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Assessment of the biogeographical variation of seed size and seed oil traits in wild *Silphium integrifolium* Michx. genotypes

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Abstract

Silphium spp. have garnered interest in Europe as a bioenergy crop and in North America as a perennial oilseed crop. However, very little has been done at this early stage of domestication to characterize wild collections for many key characteristics, including important oilseed traits. The objective of this work was to develop a basic understanding of how biogeography and associated population genetic forces have shaped seed phenotypes in plant collections across the native range of *Silphium integrifolium* Michx. (Asteraceae: Heliantheae), the primary domestication candidate for oilseed use. A collection of 53 accessions was grown in a common environment in Salina, KS, which is a location well within the native range of the species in central North America. Plants from each collection site were randomly mated by hand to produce seed representative of each accession, and the seeds subjected to seed dimensional trait, oil content and oil composition analyses. Kernel width varied along a latitudinal cline of collection site, while kernel length varied across a longitudinal cline. Palmitic and linoleic acids were inversely correlated with each other and varied along a longitudinal cline of the collection site. The results indicate that accessions collected from more southwesterly sites tended to have larger seed and those from more westerly sites had higher linoleic acid content and lower palmitic and myristic acids, which are all desirable phenotypes for an oilseed *Silphium*.

Keywords: biogeography, oil composition, oil content, seed trait, *Silphium integrifolium*

Introduction

The genus *Silphium* (L.) is a part of the Heliantheae (sunflower) tribe of the Asteraceae family and originated in the prairies of North America. The native range is from the Rocky Mountains eastward to Appalachia, and from Canada to the Gulf of Mexico (USDA – NRCS, 2019). *Silphium integrifolium* Michx. is considered a candidate for domestication to generate a new perennial oilseed crop due to its large seeds and resistance to drought and heat stress (Vilela *et al.*, 2018). In the early 2000s, work on *S. integrifolium* was initiated independently in Kansas, USA, for oilseed production and Poland for biomass production (Kowalski and Wiercinski, 2004; Van Tassel *et al.*, 2014). Despite the fact that domestication of *S. integrifolium* began just a few breeding cycles ago, recent reports show advances towards domestication (Van Tassel *et al.*, 2017). However, intensified research over the last several years did not include analysis of the natural variation in seed size and seed oil traits in this species.

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Increased seed size is a prominent part of the domestication of many crops, likely because larger seeds are better adapted to deeper sowing depths facilitated by mechanical tillage, and deep sowing provides more consistent access to available moisture in the soil to begin germination. Seed morphology traits can also play a role in the ease of threshing (Fuller, 2007). In the contribal relative wild sunflower (*Helianthus annuus* L.) as well as wild relatives of the confamilial safflower (*Carthamus oxyacanthus* Bieb.), heritable variation in seed size correlated with the geographical region of origin of the plants (Cantamutto *et al.*, 2008; Sabzalian *et al.*, 2009; Nooryazdan *et al.*, 2010; Majidi and Zadhoush, 2014). The differences in seed size among accessions seem to be the effect of local adaptation to different environments to increase survival of seedlings (Metz *et al.*, 2010).

Oil content and composition are essential to an oilseed crop's utility. Reports from a diverse set of plant species, including domesticated sunflower, indicate that the total seed oil content and seed oil composition of a plant accession are affected by the climate of the environment of origin as a result of adaptation to each environment (Sanyal and Decocq, 2016) and thus, are explained mostly by plant genotype. For instance, higher proportions of unsaturated fats allow for faster germination at colder temperatures (Linder, 2017). However, ambient temperature during seed development is also known to have effects on the seed oil content and composition in several species, with high temperatures during seed development reducing total oil content and linoleic acid in sunflower, so some environmental plasticity occurs in these traits (Harris *et al.*, 1978; Kizil *et al.*, 2008). Thus, it is important to study accessions collected from diverse environments by growing them in a common environment.

Our objective was to assess the phenotypic distribution of seed size and seed oil traits for a wild *S. integrifolium* collection in a common environment to guide future collection activities as well as structured crosses in breeding programmes.

Material and methods

Plant material

Seeds from wild *S. integrifolium* populations were obtained from several sources. First, The Land Institute employees collected seeds from plants found along roadsides and in other public places. Second, an appeal was made to supporters of The Land Institute to look for native *Silphium* on their private property and lands operated by their organizations. These volunteers were given instructions about when seeds are mature, and asked to harvest seeds from a single stalk per clump and to sample only clumps about 1 m apart from each other, to ensure that each sample was from an individual genotype. Volunteers recorded the location of the population or individual plants by taking photos of them using smart phones. The seeds and photos were sent to The Land Institute and the GPS coordinates of the populations were extracted from the photo metadata. Finally, some seeds were donated from organizations that had made similar collections previously and kept remnant seeds in cold storage. Information about the populations used in this study is presented in Fig. 1 and online Supplementary Table S1.

Heads from collected samples were threshed using a BT14 belt thresher (Almaco, Nevada, IA, USA) in December 2016 and cleaned using an STS-WM2 aspirator (SeedTech Systems, Wilton, CA, USA) and hand sieves. In February 2017, achenes were placed on germination paper that was laid on top of a cotton towel on a tray. The paper and towel were moistened, and trays were placed in a cooler (4°C) for 6 weeks. At the end of this cold-moist stratification treatment, seeds were allowed to germinate in a warm place (approximately 24°C). Trays were kept moist and covered, and checked daily. As soon as radicles could be seen emerging from an achene, it was placed in the dimple of a hydrated 50 × 95 mm peat pellet (Jiffy Products of America, Lorain, OH, USA) and covered with turface (Turface MVP, Profile Products LLC, Buffalo Grove, IL, USA). Seedlings were raised in an unheated space and watered as often as necessary to keep the pellets wet with Miracle-Gro Water Soluble All Purpose Plant Food (The Scotts Miracle-Gro Company, Marysville, OH, USA) diluted according to the label, weekly or as necessary to maintain dark green foliage. Seedlings were transplanted to the field in May 2017. The field had been prepared by tillage and the seeding of tall fescue (*Festuca arundinacea*, Scotts Sta-green, Scotts Miracle-Gro Company), as a ground cover. The fescue had germinated prior to transplanting, so shallow furrows were made in a 1 m × 2 m grid to kill the grass within the furrow and to indicate where seedlings would be planted. Pellets were buried at the intersection of the furrows. Transplants were hand watered immediately after transplanting and every few days for the next two weeks, as needed. The grass intercrop was managed by mowing with standard lawn equipment. Annual weeds were controlled by manual pulling. The whole field was fertilized each June with 50 kg/ha nitrogen in the form of urea.

In 2018, the plants flowered for the first time, beginning in late June. Plants from the same population were flagged and bagged with cotton bags to allow controlled pollination as they began to flower. Any heads with florets in anthesis or older were removed prior to bagging. Generally, two related plants were treated as females and a third plant from the same population was used as a pollen parent. Pollen parents were also bagged with cotton or nylon

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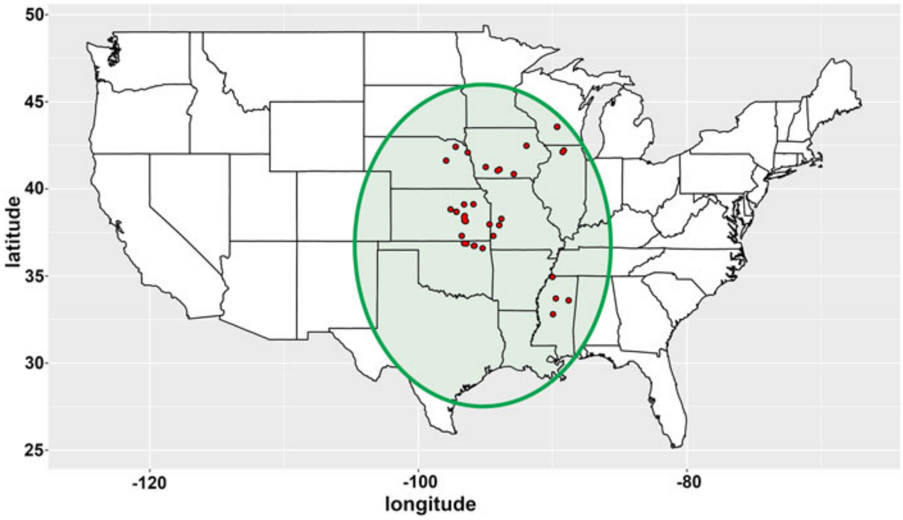

Fig. 1. Map of the USA adjusted in scale to fit on a latitude/longitude grid, with collection site overlay for the 53 accessions of *Silphium integrifolium* Michx. The green shaded area is its native range.

mesh bags to protect the pollen from predation. Heads from the pollen parents were detached, if they had a lot of fresh pollen visible, and the detached heads were rubbed on the female parent heads (after temporarily removing the cotton bags). In September, the seed was harvested from the bagged heads after heads had matured (turned brown) and further dried at room temperature. Crossed seed was threshed and cleaned as described above. Seed from these plant materials is not kept in stock, but can be requested from The Land Institute with ample notice to allow for seed production.

Phenotyping

Ten seeds sampled from several sib-mated plants of each accession were laid out in a straight line and scanned using an Epson Perfection V800 Photo flatbed scanner (Seiko Epson Corporation, Suwa, Japan). The seeds were scanned as negatives to increase the contrast between hull wings and actual seed. Image processing was done with a custom modification of functions within the R package *GiNA* (online Supplementary File 1; Diaz-Garcia *et al.*, 2016). Briefly, background extraction and object (e.g. kernel) identification was performed using threshold-based segmentation in the blue colour channel. Next, for each seed, we performed an additional round of thresholding to identify the wings and the achene boundaries, and then used functions in *GiNA* to measure various size parameters of the achene anatomy (Fig. 2).

For the oil content analysis, 8 ml of clean *Silphium* seeds (with empty hulls and chaff removed) from several sib-mated plants of each accession were weighed in an 18 mm glass cuvette and subjected to nuclear magnetic resonance (NMR) analysis on an Oxford MQC NMR fitted with a 18 mm probe (Oxford Instruments, Abingdon, UK). The calibration curve used for this instrument and probe size is specific for *Silphium* spp. and was developed from a continuum of samples with different oil contents as quantified with the AOAC method 2003.05 analysis with petroleum ether as solvent (Minnesota Valley Testing Labs, New Ulm, MN, USA; Joel Sieh, pers. comm., 2017). Oil content is expressed in g oil per kg total achene mass.

The seed fatty acid composition was analysed according to the sunflower method described by Hulke *et al.* (2010), with the following adaptations. Each *Silphium* accession was analysed in a subsample of 20 seeds harvested from several sib-mated plants of each accession. Subsamples were pulverized using an IKA Tube Mill (IKA Works, Inc, Wilmington, NC, USA). The samples were then derivatized in 3 ml of hexane-chloroform-0.5 M sodium methoxide in methanol (75:20:5, v/v). After 10 s of vortexing, 1 ml of the sample solution was transferred to a 2 ml gas chromatograph autosampler vial. To confirm the identity of detected fatty acids, we analysed the molecules of five samples using gas chromatography-mass spectrometry (GC-MS) at the North Central Soil Conservation Research Laboratory (USDA-ARS, Morris, MN, USA; Russ Gesch, pers. comm., 2019). Samples were analysed according to the procedure described by Walia *et al.* (2018). Fatty acid composition fractions are expressed as g fatty acid per kg total oil.

Statistical analysis

For each trait, observations were averaged across technical replications to produce mean values. On the mean values, we calculated the Pearson correlation for kernel length

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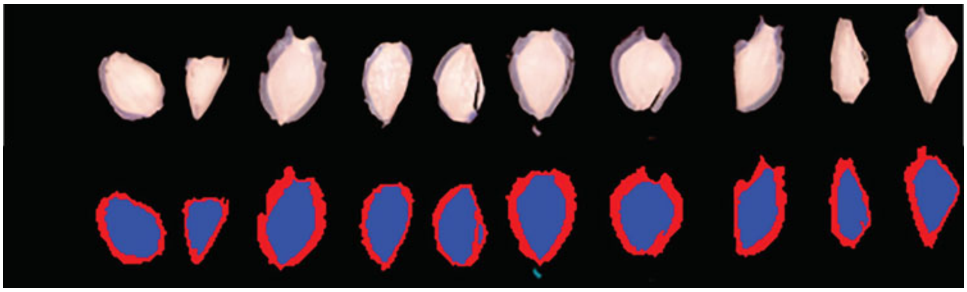

Fig. 2. Example of the seed scan used for seed size trait phenotyping. The kernel is highlighted in blue and the wing (hull) of the seed in red. The kernel and wing together form the achene.

(KL), kernel width (KW), kernel area (KA), wing area (WA), achene area (AA), kernel area-wing area ratio (KA/WA), seed oil content (Oil), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2), by using the 'rcorr' function implemented in the Hmisc R package (R Core Team, 2018).

We conducted a regression analysis using the GLM procedure implemented in SAS v. 9.4 (SAS Institute, Cary, NC) that included the geographical coordinates (latitude and longitude) of the collection sites for each of the 53 genotypes to estimate clines in phenotypic variation across the native range of the species. From the linear variables, two-dimensional contour plots were developed to graphically show the geographical clines for each of the traits.

Results

To identify variation in the seed size traits, seed oil content and the seed oil composition of *S. integrifolium*, we analysed 53 genotypes from a wild collection in a common garden experiment, collected across the native range in North America. The phenotypic values of seed size and seed oil traits revealed considerable variation among analysed genotypes (Fig. 3, online Supplemental Table S1). For seed size traits, the ranges were: KW (3.3 ± 0.2 mm to 6.7 ± 0.5 mm), KL (6.4 ± 0.2 mm to 13.9 ± 0.3 mm), KA (17.8 ± 1.1 mm^2 to 67.2 ± 4.1 mm^2), WA (12.6 ± 1.0 mm^2 to 39.9 ± 1.9 mm^2), AA (31.4 ± 1.4 mm^2 to 97.8 ± 4.1 mm^2) and KA/WA (1.1 ± 0.1 to 3.0 ± 0.2). Similarly, large variation had been detected for seed oil traits with oil (118–253 g/kg), 14:0 (17–44 g/kg), 16:0 (77–116 g/kg), 18:0 (14–44 g/kg), 18:1 (156–280 g/kg) and 18:2 (562–699 g/kg.

In order to analyse the effects of biogeography, we performed regression analyses. We incorporated the latitude and longitude as linear variables in the regression analysis, which revealed significant differences among seed and oil traits due to latitude and/or longitude (Table 1). Among the 12 traits, eight traits were significantly influenced by the origin of the genotype. Longitude affected seed size and seed oil traits more often than latitude. To graphically demonstrate these effects, we developed contour plots scaled to latitude/longitude coordinates for traits which were significantly influenced by latitude and/or longitude (Fig. 4). Kernel area was affected by latitude and longitude, which generally results in increased kernel area in genotypes from the southwestern regions. The average estimated effects of latitude and longitude on the KA were −1.2 mm^2/°latitude and −1.6 mm^2/°longitude, respectively (Table 1). Traits KW and KA/WA were only affected by latitude, where genotypes collected in the southern areas of the origin of *S. integrifolium* showed an increase in KW. The effect of latitude on KW was −0.1 mm (Table 1) for each latitudinal degree north. The other seed size traits (KL, WA and AA) were influenced by longitude only. Genotypes from collection sites in the western regions of *S. integrifolium* were larger in size for these traits compared to genotypes collected in eastern regions. For each longitudinal degree east, KL, WA and AA varied by −0.4 mm (KL), −1.2 mm^2 (WA) and −2.8 mm^2 (AA) (Table 1). However, among oil traits, only longitude influenced phenotypes, with significant effects for 16:0 and 18:2. The share of the oil composition for 16:0 decreased but 18:2 increased with more westerly collection site. The average effect estimates for the oil traits were 2 g/(kg · °longitude) (16:0) and −4 g/(kg · °longitude) (18:2, Table 1).

Further, we identified correlations between seed size and oil traits, which were supported by the biogeographic distribution (Table 2). Interestingly, the correlation analysis revealed a moderate, positive correlation of 18:2 with KL ($r = 0.31$) and WA ($r = 0.28$) but negative, moderate correlations of 16:0 with KW ($r = -0.35$), KL ($r = -0.55$), KA ($r = -0.44$), WA ($r = -0.54$) and AA ($r = -0.51$). Among all measured oil traits, only 14:0 ($r = -0.31$) and 16:0 ($r = -0.37$) revealed a correlation with oil content. The negative correlation between 16:0, the dominant saturated fat, and 18:2, the dominant unsaturated fat, while not surprising, could be beneficial for optimizing for either saturated fat or unsaturated fat content in *Silphium* breeding. The seed dimensional traits are generally moderately to highly correlated with one another, with the noteworthy exception that KA/WA ratio is not correlated with WA, indicating that change in KA largely drives that ratio.

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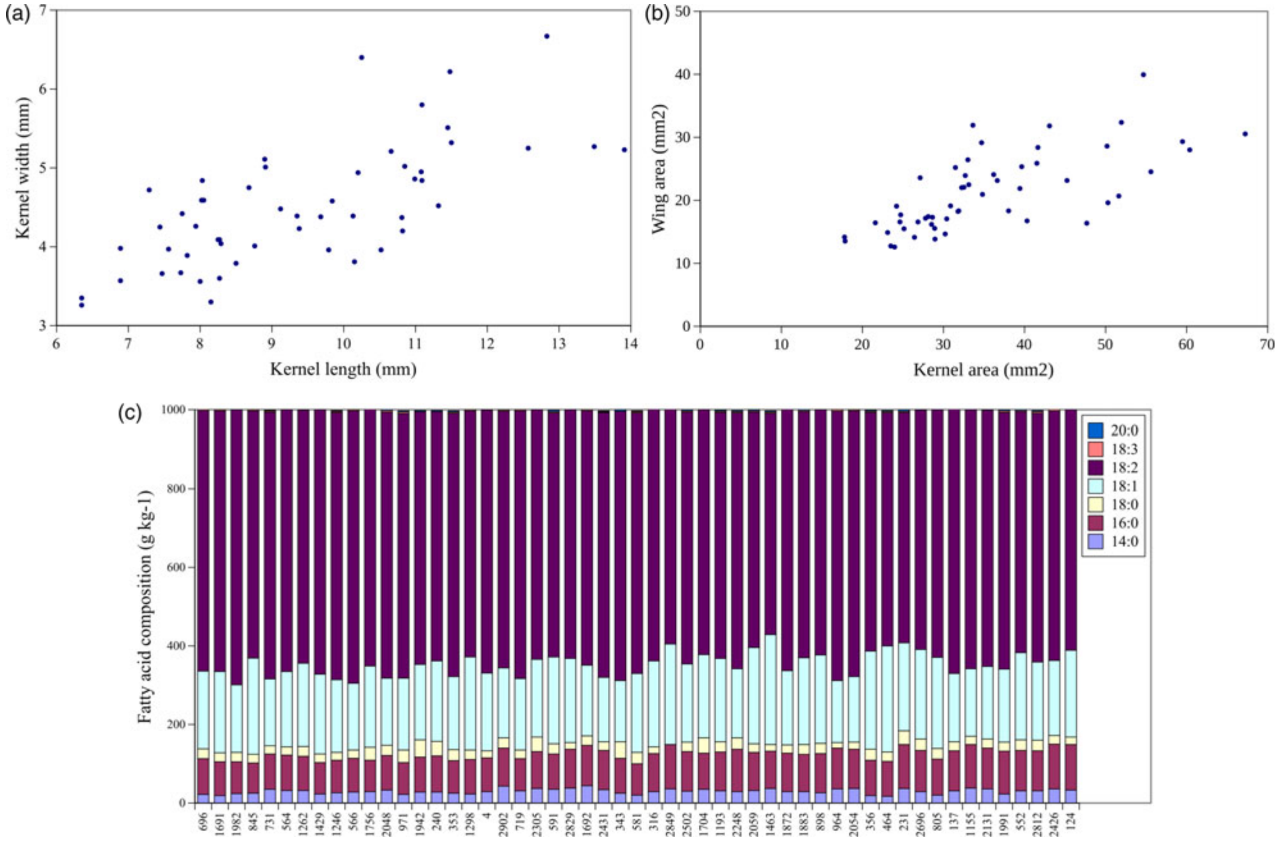

Fig. 3. Graphical representations of seed trait data from 53 wild accessions of *Silphium integrifolium*. (a) Scatterplot of accession mean kernel length versus kernel width, (b) scatterplot of mean kernel area versus wing area, (c) cumulative bar plot of fatty acid composition organized by the longitude of collection site, west (left) to east (right).

Discussion

Phenotypic variation for seed traits such as seed size and seed oil can vary among wild-collected accessions due to natural selection for adaptation (Sabzalian *et al*., 2009; Safavi *et al*., 2010). Agronomically, seed size, oil content and composition are also critical traits for the success of *S. integrifolium* as a sustainable oilseed crop. Therefore, the analysis of the natural variation for these traits in a common environment is necessary to guide plant collection and breeding.

Kernel size, and whole seed size in general, has increased during domestication of sunflower and most other crops. This is generally understood to be the effect of either direct or indirect selection for larger seeds, which are more adapted to the deeper sowing that occurred in primitive agricultural systems versus wild conditions (Fuller, 2007). Deeper sowing of seeds is still encouraged today in general agronomic recommendations because access to soil-water is more consistent, resulting in consistent germination. Increasing kernel size and achene size also improves threshing and mechanical separation of seeds from the chaff (Fuller, 2007). The wing area itself in *Silphium* can be almost the same size as the kernel area, which increases surface area and can dramatically reduce efficiency in separating filled from unfilled seeds using the forced air columns in commercial harvesting equipment. Reducing wing area while increasing kernel area (and accordingly, kernel mass) should improve harvestability and separation. Kernel area to wing area ratio is positively correlated to achene area and, to a greater extent, kernel area and kernel width (Table 2). A closer look at correlations indicates that wing area is also positively correlated with achene area, kernel area and kernel width, but not kernel area to wing area ratio, indicating that while larger seeds also have larger wing areas than smaller seeds, the wing size increases at a lesser rate than kernel size as achene size increases. This is a favourable result, and due mainly to increases in kernel width over kernel length.

Interestingly, seed size traits in *Silphium*, including the kernel area to wing area ratio, are significantly affected by a southwestern cline, such that we identified seeds with increased kernel size and high kernel area to wing area ratios

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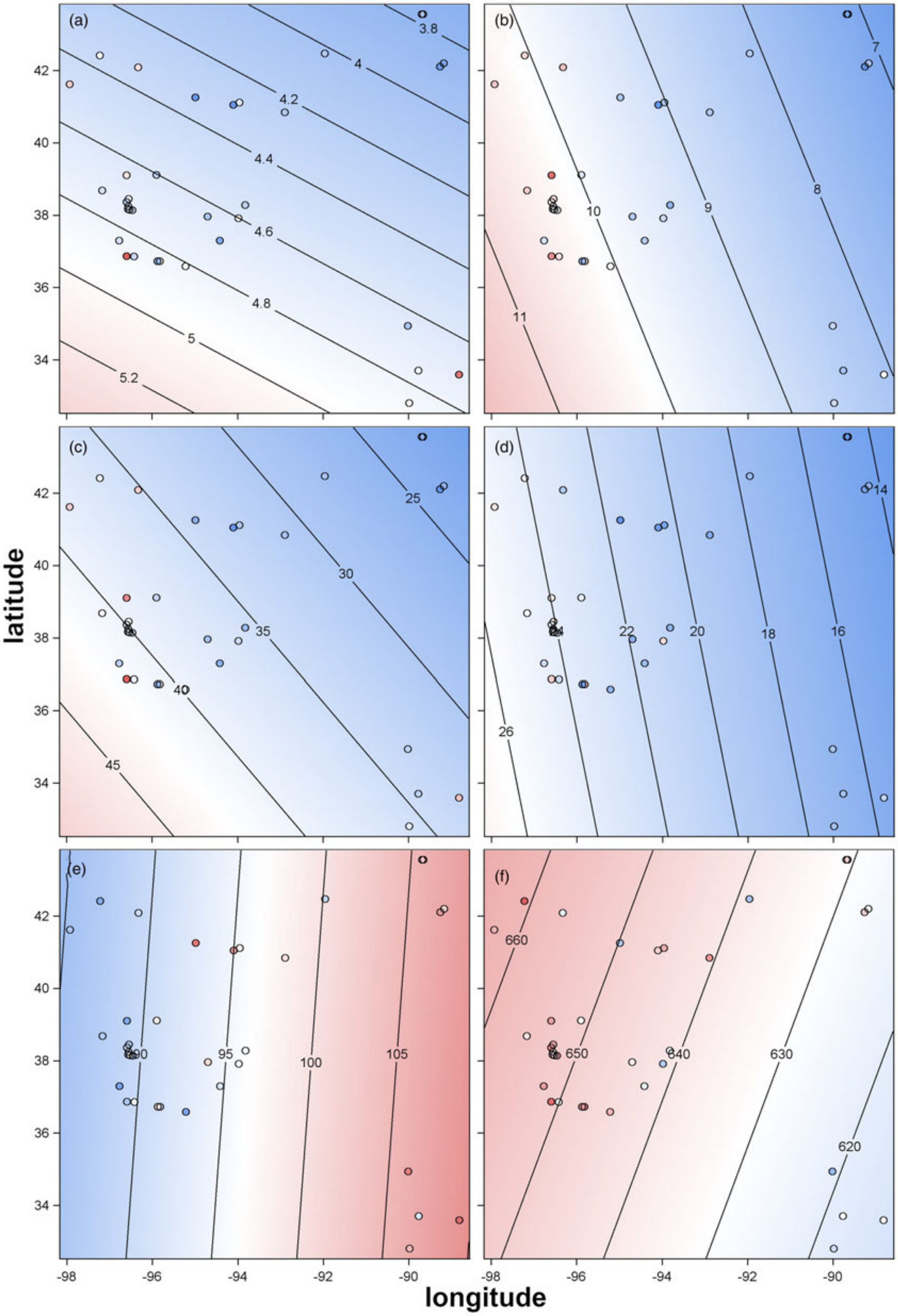

Fig. 4. Contour plots showing the trends of seed traits over the latitude/longitude gradient of the collection site. Small circles denote an accession data point based on latitude/longitude coordinates, and the colour within each circle indicates the phenotypic value of the accession on the gradient colour scale. (a) Kernel width, (b) kernel length, (c) kernel area, (d) wing area, (e) palmitic acid and (f) linoleic acid.

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Table 1. ANOVA and effect estimate table for regression analyses of seed and oil traits using geographical coordinates of the origin of analysed genotypes as linear covariates

Trait	Source	Estimate	Type III SS	*F* value	Pr > *F*
Kernel width	Long	−0.1	1.72	3.6	ns
	Lat	−0.1	3.76	7.5	<0.01
Kernel length	Long	−0.4	58.21	27.6	<0.001
	Lat	−0.1	6.08	2.9	ns
Kernel area	Long	−1.6	1136.70	11.4	<0.01
	Lat	−1.2	521.10	5.1	<0.05
Wing area	Long	−1.2	637.00	24.8	<0.001
	Lat	−0.2	16.02	0.6	ns
Achene area	Long	−2.8	3475.55	18.5	<0.001
	Lat	−1.4	719.88	3.8	ns
Kernel area to Wing area ratio	Long	0.0	0.10	0.6	ns
	Lat	0.0	0.81	5.4	<0.05
Total seed oil content	Long	−1	494.95	0.8	ns
	Lat	0	3.86	0.0	ns
Myristic acid	Long	0	29.49	0.8	ns
	Lat	0	28.07	0.7	ns
Palmitic acid	Long	2	2702.36	54.5	<0.001
	Lat	0	8.97	0.2	ns
Stearic acid	Long	0	15.06	0.4	ns
	Lat	−1	98.90	2.5	ns
Oleic acid	Long	2	1047.14	1.3	ns
	Lat	0	31.70	0.0	ns
Linoleic acid	Long	−4	7506.04	9.7	<0.01
	Lat	1	659.41	0.9	ns

Long, longitude; Lat, latitude; ns, not significant.

in the southwestern part of the range of *S. integrifolium* (Table 1, Fig. 4). Similar to our findings, Sabzalian *et al.* (2009) showed in wild safflower species, *Carthamus oxyacanthus* Bieb., that agro-morphological traits such as seed size were correlated with the site of collection. It is noteworthy to mention that yearly average precipitation decreases from east to west and temperature increases from north to south, such that the hottest, driest conditions occur in the southwest. These differences among sites may have encouraged natural selection for seeds with increased reserves during the sensitive germination and seedling stages.

Similarly, the oil composition is influenced by longitude, where the palmitic acid content decreases and the linoleic acid content, on average, increases with each degree longitude west. For a domestication goal to generate an oilseed crop for human consumption similar to oilseed sunflower, a seed oil profile with low palmitic acid content should be achieved. Arslan (2007) showed that differences in seed oil content and seed oil composition among safflower cultivars as well as wild safflower relatives can be explained by their geographic origin. He revealed differences in oil composition, ranging from 41 to 79 g/kg total oil for palmitic acid and 72 to 773 g/kg total oil for linoleic acid, are influenced by biogeography. Moreover, it has been shown that palmitic acid is positively correlated to the total seed oil content in safflower (Arslan, 2007). In contrast to safflower, in *S. integrifolium*, total oil content and palmitic acid are negatively correlated, which is in line with correlations of oil content and palmitic acid in sunflower (Petakov *et al.*, 1993). Taxonomically, the *Silphium* genus is more closely related to sunflower than safflower, which may explain such trends.

A surprising result is the presence of myristic acid in meaningful quantities in *Silphium* seed oil. Given the close relationship to sunflower, we expected to see a very similar oil composition to wild-type sunflower, which lacks myristic acid and is rich in palmitic, stearic, oleic and linoleic acids, particularly the latter two (White, 2000). While in both *Helianthus* and *Silphium*, linoleic

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Table 2. Pearson correlations among seed size traits, oil content and oil composition for a wild *Silphium integrifolium* collection

	KW	KL	KA	WA	AA	KA/WA	Oil	14:0	16:0	18:0	18:1
KL	0.70***										
KA	0.90***	0.91***									
WA	0.53***	0.83***	0.70***								
AA	0.83***	0.95***	0.96***	0.86***							
KA/WA	0.59***	0.29*	0.53***	ns	0.29*						
Oil	ns	ns	ns	ns	ns	ns					
14:0	−0.29*	ns	ns	ns	ns	ns	−0.31*				
16:0	−0.35**	−0.55***	−0.44**	−0.54***	−0.51***	ns	−0.37**	0.53***			
18:0	ns	ns	ns	ns	ns	ns	ns	ns	ns		
18:1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
18:2	ns	0.31*	ns	0.28*	ns	ns	ns	ns	−0.38**	ns	−0.88***

KW, kernel width; KL, kernel length; KA, kernel area; WA, wing area; AA, achene area; KA/WA, kernel area to wing area ratio; Oil, total seed oil content; 14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid. Significance thresholds indicated as ns = not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

acid is the dominant fatty acid in wild-type plants, the total saturated fat content of *Silphium* can be nearly twice as high as sunflower, with the majority of the saturated fat as palmitic acid, a fatty acid positively correlated with myristic acid in this study. Increased consumption of myristic, palmitic and stearic acids in the human diet has been shown to increase the risk of cardiovascular disease in long-term dietary studies (Zong *et al.*, 2016). Higher saturated fat content compared to similar vegetable oils reduces the desirability of wild-type *Silphium* oil as a vegetable oil for human consumption, based solely on health virtues, and the oil product appears to be too heterogeneous to fit into other potential uses independent of the health virtues. However, two strategies can provide hope through selective breeding. First, considerable variation exists for fatty acid content among accessions and oil composition is affected by biogeography, indicating that careful parent selection and monitoring of fatty acid content in breeding lines can reduce total saturated fats in favour of linoleic acid, a polyunsaturated fat considered to be a healthy substitute for saturated fatty acids in the human diet (Maki *et al.*, 2018). Second, mutation breeding on favourable parental lines could radically change the fatty acid composition with alterations in single genes. This has been the case in sunflower, where *FAD2-1* was mutated to reduce saturated fats and convert most of the linoleic acid to oleic acid, in an effort to balance oxidative stability of the oil with health characteristics (Schuppert *et al.*, 2006). Similar work has also increased stearic acid content in sunflower to around 180 g/kg, which is used as an identity-preserved palm oil substitute from which a solid or semi-solid fat at room temperature can be produced for certain food applications where solid fats are required (Anushree *et al.*, 2017). Determining an ideal fatty acid composition for a crop is a moving target, but many prominent oilseeds have multiple, identity-preserved oil types suited to different end markets, usually optimizing attributes such as price, healthiness, oxidative stability, flavour and whether it is solid or liquid at room temperature, depending on end use. In the current vegetable oil market, a heterogeneous oil such as wild-type *Silphium* will need to be directed through breeding into one or more desirable profiles, with the most obvious one to increase linoleic acid for a household cooking or commercial food preparation oil. Conversely, increasing palmitic or stearic acid may also be useful for developing a solid fat alternative to palm oil.

An interesting result is the correlation between seed size traits (KW, KL, KA, WA, AA) and seed oil traits (16:0, 18:2). The present study revealed negative correlations of palmitic acid to KW, KL, KA, WA, AA and linoleic acid. Genotypes collected in more southwesterly regions possess a lower palmitic acid content but a higher linoleic acid content as well as increased KW, KL, KA, WA, AA and KA/WA. Similar to oilseed sunflower, higher yield, higher kernel to wing ratio, lower palmitic and higher linoleic acid will likely be the preferred direction of improvement in *Silphium*. To obtain resources for such a breeding goal, we should focus on genotypes from southwestern regions. But focusing on southwestern genotypes solely could generate genetic bottlenecks due to limited diversity in small populations. These bottlenecks will increase the proportion of deleterious variants, negatively impacting plant fitness (Makino *et al.*, 2018). More importantly, we could lose genetic variation for other traits of agronomic importance by eliminating northeasterly accessions from breeding programmes, such as the biotic stress resistance that has been seen in such accessions. Acknowledging the

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strengths and weaknesses of potential parent stocks will be the key to developing robust breeding programmes that adequately balance necessary traits.

We identified the phenotypic variation of seed size and seed oil traits in wild *S. integrifolium*. The variation among accessions is affected by geographical clines, with the east-west cline producing the most important variation for oilseed suitability. This study is the first assessment of seed size and seed oil variation in the domestication candidate *S. integrifolium* and is a starting point to understanding how variation in the wild accessions can be best utilized for domestication. Future analyses of flowering time and disease resistance, for example, are needed to obtain sufficient information to guide future plant collection and improvement objectives.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262119000248

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