation in most cases), a larger amount of energy is recovered from saturated FAs than from unsaturated FAs, again because of extra steps and enzymes required to process unsaturated FAs (Lehninger 1993). Each *cis* double bond must be converted to a *trans* double bond before the carbons associated with them can be oxidized by the same pathway as saturated FAs. In addition, there are fewer hydrogens attached to the double-bonded carbons, which means these carbons ultimately contribute fewer electrons to the respiratory chain than saturated carbons (Murray et al. 1996).

Production of TAGs with only saturated FAs would allow mother plants to produce either the most or the largest seeds with a given amount of energy and material, and it would afford germinating seeds the maximum amount of energy for growth. However, some seed TAGs are remarkably low in saturated FAs (e.g., Eckey 1954), suggesting that something other than selection for maximum energy storage is affecting the proportion of saturated FAs in TAGs for at least some species of plants.

Differences in Melting Points

Saturated and unsaturated FAs of the same chain length also differ dramatically in their melting points. Due to the rigid 30° bend at each *cis* double bond, unsaturated FAs have melting points much lower than those of saturated FAs (fig. 1; Eckey 1954; Hilditch and Williams 1964). The single-bonded carbons in saturated FAs rotate freely and are, therefore, able to achieve a lowest-energy configuration where the chains are straight. This allows these nonpolar molecules to pack so that van der Waals forces play a significant role along much of their length. In contrast, unsaturated FAs pack less closely, producing the lower melting points. For example, the single degree of unsaturation that produces oleic acid from stearic acid reduces the melting point of this 18-carbon FA to a greater extent than shortening the saturated FA chain by 10 carbons (fig. 1).

The melting points of pure FAs do not correspond precisely to the melting points of TAGs because different combinations of FAs can be attached to the glycerol backbone (Larsson 1986). Roughly, the melting point of a TAG is the average of the melting points of the FAs of which it is composed (Malkin 1954), but there are further com-

Saturated Fatty Acid	Melting Point (°C)	Unsaturated Fatty Acid	Melting Point (°C)
Caprylic (8:0)	16.7		
Capric (10:0)	31.6		
Lauric (12:0)	44.2		
Myristic (14:0)	54.4		
Palmitic (16:0)	62.9	Palmitoleic (16:1)	-0.5
Stearic (18:0)	69.6	Oleic (18:1)	13.4
		Linoleic (18:2)	-5.0
		Linolenic (18:3)	-10.0
Arachidic (20:0)	75.3	Eicosenoic (20:1)	25.0
		Arachidonic (20:4)	-49.5
Behenic (22:0)	79.9	Erucic (22:1)	33.5

Figure 1: Melting points of some common saturated and unsaturated seed oil fatty acids. Values in parentheses preceding the colon are the number of carbons in the fatty acid chain. Values following are the number of double bonds.

plications because the oleosomes, in which TAGs are stored in plants (Huang 1992), generally consist of mixtures of TAGs having different combinations of the FAs characteristic of a given species (Stumpf 1980). Nonetheless, it remains true that the higher the proportion of unsaturated FAs incorporated into TAGs, the lower the melting point of the mixture (Larsson 1986). Therefore, if selection favors lower-melting-point TAGs under certain conditions, incorporation of a higher proportion of unsaturated FAs is a very effective mechanism to achieve this result.

Selection by Germination Temperature

Evidence suggests that the lipases that catalyze the removal of FAs from glycerol prior to β-oxidation operate more rapidly on liquid substrates (Huang 1992; Miquel 1994; Thompson and Li 1997). Under cooler temperature conditions high-oleate *Arabidopsis thaliana* seeds germinated later than wild-type seeds containing a higher proportion of lower-melting-point polyunsaturated FAs (Miquel 1994). Canola (*Brassica napus*), genetically engineered to produce high levels of high-melting-point stearic acid (18 : 0) in its TAGs (Knutzon et al. 1992), germinated poorly and grew more slowly than its untransformed parent (Linder and Schmitt 1995; Thompson and Li 1997; Linder 1998). Similar problems were not seen in a transgenic canola that produced high levels of the lower-melting-point lauric acid (12 : 0; Linder 1998), although the canola results must be interpreted carefully since the high-laurate and high-stearate canolas were produced from different parental lines.

Hence, at cool germination temperatures, seeds within a population that have a higher proportion of unsaturated oils may germinate earlier and/or more rapidly than seeds that are higher in saturated FAs. All other things being equal, such seeds should gain a competitive advantage and, therefore, be larger at time of reproduction. At cool germination temperatures, the extra potential energy in seeds with higher proportions of saturated oils would be wasted as a result of later and slower germination. On the other hand, at warm temperatures, seeds with higher proportions of saturated oils would be favored because they would have more energy for growth without delaying or slowing germination.

It is plausible that the saturated-unsaturated ratio of seed oils is optimized to provide the earliest and/or most rapid growth at the mean daily germination temperature most often experienced by the germinating seed. Mean daily germination temperature integrates the temperatures at which metabolization of seed oils for germination occurs. Although minimum and maximum daily temperatures are often important thresholds for maintaining dormancy or initiating germination (see Baskin and Baskin 1998 for discussion and examples), they are only transient temperatures during which seed oils are metabolized for germination.

If mean daily germination temperature is the selective force driving the relative proportions of saturated and unsaturated FAs in seed TAGs, two clear biogeographical patterns are predicted. The relative proportion of unsaturated FAs in TAGs should increase from low to high latitudes and from low to high altitudes. Only the latitudinal gradient is explored in detail here.

Lowland tropical areas have minimum annual temperatures that are higher than many germination temperatures in temperate areas (temperature data from www.ncdc.noaa.gov, germination temperatures from Baskin and Baskin 1998), so mean daily germination temperatures at tropical sites must necessarily be warmer than those at temperate sites. For example, coastal Belize has a minimum annual temperature of 21°C, whereas several species common in Wisconsin germinate at 10°C (Baskin and Baskin 1998). As a general rule, minimum temperatures within the Tropics also decrease with increasing latitude (Strahler and Strahler 1992); however, local ecological considerations and the life histories of individual species will also influence when germination occurs and, hence, germination temperatures. In the humid Tropics, for plants lacking seed dormancy, germination temperature will correspond closely to the temperature at the time seeds are dispersed, whereas seeds with dormancy (Vázquez-Yanes and Orozco-Segovia 1993) may experience warmer conditions as a result of delaying germination until a light gap is produced. Areas with pronounced wet-dry seasons should have oil compositions optimized for temperatures when the wet season begins. It should also be pointed out that since tropical areas do not experience freezing temperatures, and in many cases never approach freezing, selection in cooler areas of the Tropics may act for shorter saturated FAs rather than increased proportions of unsaturated FAs. This could explain the prominence of tropical species that are high in lauric (12 : 0) and myristic (14 : 0) acids (Eckey 1954; Hilditch and Williams 1964) and the evolution of short-chain FAs in *Cuphea* (Miller et al. 1964*b*).

Within the temperate zone, the situation is more complex, but the same argument regarding mean daily germination temperature holds. Although the threshold temperature required to initiate germination for many temperate plants is a maximum diurnal value (reviewed in Baskin and Baskin 1998), which causes populations of a species at different temperate latitudes to germinate at the same thresholds, the mean daily temperature is higher at lower latitudes when the threshold is exceeded. For example, analysis of mean daily temperatures in Austin, Texas, and Fargo, North Dakota, from 1989 to 1998 (data

from www.ncdc.noaa.gov) showed that when the germination temperature threshold for *Helianthus annuus* (10°C daily maximum) was exceeded during periods of seed germination (January and February in Austin and April and May in Fargo), mean temperatures were 3.6°C higher in Austin.

Predictions Following from the Theory

Several verifiable microevolutionary predictions follow from the theory that germination temperature selects an optimal saturated-unsaturated FA ratio in seeds. First, species with broad latitudinal or altitudinal distributions should exhibit patterns of saturated-unsaturated ratios such that higher-latitude and higher-altitude populations have lower proportions of saturated FAs. Second, at cooler temperatures, seeds lower in saturated FAs should germinate earlier and/or grow more rapidly prior to photosynthesis than seeds higher in saturated FAs that are otherwise genetically similar. The opposite should be true at warmer germination temperatures. Third, when growing with competitors and germinating at lower temperatures, seeds with a lower proportion of saturated FAs should be competitively superior to seeds higher in saturated FAs, and this competitive advantage should lead to higher relative fitness for the low-saturated FA plants. Finally, at cooler temperatures, TAGs lower in saturated FAs should be catabolized more rapidly.

Given sufficient genetic variation for seed oil composition, the operation of selection at the microevolutionary level should produce predictable macroevolutionary patterns. Within a clade, species that germinate at cooler temperatures should have lower proportions of saturated FAs when the composition of seed oils is analyzed using comparative methods (e.g., Felsenstein 1985; Purvis and Rambaut 1995). When the association between the proportion of saturated FAs and germaination temperature does not hold, there should be either lack of genetic variation for oil composition within one or more species in the clade or other selection processes for alternate oil compositions. Finally, if the hypothesis is correct, constraints on the evolution of the saturated-unsaturated ratio may play a role in explaining species' ranges. Species lacking genetic variation for the saturated-unsaturated ratio may be prevented from expanding their latitudinal or altitudinal range.

In the remainder of this article, I show that the macroevolutionary biogeographical distribution of the ratio of saturated and unsaturated seed oils varies as predicted for a taxonomically broad group of tropical and temperate seed plants and within the temperate genus *Helianthus*. I also present microevolutionary experimental evidence from *Helianthus* that is compatible with the hypothesis.

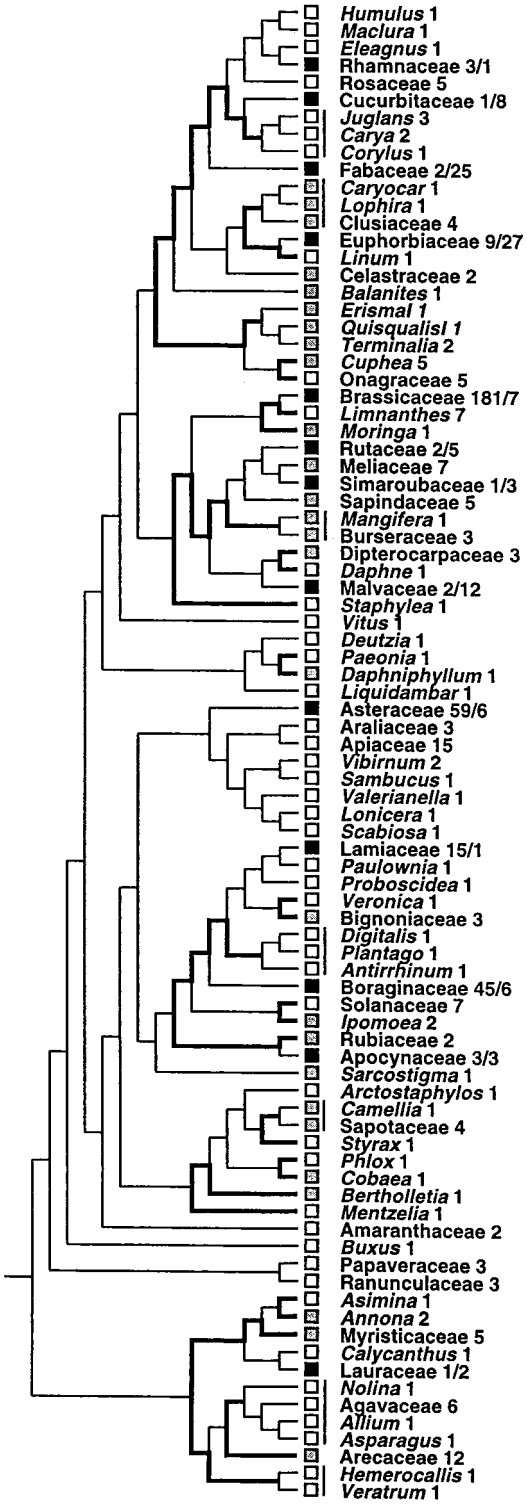
Material and Methods

Broad-Scale Pattern of the Relative Proportions of Saturated and Unsaturated Seed Oils

I used published seed oil compositions for more than 700 species of angiosperms (see table 2 for references) to determine whether temperate plants consistently had higher proportions of unsaturated FAs in seed TAGs than tropical plants. I classified the species as either strictly tropical, strictly temperate, latitudinally cosmopolitan, or uncertain using information in the references or in the literature (Heywood 1978; Mabberley 1997). More precise latitudinal specifications were not possible because references generally did not provide precise locations for the accessions from which oils were extracted and analyzed. For this reason, only the strictly tropical and strictly temperate species were included in the analyses. Six hundred eight species could be classified as strictly tropical or strictly temperate, and, of these, 391 were in genera that had two or more species sampled. These 391 species spanned 123 genera and 74 families. Five genera and 17 families had both tropical and temperate species within them, whereas 118 genera and 57 families were strictly tropical or strictly temperate.

There has been considerable debate concerning when it is necessary to analyze comparative data in a phylogenetic context (e.g., Ricklefs 1996; Ricklefs and Starck 1996; Price 1997; Ackerly and Donoghue 1998; Ackerly and Reich 1999). An adaptive trait possessing sufficient genetic variation may change so rapidly under selection that the phylogenetic constraints may be effectively erased. However, it is impossible to know a priori whether one is examining such a trait. I therefore analyzed seed oil composition with and without accounting explicitly for phylogeny. For the nonphylogenetic analyses, I conducted a *t*-test comparing the relative proportions of saturated oils in all tropical and temperate species; two-way ANOVAs for genera and families that had both tropical and temperate members, with latitude (tropical, temperate) and taxon (either family or genus) as main effects (Wilkinson 1998, SYSTAT: ANOVA module); and nested ANOVAs (Wilkinson 1998, SYSTAT: GLM) with genera or families nested within latitude for genera and families that had strictly tropical or strictly temperate members. For all analyses, the arcsine-square-root-transformed proportion of saturated oil was the dependent variable (Sokal and Rohlf 1981). For generic level analyses, species values of the transformed total proportion of saturated oil were used. For family level analyses, generic averages of the transformed proportion of saturated oil were used to reduce bias from genera in which a large number of species were represented.

For the phylogenetic analyses, I used the recently published parsimony ratchet angiosperm phylogeny of Soltis



et al. (1999) in conjunction with the expanded trees presented on www.wsu.edu/~soltilab/three_gene_trees/nature.html. For each of the species for which I had seed oil composition data, I determined the lowest monophyletic taxonomic level available on the tree (fig. 2). For example, the seed oil data included many species in the family Brassicaceae, whereas the tree only included three species in the Brassicaceae, one each from separate genera. In this case, the species data were treated as samples within the Brassicaceae. The only exceptions to this procedure were the species in the Scrophulariaceae, which is highly paraphyletic on the tree. In this case, I used only the genera that could be unambiguously placed (*Digitalis*, *Plantago*, *Antirrhinum*). After species were assigned to monophyletic groups, the tree was pruned of groups for which oil composition data were lacking. The pruned tree had 86 terminal taxa of which 44 were species, nine genera, and 33 families. Of these clades, 27 had only tropical samples, 46 had only temperate samples, and 13 had both tropical and temperate samples. I conducted my analyses using only the strictly tropical and strictly temperate clades, as the clades that had both tropical and temperate representatives (consisting entirely of families) had already been analyzed in the nonphylogenetic analysis.

Since there were fewer tropical clades than temperate clades, tropical clades with two or more species for which seed oil compositions were known were paired with temperate clades that also had two or more species for which oil compositions were known. Clades were paired according to the rules illustrated in Kelly and Purvis (1993) such that the evolutionary path connecting the clades on the branches of the cladogram did not share any branches with any other paired set of clades. This ensured that each comparison was phylogenetically independent of all the others. Pairings were also made to allow the maximum number of comparisons and, whenever possible, to pair

Figure 2: Pruned parsimony ratchet cladogram of monophyletic taxa for which seed oil compositions were available in the literature. Open boxes represent clades for which only temperate oil compositions were available, lightly shaded boxes represent clades for which only tropical oil compositions were available, and black boxes represent clades for which both tropical and temperate oil compositions were available. The number following each taxon indicates the number of species for which oil composition data were available. For the taxa for which both tropical and temperate oil composition data were available, the number before the slash is the number of temperate species and the number following is the number of tropical species. Thicker branches on the phylogeny connect the 16 pairs of strictly tropical and strictly temperate clades that were used in analyses of the mean proportions of saturated FAs. Horizontal lines indicate strictly tropical or strictly temperate clades that included more than one taxon on the tree.

Table 1: Taxa in the genus *Helianthus* used to study the macroevolution of the saturated-unsaturated ratio

Species	Population	% saturated fat ($\bar{X} \pm$ SD)	Latitude
Helianthus annuus	PI 468486	7.8 ± .5	35°30′
H. atrorubens	PI 503202	8.4 ± .6	36°30′
H. bolanderi	Bol 27	10.3 ± 1.7	38°30′
H. cusickii	PI 531039	5.8 ± .4	44°00′
H. debilis cucumerifolius	PI 494583	10.8 ± 1.2	28°30′
H. debilis silvestris	PI 494588	8.2 ± .7	31°00′
H. debilis tardiflorus	PI 468689	12.2 ± 1.1	28°00′
H. debilis vestitus	PI 468693	12.5 ± 1.2	27°30′
H. divaricatus	PI 547173	6.9 ± .8	39°00′
H. giganteus	PI 547180	5.8 ± .4	45°00′
H. maximiliani	Ames 17965	6.3 ± .4	43°00′
H. mollis	PI 478309	7.8 ± .5	38°00′
H. neglectus	PI 468767	7.8 ± .6	32°30′
H. niveus tephrodes	Ames 6852	11.0 ± .3	32°00′
H. nuttallii nuttallii	PI 531048	5.9 ± .4	45°00′
H. petiolaris fallax	PI 468816	7.0 ± .6	38°00′
H. petiolaris petiolaris	Ames 17987	6.3 ± .7	41°30′
H. praecox hirtus	PI 468849	10.8 ± .9	28°30′
H. pumilis	PI 531059	6.9 ± .8	41°30′

Note: For all species except *Helianthus bolanderi*, the population designation refers to the accession number in the National Plant Germplasm Collection. For *H. bolanderi*, the value is from the private collection of Loren Rieseberg. Latitudes given to the nearest half degree.

clades with their least evolutionarily distant clade from the opposite latitude. Ten pairs of tropical and temperate clades were produced in this manner. For three of these pairs, there were at least three different clades with which one of the clades could be paired. In these cases, tests were performed for all the combinations to test the sensitivity of the analysis to the pairs chosen. For each pair, a *t*-test was conducted using unequal variances for the two categories (Wilkinson 1998).

To take advantage of clades containing only a single species, a final test was conducted. Tropical and temperate clades were paired as above to produce 16 pairs (fig. 2). Mean values were produced for each clade as an estimate of the value of the common ancestor for the clade, and a χ^2 test was conducted to determine whether there was a larger number of pairs where the temperate clade had a lower average proportion of saturated oils in its seeds than would be expected by chance.

Seed Oil Evolution in Helianthus

Interspecific Variation. I undertook a study of the temperate genus *Helianthus* to test whether the pattern of saturated-unsaturated ratio evolution is concordant with prediction over a limited latitudinal range and using a quantitative predictor variable (latitude) rather than discrete ones (tropical/temperate). Technically, it is much simpler to work with latitude as a surrogate for germination temperature since latitude can be determined very simply for any population, although this can lead to problems where latitude and temperature are decoupled. Use of latitude as a surrogate for germination temperature is justified for the species in this study by the narrow range of altitudes over which they grow (<300 m), the fact that all of them except *Helianthus bolanderi* grow in the Great Plains or Florida, and that they all germinate in the spring in herbaceous or disturbed herbaceous communities (Heiser et al. 1969; Rogers et al. 1982). *Helianthus bolanderi* grows in the Mediterranean climate of California and germinates in the fall at the start of the wet season. I deal with this anomaly below.

Seeds from the U.S. Department of Agriculture National Plant Germplasm Collection and from the collections of Loren Rieseberg of 19 *Helianthus* taxa (table 1) were grown in the Indiana University greenhouse. Locations of the populations from which the taxa had been collected were known, although in some cases only the state from which the populations had been collected was recorded. For those

taxa where only the state of collection was known, a two-step process was employed to determine assignment of latitude. Distribution maps (Heiser et al. 1969; Rogers et al. 1982) were examined. If the taxon was found in a limited range within the state, the center of the range was used to determine latitude. If the taxon was found throughout the state, the central latitude of the state was used. Common garden conditions included the soil mixture (two-thirds Indiana University compost and one-third coarse sand), light, and temperature. All plants were kept watered at all times and were fed a half-strength nutrient solution of nitrogen-phosphorous-potassium and micronutrients weekly. Seven to nine plants were grown per taxon. When the plants of a taxon began to flower, controlled reciprocal crosses were performed among all members of the taxon. Hence, 42–72 reciprocal crosses were performed for each taxon. All of the achenes (dry fruits) in a head were from a single cross. When heads matured, they were collected and 25 crosses per taxon were selected at random for seed oil composition analysis. For each cross, total seed oil was extracted from five to 10 seeds (depending on seed size; Metcalfe and Wang 1981). Oil composition for each cross was determined by gas chromatography (Christie 1982). Relative proportions of the four major seed oil FAs (16 : 0, 18 : 0, 18 : 1, 18 : 2; summing to >98% of total seed oil) were determined using Hewlett-Packard's Chemstation software (Hewlett-Packard 1996). Values for the 16 : 0 and 18 : 0 FAs were summed to get the total proportion of saturated FAs in a sample. Spot checks on the repeatability of results within a sample revealed that the average within-sample error was <2% of the value of any FA. For each taxon, the mean and standard deviation of the proportion of total saturated FAs were calculated from the 25 samples (table 1). In general, variation around the means was low, so only taxon means were used for comparative analysis of the evolution of oil composition.

Phylogenetic relationships within *Helianthus* are complicated and are not fully resolved (Schilling and Heiser 1981; Rieseberg 1991; Schilling 1997; Schilling et al. 1998). The genus contains 11 polyploid species (Heiser et al. 1969) and at least three diploid hybrid species (Rieseberg 1991). Even among the nonhybrid diploid taxa there is controversy about the relationships among the taxa because chloroplast and nuclear phylogenetic reconstructions produce incongruent tree topologies (Rieseberg 1991; Schilling 1997; Schilling et al. 1998), and the morphological and molecular data provide insufficient resolution (Schilling and Heiser 1981; Rieseberg 1991; Schilling 1997; Schilling et al. 1998).

Only nonhybrid diploid taxa were included in the analyses because current comparative phylogenetic methods for continuous traits assume no reticulation. To deal with phylogenetic uncertainties among the remaining taxa, analyses using four different phylogenetic trees were undertaken (fig. 3): first, the strict consensus tree based on parsimony analysis of DNA sequence data from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) repeat (Schilling et al. 1998); second, the most parsimonious chloroplast DNA (cpDNA) tree based on the restriction fragment length polymorphism (RFLP) data for the perennial (Schilling 1997) and annual (Rieseberg 1991) taxa; third, the most parsimonious rDNA RFLP tree for the species in Section *Helianthus* (Rieseberg 1991); and finally, the 50% majority rule consensus tree based on parsimony analysis of the DNA sequence from the ITS region of the nuclear rDNA repeat (Schilling et al. 1998).

Because all four trees have polytomies, they provide fewer independent contrasts than fully resolved trees and, therefore, less power for rejecting the null hypothesis. To extract maximum power from the phylogenetic analyses, sets of trees with randomly resolved polytomous nodes (Martins 1996) were generated for all trees using the Random Resolve command in MacClade 3.04 (Maddison and Maddison 1992). For the rDNA RFLP tree, there were only 15 unique resolutions of the polytomies, whereas for the other three trees, there were many thousands. For each of those trees, 2,000 random resolutions were created followed by removal of duplicate resolutions. This yielded 2,000 resolutions of the ITS strict consensus tree, 1,953 resolutions of the ITS majority rule tree, and 1,994 resolutions of the cpDNA RFLP tree. All of the resolved trees were given equal length branches with values of 1. It has been shown that this model produces uninflated Type I errors better than other branch-length assumptions when the true branch lengths are unknown (Purvis et al. 1994; D. Ackerly, personal communication). Each of the fully resolved trees were analyzed using ACAP (Ackerly 1998), which easily implements Felsenstein's (1985) method of independent contrasts on multiple trees having all branches with lengths of 1. In each analysis, average proportion of saturated seed oil of each taxon was the dependent variable, and latitude (to the nearest half degree) of the population for each taxon was the independent variable. The regression coefficients for the analyses of each set of resolved trees were ordered and the proportion of nonsignificant regression coefficients was assessed. All analyses were conducted twice: once with all taxa and once with *H. bolanderi* excluded from the analysis.

In addition to the phylogenetic analyses, a nonphylogenetic analysis was conducted by regressing the average proportion of saturated FAs in a species' seed oil on latitude.

Intraspecific Variation. To test whether the latitudinal pat-

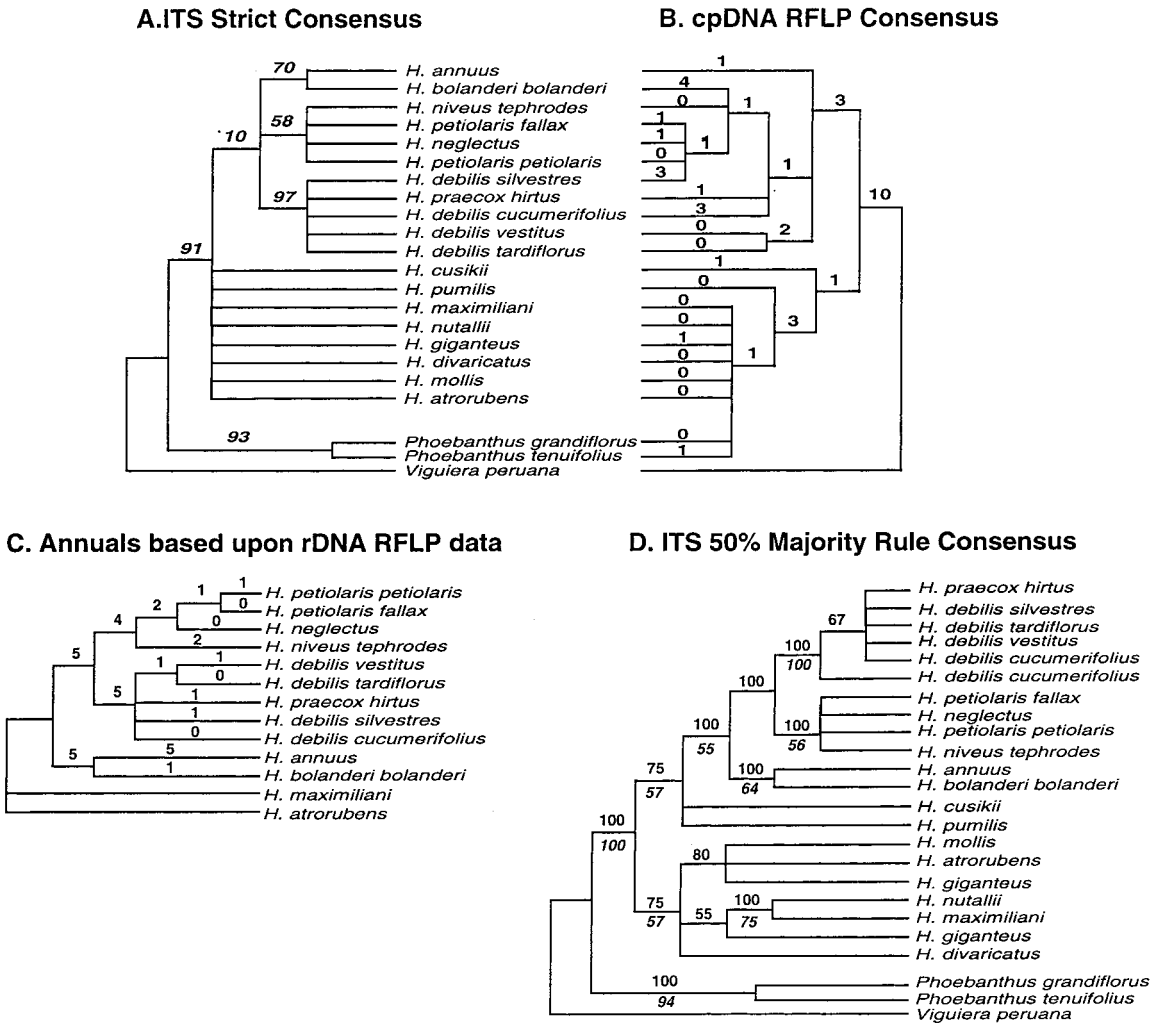


Figure 3: Polytomous phylogenetic trees from which fully resolved trees were generated for analyses of the evolution of the saturated-unsaturated ratio in *Helianthus*. *A*, Strict consensus tree produced by a maximum parsimony analysis of the ITS region of the nuclear rDNA repeat. Italicized values on branches are bootstrap support percentages based on 100 bootstrap replicates. *B*, Strict consensus tree produced by a maximum parsimony analysis of cpDNA RFLP data. Values on branches are the number of changes on a branch. The *Phoebanthus* and *Viguiera* species were not included in the comparative analyses. *C*, Single most parsimonious tree of Section *Helianthus* produced by analysis of RFLP data from the nuclear rDNA repeat. Values on branches represent the number of changes on a branch. *Helianthus maximiliani* and *H. atrorubens* are out-groups and are not included in the comparative analyses. *D*, 50% majority rule consensus tree produced by a maximum parsimony analysis of the same DNA sequence data in *A*. Values above a branch are the proportion of trees that produced that branch. Italicized values below branches are the bootstrap support percentages for a branch. Only bootstrap values >50% are given. The *Phoebanthus* and *Viguiera* species were not included in the comparative analyses.

tern of the saturated-unsaturated ratio is concordant with prediction within species, I examined variation in the saturated-unsaturated ratio along a latitudinal gradient for two species of sunflowers (*Helianthus annuus* and *Helianthus maximiliani*) that occur from southern Texas to southern Canada, nearly the complete latitudinal range of the genus (Heiser et al. 1969; Rogers et al. 1982). For both species, seeds from two populations in Texas and two in southern Canada were grown under common garden conditions in the University of Texas greenhouses. Also, *H. annuus* seeds from Oklahoma were grown. Plants were provided with adequate water at all times and were fertilized once a week with a half-strength NPK plus micronutrients solution. When plants flowered, controlled, reciprocal crosses were made between all plants within a population. Seeds were harvested when heads matured. Twenty-five crosses from each population were selected at random, and the FA composition of the TAGs from five

seeds/cross was determined by gas chromatography (Metcalfe and Wang 1981; Christie 1982).

Germination Performance in H. annuus

To determine whether germination performance of seeds with different saturated-unsaturated ratios was affected by germination temperature as predicted, *H. annuus* seeds from Texas (10.9% ± 1.0% saturated oil) and Canada (6.1% ± 0.6%) were used in a fully factorial experiment that measured seed performance at three biologically relevant germination temperatures (10°/4°C [low], 17°/10°C [medium], and 24°/17°C [high], 12L : 12D) in a completely randomized design. Seeds used in the experiment were harvested from controlled crosses similar to those described above. Surface-sterilized seeds (10 min in a 2% bleach solution [v/v]) were placed in individual wells of 48-well trays (Falcon, Lincoln Park, N.J.). To ensure the oil composition of the Texas and Canadian seeds used in the experiment were significantly different from one another, the very tip of the cotyledons of each seed was removed (approximately one-fourth of the total seed), and the oils were extracted (Metcalfe and Wang 1981) and determined by gas chromatography (Christie 1982). The small amount of tissue sacrificed to determine seed oil composition did not affect timing of germination or the rate of radicle and hypocotyl growth (C. Linder, unpublished data). Each clipped seed was provided with 300 μL of sterile water. Immediately after adding water, trays were wrapped in aluminum foil and placed at 4°C for 24 h. At the end of 24 h, the fruit and seed coats were removed and seeds were placed at the appropriate temperature under a safe-green light to simulate light conditions equivalent to burial. Thirty-two seeds of each oil type were placed in each temperature regimen for a total of 96 seeds per type. Each day, the length of the radicle and hypocotyl of each seed was recorded on a high-resolution camcorder. The safe-green lighting was maintained during image capture. Images were uploaded to an Apple Macintosh G3 computer, and the lengths of hypocotyls and radicles were measured using NIH Image software (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Measurements were made once a day for 11 d following initiation of temperature treatments, except for low temperature where measurements were made for 15 d because of retarded germination and slower growth. Measurements were approximately 24 h apart. Since we took images of the seeds at day 0 (the day they were placed in the growth chamber), it was possible to determine timing of initiation of germination by the first image when the radicle-hypocotyl combination showed clear elongation. Seeds that did not germinate during the experiment were tested with tetrazolium (Delouche et al. 1962) to determine whether they were viable. A small proportion of seeds failed to germinate (<10%), and most of these were unviable (>95%). Based on these data, only germinated seeds were included in the analyses.

Timing of germination (hours since the start of the temperature treatment) was log transformed after examining the distribution of the data on probability plots and the residuals of the data for heteroscedasticity (Wilkinson 1998, graph functions). Data were then analyzed in a two-way ANOVA with saturation (high, low) and temperature (high, medium, low) as main effects. To test whether the rate of growth for the hypocotyl and radicle combined was affected by the proportion of saturated oil at different temperatures, the total length of hypocotyl and radicle (HRL) was regressed on time since germination. Plots of the growth of HRL suggested that after the initial period of recruitment into the pool of germinated seeds, growth was linear. Seeds recruited into the germinated pool later at lower temperatures, so linear regressions were performed on data taken after 45, 60, and 100 h for high- , medium- , and low-temperature treatments, respectively. Slopes were compared to determine whether growth rates differed significantly.

Results

Broad-Scale Pattern of the Relative Proportions of Saturated and Unsaturated Seed Oils

Nonphylogenetic Analyses. Overall, tropical species had significantly higher proportions of saturated oils in their seeds than temperate species ($t = 16.07$, df $= 200$ [unequal variances], $P = \ll .001$; see fig. 4). On average, temperate species had 7.9% saturated oil and tropical 35.9%. Temperate members of families and genera that had both tropical and temperate representatives had lower proportions of saturated oils than those in tropical taxa on average (tropical genera $= 0.204$, temperate genera $= 0.100$, and tropical families $= 0.242$, temperate families $= 0.085$); however, there was significant variation among taxa (table 2). For 16 of 17 families, the temperate genera had a lower average proportion of saturated oil than tropical genera ($\chi^2 = 13.24$, df $= 1$, $P < .001$, null hypothesis: random distribution of proportions of saturated oil; see table 3). The sole exception was Lauraceae, for which the tropical and temperate genera were nearly identical. Orthogonal contrasts for each family (table 3) revealed that the differences in proportions of saturated oil were significant for 10 of the families and that the differences were nearly significant for another four families. At the generic level, four of five genera had lower average proportions of saturated oil in their temperate species. However, this dis-

tribution was not significantly different from a random distribution of proportions of saturated oils because of the low power of the test. Orthogonal contrasts within genera showed that for three of the five genera, the tropical species had significantly higher proportions of saturated FAs than temperate species. In no case had members of tropical families or genera evolved significantly lower proportions of saturated FAs in their seed oils than temperate ones.

Strictly temperate families and genera consistently had lower proportions of saturated oil in their seeds than ones that were strictly tropical (fig. 4; table 4). Thirty of 31 strictly temperate families had average proportions of saturated FA below the overall mean family proportion of saturated FAs ($\bar{X} = 0.173$), whereas only one of 26 strictly tropical families did so ($\chi^2 = 49.2$, df $= 1$, $P \ll .001$). Among the strictly temperate families, the maximum family average was 0.220 (Dipsacaceae) with an overall temperate family average of 0.079. The Dipsacaceae was a bit of an outlier with the next highest temperate family average being 0.159 (Symplocaceae). Only two strictly tropical families had average values less than the Dipsacaceae. Of these, only the Theaceae had a value (0.069) that approached that of the temperate average. The other (Rubiaceae) had a value (0.187) that was close to the maximum temperate family average. The overall tropical family average for saturated FAs was 0.462.

Seventy-eight of 79 strictly temperate genera had average proportions of saturated FAs below the overall mean generic proportion of saturated FAs ($\bar{X} = 0.143$), whereas only four of 39 strictly tropical genera did so ($\chi^2 = 96.4$, df $= 1$, $P \ll .001$). Among the strictly temperate genera, the maximum generic average proportion of saturated seed oils was 0.152 with an overall temperate generic average of 0.077. The next highest temperate generic average was 0.130. Only six strictly tropical genera had average values less than the maximum temperate generic average, and only two were less than the value of the second highest temperate genus. The overall tropical generic average was 0.416. None of the tropical generic averages for proportion of saturated FA approached the overall temperate generic average.

Table 2: ANOVAs of the arcsine square root–transformed proportion of saturated fatty acids in the seed oils of families and genera that have both tropical and temperate species for which seed oil composition has been determined

	df	MS	*F*	*P*
Families:[a]				
Family	16	.11163	13.96	$\ll .001^{***}$
Latitude	1	.90057	112.65	$\ll .001^{***}$
Family × latitude	16	.04188	5.24	$\ll .001^{***}$
Error	131	.00799		
Genera:[b]				
Genus	4	.01987	7.28	$.009^{**}$
Latitude	1	.10366	38.00	$< .001^{***}$
Genus × latitude	4	.02621	9.61	$.004^{**}$
Error	8	.00273		

Note: Saturated oil values for these analyses and those in tables 3, 4, and 5 are from Eckey 1954; Earle et al. 1959, 1960*a*, 1960*b*, 1962, 1964; Earle 1960; Mikolajczak et al. 1961; Mikolajczak 1962; Kleiman et al. 1964, 1965; Miller et al. 1964*a*, 1964*b*, 1965, 1968.

[a] $r^2 = .813$.

[b] $r^2 = .919$.

** $P \leq .01$.

*** $P \leq .001$.

Phylogenetic Analyses. For the pairs of strictly tropical and

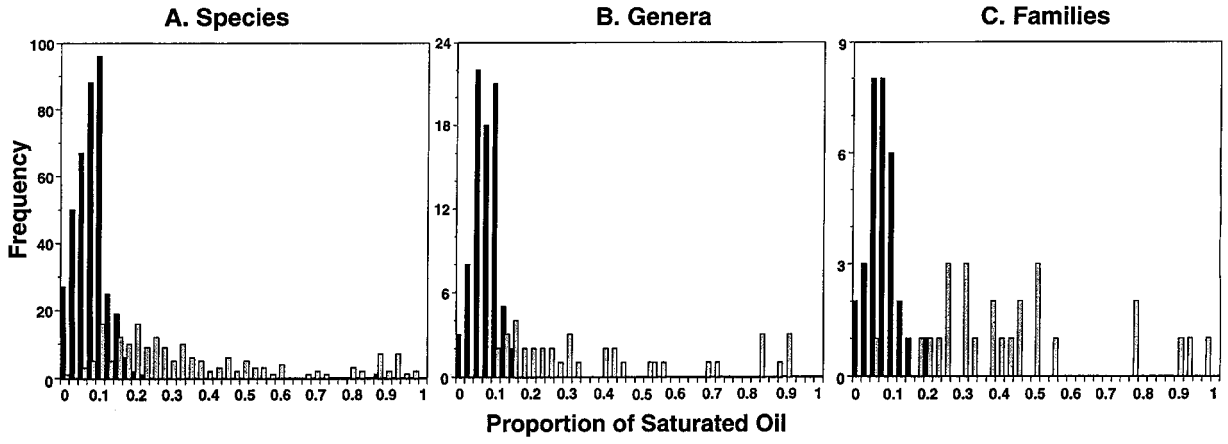


Figure 4: Histograms of the average proportions of saturated FAs in seed oils of species, genera, and families that are strictly tropical or strictly temperate. Tropical species, genera, and families are represented by lightly shaded bars, and temperate species, genera, and families are represented by black bars.

Table 3: Orthogonal contrasts testing whether the tropical and temperate members of genera and families that have both tropical and temperate representatives have different proportions of saturated fatty acids in their TAGs

	Contrast		Mean proportion saturated oil (*N*)	
	F^a	*P*	Tropical	Temperate
Family:				
Annonaceae	7.38	.007**	.31 (1)	.06 (1)
Apocynaceae	6.98	.009**	.21 (1)	.02 (1)
Asclepiadaceae	2.92	.09	.20 (1)	.06 (1)
Asteraceae	25.77	≪.001***	.23 (6)	.09 (37)
Boraginaceae	8.42	.069	.14 (3)	.11 (22)
Capparaceae	16.06	.015*	.34 (5)	.08 (3)
Cucurbitaceae	3.90	.05*	.24 (5)	.09 (1)
Euphorbiaceae	7.33	.015*	.15 (14)	.10 (5)
Fabaceae	6.62	.011*	.27 (17)	.14 (2)
Lamiaceae	3.24	.074	.12 (1)	.04 (13)
Lauraceae	.07	.79	.87 (1)	.89 (1)
Malvaceae	2.79	.097	.24 (3)	.13 (2)
Polemoniaceae	7.50	.007**	.22 (1)	.02 (1)
Rhamnaceae	2.38	.12	.25 (1)	.10 (1)
Rutaceae	17.36	<.001***	.29 (2)	.04 (2)
Scrophulariaceae	2.44	.12	.16 (1)	.07 (7)
Simaroubaceae	79.12	≪.001***	.94 (1)	.04 (1)
Genus:				
Abutilon	.29	.61	.19 (1)	.16 (1)
Capparis	49.33	<.001***	.45 (2)	.08 (2)
Cleome	7.20	.028*	.18 (2)	.08 (2)
Croton	.03	.87	.10 (2)	.10 (2)
Hibiscus	10.61	.011*	.27 (2)	.11 (2)

Note: Sample sizes for the family contrasts are the number of generic averages used in the full family analysis. Sample sizes for generic contrasts are the number of species used in the full generic analysis.

[a] $df = 1, 131$.

* $P \leq .05$.

** $P \leq .01$.

*** $P \leq .001$.

strictly temperate clades (fig. 2), 15 of the 16 temperate clades had lower average proportions of saturated oils than tropical clades ($\chi^2 = 7.57$, df $= 1$, $P = .006$). In addition, for the 10 pairs where oil composition values were available for at least two species in both latitudinal types, eight of the temperate clades had significantly lower proportions of saturated seed oils than their tropical counterpart (table 5). For two of the three cases where multiple combinations were tried, all of the combinations revealed temperate clades with significantly lower proportions of saturated seed oil. For the third case, two of three comparisons were significant, and the third was marginally nonsignificant (see the last entry in table 5). Only the comparison of the Rubiaceae and the Scrophulariaceae was highly nonsignificant. In no case did a tropical clade have a significantly lower proportion of saturated seed oils.

Seed Oil Evolution in Helianthus

For the nonphylogenetic analysis, there was a highly significant relationship between latitude and the proportion of saturated FAs in *Helianthus* seed oils ($r = 0.879$, df $= 18$, $P < .001$; see fig. 5). On average, the proportion of saturated seed oil decreased 0.3% per degree of latitude.

Phylogenetic Analyses. With the exception of the rDNA RFLP tree, every fully resolved tree that included *Helianthus bolanderi* produced a negative correlation coefficient having a *P* value of .02 or less. All but three of the 5,947 trees produced *r*'s having *P* values of <.01. As anticipated, when *H. bolanderi* was excluded, the significance of the relationship between latitude and proportion of saturated FAs increased in all cases. These results strongly suggest that whatever the correct resolutions are for these trees, there will be a significant inverse relationship between latitude and proportion of saturated FAs in the seed TAGs of *Helianthus*. For the rDNA RFLP tree, when *H. bolanderi* was included in the resolved trees, none were significant at $\alpha = 0.05$, whereas with *H. bolanderi* excluded, all of the resolutions were significant at $\alpha < 0.05$. This result is not surprising since the rDNA RFLP tree has the fewest number of contrasts (10 with *H. bolanderi* and nine without) of the four phylogenies. The greater number of contrasts in the larger trees diminishes the effect of including *H. bolanderi* in their analyses.

Intraspecific Pattern. The latitudinal pattern of oil compositions was entirely concordant with expectation for both species of sunflower (table 6). Texas populations had significantly higher proportions of saturated FAs in their TAGs than Canadian populations ($P < .01$ for all combinations of Texas and Canadian *Helianthus annuus* populations, and $P < .005$ for all similar combinations of *Helianthus maximiliani*). The Oklahoma population of *H.*

Table 4: Nested ANOVAs for families and genera that were either strictly tropical or strictly temperate

	df	MS	*F*	*P*
Family:[a]				
Latitude	1	4.38657	56.77	≪.001***
Family within latitude	55	.07727	10.18	≪.001***
Error	125	.00758		
Genus:[b]				
Latitude	1	10.32086	106.27	≪.001***
Genus within latitude	116	.09712	20.16	≪.001***
Error	250	.00482		

Note: The dependent variable is the arcsine square root–transformed proportion of saturated fatty acids in seed oil.

[a] $r^2 = .934$.

[b] $r^2 = .950$.

*** $P \leq .001$.

annuus was intermediate between the Texas and Canadian *H. annuus* populations. Populations within a region did not differ from one another ($P > .4$ in all cases).

Germination Performance in H. annuus

The significant interaction of saturation and temperature (table 7) indicated that the timing of germination for the high- and low-saturated-oil seeds differed at different germination temperatures (fig. 6). Orthogonal contrasts of high- and low-saturated-oil seeds within temperature treatments showed that at low temperature, low-saturated-oil seeds germinated earlier than high-saturated-oil seeds ($MS = 0.10631$, $F = 4.692$, $df = 1, 53.5$, $P = .035$). At medium and high temperatures, germination occurred at statistically indistinguishable times, albeit at high temperatures high-saturated-oil seeds almost germinated significantly earlier than low-saturated-oil seeds ($MS = 0.77949$, $F = 3.50$, $df = 1, 51.4$, $P = .067$).

At high temperature, high-saturated-oil seeds grew significantly more rapidly than low-saturated-oil seeds ($t = 2.516$, $P < .025$; see fig. 6). At medium and low temperatures, the growth rates of the high- and low-saturated-oil seeds were indistinguishable ($P > .2$ for both temperatures) although there was a trend toward faster growth for low-saturated-oil seeds at low temperatures.

Discussion

Macro- and Microevolutionary Data Support the Hypothesis

The results of both the macro- and the microevolutionary studies presented here strongly support the hypothesis that selection has altered the relative proportions of saturated and unsaturated FAs in seed TAGs along a latitudinal gradient. Both the broad-scale study and the more limited macroevolutionary study within *Helianthus* indicate a selective pressure correlated with latitude has acted on the balance between saturated and unsaturated seed oils. The results are robust whether the analyses are undertaken within a phylogenetic context or on the assumption that selection has erased the effects of phylogenetic history. The broad-scale results make it clear that the pattern holds broadly within angiosperms. Only the basal-most orders were unrepresented in the analyses (Qiu et al. 1999; Soltis et al. 1999). Still, there are some taxonomic groups for which phylogenetic constraints or other factors have kept tropical and temperate proportions of saturated oil in seed oils nearly identical (tables 3, 5). Further study is required

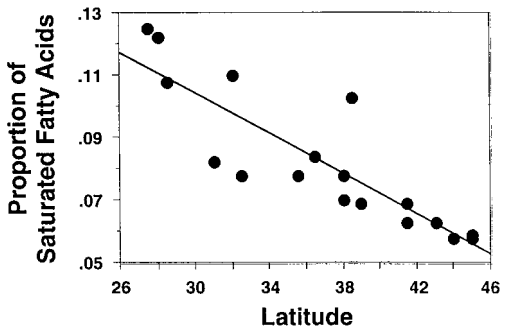


Figure 5: A nonphylogenetic regression of the proportion of saturated FAs in the seed oils of 19 species of *Helianthus* on latitude. The line is the least squares best fit to the data.

Table 5: Phylogenetically constructed *t*-tests of independent strictly tropical and strictly temperate clades (see fig. 2)

Clades (temperate-tropical)	df	*t* value	*P* value
Asparagales-Arecales	14.6	22.86	$\ll .001^{***}$
Liliales-Myristicaceae	5.0	8.26	$< .001^{***}$
Amaranthaceae-Sapotaceae/Theaceae	5.0	3.12	$.026^{*}$
Scrophulariaceae-Rubiaceae	1.1	1.19	.427
Solanaceae-Convolvulaceae	1.4	7.03	$.05^{*}$
Proboscidea/*Paulownia*-Bignoniaceae	3.0	3.36	$.044^{*}$
Limnanthes-Meliaceae	6.1	7.96	$< .001^{***}$
Onagraceae-Lythraceae	5.8	17.48	$\ll .001^{***}$
Humulus/*Maclura*/*Eleagnus*-Clusiaceae/*Lophira*/*Caryocar*	2.3	6.28	$.017^{*}$
Humulus/*Maclura*/*Eleagnus*-Combretaceae/Vochysiaceae	3.9	2.51	.07

Note: The first clade is always the temperate clade. *Limnanthes* was also paired with Sapindaceae, Burseraceae/*Mangifera*, and Dipterocarpaceae. Each *t*-test with these families also had *P* values <.001. The least significant of three pairings of the Clusiaceae/*Lophira*/*Caryocar* clade with temperate clades is presented. Both of the other pairings (*Juglans*/*Carya*/*Corylus* and Rosaceae) had *P* values <<.001. The least significant of three pairings of the Combretaceae/Vochysiaceae clade with temperate clades is presented. Both of the other pairings (*Juglans*/*Carya*/*Corylus* and Rosaceae) had *P* values of ≤.02.

* $P \leq .05$.

*** $P \leq .001$.

Table 6: Locations and proportions of saturated fatty acids in the seed oils of populations of *Helianthus annuus* and *Helianthus maximiliani*

Species population	Location	Latitude	Proportion saturated fat ($\bar{X} \pm$ SD)
Helianthus annuus:			
PI 468519	Texas, U.S.A.	27°30′	12.0 ± 1.3
PI 468517	Texas, U.S.A.	28°00′	11.1 ± 1.0
PI 468486	Oklahoma, U.S.A.	35°25′	8.8 ± .6
PI 592316	Saskatchewan, Canada	50°39′	5.9 ± .7
PI 592311	Saskatchewan, Canada	50°23′	5.4 ± .7
Helianthus maximiliani:			
AT-4	Texas, U.S.A.	30°10′	10.0 ± .8
AT-1	Texas, U.S.A.	30°15′	9.3 ± .5
PI 592336	Manitoba, Canada	49°44′	6.6 ± .6
PI 592339	Manitoba, Canada	49°44′	6.4 ± .5

Note: Populations beginning with PI are from the National Plant Germplasm collection and are designated by their accession numbers. Populations beginning with AT are from my personal collections.

to determine why these anomalies exist and whether they constitute exceptions that can be accounted for within the theory, for example, delayed germination at warmer temperatures or noncompetitive circumstances during germination and establishment.

The results from *Helianthus*, a wholly temperate clade, make it clear that the pattern seen throughout the angiosperms is not a discrete tropical-temperate phenomenon but a continuous pattern that holds within more constrained latitudinal bounds. In addition, the effect of *Helianthus bolanderi* on the significance of the analysis of the rDNA RFLP phylogeny supports the hypothesis that germination temperature is the selective agent affecting the balance between saturated and unsaturated FAs in TAGs (see below). The intraspecific pattern of seed oil compositions in *Helianthus annuus* and *Helianthus maximiliani* supports the hypothesis that there can be sufficient genetic variation within species to allow seed oil composition characteristics to become adapted to local conditions. It also indicates that microevolutionary selection could account for the observed macroevolutionary pattern of relative proportions of saturated and unsaturated FAs in seed oils.

With respect to the hypothesis that germination temperature is the primary selective agent responsible for the latitudinal pattern, two lines of support emerge from the results. First, as predicted, *H. bolanderi* does not fit the pattern seen for the other taxa of *Helianthus* examined in this study. The germination temperature regimen to which it is subjected in the Mediterranean climate of California decouples it from the latitudinal temperature gradient to which the other species in the study are subject. As expected, *H. bolanderi* has a higher proportion of saturated FAs than other species of *Helianthus* growing at similar latitudes in the Great Plains. If field data could be gathered for the germination temperatures of the taxa in this study, it would be interesting to know whether the relationship between germination temperature and the proportion of saturated oil would be significant even when *H. bolanderi* is included in the rDNA RFLP phylogeny analysis.

Second, the *H. annuus* germination performance results are particularly supportive of the hypothesis that cooler germination temperatures have selected lower proportions of saturated FAs and that warmer germination temperatures have selected higher proportions. At low temperatures, *H. annuus* low-saturated-oil seeds germinated earlier but did not grow faster than high-saturated-oil seeds, whereas at high temperatures, high-saturated-oil seeds grew significantly faster and possibly germinated earlier than low-saturated-oil seeds. At intermediate temperatures, the seed performance of high- and low-saturated-oil seeds was indistinguishable. However, selection has likely altered other characters in Texas and Canadian seeds that are correlated with the saturated-unsaturated ratio.

Table 7: ANOVA of the log-transformed germination times of *Helianthus annuus* seeds from Canada and Texas at three germination temperature regimens

	df	MS	*F*	*P*
Saturation	1	.06022	.61	.43
Temperature	2	16.89676	172.11	$\ll .001^{***}$
Saturation × temperature	2	.30563	3.11	$.043^{*}$
Error	166	.09817		

Note: $r^2 = .685$.
* $P \leq .01$.
*** $P \leq .001$.

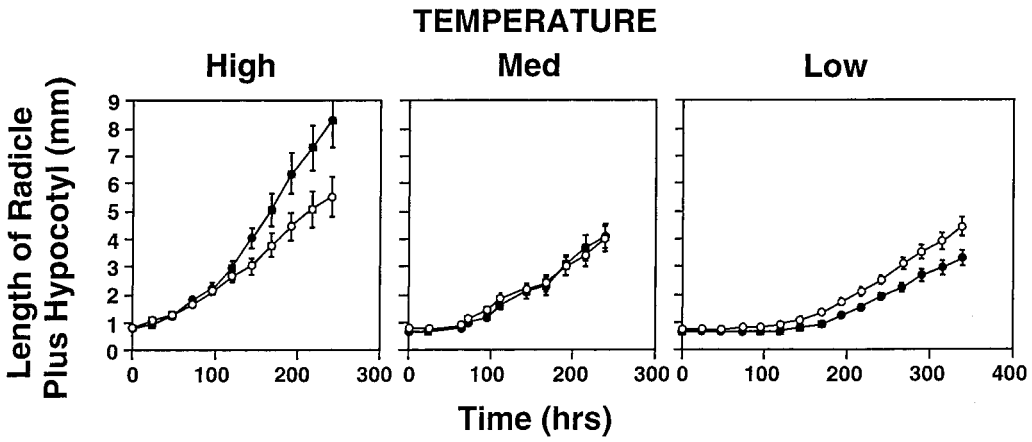


Figure 6: Length of the radicle plus the hypocotyl (mm) over time (h) of high- (*filled circles*) and low- (*open circles*) saturated-oil seeds germinating at three temperature regimens (high, medium, and low; see text for the temperatures associated with these designations). Each point is the mean length of the hypocotyl plus the radicle for a saturated-oil type by temperature combination at a sample time. Bars around each point represent the standard errors of the means.

Some part of the seed performance differences seen in this study may be due to those characters rather than the saturated-unsaturated ratio. Work is under way in my laboratory to breed sunflowers that differ in their oil compositions but that are otherwise genetically similar so that unambiguous tests of the effect of seed oil composition on seed germination performance can be made. Using these same lines, I also will test whether performance differences translate into fitness differences that could account for the adaptive evolution of saturated-unsaturated ratios.

Alternative Hypotheses

Other factors besides germination temperature that covary with latitude might select for lower saturated-unsaturated ratios at higher latitudes, but these factors are less plausible selective agents than germination temperature. Two important nontemperature factors that covary with latitude are herbivory and photoperiod. It has been suggested that herbivory increases at lower latitudes (Janzen 1970; Connell 1971; Seigler 1979), so one would expect stronger selection for unpalatable oils at lower latitudes. However, oils known to be unpalatable to herbivores are unsaturated rather than saturated (Seigler 1979)—for example, erucic acid (22 : 1) in the Brassicaceae (Gurr 1980; Stefansson and Downey 1995). Thus, the observed latitudinal pattern of the proportion of saturated oils is the opposite of what is expected. Photoperiod at higher latitudes is longer in the summer and shorter during the winter; however, no mechanism is known or has been suggested by which different photoperiods might favor particular oil compositions. A survey of the literature on photoperiod did not produce any plausible connection between it and oil composition.

Another possibility is that temperature during seed dormancy, rather than germination, is the primary selective pressure. Higher-latitude plants experience colder dormancy conditions, so perhaps lower proportions of saturated oil are selected at higher latitudes to act as an antifreeze. This explanation is unlikely because winter soil temperatures routinely fall below the freezing point of oils stored in many species' seeds. For example, wild *H. annuus* in southern Canada routinely experiences winter soil temperatures $<-5°C$, the freezing point of its lowest melting-point oil (linoleic acid, 18 : 2). Under such conditions, unsaturated oils are not likely to be more effective than saturated oils as antifreezes. In addition, because TAGs are sequestered in oleosomes (Huang 1992) during dormancy, they are not dispersed in the cytoplasm in an optimal fashion to act as antifreeze.

Finally, it is known that plants produce a higher proportion of unsaturated FAs for incorporation into phospholipids at colder temperatures (e.g., Nishida and Murata 1996). Perhaps the increase in unsaturated oils at higher latitudes is a pleiotropic effect caused by production of more unsaturated FAs for phospholipids during seed development. Three lines of evidence make this unlikely. First, seeds of most temperate species develop during summer when there is little, if any, need for cold-tolerant phospholipids. Second, incorporation of FAs into seed oils is often decoupled from their incorporation into phospholipids (e.g., Gurr 1980). Seeds of many plant species have FAs in their oils that are not found in phospholipids.

Finally, populations of at least some broadly distributed species are not plastic for differences in their saturated-unsaturated ratios when grown under common garden conditions. Such plasticity would be expected because the physiological response to cooler temperatures is to produce phospholipids lower in saturated FAs.

Although there may be other explanations, as yet unconsidered, for the nearly ubiquitous biogeographical pattern of saturated-unsaturated FA ratios in seed TAGs, selection by germination temperature to optimize the rate and quantity of energy stores is best supported by current evidence. Critical tests of shortcomings in the current evidence will be addressed by the experiments mentioned above. It is also desirable to test directly whether seeds with lower proportions of saturated FAs in their seed oils metabolize them more rapidly at cool temperatures than seeds higher in saturated FAs. If the theory can be confirmed, it would lead to the possibility of determining the underlying molecular genetic differences on which selection has acted, since a great deal is known about the genetics of seed oil synthesis (Browse and Somerville 1991; Ohlrogge et al. 1991, 1993; Ohlrogge and Browse 1995; Harwood 1996; Ohlrogge and Jaworski 1997). Also, comparison of distantly related groups could provide a detailed picture of how selection has acted on the genes responsible for the saturated-unsaturated ratio in different groups of plants.

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