

Intraspecific and interspecific adaptive latitudinal cline in Brassicaceae seed oil traits

Anushree Sanyal^{1,3,4}, Jonathan Lenoir¹, Carmel O'Neill², Frederic Dubois¹, and Guillaume Decocq¹

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¹ Unité de recherche "Ecologie et Dynamique des Systèmes Anthropisés" (EDYSAN, UMR 7058 CNRS-UPJV), 1 rue des Louvels, FR-80037, Amiens Cedex, France

² John Innes Centre, Norwich, NR4 7UH, United Kingdom

³ Present address: Department of Organismal Biology, Systematic Biology, 18D Norbyvagen, Uppsala University, 75236, Uppsala, Sweden

⁴ Author for correspondence (e-mail: anushree.sanyal@ebc.uu.se)

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PREMISE OF THE STUDY: Nearly all seed plants rely on stored seed reserves before photosynthesis can commence. Natural selection for seed oil traits must have occurred over 319 million years of evolution since the first seed plant ancestor. Accounting for the biogeographic distribution of seed oil traits is fundamental to understanding the mechanisms of adaptive evolution in seed plants. However, the evolution of seed oils is poorly understood. We provide evidence of the adaptive nature of seed oil traits at the intraspecific and interspecific levels in Brassicaceae—an oilseed-rich and economically important plant family.

METHODS: Univariate statistics, Pearson's correlation, multiple regression, generalized linear mixed model analysis, and phylogenetic autocorrelation tests on seed oil traits of 360 accessions of *Arabidopsis thaliana* and 216 Brassicaceae species helped provide evidence of the adaptive nature of seed oil traits.

KEY RESULTS: At higher latitudes, both seed oil content and unsaturated fatty acids have selective advantages in *Arabidopsis thaliana* (intraspecific-level), while only unsaturated fatty acids have selective advantages across 216 Brassicaceae species (interspecific-level). The seed oil patterns fit within the theoretical framework of the gradient model. Seed oil content increases significantly from temperate to subtropical to tropical regions in Brassicaceae herbs. Absence of phylogenetic signals for seed oil traits and high seed oil content in four tribes of Brassicaceae were observed.

CONCLUSIONS: Multiple seed oil traits are adaptive in nature and follow a gradient model. Consistent evolutionary patterns of seed oil traits were observed at the intraspecific and interspecific levels in Brassicaceae. Seed oil traits change with latitude and across biomes, suggesting selection. The absence of a phylogenetic signal for seed oil traits and the occurrence of high seed oil content in four Brassicaceae tribes provides evidence of the adaptive nature of seed oil traits in Brassicaceae.

KEY WORDS Seed oil content; latitudinal cline; adaptation; habitat; biomes; seed oil composition; Brassicaceae; phylogenetic signal; biogeographical distribution; temperate; tropical; subtropical.

The primary functions of seeds are energy storage, dispersal, and perennation (Levin, 1974). During seed germination, prior to initiation of photosynthesis, nearly all seed plants depend on the energy reserves stored in the seeds. The quantity and quality of seed reserves are very critical because of their role in germination, which often plays a crucial role in determining plant fitness (Rees and Long, 1992; Westoby et al., 1992) especially in annuals (Linder, 2000). Energy and carbon reserves in seeds are starches, proteins, or triacylglycerols (TAGs), which are the fats and oils. The majority (>80%) of plant species rely almost exclusively on TAGs in their seeds (Harwood, 1980). Earliness of germination was positively correlated with seed lipid content and the seed area-to-mass

ratio (Gardin et al., 2011). A germinating seed has two critical requirements, namely, a source of carbon skeleton precursors and an energy source (adenosine triphosphate, ATP) to assemble the precursors. The ATP is needed for the biogenesis of new cellular constituents, synthesis of proteins, and subsequently enzymes for the degradation and conversion of storage substances. Lipids, the principal reserve materials in most seeds, meet these two requirements most effectively (Mayer and Poljakoff-Mayber, 1963).

Lipids are superior to carbohydrates as energy storage products because on a unit weight basis the oxidation of the former yields about twice as much ATP as the latter (Spector, 1956). The quantity and quality of stored reserves in seeds is associated with germination

characteristics (energy availability, seed viability, and timing and speed of germination) and seedling growth, and play an important role in plant fitness and are therefore under selection (Levin, 1974; Linder, 2000). During germination, two distinct patterns of mitochondrial development are dependent on the nature of the stored reserves. In starch-storing seeds, repair and activation of preexisting organelles dominate, while oil-storing seeds typically produce new mitochondria (Morohashi and Bewley, 1980; Morohashi, 1986; Ehrenshaft and Brambl, 1990). Furthermore, the carbons in fatty acids (FAs) are highly reduced and the oxidation of stored oils in germinating seeds yield twice as much energy as stored proteins and carbohydrates on a per gram basis thus facilitating germination (Baud and Lepiniec, 2010; Lüttge, 2012; Theodoulou and Eastmond, 2012).

The seed oil content (SOC) and seed oil composition varies widely within a single species (O'Neill et al., 2003), within genera, among genera of the same family, and among families (Levin, 1974). For example, the SOC within the genus *Chrozophora* varies from 8.1–56.5% (Kleinman et al., 1965; Barclay and Earle, 1974; Kapoor et al., 1986); genera *Balanites* and *Zygophyllum* of the family Zygophyllaceae have mean SOC of 7.9% and 40%, respectively (Matthäus, 2012); mean SOC of the families Polygonaceae and Chrysobalanaceae are 3% and 57.7%, respectively (Matthäus, 2012); and the SOC among genotypes of *Arabidopsis thaliana* varies from 28–44% and the seed oil composition varies from 31–45% (O'Neill et al., 2003). The heritability of SOC typically exceeds 50% (Johnson and Bernard, 1962; Barker and McKenzie, 1972), and most species are relatively insensitive to differences in population density, climatic regime, and mineral levels in the soil (Genter et al., 1956; Comstock et al., 1969; Kittock and Williams, 1970). The genetic variability and genetic control of SOC show that SOC may be subject to evolutionary change (Levin, 1974). Selection may act on different proportions of SOC in different biogeographic regions of the world. Some work has been done on the evolution of seed oil composition (constituent FAs in the seed oil) (Linder, 2000; Sanyal and Linder, 2013); however, only one study has attempted to study the selective pressure on SOC (Levin, 1974).

Previous studies have shown that multigene (*DGAT1*, *WRI1*, and *SDP1*) engineering of TAG metabolism increased SOC in *A. thaliana* (van Erp et al., 2014). Overexpression of *DGAT1* in transgenic lines of *Brassica napus* increased the SOC by 14% and prevented reduction of SOC during drought (Weslake et al., 2008). Furthermore, the following genes and transcription factors are all regulators of SOC: *WRINKLED1*, *LEAFY COTYLODON 1* (*LEC1*), *TRIACYLGLYCEROL BIOSYNTHESIS DEFECT1* (Katavic et al., 1995; Focks and Benning, 1998; Jako et al., 2001; Tan et al., 2011), *LEC1*-like (*LIL*), *LEC2*, *PICKLE* (Bao and Ohlrogge, 1999; Ogas et al., 1999; Dean Rider et al., 2003; Baud et al., 2007), *GLABRA2* (Shen et al., 2006), *SUGAR-DEPENDENT1* (Kim et al., 2014; Kelly et al., 2013a,b), *ABSCISSIC ACID INSENSITIVE 3*, *FUSCA3* (Santos-Mendoza et al., 2008), *DNA BINDING WITH ONE FINGER4* and *DOF11* (Wang et al., 2007), Acetyl CoA carboxylase (Ohlrogge and Jaworski, 1997), and expressed yeast gene encoding cytosolic glycerol-3-phosphate dehydrogenase and yeast sn-2 acyltransferase gene in *B. napus* (Zou et al., 1997; Vigeolas et al., 2007). Hence, selection could be acting on the variation of the genes responsible for controlling SOC caused by their possible role in plant adaptation.

Baker (1970) suggested that greater packing of species and competitive interactions among tropical plants may select for a greater quantity of energy reserve in seeds, and the amount of SOC could change with biomes. In this study, we will examine whether the nature of the seed oil patterns fits the framework of the gradient

model. In the gradient model, the traits of species are observed to change monotonically along positional gradients (latitude and altitude) across the geographical range, which is usually a consequence of the response of species to a particular environmental factor (Gaston et al., 2008). A study by Levin (1974) on the evolutionary pressures acting on SOC, explored the relationships between SOC and plant habit (herbs, shrubs, and trees) in Fabaceae, as well as SOC and biomes, in 1400 species from 113 families. A significant relationship between SOC and habit was observed in Fabaceae: an increase in woodiness was accompanied by a shift in favor of a more efficient storage product. However, no significant difference was found between the SOC of plants of the same habit (herbs, shrubs or trees) in the temperate, tropical, and subtropical regions; the exception to this was the herbs from the subtropical region, which had greater SOC than the herbs from the other two regions. A recent study revealed a significant positive relationship between SOC and latitude in the families Sapindaceae and Sapotaceae, and a negative relationship between SOC and latitude in Papaveraceae (Sanyal and Decocq, 2016). Two studies explored the relationship between seed oil composition and latitude in *A. thaliana* and sunflower where the proportions of saturated FAs decreased significantly with increasing latitude (Linder, 2000; Sanyal and Linder, 2013). A positive correlation between SOC and polyunsaturated fatty acids (PUFAs) has been observed (Bretagnolle et al., 2016; Sanyal and Decocq, 2016) and lighter and smaller seeds have also been observed to have greater SOC (Bretagnolle et al., 2016).

Although the Fabaceae family is large with several crop species, the family is atypical in having low SOC and high protein content. Brassicaceae, however, is a large family of 338 genera and 3710 species (Beilstein et al., 2008) comprising several oilseed-rich species and crops of major economic importance. Here, we explore the relationships between seed oil traits (SOC and seed oil composition) and life cycle strategies (annual, biennial, perennial) in the oilseed-rich Brassicaceae family, and the relationship between seed oil traits (SOC and seed oil composition) to latitude and biomes (temperate, subtropical, and tropical) to account for the biogeographical distribution of the species. Because seed oils contain FAs, examining the relationship between SOC and its constituent FAs will be crucial in determining the evolutionary potential of each of these seed oil traits. We examined the relationship and patterns of SOC and seed oil composition at both the intraspecific level in *A. thaliana* and at the interspecific level across Brassicaceae species to determine the effect and interactions of multiple seed oil traits and their possible roles in plant adaptation. Previous studies have shown that the proportions of unsaturated FAs increase with latitude and increase plant fitness (Linder, 2000), but no relationship between SOC and latitude was observed (Levin, 1974). Hence, we examined the relationship of both SOC and FAs with changing latitude. Our study will also try to determine if the proportions of certain FAs increase or decrease with SOC to understand their independent or combined role in plant adaptation. Finally, we also examined SOC in the Brassicaceae family within a phylogenetic context.

MATERIALS AND METHODS

Seed oil content and seed oil composition

Published SOC and seed oil composition data for 216 species and subspecies of 88 genera of the Brassicaceae family were obtained

from the Seed Oil Fatty Acids database (Matthäus, 2012) and from the literature (El Bassam, 2010) (Appendix S1, see the Supplemental Data with this article). The SOC and seed oil composition for a given species was measured as the mean and median across all the percent SOC and seed oil composition values available for that given species. The SOC of 360 ecotypes of *A. thaliana* was obtained from O'Neill et al. (2003) (Appendix S2), and the seed oil composition (individual FA proportions) was obtained from O'Neill (Appendix S3).

Life history strategies and distribution of Brassicaceae herbs

Life history strategies data (annual, biennial, perennial) of the 216 species and subspecies of Brassicaceae herbs was taken from the Plants for a Future database (<http://www.pfaf.org/user/default.aspx>) and from the literature (Warwick et al., 2009). The classification of the 216 species and subspecies based on their geographical distribution into tropical, temperate, or subtropical was done based on the information available in the Germplasm Resources Information Network (<http://www.ars-grin.gov/>) from the USDA, the Euro+Med PlantBase (<http://www.emplantbase.org/home.html>), the Plants for a Future database (<http://www.pfaf.org/user/default.aspx>), and from published works of local and regional flora (Lambinon et al., 2004). The latitude information, occurrence records, and counts of occurrence records of the 216 species and subspecies was extracted from the Global Biodiversity Information Facility (GBIF) (<http://www.gbif.org/>) by using the 'rgbif' package (Chamberlain et al., 2014) in the R programming software (R Development Core Team, 2013). The occurrence data of the 319 genotypes of *A. thaliana* were obtained from The Arabidopsis Information Resource database (<http://www.arabidopsis.org/>) and from the literature (Sanyal and Linder, 2013).

Statistical analyses

Does seed oil content and seed oil composition in Brassicaceae herbs change with geographical regions and life cycle strategies?— The differences in the SOC and the nine constituent FAs of herbs in different biomes (temperate, subtropical, and tropical) and herbs with different life cycle strategies (annual, biennial, and perennial) in Brassicaceae were examined. To compare the SOC and the nine constituent FAs among herbs in different biomes, a multiple regression was performed where herbs in temperate, subtropical, and tropical regions were treated as a fixed main effect and seed oil traits as dependent variables. Multiple regression was performed by using the model: $\text{lm}(\text{seed oil traits} \sim \text{temperate} + \text{subtropical} + \text{tropical})$. Multiple regression was performed to test for differences in SOC and the nine constituent FAs in Brassicaceae herbs with different life cycle strategies (annual, biennial, perennial). SOC and the individual FAs were the dependent variables, and annual, biennial, and perennial were the three independent variables. Multiple regression was performed by using the model: $\text{lm}(\text{seed oil traits} \sim \text{annual} + \text{biennial} + \text{perennial})$.

We used general linear mixed models (GLMMs) to account for the phylogenetic information when correlating SOC, and the nine constituent FAs with life cycle strategies. In the GLMM model, the seed oil traits (SOC and nine FAs) were the response variables; life cycle strategies (annual, biennial, or perennial) were set as fixed effect variable; and tribe, genus, and species as the random effect variables. We also used GLMMs to account for the phylogenetic information when correlating SOC and the nine constituent FAs with biomes. In the GLMM model, the seed oil traits (SOC and nine

FAs) were the response variables; biomes (temperate, subtropical, or tropical) were set as the fixed effect variable, and tribe, genus, and species as the random effect variables.

Do seed oil content and seed oil composition change with latitude?— The effect of latitude on SOC and individual FA proportions within *A. thaliana* and across Brassicaceae was examined. At the intraspecific level, we carried out an ordinary least-square (OLS) regression with 323 ecotypes of *A. thaliana* with the mid-latitudinal position of each genotype as the explanatory variable, and the SOC of each genotype as the response variable. At the interspecific and family level, an OLS regression was performed with 216 Brassicaceae species and subspecies with species' mid-latitudinal range as the explanatory variable, and species' SOC as the response variable. Because the mid-latitudinal range position of a species is constrained between the poles and depends on its latitudinal range, it is likely that the relationship between species' mid-latitudinal range and species' SOC is affected by species' latitudinal range. Hence, we included species' latitudinal range as a covariate in all our models at the family level. Mid-latitudinal range position was computed using the following formula: $(\text{minimum latitude} + \text{species' latitudinal range}/2)$, where species latitudinal range is the maximum latitude minus the minimum latitude.

Do we see correlations between seed oil content and fatty acid composition?— The correlation between seed oil content and seed oil composition in *A. thaliana* and across Brassicaceae species was examined. Pearson's correlation coefficient (r) was performed for pairwise comparisons of SOC and nine constituent FAs (16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:2, 22:1) to evaluate their relationships. All tests were performed using R 3.0.0 (R Development Core Team, 2013) and Systat 13 (Wilkinson, 2010).

Phylogenetic analyses

Mapping seed oil content and seed oil composition data onto a phylogenetic tree— For the phylogenetic analyses, the most recent phylogenies obtained from phytochrome A (*PHYA*) and *ndhF* gene regions (Belstein et al., 2008; Liu et al., 2012) has been used. The major clades can be closely compared with the nrDNA ITS phylogeny (Bailey et al., 2006). The SOC and seed oil composition data available for 26 of the 100 species included in the phylogeny were mapped onto the phylogeny to test for phylogenetic signals for the SOC and seed oil composition trait in Brassicaceae. This was to explore if certain clades evolved towards high or low SOC and individual FA proportions, how close they were phylogenetically, and whether the clades had primarily temperate or tropical species to determine the nature of seed oil evolution.

Phylogenetic autocorrelation

Phylogenetic autocorrelation was estimated to measure the extent to which the phenotypic trait values correlate throughout the phylogeny. Phylogenetic distance between the species was obtained from the Brassicaceae phylogeny (Belstein et al., 2008). The DNA sequences were aligned, and rooted phylogenetic trees were obtained by using the neighbor-joining method and the unweighted pair group method. Residuals from the autoregressive models were tested for independence (Gittleman and Kot, 1990). The residual values were considered as the dependent variable and the

phylogenetic distance as the independent variable. Phylogenetic signal for seed oil content and seed oil composition was estimated by performing Abouheif's C_{mean} and Moran's I test (Münkemüller et al., 2012). Trait evolution was measured under a pure Brownian motion model, which defines the phylogenetic relationship as the only predictor of trait similarity among species. All tests were performed within R 3.0.0 (Chamberlain et al., 2014).

RESULTS

Relationship between seed oil content and the biogeographical distribution and the life cycle strategies of Brassicaceae herbs

A multiple regression analysis showed significant differences ($F_{3,154} = 3.763, P = 0.012, R^2 = 0.07$) in the SOC of herbs in the different biomes (temperate, subtropical, and tropical). Significant differences in the proportions of the FAs 18:0 ($F_{3,161} = 2.808, P = 0.04, R^2 = 0.05$), 18:3 ($F_{3,161} = 4.099, P = 0.008, R^2 = 0.071$), 20:0 ($F_{3,157} = 2.891, P = 0.037, R^2 = 0.05$) were observed between species in different biomes. The proportions of SOC increased from the temperate (26.4%) to the subtropical (28.6%) to the tropical (30%) regions (Table 1). The proportions of saturated FAs 18:0 and 20:0 decreased from the temperate to the subtropical and tropical regions, and the proportions of unsaturated FA 18:3 decreased from the temperate to the subtropical and tropical regions (Table 1).

Multiple regression analysis showed that there is no significant relationship ($P = 0.17$) between SOC and the different life cycle strategies (annual, biennial, and perennial). Significant differences in the proportions of the FAs 16:0 ($F_{3,161} = 3.5, P = 0.017, R^2 = 0.06$), 18:0 ($F_{3,161} = 3.327, P = 0.02, R^2 = 0.06$), 18:2 ($F_{3,161} = 11.24, P = 9.774e-07, R^2 = 0.17$), 18:3 ($F_{3,161} = 3.584, P = 0.015, R^2 = 0.06$), 20:0 ($F_{3,157} = 3.561, P = 0.015, R^2 = 0.064$), 20:1 ($F_{3,161} = 4.088, P = 0.008, R^2 = 0.07$) were observed between species with different life cycle strategies.

A GLMM model accounting for phylogenetic information of the tribes, genus, and species showed a significant relationship between SOC and life cycle strategies ($P = 0.01$) and the relatedness of the species. A significant relationship was also observed for oleic ($P \leq 0.01$), linoleic ($P < 0.0001$), linolenic ($P = 0.02$), eicosenoic ($P \leq 0.03$), and erucic ($P < 0.0001$) acids (Appendix S4).

TABLE 1. Seed oil content of Brassicaceae species in reference to their ecological and geographical parameters.

Biomes	N	Min	Max	Mean	SD	SE
Herbs	Oil Content (%)					
Temperate	214	4	48.7	26.4	8.3	0.568
Subtropical	39	6	40.4	28.6	7.9	1.272
Tropical	31	12.2	42	30	7.1	1.278
	18:0 (%)					
Temperate	162	0.1	4.91	1.68	1.0	0.08
Subtropical	33	0.3	4.91	1.87	1.3	0.231
Tropical	27	0.2	4.91	1.69	1.0	0.186
	18:3 (%)					
Temperate	153	1.23	69.8	27.2	15.93	1.288
Subtropical	27	1.23	56.4	20.94	12.84	2.426
Tropical	22	1.23	66	23.11	19.1	3.982
	20:0 (%)					
Temperate	73	0.2	3.6	1.25	0.713	0.083
Subtropical	22	0.49	3	1.29	0.725	0.155
Tropical	14	0.4	2.1	1.33	0.599	0.16

Effect of latitude on seed oil content and individual fatty acids within and between Brassicaceae species

A significant increase in the SOC ($P = 0.025, R^2 = 0.015$, Fig. 1A) with increasing latitude and a significant decrease in the proportions of saturated palmitic acid ($P = 0.027, R^2 = 0.016$, Fig. 1B) with increasing latitude was observed in *A. thaliana*. The proportions of unsaturated oleic, linolenic, eicosenoic, and eicosadienoic acids increased with latitude although the relationships were not significant. The proportions of saturated arachidic acid decreased with increasing latitude while a reverse trend was observed for stearic acid, but none of the responses were significant. No change was observed in the proportions of linoleic and erucic acids.

The OLS regression analysis of the SOC of the 216 Brassicaceae species did not reveal a significant relationship between SOC and latitude. However, significant relationships between unsaturated oleic ($P = 0.016, R^2 = 0.033$) and linolenic ($P = 0.004, R^2 = 0.052$) acids and latitude (Fig. 1C and D), and a nearly significant relationship between linoleic ($P = 0.084$) acid and latitude were observed where their proportions increased with increasing latitude, as observed in *A. thaliana*. The trends observed for saturated palmitic, stearic, and arachidic acids were decreased proportions with increasing latitude, and for unsaturated eicosenoic acid a reverse trend was observed, although these relationships were not significant.

A GLMM model accounting for phylogenetic information of the tribes, genus, and species showed a significant relationship between SOC and biomes ($P = 0.01$) and the relatedness of the species. A significant relationship was also observed for linoleic ($P \leq 0.04$), eicosenoic ($P \leq 0.01$), and erucic ($P < 0.008$) acids (Appendix S5). The degrees of freedom for GLMMs were not reported because no consensus exists on the way to compute them (Bolker et al., 2008).

Correlation analysis between SOC and FAs in A. thaliana and Brassicaceae species

In *A. thaliana*, significant negative correlations between SOC and palmitic ($r = 0.25, P < 0.0001$) and linoleic ($r = 0.248, P < 0.0001$) acids were observed. There was a significant positive relationship between SOC and oleic (18:1, $r = 0.226, P = 0.001$), eicosenoic (20:1, $r = 0.249, P < 0.0001$), and stearic (18:0, $r = 0.178, P = 0.04$) acids. Negative relationships observed between SOC and eicosadienoic (20:2, $r = -0.101$), and erucic (22:1, $r = -0.135$) acids, and a positive relationship with arachidic acid (20:0, $r = 0.011$) were not significant, the relationship between linolenic acid and SOC was nearly significant at $P = 0.087$ (Table 2, Fig. 2). Across Brassicaceae species, a significant negative correlation was observed only between linoleic acid and SOC.

Phylogenetic signals for the seed oil content and seed oil composition traits in Brassicaceae

Phylogenetic autocorrelation analysis of the 26 species for which SOC data was available did not detect any phylogenetic signals for the SOC ($P = 0.94$) and seed oil composition (FA) traits (palmitic ($P = 0.93$), stearic ($P = 0.79$), oleic ($P = 0.13$), linoleic ($P = 0.8$), linolenic ($P = 0.91$), eicosenoic ($P = 0.23$), and erucic ($P = 0.43$) acids). The SOC data of 25 of the 26 species were from 16 tribes of Brassicaceae (Table 3, Fig. 3) with the exception of *Lunaria annua*, which does not belong to any of the tribes. In the monophyletic Camelinae tribe, all the three species' *Camelina microcarpa* (31.2%), *Capsella*

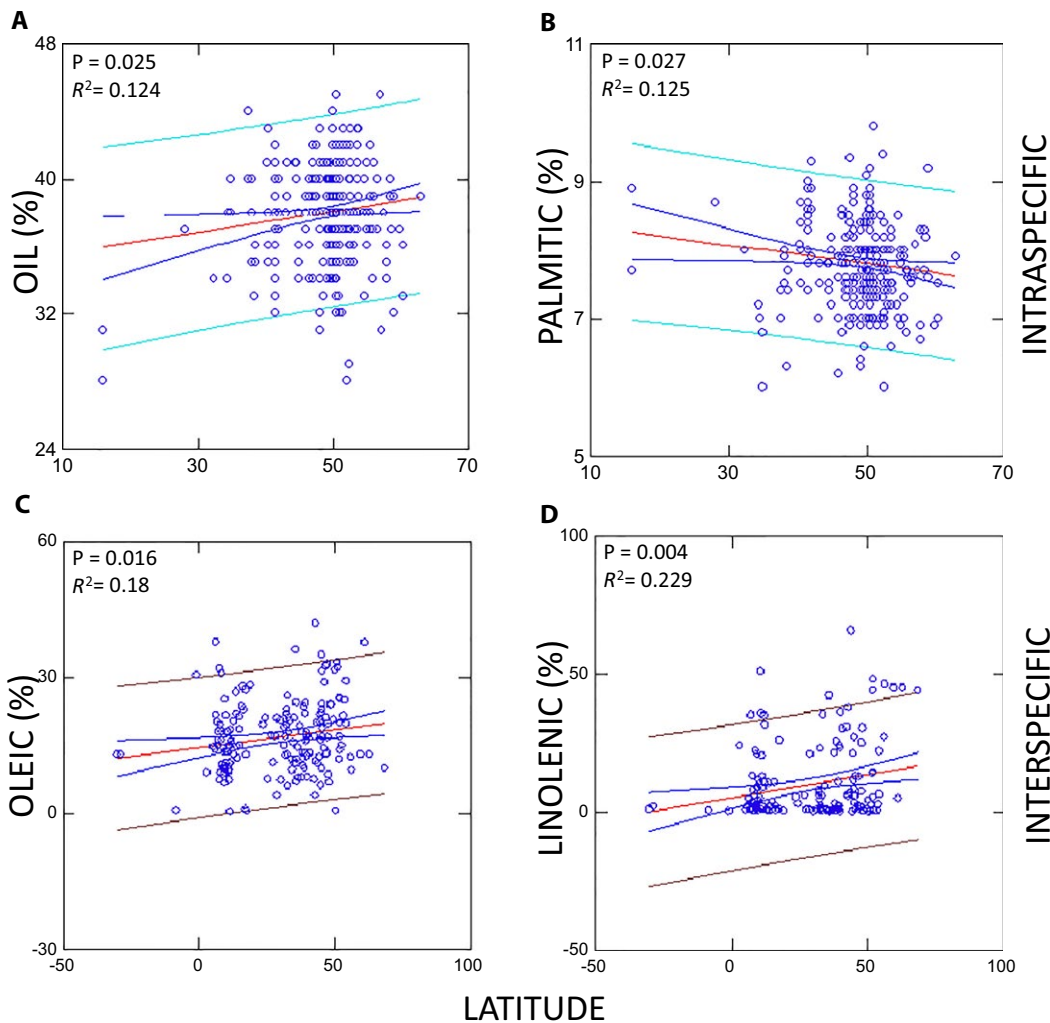


FIGURE 1. (A) Regression of seed oil content on latitude in *Arabidopsis thaliana*. (B) Regression of palmitic acid proportions on latitude in *Arabidopsis thaliana*. The red line is the best-fitted line for the regression model. The dark blue line shows the 95% confidence intervals, and the light blue line indicates the 95% prediction interval. (C) Regression of oleic acid proportions on latitude in Brassicaceae. As in (B), the red line is the best-fitted line for the regression model, and the dark blue line shows the 95% confidence intervals. The brown line indicates the 95% prediction interval.

bursa-pastoris (31.8%), and *A. thaliana* (39.8%) have high SOC (>31%). In the monophyletic Cardamineae, both the taxa *Barbarea vulgaris* (28.6%) and *Planodes virginicum* (25.5%) have high SOC. In the monophyletic Thlaspidaceae, the two taxa *Alliaria petiolata* (29%) and *Thlaspi arvense* (29.5%) have very similar and high SOC (Table 3, Fig. 3). Furthermore, in the Brassiceae tribe, the SOC of *Brassica oleracea* and *Cakile maritima* are high (33% and 44.7%). The SOC of *Hirschfeldia incana* is 19.1%, but the variation of the SOC of the species ranges from 5.4–32%, and the median value was high at 22.6%. In the tribe Isatideae, no consistent pattern was detectable from the SOC proportions in the two taxa *Isatis tinctoria* (23.3%) and *Myagrum perfoliatum* (10.9%). Two closely related tribes—Hesperideae and Anthonieae—were each represented by a single species, *Hesperis matronalis* and *Bunias orientalis*, but the SOC of the two species in the two tribes were very different at 29.8% and 11.1%, respectively (Table 3, Fig. 3). The SOC pattern of eight tribes (Descurainieae, Lepideae,

Hesperideae, Anthonieae, Sisymbrieae, Arabideae, Calepineae, Aethionomeae, Table 3, Fig. 3) could not be determined because SOC data of only a single species were available for these tribes. Eleven tribes were unrepresented in this study because of lack of SOC data. Thus, this study revealed that high SOC (>25%) evolved independently in four tribes: Cardamineae (27.1%), Camelinae (34.3%), Brassiceae (33.4%), and Thlaspidaceae (29.3%) (Table 3, Fig. 4).

DISCUSSION

This study determined a significant relationship between seed oil traits and biomes (temperate, subtropical, and tropical) and changing latitude in the Brassicaceae family. The study also revealed a significant relationship between seed oil composition (FA composition) and the life cycle strategies (annual, biennial, perennial) in Brassicaceae. The study showed that no phylogenetic signal was identified for seed oil traits, but reports that high SOC evolved independently in four tribes of the Brassicaceae family suggesting that comparing topologies of gene trees of genes regulating seed oil traits can help understand the seed oil evolutionary patterns.

Evidence of differences in seed oil content and seed oil composition within herbs in different geographical regions and with different life cycle strategies

Characteristics of seeds and seedlings require precise integrations and coordination between different functions for successful reproduction. Oils are found in at least 83 (30%) of the 277 plant families listed by Engler and Prantl (McNair, 1930), and of these 83 families, up to 48.1% are mainly tropical or subtropical, and up to 18% are mainly temperate in habitat (McNair, 1930). Hence, oils are found more frequently in plants in tropical and subtropical regions than in temperate regions. The adaptive nature of seed oil composition in temperate and tropical regions has been shown in previous studies where plants with greater proportions of unsaturated fats germinate earlier and compete better at higher latitudes (Linder, 2000).

TABLE 2. Correlation between seed oil content and constituent fatty acids in seed oil in *A. thaliana*.

Oil	16:0	18:0	18:1	18:2	18:3	20:0	20:1	20:2
Oil								
16:0	−0.25							
18:0	0.178	0.28						
18:1	0.226	−0.186	0.179					
18:2	−0.248	−0.047	−0.263	−0.106				
18:3	0.168	−0.122	−0.099	−0.246	−0.275			
20:0	0.011	0.408	0.602	−0.148	−0.212	0.009		
20:1	0.249	0.105	0.354	0.078	−0.446	0.152	0.511	
20:2	−0.101	−0.066	−0.183	−0.583	0.362	0.207	0.123	0.102
22:1	−0.135	−0.063	−0.080	−0.473	−0.073	0.44	0.287	0.381
								0.462

Numbers in bold indicate that correlation is significant at $P = 0.05$.

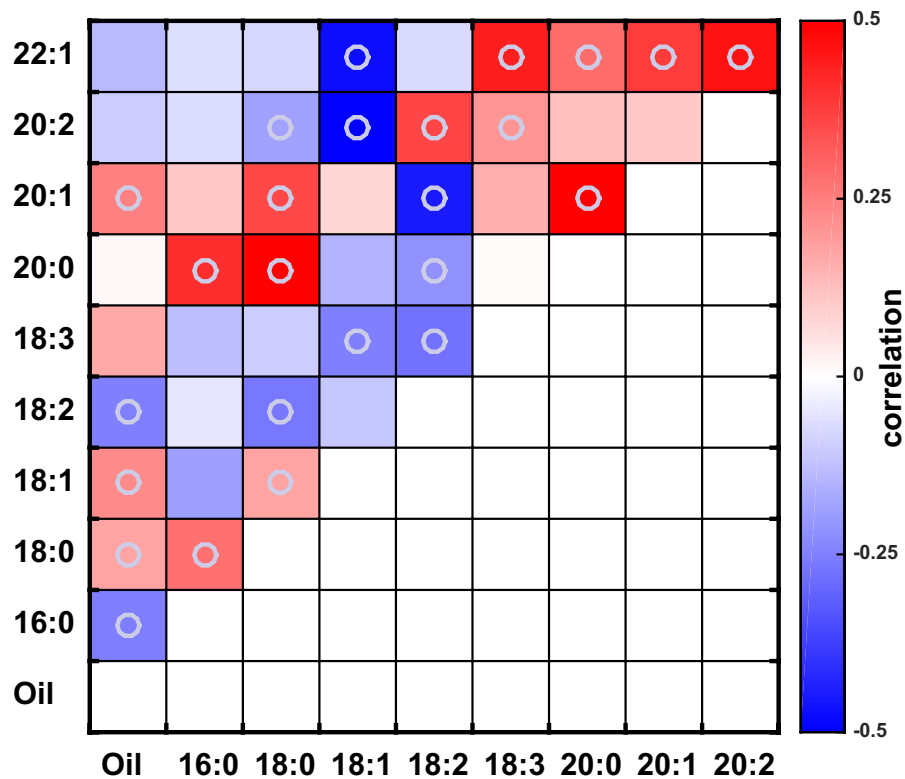


FIGURE 2. Correlation between SOC and the constituent fatty acids. Circles in the cells indicate significant negative and positive correlations at p -value = 0.05. The numerical bins for the correlations corresponding to the shading are shown in the bar to the right.

An increase in proportions of PUFAs and decrease in seed size and weight has been observed with increasing SOC (Bretagnolle et al., 2016; Sanyal and Decocq, 2016). It is also known that high temperature increases SOC proportions (Hartwig, 1973).

The majority of Brassicaceae species are oilseed rich with great variation in SOC (3–48%) which could potentially be under selection. In our study, we observed a significant increase in SOC in the Brassicaceae herbs from temperate (26.4%) to the subtropical (28.6%) to the tropical (30%) regions (Table 1). This increase in SOC in herbs in subtropical and tropical regions when compared to the temperate herbs possibly helps in adaptation as greater packing of species and greater competitive interactions in the tropics may select for a more efficient and higher energy-containing storage product like oil in the seeds (Baker, 1970). The proportions of the saturated FAs (18:0 and 20:0) increase significantly from the

temperate to the subtropical and tropical regions, whereas the proportions of the unsaturated FA (18:3) significantly decreases from the temperate to the subtropical and tropical regions (Table 1). This trend follows the pattern predicted by the adaptive theory where the proportions of unsaturated FAs increase with increasing latitude, and the increased proportions of SOC increase the quantity of energy and the increased proportions of unsaturated FAs with low melting points increase the proportions of the available energy for early germination, which helps plants to adapt at higher latitudes where the growing season is shorter. Thus, multiple oil traits seem to be facilitating the adaptation of plants at higher latitudes.

Furthermore, the majority of the herbs are annuals and have to germinate, establish, and reproduce within a year (Linder, 2000) so there might be increased selection pressure on seed oil traits in herbs. In our study, we did not see any significant differences in the SOC

TABLE 3. Seed oil content in different tribes, genera, and species in Brassicaceae.

Tribes	Genera	Species	Species name	Mean Oil content (%)
Camelineae	3	4	<i>Arabidopsis thaliana</i>	39.8
			<i>Capsella bursa-pastoris</i>	31.8
			<i>Camelina microcarpa</i>	31.2
			<i>Turritis glabra</i>	38
Physarieae	1	1	<i>Dimorphocarpa wislizeni</i>	3.9
Descurainieae	1	1	<i>Descurainia sophia</i>	35.7
Lepideae	1	1	<i>Lepidium draba</i>	15.9
Cardamineae	1	2	<i>Barbarea vulgaris</i>	28.6
			<i>Planodes virginicum</i>	25.5
Alysseae	1	1	<i>Alyssum canescens</i>	20.9
Euclidieae	2	2	<i>Euclidium syriacum</i>	3.4
			<i>Sterigosella africana</i>	31
Hesperideae	1	1	<i>Hesperis matronalis</i>	29.8
Anchonieae	1	1	<i>Bunias orientalis</i>	11.1
Sisymbrieae	1	1	<i>Sisymbrium altissimum</i>	35
Brassicaceae	3	3	<i>Brassica oleracea</i>	38
			<i>Hirschfeldia incana</i>	19.1
			<i>Cakile maritima</i>	44.7
			<i>Isatis tinctoria</i>	23.3
Isatideae	2	2	<i>Myagrurn perfoliatum</i>	10.9
			<i>Arabis alpina</i>	28
Thlaspidaeae	2	2	<i>Alliaria petiolata</i>	29
			<i>Thlaspi arvense</i>	29.5
Calepineae	1	1	<i>Goldbachia laevigata</i>	48
			<i>Lunaria annua</i>	34.6
Aethionemeae	1	1	<i>Aethionema saxatile</i>	21.5

between annuals, biennials, and perennials. However, we did see significant differences in the proportions of FAs (16:0, 18:0, 18:2, 18:3, 20:0, 20:1) between annuals, biennials, and perennials suggesting that seed oil composition could be playing a role in plant adaptation.

Evidence of a latitudinal cline within and across Brassicaceae species

Significant increase in SOC and decrease of saturated palmitic acid with increasing latitude in *A. thaliana* (Figs. 1A, B) shows that there could be selection for greater proportions of SOC and unsaturated FAs with increasing latitudes. The trend of increasing proportions of unsaturated FAs (oleic, linolenic, eicosenoic, and eicosadienoic) with increasing latitude also point towards the same pattern. A positive correlation between SOC and the proportions of PUFAs were also observed in Sapindaceae and several weed species (Bretagnolle et al., 2016; Sanyal and Decocq, 2016). Correlation studies also revealed that there was a significant negative correlation between SOC and saturated palmitic acid, and significant positive correlations with unsaturated oleic, linolenic, eicosenoic acids (Table 2, Fig. 2), and also saturated stearic acids. However, the proportion of stearic acid is $\leq 4\%$. Significant negative correlation between SOC and unsaturated linoleic acid suggests a tradeoff between oleic and linoleic acids because the former is the precursor of the latter in the seed oil biochemical pathway.

The pattern of increased proportions of FAs with increasing latitude has been observed previously in *Arabidopsis* (Sanyal and Linder, 2013) and sunflower (Linder, 2000). This pattern has been shown to be adaptive in nature in a previous study on sunflowers

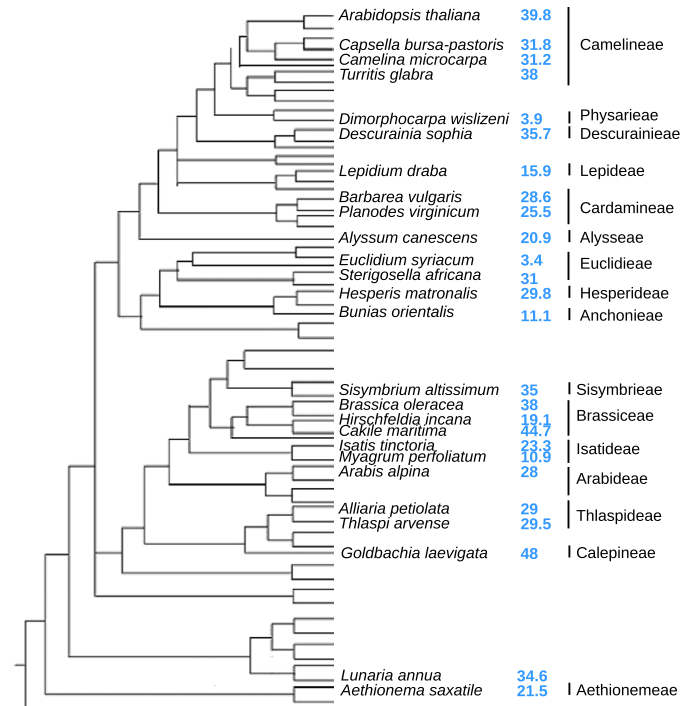


FIGURE 3. Pruned phylogenetic tree adapted from Beilstein et al. (2008) showing the SOC pattern of the species. The numbers in blue indicate the SOC of the species. Only the species for which SOC information is available has been shown on the tree.

(Linder, 2000). In our study we also observed a similar pattern. This suggests that the significant increase in SOC and unsaturated oleic, linoleic, and eicosenoic acids with latitude, and significant decrease of palmitic acid with latitude in *A. thaliana* is likely an adaptive trait because higher SOC and higher proportions of unsaturated FAs could be providing more energy quickly. Increased SOC provides greater amounts of energy and greater proportions of unsaturated FAs with low melting points, and provides the needed energy faster at cooler temperatures at higher latitudes. This increased SOC helps the species germinate quickly in shorter growing seasons at higher latitudes and gives it an advantage over other competing species (Linder, 2000). The proportions of saturated and unsaturated FAs, and subsequently their melting points, vary with latitude (Linder, 2000; Sanyal and Linder, 2013). Thus, at lower latitudes, seeds with higher proportions of saturated oils and higher melting points would be favored because they would have more energy per carbon atom for growth without delaying or slowing germination. At higher latitude and cooler germination temperatures, seeds with higher proportions of unsaturated oils may germinate earlier and/or more rapidly than seeds that are higher in saturated FAs (Linder, 2000). Thus, selection could be acting on multiple seed oil traits such as SOC and unsaturated FA proportions, both of which increase with increasing latitude; the selection is possibly helping in adaptation in *A. thaliana*. In addition, previous studies on weed, rain forest, and crop species have shown that seed size and weight and SOC are inversely proportional (Bretagnolle et al., 2016; Hamilton et al., 2013; Nagel and Börner, 2010). *Arabidopsis thaliana* also has smaller and lighter seeds with high SOC—this suggests that there could be selection for greater SOC in the small seeds of *A. thaliana* to pack more energy-rich molecules to increase

fitness. Therefore, K-selection will be favored over r-selection at the higher latitudes, which will lead to selection of energy-rich seeds. This possible selection on multiple seed oil and seed traits could be contributing towards the successful establishment of *A. thaliana* at higher latitudes.

At the interspecific level in the Brassicaceae family, a significant increase in the proportions of unsaturated oleic and linolenic acids with latitude in the 216 Brassicaceae species suggests that an adaptive pattern is seen in the seed oil composition trait where there is selection for greater proportions of unsaturated FAs at higher latitudes showing the same pattern seen in *A. thaliana*. Furthermore, the trends of decreasing saturated palmitic, stearic, and arachidic acid proportions and increasing unsaturated linoleic and eicosenoic acids with increasing latitude also point towards this adaptive theory. A significant relationship between SOC and changing latitude was not observed. Lack of precise information of the latitudinal location of the SOC data and possibly incomplete species occurrence data from GBIF due to differences in sampling efforts across the world could be obscuring our results and the exact relationship between SOC and latitude in Brassicaceae. However, a multiple regression analysis revealed a significant difference in the SOC, stearic, linolenic, and arachidic acids between species in temperate, subtropical, and tropical regions. The nature of the intraspecific and interspecific seed oil patterns fit within the theoretical framework of the gradient model (Gaston et al., 2008).

Other important nontemperature related factors, which could covary with latitude, are herbivory and photoperiod. It has been suggested that herbivory increases at lower latitudes (Janzen, 1970; Connell, 1971; Seigler, 1979) and herbivores prefer seeds with higher SOC because it provides more energy. Furthermore, wild birds have shown a preference for black-oil sunflower varieties with the highest SOC (Collins and Horn, 2012). Thus, one would expect a selection force on SOC at lower latitudes because of herbivory, which could explain the increase in SOC at higher latitudes. Photoperiod and temperature had an effect on the reproductive period and SOC in soybean (Miranda et al., 1998). The plants subjected to a long photoperiod had higher SOC than those subjected to shorter photoperiod conditions (Tianfu et al., 1997). Photoperiod at higher latitudes is longer in the summer and shorter in the winter. In addition, the level of illumination has a significant effect on SOC. In temperate herbs, it has been observed that the mean SOC is inversely proportional to illumination and SOC increases from 16.59% to 25.43% to 27.91% as the level of illumination decreases in those inhabiting open habitats, woodland borders, and shaded habitats and woodlands (Levin, 1974).

Phylogenetic signal in the seed oil content trait

The absence of phylogenetic signal for the seed oil traits in Brassicaceae was observed (Fig. 3). However, the occurrence of high SOC (>27%) proportions in four tribes: Cardamineae, Thlaspidiae, Brassiceae, and Camelinae (Table 3, Fig. 4) suggests that examining the phylogenies from genes regulating seed oil traits will help understand the underlying mechanism of the observed pattern (Sun et al., 2013). A phylogeny of Brassicaceae generated from the *FAE1* gene suggested that purifying selection is the major evolutionary force acting on the gene, which is consistent with similar findings in another fatty acid elongase gene—*EVOVL5*—responsible for encoding an enzyme involved in the biosynthesis of long-chain PUFAs in fish (Carmona-Antoñanzas et al., 2013).

Studying the phylogeny of several SOC and seed oil composition genes in Brassicaceae species will help identify the evolutionary patterns of the seed oil genes. This could help us understand the genetic basis of the adaptive nature of SOC and seed oil composition in Brassicaceae and other families. This suggests that because the *FAE1* gene is involved in TAG biosynthesis, focus on the sequence variation and phylogeny generated from regulating seed oil content and seed oil composition genes will be useful in understanding the evolutionary pattern of the SOC trait. Hence, several regulating seed oil content and seed oil composition genes in Brassicaceae and other families should be used for generating phylogenies, and a comparison of the tree topologies may reveal meaningful and robust patterns of adaptive evolution and/or phylogenetic signals for the SOC trait within and across families.

CONCLUSIONS

Our study provides evidence for the adaptive nature of the seed oil traits in plants across biomes. We show that the nature of the intraspecific and interspecific seed oil patterns fit within the theoretical framework of the gradient model, and that the same phenotypic patterns with respect to the relationship between seed oil content and biomes were observed at both the intraspecific and interspecific levels. A significant latitudinal cline for seed oil composition was observed at both the intraspecific and interspecific levels, while a significant latitudinal cline for seed oil content was observed only at the intraspecific level. The study suggests that multiple seed oil traits (seed oil content and unsaturated fatty acids) could have selective advantages at higher latitudes in *Arabidopsis thaliana* (intraspecific-level pattern) and across 216 Brassicaceae species (interspecific-level pattern). We show that there is a significant difference in the seed oil composition of the Brassicaceae species with different life cycle strategies (annuals, biennials, perennials). Phylogenetic signals for the seed oil traits (seed oil content and seed oil composition) were not detected in the Brassicaceae family. However, the occurrence of high seed oil content (>27%) was observed in four tribes: Camelinae, Thlaspidiae, Cardamineae, and Brassiceae; this suggests that phylogenetic gene trees of seed oil genes can reveal the seed oil evolutionary patterns. These seed oil evolution patterns are in accordance with the predictions of the adaptive theory. This study builds the framework for understanding the adaptive evolution of seed oil traits both at the intraspecific and interspecific levels not only in Brassicaceae, but also in other angiosperm families.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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