Title: Domestication has altered within-plant trait variability in a crop plant

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Abstract:

For over 10,000 years humans have shaped plant traits through domestication. Studies of domestication have focused on changes to trait averages; however, plants also have characteristic levels of trait variability among their repeated parts, which can be heritable and mediate critical ecological interactions. Here, we ask how domestication selection has altered among-leaf trait variability using alfalfa (*Medicago sativa*), the oldest forage crop in the world. We found that domestication changed variability more than averages for multiple traits. Relative to wild progenitors, domesticates had elevated variability in specific leaf area, trichomes, C:N, and phytochemical concentrations and reduced variability in phytochemical composition among their leaves. Our work shows that within-plant trait variability is a novel facet of the domesticated plant phenotype, constituting a novel frontier of trait diversity within crop fields. As many critical biotic interactions occur at the scale of individual plants, our findings suggest that trait variability and diversity among leaves could act to magnify or counter the depauperate trait diversity often found at higher scales in agroecosystems.

Main Text

Humans are a powerful evolutionary force on the plants we grow. Compared to wild relatives, domesticated plants often grow faster or larger, follow altered life histories, and differ in physical, nutritive, and chemical traits (*1*, *2*). Understanding these trait differences is vital to human health and agroecological management (*3*). To date, most studies of plant domestication have focused on shifts in trait means, such as mean nutritive or defensive content of fruits or leaves. However, this obscures a fundamental dimension of the plant phenotype: the level of trait *variability* among plant repeated structures. While sub-individual variability is often dismissed as noise, studies in wild plants suggest it may be a key scale of diversity: it is often greater than variability among individuals and influences critical biotic interactions including herbivory and pollination (*4*–*6*). Moreover, recent work finds that sub-individual variability can be heritable and controlled by loci independent of those shaping trait means (*7*), suggesting that variability *per se* can respond to selection and that our understanding of domestication is incomplete without this dimension of the plant phenotype. Indeed, differences in trait variability between crops and wild plants could represent yet another scale of agroecological biodiversity loss – or constitute a novel frontier of trait diversity that could be used to enhance ecosystem services (*3*).

Domestication selection could increase or decrease trait variability within plants, due to indirect or direct processes. Fixed relationships between trait means and variances, often positive (*4*, *8*), could cause selection on means to indirectly change variability. This phenomenon could make selection for higher average nutrient content lead to an increase in nutrient variability, creating a surprising side effect of crop improvement. Similarly, relationships between the concentration and richness of phytochemicals (*9*) suggest that selection on overall phytochemical production could change phytochemical diversity within and among leaves. Selection on whole-plant traits, such as growth, could indirectly alter the range of traits achieved across tissue ontogeny (*10*) or increase developmental error and trait stochasticity as leaves mature (*11*). Alternatively, variability could be directly under selection; humans may have favored homogeneity within plants to facilitate harvest and marketability (*12*), or selected for trait variability if it

52 deterred agricultural pests (*5*, *6*), promoted less costly patterns of herbivory (*13*), or allowed plants to
53 cope with novel agricultural environments via enhanced phenotypic plasticity (*14*) or adaptive noise (*15*)
54 among tissues.

55 Despite the potential for within-plant trait variability to change with domestication, we lack a basic
56 understanding of the presence or strength of shifts in this trait, as well as its relation to trait means and
57 tissue ontogeny. Such an understanding could advance agricultural sustainability by informing new ways
58 to use trait diversity for management – a key goal in agroecology (*3*) – at a scale of critical but overlooked
59 importance to both costly and beneficial ecological interactions (*16*, *17*). Indeed, fine-scale variability has
60 been shown to influence performance of herbivores (*5*, *6*) and levels of costly damage (*16*), inhibit
61 pathogen spread (*18*) and alter pollination (*4*, *17*). Here, we explore how domestication has shaped levels
62 of within-plant trait variability using alfalfa (*Medicago sativa*), a key forage crop with an 8,000-year
63 domestication history. We quantified the standard deviation of multiple important nutritive, physical, and
64 chemical traits and the beta diversity of phytochemical composition (Table S5) among 9 leaves within 30
65 domestic and 30 wild plants grown in a common garden (N=540 leaves), stratified across leaf ontogeny
66 (Fig. S2). We selected genotypes to represent an extensive range of cultivar domestication histories and
67 populations across the native range (Fig. S1; Table S4). Specifically, we ask (i) How does sub-individual,
68 among-leaf trait variability change with domestication? (ii) Can changes in trait variability be explained
69 by trait means, or are they an independent feature of the domesticated phenotype? (iii) To what degree do
70 changes in trait variability reflect changes in leaf ontogenetic trajectories, versus pervasive shifts within
71 age classes?

72 We found that domestication increased sub-individual variability in physical and chemical traits in alfalfa
73 (Fig. 1b; Table S1). Effect sizes were large; relative to wild plants, the trait variability encompassed by
74 domestic plant leaves across ontogeny was 69.6% greater for specific leaf area (SLA)($\chi^2_{[1,N=60]}$= 23.06,
75 p<.001), 37.3% greater for the concentration of individual saponins ($\chi^2_{[1,2892]}$=8.27, *p*<.01), and 53.8%

76 greater for the summed concentration of all saponins ($\chi^2_{[1,60]}$=9.82, p<.01). In contrast, we observed no
77 differences in among-leaf variability in trichome density, leaf water content, carbon, nitrogen, or C:N
78 ratio between domestic and wild plants at this scale. Chemical diversity also increased with
79 domestication: the richness of saponins encompassed by domestic plant leaves (γ) was 8.1% greater
80 ($\chi^2_{[1,60]}$=5.27, p<.05) than in wild plants. In contrast to γ diversity, variability in multivariate chemical
81 composition among those leaves (β diversity) was 16.2% lower in domestic plants (proportional
82 compound abundances: $\chi^2_{[1,60]}$=5.98, p=.01).

83 These results suggest that domestication has altered the landscape of trait variability within plants, with
84 greater variability in physical and nutritive traits and individual compound concentrations, and a
85 restructuring of chemical diversity from greater among-leaf diversity in wild plants toward greater within-
86 leaf diversity in domestic plants. Such shifts could shape plant productivity and agroecological
87 interactions in multiple ways; for example, among-leaf 'noise' in functional traits could provide a bet-
88 hedging strategy, countering unpredictable abiotic or biotic conditions across leaves (*15*). Greater trait
89 variability could reduce herbivory by increasing uncertainty and risky movement during foraging (*17*),
90 requiring frequent physiological adjustment (*19*), and increasing costly chemical synergies (*19*) and non-
91 linear averaging effects (*5*) – or increase herbivory by expanding opportunity for performance-enhancing
92 dietary mixing (*20*). As many insect herbivores complete development on one or few plant individuals,
93 trait variability at the within-plant scale may be particularly critical in shaping amount and spatial pattern
94 of damage, and associated costs to plant growth (*13*).

95 Next, we asked whether domestication effects on trait variability could be explained by changes in trait
96 means or total compound concentrations. Consistent with the plant domestication literature (*1*), we
97 observed change in the means and total levels of multiple physical, nutritive, and chemical traits (Fig. 1a;
98 Table S2). These mean shifts explained changes in variability for some traits, but for other traits
99 variability changed independently of or opposite to changes in means. Greater variability in individual

saponin compounds and higher compound richness in domestic plants were explained by their 44.4% higher average concentrations and 42.8% greater total production (Fig. 1c; Table S2-3). Proportional saponin composition, in contrast, had 14.0% lower variability in domestic plants even after accounting for the increase in total saponin production ($\chi^2_{[1,60]}$=3.96, $p < .05$). Similarly, SLA, trichomes, and C:N had 32.9%, 45%, and 16.8% higher variability in domestic plants after accounting for an increase, decrease, and no change in means, respectively ($\chi^2_{[1,60]}$=12.16, p<.001; t_{55}=-2.81, p<.01, $\chi^2_{[1,60]}$=3.81, p=.05; t_{38}=-2.21, p<.05; Table S2-3).

The variety of relationships we found between changes in trait means and variability suggest domestication can shape within-plant variability via multiple pathways and processes. When domestication has altered trait means or overall chemical production, our results suggest that we should look for corresponding shifts in trait variability and chemical diversity. However, for some traits these two properties may be de-linked, suggesting that domestication selection and plant breeding could potentially manipulate within-plant variability as a feature independent of trait means. These results parallel recent studies finding both independent and shared genetic control of trait means and variabilities within individuals (*7*). Considering these two properties as synergistic attributes of the plant phenotype is also of critical ecological relevance because the importance of trait means or variability to consumers likely depends on the magnitude of the other (*21*). Thus, understanding among-leaf distributions in traits and chemical similarity, in addition to their means and overall compound production has critical implications for plant breeders balancing crop improvement with vulnerability to pest attack.

To understand how shifts in trait variability are related to leaf ontogeny, we quantified variability among leaves in early, middle, and later stages of expansion (Fig. S2). We found that effects of domestication on variability were of similar or greater magnitude within leaf age classes as across leaf ontogeny: variability in SLA and LWC increased 18.8% and 23.9% among leaves within each age class ($\chi^2_{[1,180]}$=5.25, p<.05; $\chi^2_{[1,180]}$=7.28, p<.01), after accounting for changes in means with domestication (Fig. 2, Table S3). The

effect of domestication on variability in total saponin concentrations differed with leaf age class, reaching a 106.7% increase for young leaves of domestic compared to wild plants (t_{171}=-3.33, p=.001; Table S1) – larger than the 78.1% increase in the corresponding mean (t_{171}=-3.67,p<.001; Table S2). α chemical richness and Simpson's diversity were 8.8% higher and 4.0% lower, respectively, for young, middle, and older leaves of domestic plants, with effects associated with greater compound production in parallel to results among all leaves (Table S1&S3). β diversity in proportional compound concentrations declined by 20.4% with domestication in each leaf age class ($\chi^2_{[1,180]}$=8.04, p<.01) – an effect size of greater magnitude than that quantified across leaf ontogeny (Table S3). For other traits, domestication effects on among-leaf variability occurred within one age class but not others (C:N; Fig. 2c, Table S3). These results suggest that shifts in variability are not simply due to greater trait maxima or lower trait minima reached across leaf ontogeny in domesticated plants; rather, altered trait variability is a ubiquitous feature of domestic plant leaves, with more pronounced domestication effects at some stages of leaf development than others. Herbivores that specialize within leaf age class may experience different domestication effects, and tissue-age generalists may find leaf age or nodal position to be a noisier quality cue.

Our findings suggest that, over thousands of years of domestication, humans have not only been altering crop leaves on average but also shaping the magnitude of trait variability around these means. This finding, plus our growing appreciation for the ecological and evolutionary importance of variability *per se*, indicates that variability may be a key component missing from our understanding of domestication selection and agroecology. Indeed, modern agroecosystems have artificially low trait diversity at the landscape, community, and population scale, with cascading effects on plant damage, yield, and agroecosystem function (*3*). Our work reveals a previously overlooked scale at which trait diversity differs between agricultural and natural systems: among leaves within plants. At the same time, the result that domestication can increase within-plant variability for some traits, and in various association with trait means suggests that this novel frontier of diversity could, through cultivar selection or plant breeding, be harnessed to mitigate low diversity at other scales and enhance sustainable pest management.

149 **Materials and Methods**

150 *Selection of wild plant populations and cultivars.* Alfalfa is one of the most important agricultural plants,
151 grown across the globe for both animal forage and human consumption. It also boasts one of the longest
152 and most complex domestication histories of any forage crop, having been cultivated by humans for over
153 8,000 years (*22*) from a species complex with few barriers to gene flow (*23*, *24*). Studies of alfalfa
154 cultivars trace their domestication history to three wild subspecies (*M. sativa* ssp. *falcata*, *M. sativa* ssp.
155 *caerulea* and their naturally-occurring hybrid, *M. sativa* ssp. *hemicycla*; diploid), with a potential role for
156 several tetraploid subspecies (e.g. *M. sativa* ssp. *varia; M. sativa* ssp. *sativa*) that may have arisen in the
157 wild or through hybridization with cultivars (*25*). We used this information to acquire as broad an array of
158 wild progenitor genotypes as possible, from across the native range of Vavilov's "Near Eastern" and
159 "Central Asiatic" centers of crop origin (*26*). We focused on the three diploid subspecies (ssp. *falcata*,
160 ssp. *caerulea*, and ssp. *hemicycla*), as the origin of tetraploid subspecies is less clear and may be linked to
161 ongoing gene flow with cultivars or, if derived from evolution in nature, are also hypothesized to have
162 arisen from among those diploid progenitors (*24*, *25*, *27*) (Fig S1; Table S4). We then selected 30
163 domestic cultivars encompassing a range of domestication histories, both pre-and post-arrival to the
164 United States, using information about original agricultural introductions of the plant (*26*) (Fig S1; Table
165 S4). The goal of this broad sampling was to represent a comprehensive array of phenotypes among wild
166 progenitors as well as among cultivars, given the long history of alfalfa domestication, hybridization, and
167 cultivar development.

168

169 *Common garden and trait collection.* In late May of 2018 we imbibed and germinated 15-20 alfalfa
170 seeds/population (or cultivar) on filter paper, following scarification (100-grit sandpaper) and a bleach
171 soak (3% solution for 10min). Seedlings were established in cell trays of standard peat mix in the
172 greenhouse. After 15 days, we transplanted N = 240 healthy plants (N = 4/population) into individual pots
173 containing a mix of peat mix and field soil (50%-50%), to acclimate them to field soil conditions. In early
174 July, after ca. 30 days of growth in the greenhouse, we outplanted one individual per population/cultivar

175 (N = 60) into a common garden at Kellogg Biological Station (Hickory Corners, MI). Plants were
176 arranged in a stratified design, alternating positions between wild and domestic populations. Within this
177 'checkerboard' layout, populations or cultivars were randomly assigned to each position. We placed a
178 parallel set of plants (N = 60) into screenhouses next to the common garden, to experience similar abiotic
179 conditions as field plants, but protected from herbivores to avoid differential induction between wild and
180 domestic plants.
181
182 We collected leaf tissue for physical (specific leaf area, trichome density) and nutritive (leaf water
183 content, carbon and nitrogen content) traits in August of 2018 from plants in the common garden. To do
184 so, we haphazardly selected 3 vegetative stems per plant individual, placing them immediately into water
185 tubes. In the lab, we used the branching architecture of the alfalfa plants to select three leaves, in each of
186 three relative age classes. Specifically, for each of three peduncles (lateral, vegetative branches) we
187 selected an expanding leaf at the tip ("young"), an expanded leaf in the middle of the stem ("middle"),
188 and the basal leaf, where the peduncle joins the main stem ("old") for a total of 9 individual leaves per
189 plant (See Figure 1). We avoided leaves with evidence of physical damage, pathogens or herbivory.
190 Following removal, each leaf was immediately weighed using an analytical balance (Mettler Toledo
191 MS104TS), scanned, photographed under magnification, and dried in a coin envelope. We used ImageJ
192 image analysis software to quantify leaf area and trichome density (*28*). Specific Leaf Area (SLA) was
193 calculated following the formula $SLA = \frac{leaf\ area_{(fresh)}}{leaf\ mass_{(dry)}}$. We quantified trichome density using a transect
194 method, counting the number of intersections per mm on a line perpendicular to the midrib, using the
195 middle leaflet of each trifoliate leaf. Leaf water content was quantified as $LWC =$
196 $\frac{leaf\ mass_{(fresh)} - leaf\ mass_{(dry)}}{leaf\ mass_{(fresh)}} \times 100$. Elemental analysis of nitrogen and carbon content was assessed by
197 flash combustion by the University of Georgia Stable Isotope Ecology Laboratory. To prepare samples
198 for elemental analyses, we manually pulverized each leaf using a razor blade and placed 0.5-3.0mg of
199 homogenized tissue into aluminum capsules. Manual pulverization, rather than use of a bead mill, was

200 required due to the small size of leaves. At the time of leaf collection, we also measured plant height and
201 width for a measure of plant size (volume).
202
203 We collected leaves for chemical analysis in June of 2019 from plants maintained in screen cages to
204 exclude herbivores and avoid differential induction of chemical defenses. Leaves were removed directly
205 from plants, using the method described above to stratify among age classes (N = 9 leaves/plant, across 3
206 relative age classes), and placed in individual coin envelopes. We also collected a second set of 9 leaves
207 as a single aggregate/bulked sample per-plant, to serve as additional references for compound
208 identification. We focused on identification of saponin compounds, due to their known resistance function
209 against herbivores (*29*, *30*). All samples were then dried in a drying oven (no heat; air circulation only). In
210 the lab, leaves were weighed, homogenized using a bead mill (60sec), and extracted individually using
211 EtOH solvent containing a 100 nm digitoxin as the internal standard. For the set of bulked leaves, all
212 leaves were homogenized together. Vials were agitated at room temperature (23C) for 10min to ensure
213 contact between solvent and tissue in a ThermoMix (23C). Because leaf masses varied by orders of
214 magnitude, we adjusted the solvent volume in proportion to the tissue mass (1mg : 300uL solvent), rather
215 than choosing a single (by necessity, very low) mass to add across all vials. If leaves were > 7mg, they
216 were homogenized with a razor blade and then subsampled to < 7mg, to remain within the capacity of the
217 extraction vial. Following extraction, vials were centrifuged (10 min @ 13000 rcf) and 100 uL of
218 supernatant was transferred to an LC-MS vial containing a small-volume insert. As an additional
219 "overall" reference, a pooled sample was prepared by adding an equal volume of all extracts to one vial,
220 to be used for quality control (monitoring retention time stability and signal intensity during runs) and for
221 compound identification purposes. We also prepared solvent blanks containing 100nM EtOH and
222 digitoxin solution.
223
224 *LCMS analysis.* Ethanolic leaf extracts were analyzed by LC/MS using a Thermo Q-Exactive mass
225 spectrometer interfaced with a Thermo Vanquish UPLC system. 10 uL of sample was injected onto a

226 Waters Acquity BEH-C18 UPLC column (2.1 x 100 mm) maintained at 40C. Compounds were separated
227 using a gradient as follows: initial conditions were 95% solvent A (10 mM ammonium formate, pH 3.0;
228 adjusted with formic acid) and 5% solvent B (acetonitrile containing 0.1% formic acid) and held until 0.5
229 min, ramp to 50% B at 2 min, ramp to 99% B at 7 min and then hold at 99% B until 8 min, return to 5% B
230 at 8.01 min and hold at the initial conditions until 10 min. Ionization conditions were as follows:
231 electrospray ionization in positive ion mode, capillary voltage was 3.5 kV, capillary temperature 256C,
232 sheath gas 47.5, auxiliary gas 11.25, probe heater temperature was 412C, S-lens RF level was 50. Data
233 were acquired using a FullMS method for quantitative analysis and a data-dependent MS/MS (DDA)
234 method for generating MS/MS spectra for compound identification. The FullMS method used a scan
235 range of m/z 120-1800, resolution setting of 70,000, AGC target of 3e6 and max ion time of 200 ms. The
236 DDA method consisted of a survey scan from m/z 120-1800 at 35,000 resolution, AGC target 3e6 and
237 max ion time of 100 ms; the top 5 ions were selected for MS/MS with an isolation width of 1.2 Da,
238 resolution at 17,500, AGC target of 1e5, max ion time of 50 ms, and dynamic exclusion setting of 3
239 seconds. Solvent blank and pooled samples were run every 22 samples using the FullMS method. In
240 addition the pooled sample and multi-leaf samples were run using the DDA method.
241
242 *Peak processing.* Non-targeted data analysis was performed using Progenesis QI software (Nonlinear
243 Dynamics). Peaks were aligned across all samples but peak picking and deconvolution was done using
244 only the data from ethanol blank samples, multi-leaf samples, and pooled sample to reduce processing
245 time. An absolute ion intensity threshold of 10,000 and minimum chromatographic peak width of 0.1
246 minutes was set and peaks with retention time after 7.5 minutes were excluded from the analysis.
247 Triterpene glycoside saponins are a major class of compounds in alfalfa and were identified based on
248 accurate mass and MS/MS spectra present in the DDA data. Due to the large number of samples, the LC
249 separation method was limited to 10 minutes to reduce instrument time and cost; however, this resulted in
250 some poorly-resolved saponin peaks that Progenesis was unable to pick correctly. To generate accurate
251 peak areas for these compounds, a second targeted analysis method was performed using Quanlynx in the

Masslynx software (Waters). Raw LC-MS data for identified compounds will be made available through MetaboLights.

Normalization and filtering of peak areas. To reduce prevalence of false positives in the data, we converted all raw peak areas below 10000ppm to zero, as this is near the instrumental detection limit. We then normalized compound peak areas to the peak intensities of digitoxin in each sample. Lastly, to reduce chemical noise in the data, we subtracted the mean peak area found in blanks (N = 29 blanks) from their peak areas quantified in each sample.

Statistical analysis. For each trait, we used three model structures to explore how variability shifts with domestication, and the degree to which shifts occur jointly with or independently from changes in trait means. Specifically, we asked 1) How does trait variability shift with domestication ($\sigma_\chi \sim D$); 2) How do trait means shift with domestication ($\bar{\chi} \sim D$); and 3) Do shifts in variability occur independently of, or jointly with, shifts in trait magnitude ($\sigma_\chi \sim D + \bar{\chi}$). By comparing domestication effects across these three models, we can quantify overall shifts in both attributes of the plant phenotype, as well as the degree to which these trait outcomes occur independently or jointly.

For physical traits (SLA, trichome density), nutritive traits (LWC, C and N content, C:N ratio), and individual saponin compounds we used the standard deviation and mean trait values among leaves. For analyses of variability across ontogeny, we excluded N = 2 compounds that were not found across all leaf stages, as variability values would fail to represent comparable ranges of leaf ontogeny. For chemical diversity, we quantified richness, Shannon's, and Simpson's diversity of all saponins (N = 86) (function *diversity*, R package "vegan", version 2.5-6) (*31*) within individual leaves to represent alpha chemical diversity (α); pooled across all leaves as a measure of gamma diversity (γ); and Bray-Curtis dissimilarity in presence/absence, proportional abundance, and raw abundance among leaves (functions *vegdist* and

betadisper, R package "vegan", version 2.5-6) (*31*) to quantify beta diversity (β). For α, *γ,* and β chemical diversity, we used the peak area at the corresponding scale to represent total compound production, and parallel use of the mean for other traits (summed concentration per leaf for α diversity; average across all leaves for *γ* diversity, and average per-leaf concentration among all leaves or within leaf age class for β diversity at the whole-plant and within age-class scales, respectively). In this way, we can ask how multivariate aspects of chemical diversity are related to the overall quantity of compounds produced by plant leaves.

All metrics of physical, nutritive, and chemical variability and means were calculated at the whole-plant scale (across N = 9 leaves of all ages) as well as within young, middle, or older-aged leaves per plant (N = 3 leaves/stage). Together, these ten measures describe multiple dimensions of within-plant trait variability, generated by differences in trait amount as well as in trait identity among and within leaves of differing ontogenies.

We used generalized linear mixed models (GLMMS) to test for differences in trait means and within-plant variability between wild and domestic plants. All models were implemented using the function *glmmTMB* (R package "glmmTMB", version 1.0.0) (*32*) in the statistical computing environment *R* (version 3.6.0) (*33*). For all models, we used domestication status (wild, domestic) as a main effect to predict each response variable at two scales: among all leaves (N = 9 leaves/plant) and within each leaf age class (N = 3 leaves/plant/class). For analyses within leaf age class, we used age (young, middle, old) as an additional main effect, and tested for interactions with domestication status. We also used plant individual as a random effect in these models, as each plant yielded three trait measures (one per age class). In all models, we used plant size (volume; estimated as height X width) as a covariate. For analyses of individual compound variability (SD) among leaves, we included compound identity as a random effect, and allowed the relationship between mean and SD to vary within each compound as well within each plant individual (random slopes). For analyses of compound diversity (richness, Shannon), we

allowed the relationship between diversity and total peak area to vary within plant individual (random slopes). For each analysis, we performed model selection using AIC (function *dredge*; R package "muMIn", version 1.43.6 and function *AICctab*; R package "bbmle", version 1.0.20) (*34*, *35*).

To compare domestication effects across traits with such a diverse array of measurement units (e.g. from trichome densities to Bray-Curtis distances), we converted all effect sizes to percent change in response variables (means or variability) with domestication. For each trait and model structure, we obtained 95% CIs for the effect of domestication using the function *bootMer* (R package "lme4"; nsim=500) (*36*). We then calculated predicted values for wild plants using the *predict* function (*33*) implemented via the *ggpredict* function (R package "ggeffects", version 0.13.0) (*37*), and calculated the corresponding percent changes associated with beta and its 95% CI. Thus, positive % change values represent an increase with domestication, and negative values represent a decline with domestication. We obtained p-values for domestication effects using the *Anova* function (R package "car", version 3.0-6) (*38*). For post-hoc contrasts (e.g. effects of domestication within leaf age class), we conducted t-tests for using the function *pairs* (R package "emmeans", version 1.4.1) (*39*). Data visualization was performed using ggplot2 (version 3.2.0) (*40*).

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Figures:

Fig. 1. Effects of domestication on within-plant trait variability, and the relationship with trait means. Y-axes show model-estimated domestication effects (% change) and 95% CIs. Righthand y-axis is for chemical diversity traits. Asterisks show significance at the <.05, <.01, and <.001 levels. Top right: schematic of sampling design within each plant and metrics of variability.

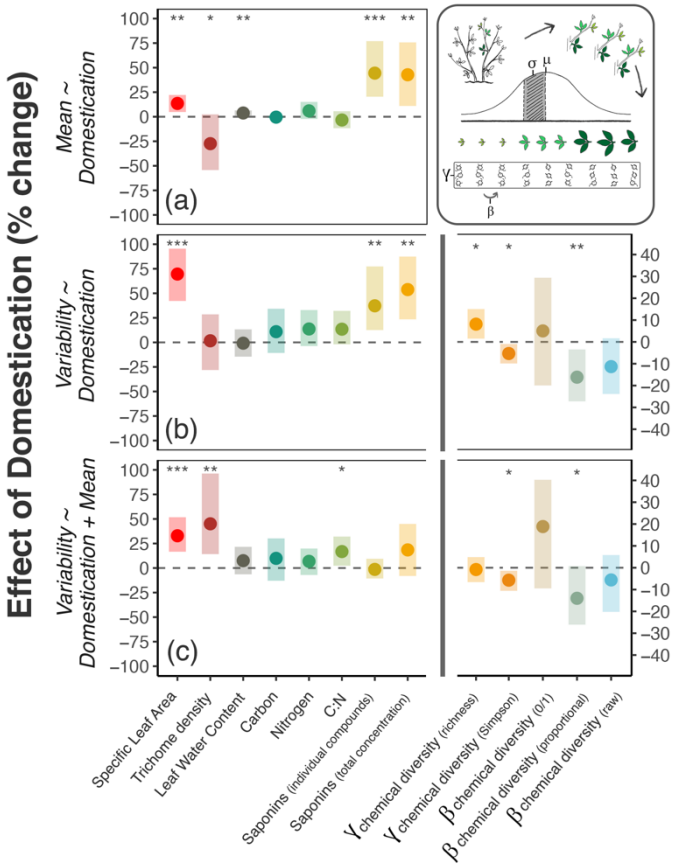
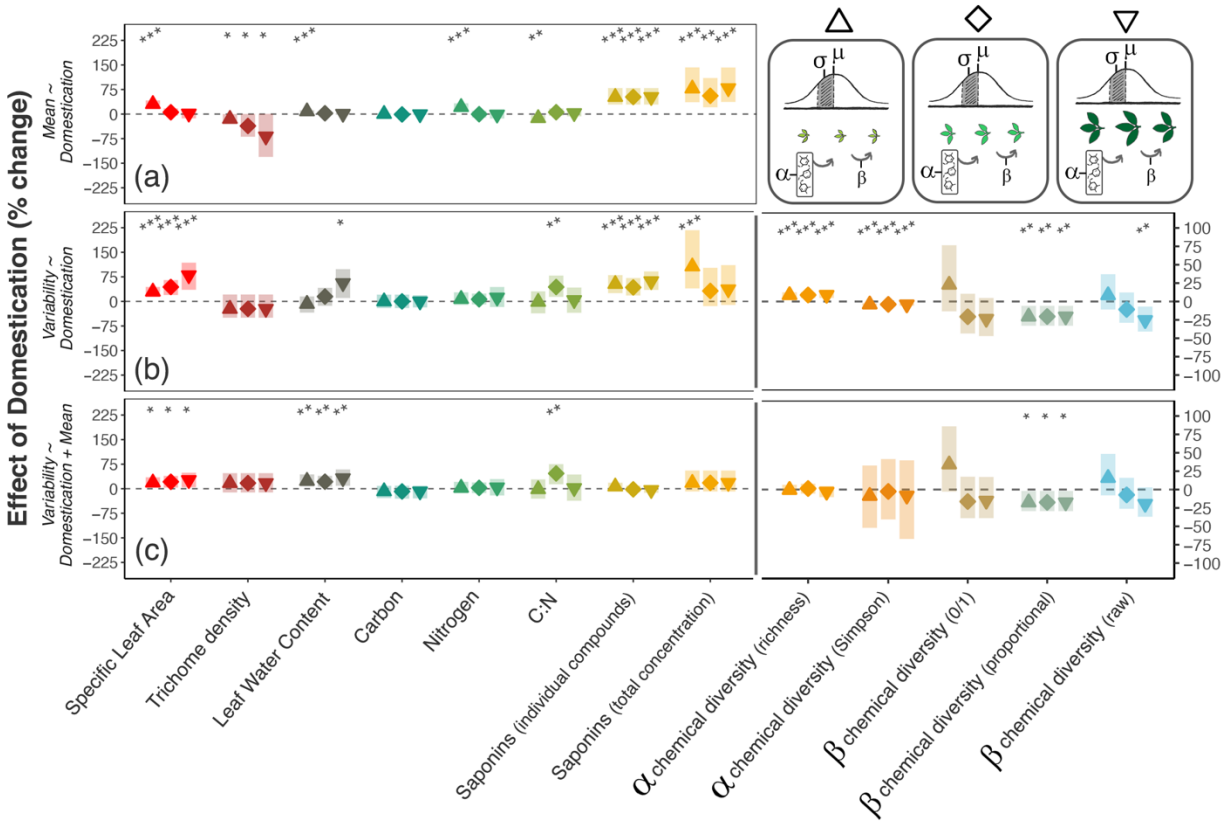


Fig. 2. Shifts in trait variability across leaf ontogeny. Points indicate leaf age class: young/expanding (△), expanded (◇), and older/expanded (▽). See Fig. 1 for definitions.



Supplementary information for

Domestication has altered within-plant trait variability in a crop plant

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This Supplement includes:

Figure S1-S4

Tables S1-S5

Supplemental Figures:

Figure S1. Schematic of *Medicago sativa* domestication, used to select wild and domestic plants. We used our historical understanding of alfalfa's domestication history (*25*, *26*) to select wild plants representing a broad geographic extent and population/subspecies structure, and domestic cultivars to represent an array of domestication histories among those subspecies. Wild populations were selected to include three wild progenitor subspecies (*Medicago sativa* ssp. *caerulea*, ssp. *hemicycla*, and ssp. *falcata*) across a broad geographic extent of the native range. Commercial cultivars were selected to encompass different breeding histories within and among the wild subspecies, as well as various introductions of alfalfa germplasm into the United States. Table S4 provides geographic information for wild populations and backgrounds of cultivars used in this study, selected from the USDA GRIN repository.

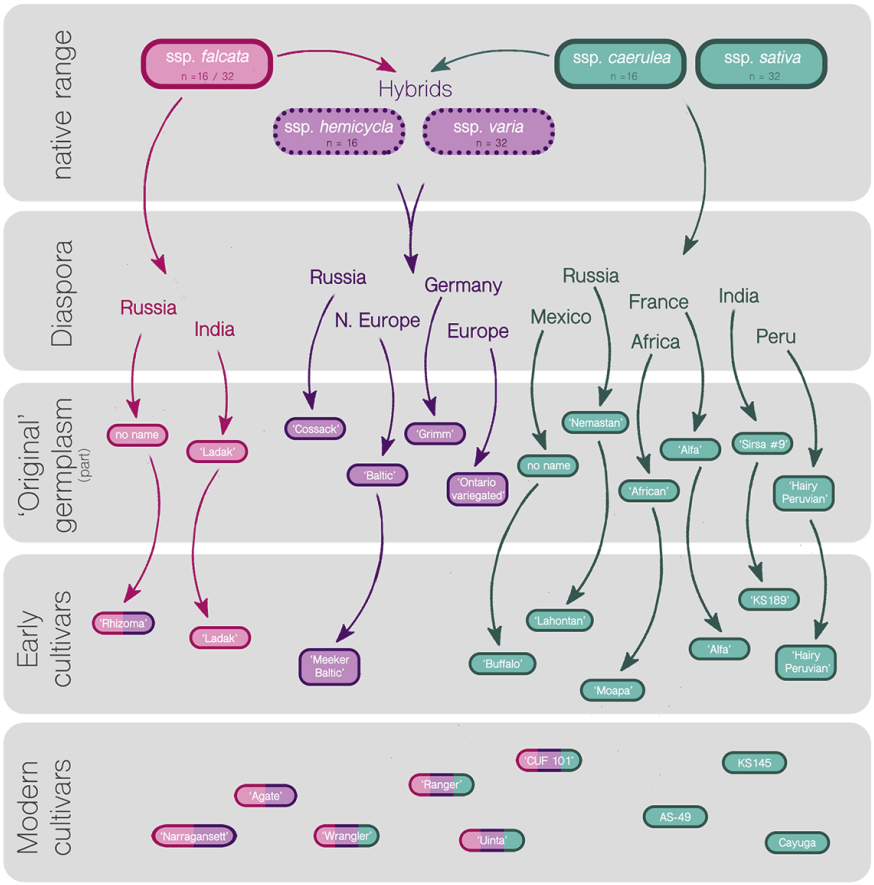


Figure S2. Cartoon showing experimental and sampling design. We grew a single individual plant from each wild population or cultivar (N = 60 total plants), as our goal was to estimate levels of within-plant variability across as great a breadth of wild progenitor and domestic phenotypes as possible, rather than characterize this trait for any particular wild population or cultivar, and in a similar design to other work (*41*). Leaf sampling leveraged the modular structure of alfalfa plants; the multiple main stems produce lateral stems called 'peduncles', each of which is characterized by multiple leaves at various stages of expansion, from a single older basal leaf that defines the base of the peduncle to young expanding leaves at the apex. We used this organization to select leaves at differing stages of expansion: for each plant we selected three peduncles, and from each peduncle we collected the expanding leaf at the apical tip of the peduncle (early in expansion but with all three trifoliate leaflets visible); the leaf at the middle node, and the basal leaf (largest/fully expanded). We processed each leaf sample individually for physical, nutritive, and chemical traits, and quantified trait means and variability at the whole-plant scale (N=9 leaves/plant) and within leaves in each age class (N = 3 leaves /age class/plant).

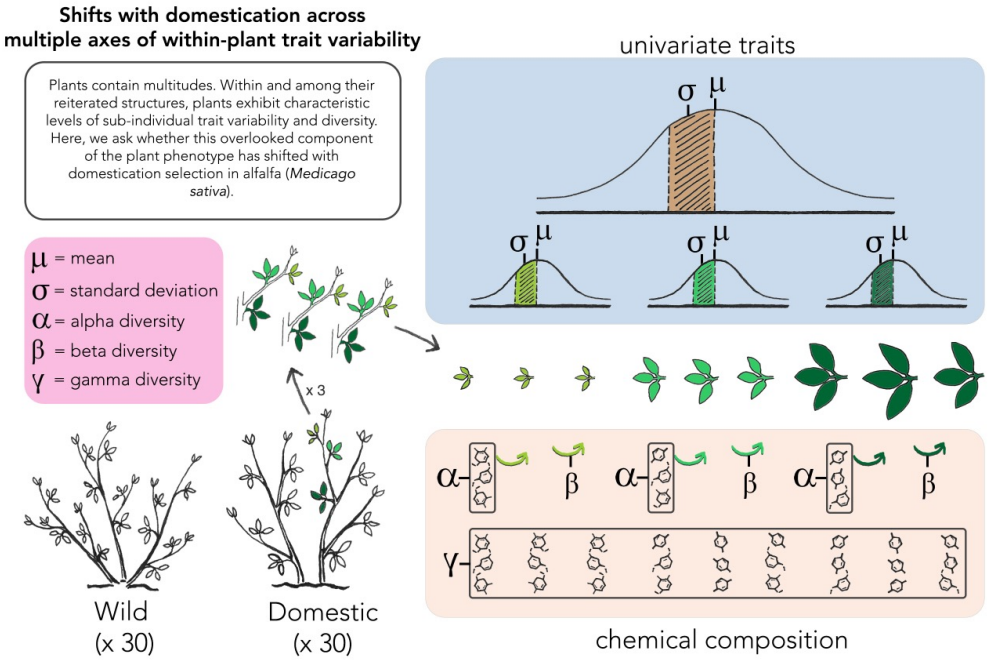


Figure S3. Raw data showing measures of within-plant variability of wild and domestic plants. Variability is quantified as the standard deviation in physical traits (Specific Leaf Area, trichome density), nutritive traits (Leaf Water Content, Carbon, Nitrogen, and C:N ratio), and concentrations of N = 84 saponin compounds (data shown for of 5 random compounds). We quantified chemical richness, Shannon, and Simpson's diversity within single leaves (alpha diversity) and aggregated at the whole-plant scale (gamma diversity). We also quantified variability in terms of dissimilarity in chemical composition among leaves (beta diversity). Plots show levels of variability within leaves of young (up-triangle), middle (diamond) and older (down-triangle) age classes, as well as variability encompassed by leaves of all ontogenetic stages (circles). For chemical diversity, triangle/diamond points show the average alpha diversity of leaves within those age classes, and circles show the average gamma diversity of entire plants. For chemical dissimilarity, triangles/diamonds are the average dispersions within/among leaves of each age class, and circles show the average dispersion among all leaves, across ontogeny.

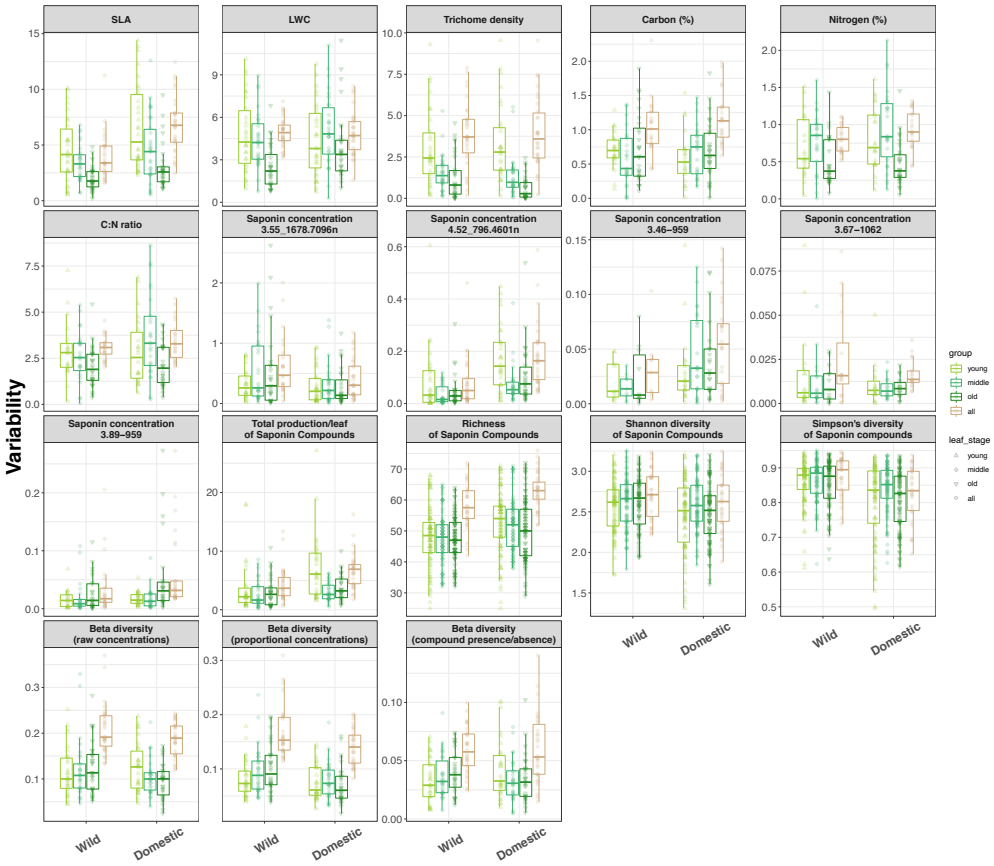
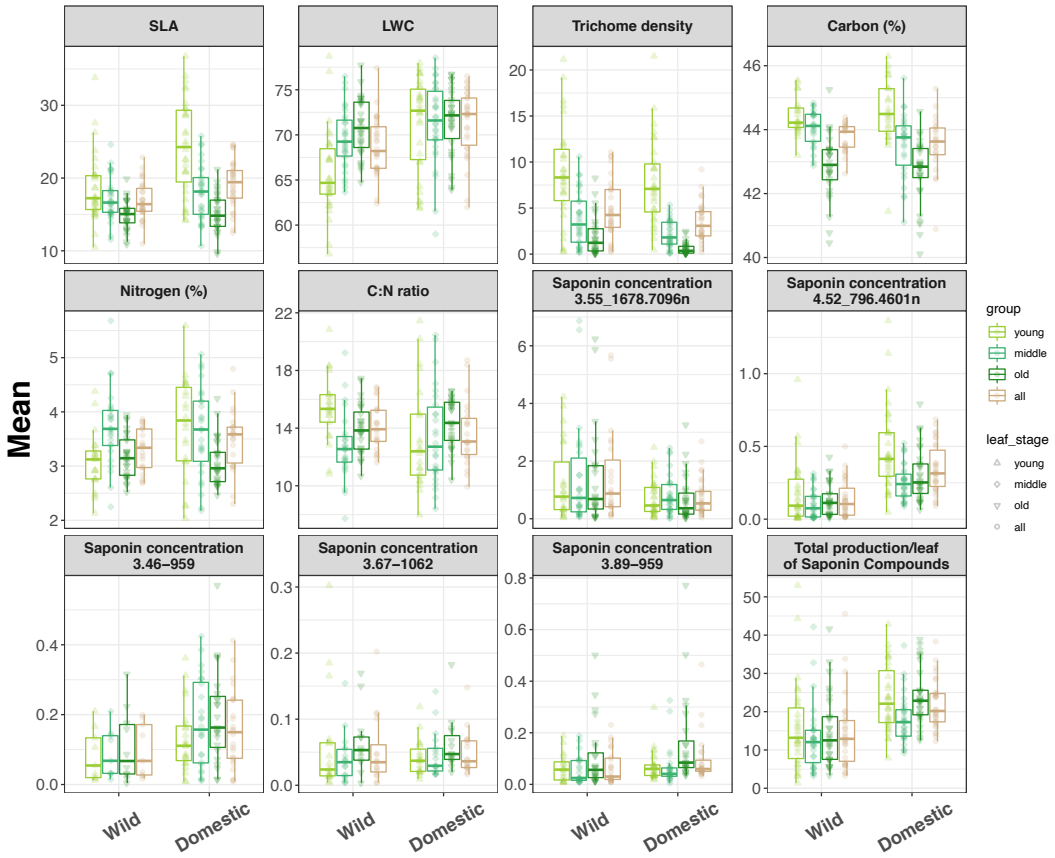


Figure S4. Raw data showing measures of within-plant trait means for wild and domestic plants. Means shown correspond to the metrics of variability plotted above. For Specific Leaf Area, trichome density, Leaf Water Content, Carbon, Nitrogen, C:N ratio, concentrations of single saponin compounds, and total production of saponins, this is the trait mean that directly accompanies the trait standard deviation shown in Fig S3 (trait mean/sd among N = 3 leaves within age class, or across all N = 9 leaves/plant). Means for total compound production correspond to the covariates for compound production used in models of gamma and beta chemical diversity. For chemical richness, Shannon, and Simpson's diversity within single leaves (alpha diversity), covariates in models were the corresponding total compound concentrations per leaf (not shown).



506 **Supplemental Tables:**

507

508 **Table S1. Effects of domestication on sub-individual trait variability in alfalfa.** Models test the
509 hypothesis that levels of within-plant, among-leaf trait variability differ between domestic plants and their
510 wild progenitors. Variability is quantified among all leaves per plant (whole-plant variability) as well as
511 within leaves of different ontogenetic stages (young, middle, older age classes). Response variables are
512 the standard deviation among leaves for physical traits (SLA, Trichome density), nutritive traits (LWC,
513 Carbon, Nitrogen, C:N ratio) and concentrations of saponin compounds (concentration of single
514 compounds & total concentration per leaf); the diversity of saponin compounds within individual leaves
515 (saponin richness, Shannon diversity, and Simpson's diversity); and dissimilarity of saponin chemistry
516 among leaves (beta diversity) in compound identity (presence/absence); proportional concentrations; and
517 raw concentrations. In cases of significant interactions (Domestication × Leaf stage), significance is
518 shown for post-hoc contrasts (t-tests) within leaf age class. Hyphens indicate that effects are consistent for
519 leaves within each age class (no interactions). Significance levels are indicated by asterisks: $p \leq .05$ (*), p
520 $\leq .01$ (**), and $p \leq .001$ (***).

521

Trait / Response	Leaf age class	N	*df* (residual)	β (± 95% CI)	% change (± 95% CI)	Test statistic	p-value	Sig.
SLA	all	60	57	2.8 (1.7, 3.84)	69.6% (42.2%, 95.4%)	$\chi^2 = 23.06$	**< .001**	***
SLA	young	180	174	1.44 (0.64, 2.14)	29.8% (13.3%, 44%)	$\chi^2 = 14.64$	**< .001**	***
SLA	middle	-	-	-	-	-	-	***
SLA	old	-	-	-	-	-	-	***
Trichome density	all	60	57	0.06 (-1.09, 1.1)	1.6% (-28.1%, 28.5%)	$\chi^2 = 0.01$	0.905	
Trichome density	young	180	174	-0.26 (-0.69, 0.19)	-22.7% (-49.6%, 21.2 %)	$\chi^2 = 1.16$	0.282	
Trichome density	middle	-	-	-	-	-	-	
Trichome density	old	-	-	-	-	-	-	
LWC	all	60	57	-0.04 (-0.71, 0.65)	-0.8% (-14.5%, 13.3%)	$\chi^2 = 0.01$	0.903	
LWC	young	180		-0.36 (-1.62, 0.71)	-7.6% (-34.1%, 15%)	$t_{172} = 0.62$	0.534	

LWC	middle	180		0.65 (-0.59, 1.83)	14.8% (-13.3%, 41.4%)	t_{172} = -1.12	0.263	
LWC	old	180		1.4 (0.26, 2.46)	55.8% (10.4%, 98.4%)	t_{172} = -2.41	**0.017**	*
C	all	43	40	0.11 (-0.11, 0.36)	10.9% (-10.7%, 34.3%)	χ^2 = 0.97	0.324	
C	young	151	145	0 (-0.13, 0.13)	0% (-20.2%, 20.6%)	χ^2 = 0.00	0.998	
C	middle	-	-	-	-	-	-	
C	old	-	-	-	-	-	-	
N	all	43	39	0.11 (-0.03, 0.27)	13.6% (-3.7%, 32.9%)	χ^2 = 2.47	0.116	
N	young	151	144	0.05 (-0.08, 0.2)	7.4% (-10.8%, 27.6%)	χ^2 = 0.56	0.456	
N	middle	-	-	-	-	-	-	
N	old	-	-	-	-	-	-	
C:N	all	43	40	0.41 (-0.06, 0.98)	13.4% (-1.9%, 32.1%)	χ^2 = 2.34	0.126	
C:N	young	151		-0.04 (-1.06, 0.89)	-1.5% (-36.4%, 30.6%)	t_{143} = 0.09	0.927	
C:N	middle	151		1.13 (0.36, 2.03)	43.9% (13.9%, 78.8%)	t_{143} = -2.62	**0.01**	*
C:N	old	151		0.08 (-0.7, 0.86)	4.1% (-34.6%, 42.2%)	t_{143} = -0.2	0.839	
Saponins (single compounds)	all	2892	2885	0.32 (0.12, 0.57)	37.3% (12.5%, 77.3%)	χ^2 = 8.27	**0.004**	**
Saponins (single compounds)	young	8939		0.43 (0.22, 0.59)	53.0% (25.2%, 80.3%)	t_{8929} = -4.75	**< .001**	***
Saponins (single compounds)	middle	8939		0.35 (0.17, 0.54)	42.6% (18.1%, 71.2%)	t_{8929} = -3.95	**< .001**	***
Saponins (single compounds)	old	8939		0.48 (0.3, 0.65)	61.7% (35.1%, 91.4%)	t_{8929} = -5.34	**< .001**	***
Saponins (total concentration)	all	60	57	2.35 (0.85, 3.93)	53.8% (19.6%, 90.0%)	χ^2 = 9.82	**0.002**	**
Saponins (total concentration)	young	180		0.73 (0.33, 1.15)	106.7% (39.5%, 217.4%)	t_{171} = -3.33	**0.001**	**
Saponins (total concentration)	middle	180		0.28 (-0.15, 0.70)	31.9% (-14.2%, 102.4%)	t_{171} = -1.27	0.206	
Saponins (total concentration)	old	180		0.31 (-0.12, 0.74)	36.9% (-11.0%, 110.6%)	t_{171} = -1.44	0.151	
Richness	all (γ)	60	54	4.63 (0.56, 8.56)	8.1% (1.5%, 15.0%)	χ^2 = 5.27	**0.022**	*
Richness	young (α)	540	532	4.06 (2.18, 5.86)	8.8% (4.7%, 12.7%)	χ^2 = 20.0	**<.001**	***
Richness	middle (α)	-	-	-	-	-	**-**	
Richness	old (α)	-	-	-	-	-	**-**	
Shannon diversity	all (γ)	60	56	-0.11 (-0.26, 0.11)	-4.2% (-10.9%, 2.9%)	χ^2 = 1.46	0.227	
Shannon diversity	young (α)	540	532	-0.06 (-0.14, 0.01)	-2.6% (-5.7%, 0.3%)	χ^2 = 3.34	**0.07**	
Shannon diversity	middle (α)	-	-	-	-	-	-	

Shannon diversity	old (α)	-	-	-	-	-	-	
Simpson diversity	all (γ)	60	56	-0.05 (-0.09, -0.01)	-5.3% (-9.9%, -1.0%)	$\chi^2 = 5.42$	**0.020**	*
Simpson diversity	young (α)	540	533	-0.03 (-0.05, -0.02)	-4.0% (-5.7%, -2.4%)	$\chi^2 = 22.39$	**< .001**	***
Simpson diversity	middle (α)	-	-	-	-	-	-	
Simpson diversity	old (α)	-	-	-	-	-	-	
Beta diversity (presence/absence)	all	60	57	-0.00 (-0.01, 0.02)	-5.03% (-20.7%, 28.4%)	$\chi^2 = 0.16$	0.69	
Beta diversity (presence/absence)	young	180		0.21 (-0.13, 0.53)	22.9% (-12.2%, 70.4%)	$t_{171} = -1.21$	0.227	
Beta diversity (presence/absence)	middle	180		-0.23 (-0.53, 0.08)	-20.7% (-40.9%, 8.1%)	$t_{171} = 1.37$	0.173	
Beta diversity (presence/absence)	old	180		-0.27 (-0.61, 0.03)	-23.6% (-45.5%, 2.9%)	$t_{171} = 1.59$	0.114	
Beta diversity (proportional)	all	60	56	-0.18 (-0.31, -0.04)	-16.2% (-26.5%, -4.4%)	$\chi^2 = 5.98$	**0.014**	*
Beta diversity (proportional)	young	180	173	-0.23 (-0.39, -0.07)	-20.4% (-32%, -7%)	$\chi^2 = 8.04$	**0.005**	**
Beta diversity (proportional)	middle	-	-	-	-	-	-	
Beta diversity (proportional)	old	-	-	-	-	-	-	
Beta diversity (raw)	all	60	56	-0.02 (-0.05, 0)	-11.3 % (-24.8%, 2.2%)	$\chi^2 = 3.02$	0.082	
Beta diversity (raw)	young	180		0.08 (-0.17, 0.33)	8.6 % (-15.5%, 39.3%)	$t_{171} = -0.69$	0.490	
Beta diversity (raw)	middle	180		-0.11 (-0.34, 0.09)	-10.8 % (-28.9%, 9.8%)	$t_{171} = 0.97$	0.336	
Beta diversity (raw)	old	180		-0.29 (-0.53, -0.05)	-25.4 % (-41%, -5.2%)	$t_{171} = 2.46$	**0.015**	*

522

Table S2. Effects of domestication on among-leaf trait means in alfalfa. Models test the hypothesis that traits means differ between domestic plants and their wild progenitors. Trait means are quantified among all leaves per plant (plant-level average) as well as across leaf ontogeny (within three leaf age classes). Response variables are the among-leaf average for physical traits (SLA, Trichome density), nutritive traits (LWC, Carbon, Nitrogen, C:N ratio) and concentrations of saponin compounds (concentration of single compounds & total concentration per leaf). In cases of significant interactions (Domestication × Leaf stage), significance is shown for post-hoc contrasts (t-tests) within leaf age class. Hyphens indicate that effects are consistent for leaves within each age class (no interactions). Significance levels are indicated by asterisks: $p \leq .05$ (*), $p \leq .01$ (**), and $p \leq .001$ (***).

Trait / Response	Leaf age class	N	*df* (residual)	β (± 95% CI)	% change (± 95% CI)	Test statistic	p-value	Sig.
SLA	all	60	57	2.29 (0.81, 3.74)	13.5% (4.8%, 22.2%)	$\chi^2 = 9.3$	**0.002**	**
SLA	young	180		5.68 (3.73, 7.77)	30.5% (20%, 41.8%)	$t_{172} = -5.5$	**< .001**	***
SLA	middle	180		0.94 (-0.78, 2.96)	5.5% (-4.6%, 17.3%)	$t_{172} = -0.92$	0.361	
SLA	old	180		0.23 (-1.98, 2.32)	1.6% (-13.3%, 15.5%)	$t_{172} = -0.23$	0.821	
Trichome density	all	60	57	-1.33 (-2.63, 0.12)	-27.4% (-54.3%, 2.4%)	$\chi^2 = 3.81$	**0.051**	*
Trichome density	young	180	174	-1.33 (-2.53, 0.01)	-14.9% (-28.4%, 0.2%)	$\chi^2 = 3.82$	**0.051**	*
Trichome density	middle	-	-	-	-	-	-	*
Trichome density	old	-	-	-	-	-	-	*
LWC	all	60	57	2.61 (0.77, 4.43)	3.8% (1.1%, 6.4%)	$\chi^2 = 8.68$	**0.003**	**
LWC	young	180		5.45 (3.52, 7.65)	8.3% (5.4%, 11.6%)	$t_{172} = -5.14$	**< .001**	***
LWC	middle	180		1.78 (-0.24, 4.15)	2.6% (-0.3%, 6%)	$t_{172} = -1.68$	0.095	
LWC	old	180		0.61 (-1.4, 3.09)	0.9% (-2%, 4.4%)	$t_{172} = -0.57$	0.567	
C	all	43	39	-0.18 (-0.62, 0.3)	-0.4% (-1.4%, 0.7%)	$\chi^2 = 0.62$	0.433	
C	young	151		0.18 (-0.31, 0.75)	0.4% (-0.7%, 1.7%)	$t_{143} = -0.7$	0.486	
C	middle	151		-0.47 (-1, 0.01)	-1.1% (-2.3%, 0%)	$t_{143} = 1.88$	0.062	
C	old	151		-0.17 (-0.63, 0.29)	-0.4% (-1.5%, 0.7%)	$t_{143} = 0.71$	0.478	

N	all	43	40	0.2 (-0.07, 0.5)	6% (-2.2%, 15.1%)	$\chi^2 = 1.78$	0.182	
N	young	151		0.66 (0.25, 1.01)	21.4% (8.2%, 32.8%)	$t_{142} = -3.3$	**0.001**	***
N	middle	151		-0.01 (-0.4, 0.37)	-0.4% (-10.8%, 10.1%)	$t_{142} = 0.07$	0.942	
N	old	151		-0.04 (-0.43, 0.32)	-1.4% (-13.6%, 10%)	$t_{142} = 0.24$	0.812	
C:N	all	43	40	-0.47 (-1.64, 0.79)	-3.3% (-11.6%, 5.6%)	$\chi^2 = 0.63$	0.428	
C:N	young	151		-1.9 (-3.39, -0.49)	-12.4% (-22.3%, -3.2%)	$t_{143} = 2.52$	**0.013**	**
C:N	middle	151		0.73 (-0.62, 2.21)	5.7% (-4.9%, 17.4%)	$t_{143} = -1.05$	0.297	
C:N	old	151		0.43 (-0.96, 1.9)	3.1% (-6.9%, 13.7%)	$t_{143} = -0.65$	0.515	
Saponins (single compounds)	all	2892	2885	0.37 (0.19, 0.57)	44.4% (20.5%, 77%)	$\chi^2 = 14.8$	**< .001**	***
Saponins (single compounds)	young	8939	8931	0.42 (0.26, 0.58)	52.2% (29.3%, 79.3%)	$\chi^2 = 26.78$	**< .001**	***
Saponins (single compounds)	middle	-	-	-	-	-	-	***
Saponins (single compounds)	old	-	-	-	-	-	-	***
Saponins (total concentration)	all	60	56	6.42 (1.93, 10.75)	42.8% (12.8%, 71.6%)	$\chi^2 = 7.56$	**0.006**	**
Saponins (total concentration)	young	180		0.58 (0.31, 0.89)	78.1% (36.3%, 142.3%)	$t_{171} = -3.67$	**< .001**	***
Saponins (total concentration)	middle	180		0.44 (0.19, 0.74)	55.8% (21.0%, 110.1%)	$t_{171} = -2.82$	**0.005**	**
Saponins (total concentration)	old	180		0.58 (0.32, 0.88)	79.0% (37.1%, 142.1%)	$t_{171} = -3.71$	**<.001**	***

Table S3. Effects of domestication on within-plant variability, accounting for shifts in associated means or total compound production. Models test the hypothesis that differences within-plant trait variability between domestic plants and their wild progenitors are explained by shifts in associated trait means, or shifts in overall production of secondary metabolites – e.g. the relationship between trait variability (Table S1) and trait means or magnitudes (Table S2). Response variables are the standard deviation among leaves for physical traits (SLA, Trichome density), nutritive traits (LWC, Carbon, Nitrogen, C:N ratio) and concentrations of saponin compounds (concentration of single compounds & total concentration per leaf); the diversity of saponin compounds within individual leaves (saponin richness, Shannon diversity, and Simpson's diversity); and dissimilarity of saponin chemistry among leaves (beta diversity) in compound identity (presence/absence); proportional concentrations; and raw concentrations. For chemical diversity, we used the peak area at the corresponding scale as a covariate to parallel use of the mean for other traits (total concentration per leaf for α diversity; and averaged across all leaves or within leaf age class for γ and β diversity at the whole-plant and within age-class scales, respectively). In cases of significant interactions (Domestication × Leaf stage or Domestication × Mean), significance is shown for post-hoc contrasts (t-tests) within leaf age class and/or holding the mean constant at its average value. Hyphens indicate that effects are consistent for leaves within each age class (no interactions). Significance levels are indicated by asterisks: $p \leq .05$ (*), $p \leq .01$ (**), and $p \leq .001$ (***).

Trait / Response	Leaf age class	N	*df* (residual)	β (± 95% CI)	% change (± 95% CI)	Test statistic	p-value	Sig.
SLA	all	60	56	1.53 (0.77, 2.41)	32.9% (16.6%, 51.7%)	$\chi^2 = 12.16$	**< .001**	***
SLA	young	180	173	0.79 (0.13, 1.46)	18.8% (3.1%, 34.8%)	$\chi^2 = 5.25$	**0.022**	**
SLA	middle	-	-	-	-	-	-	**
SLA	old	-	-	-	-	-	-	**
Trichome density	all	60	-	0.37 (0.13, 0.67)	45.0 % (14.2%, 96.2%)	$t_{55} = -2.81$	**0.007**	**
Trichome density	young	180	173	0.16 (-0.12, 0.39)	17.2% (-11.3%, 47.4%)	$\chi^2 = 1.94$	0.163	

Trichome density	middle	-	-	-	-	-	-	
Trichome density	old	-	-	-	-	-	-	
LWC	all	60	56	0.35 (-0.3, 1.02)	7.5% (-6.4%, 21.6%)	$\chi^2 = 1.19$	0.274	
LWC	young	180	173	0.93 (0.26, 1.7)	23.9% (6.8%, 43.8%)	$\chi^2 = 7.28$	**0.007**	**
LWC	middle	-	-	-	-	-	-	**
LWC	old	-	-	-	-	-	-	**
C	all	43	39	0.1 (-0.14, 0.32)	9.9% (-12.9%, 30.2%)	$\chi^2 = 0.79$	0.374	
C	young	151	143	-0.06 (-0.2, 0.06)	-7.8% (-28.5%, 8.7%)	$\chi^2 = 0.61$	0.435	
C	middle	-	-	-	-	-	-	
C	old	-	-	-	-	-	-	
N	all	43	38	0.06 (-0.06, 0.17)	6.8% (-7.1%, 19.8%)	$\chi^2 = 0.92$	0.338	
N	young	151	143	0.02 (-0.11, 0.15)	2.8% (-14.5%, 20.8%)	$\chi^2 = 0.09$	0.764	
N	middle	-	-	-	-	-	-	
N	old	-	-	-	-	-	-	
C:N	all	43		0.51 (0.07, 0.97)	16.8% (2.4%, 32%)	$t_{38} = -2.21$	**0.033**	*
C:N	young	151		-0.05 (-0.91, 0.86)	-1.5% (-30.2%, 28.5%)	$t_{137} = 0.09$	0.925	
C:N	middle	151		1.23 (0.36, 1.98)	46.7% (13.7%, 75%)	$t_{137} = -2.94$	**0.004**	**
C:N	old	151		0.04 (-0.73, 0.87)	2% (-36.5%, 43.3%)	$t_{137} = -0.11$	0.915	
Saponins (single compounds)	all	2892	2880	-0.01 (-0.11, 0.09)	-1.4% (-10.6%, 9.4%)	$\chi^2 = 0.07$	0.786	
Saponins (single compounds)	young	8939		0.06 (-0.04, 0.17)	6.6% (-3.9%, 18.3%)	$t_{8939} = -1.24$	0.217	
Saponins (single compounds)	middle	8939		-0.02 (-0.11, 0.09)	-1.9% (-10.3%, 9.2%)	$t_{8939} = 0.38$	0.707	
Saponins (single compounds)	old	8939		-0.04 (-0.13, 0.07)	-3.9% (-12.3%, 6.8%)	$t_{8939} = 0.76$	0.447	
Saponins (total concentration)	all	60	56	0.94 (-0.3, 2.22)	18.5% (-5.9%, 43.7%)	$\chi^2 = 1.79$	0.181	
Saponins (total concentration)	young	180	172	0.16 (-1.0, 0.48)	17.9% (-8.3%, 55.5%)	$\chi^2 = 1.32$	0.25	
Saponins (total concentration)	middle	-	-	-	-	-	-	
Saponins (total concentration)	old	-	-	-	-	-	-	
Richness	all (γ)	60	53	-0.51 (-4.03, 2.96)	-0.8% (-6.6%, 4.8%	$\chi^2 = 0.09$	0.768	
Richness	young (α)	540		-0.21 (-3.68, 3.26)	-0.4% (-7.4%, 6.5%)	$t_{526} = 0.12$	0.904	
Richness	middle (α)	540		0.70 (-2.79, 4.01)	1.4% (-5.6%, 8.0 %)	$t_{526} = -0.41$	0.685	

Richness	old (α)	540		-1.45 (-5.00, 2.24)	-3.0% (-10.3%, 4.60%)	$t_{526} = 0.80$	0.423	
Shannon diversity	all (γ)	60	55	-0.11 (-0.30, 0.09)	-4.1% (-11.0%, 3.4%)	$\chi^2 = 1.23$	0.268	
Shannon diversity	young (α)	540		-0.09 (-0.24, 0.09)	-3.5% (-9.4%, 3.6%)	$t_{524} = 1.06$	0.290	
Shannon diversity	middle (α)	540		0.06 (-0.09, 0.24)	2.3% (-3.6%, 9.6%)	$t_{524} = -0.71$	0.477	
Shannon diversity	old (α)	540		-0.04 (-0.18, 0.14)	-1.5% (-6.9%, 5.6%)	$t_{524} = 1.06$	0.290	
Simpson diversity	all (γ)	60	53	-0.05 (-0.09, -0.01)	-5.7% (-10.6%, -1.4%)	$\chi^2 = 5.59$	0.018	*
Simpson diversity	young (α)	540		-0.07 (-0.45, 0.28)	-8.6% (-52.0%, 32.7%)	$t_{524} = 0.39$	0.695	
Simpson diversity	middle (α)	540		-0.02 (-0.35, 0.36)	-2.6% (-40.5%, 41.5%)	$t_{524} = 0.12$	0.901	
Simpson diversity	old (α)	540		-0.07 (-0.57, 0.34)	-8.3% (-67.1%, 39.7%)	$t_{524} = 0.32$	0.752	
Beta diversity (presence/absence)	all	60	55	0.01 (-0.00, 0.02)	18% (-7.7%, 45.0%)	$\chi^2 = 2.0$	0.156	
Beta diversity (presence/absence)	young	180		0.29 (0.01, 0.6)	34.2% (1.1%, 82.3%)	$t_{170} = -1.76$	0.080	
Beta diversity (presence/absence)	middle	180		-0.18 (-0.48, 0.14)	-16.3% (-38.1%, 14.8%)	$t_{170} = 1.08$	0.280	
Beta diversity (presence/absence)	old	180		-0.17 (-0.51, 0.16)	-15.2% (-40.1%, 17.5%)	$t_{170} = 0.98$	0.329	
Beta diversity (proportional)	all	60	55	-0.15 (-0.30, -0.01)	-14.0% (-25.6%, 0.96%)	$\chi^2 = 3.96$	**0.047**	*
Beta diversity (proportional)	young	180	172	-0.19 (-0.35, -0.04)	-17.5% (-29.7%, -3.9%)	$\chi^2 = 5.5$	**0.019**	*
Beta diversity (proportional)	middle	-	-	-	-	-	-	*
Beta diversity (proportional)	old	-	-	-	-	-	-	*
Beta diversity (raw)	all	60	55	-0.01 (-0.04, 0.02)	-5.6% (-20.1%, 8.0%)	$\chi^2 = 0.69$	0.405	
Beta diversity (raw)	young	180		0.15 (-0.08, 0.4)	15.8% (-7.9%, 48.5%)	$t_{170} = -1.23$	0.219	
Beta diversity (raw)	middle	180		-0.08 (-0.3, 0.15)	-7.3% (-26.1%, 16%)	$t_{170} = 0.65$	0.520	
Beta diversity (raw)	old	180		-0.22 (-0.46, 0.03)	-19.5% (-37%, 3%)	$t_{170} = 1.81$	0.073	

566 **Table S4.** Plant populations and cultivars used in this study. Wild populations were selected to
567 encompass multiple progenitor subspecies, across each subspecies' geographic range (Fig S2). Domestic
568 cultivars were selected to include a variety of domestication histories, both pre- and post-arrival into the
569 United States (*26*) (Table S5).
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Population / cultivar ID	GRIN Accession ID	Domestication status	wild ssp or cultivar name	Source population*	Estimated % of each wild ssp		
					ssp. *falcata*	ssp. *hemicycla*	ssp. *caerulea*
01_W_FAL	PI 464729	W	ssp. falcata	Turkey	100	0	0
02_W_FAL	PI 577555	W	ssp. falcata	Ukraine	100	0	0
03_W_FAL	PI 634106	W	ssp. falcata	Ukraine	100	0	0
04_W_FAL	PI 631811	W	ssp. falcata	Kazakhstan	100	0	0
05_W_FAL	PI 641544	W	ssp. falcata	Mongolia	100	0	0
06_W_FAL	PI 494662	W	ssp. falcata	Romania	100	0	0
07_W_FAL	PI 577558	W	ssp. falcata	Russia	100	0	0
08_W_FAL	PI 258752	W	ssp. falcata	Russia	100	0	0
09_W_FAL	PI 325387	W	ssp. falcata	Russia	100	0	0
10_W_FAL	PI 631707	W	ssp. falcata	China	100	0	0
11_W_HEM	PI 631814	W	ssp. hemicycla	Russia	0	100	0
12_W_HEM	PI 315460	W	ssp. hemicycla	Russia	0	100	0
13_W_HEM	PI 464727	W	ssp. hemicycla	Turkey	0	100	0
14_W_HEM	PI 464728	W	ssp. hemicycla	Turkey	0	100	0
15_W_HEM	PI 577543	W	ssp. hemicycla	Georgia	0	100	0
16_W_HEM	PI 634176	W	ssp. hemicycla	Kazakhstan	0	100	0
17_W_HEM	PI 634136	W	ssp. hemicycla	Kazakhstan	0	100	0
18_W_HEM	PI 641615	W	ssp. hemicycla	Kazakhstan	0	100	0
19_W_HEM	PI 641619	W	ssp. hemicycla	Kazakhstan	0	100	0
20_W_HEM	PI 641601	W	ssp. hemicycla	Kazakhstan	0	100	0
21_W_CAER	PI 577549	W	ssp. caerulea	Georgia	0	0	100
22_W_CAER	PI 440500	W	ssp. caerulea	Kazakhstan	0	0	100
23_W_CAER	PI 641606	W	ssp. caerulea	Kazakhstan	0	0	100
24_W_CAER	PI 577541	W	ssp. caerulea	Kazakhstan	0	0	100
25_W_CAER	PI 464713	W	ssp. caerulea	Turkey	0	0	100
26_W_CAER	PI 464721	W	ssp. caerulea	Turkey	0	0	100

27_W_CAER	PI 464723	W	ssp. caerulea	Turkey	0	0	100
28_W_CAER	PI 210367	W	ssp. caerulea	Iran	0	0	100
29_W_CAER	PI 314267	W	ssp. caerulea	Uzbekistan	0	0	100
30_W_CAER	PI 505871	W	ssp. caerulea	Former Soviet Union	0	0	100
31_D_RHI	W6 2525	D	Rhizoma	Europe	50	50	0
32_D_LAD	W6 2502	D	Ladak	India	90	5	5
33_D_MEE	PI 672756	D	Meeker Baltic	Europe	0	100	0
34_D_LAH	W6 2516	D	Lahontan	Russia, Middle East	5	5	90
35_D_ALF	W6 2506	D	Alfa	France	0	0	100
36_D_BUF	W6 2498	D	Buffalo	Mexico / South America	0	0	100
37_D_HAI	PI 672744	D	Hairy Peruvian	Peru	0	0	100
38_D_KS189	PI 672752	D	KS189	India	0	0	100
39_D_MOA	W6 2518	D	Moapa	North Africa	0	0	100
40_D_KS145	PI 672750	D	KS145	mix	0	0	100
41_D_UIN	PI 672766	D	Uinta	mix	12	51	37
42_D_AGA	W6 2505	D	Agate	mix	47	40	13
43_D_NAR	W6 2519	D	Narragansett	mix	25	75	0
44_D_WRA	PI 601132	D	Wrangler	mix	10	20	70
45_D_AS49	PI 672732	D	AS-49	mix	0	0	100
46_D_CUF	PI 517240	D	CUF 101	mix	1	2	97
47_D_CAY	W6 2511	D	Cayuga	mix	11	15	74
48_D_SAR	W6 2526	D	Saranac AR	mix	2	7	91
49_D_WIN	PI 608671	D	Winema	mix	33	33	33
50_D_ATL	PI 672733	D	Atlantic	mix	15	50	35
51_D_RAN	PI 612887	D	Ranger	mix	10	45	45
52_D_VER	NSL 4099	D	Vernal	mix	33	50	17
53_D_ZIA	PI 672767	D	Zia 16-2	mix	0	0	100
54_D_TET	PI 672768	D	Teton	mix	50	0	50
55_D_FLO	PI 672743	D	Florida 77	mix	33	33	33
56_D_ARC	W6 2507	D	Arc	mix	6	31	63
57_D_MAR	W6 2517	D	Mark II	mix	25	75	0
58_D_GUA	PI 639220	D	Guardsman II	mix	33	33	33
59_D_CAL	PI 672735	D	Caliente	mix	0	0	100
60_D_WL202	PI 672776	D	WL 202	mix	33	51	16

* For wild plants, this column indicates location (country) of the wild population. For domestic plants, country names indicate where plants were cultivated before being brought to the United States (*26*). These original nine germplasm 'sources' therefore represent unique domestication histories, in different parts of the world. The remaining 21 domestic cultivars ("mix") were created by hybridizing among these original nine cultivars. I estimated the percent contribution of each wild subspecies to each cultivar using Barnes (1977).

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575 **Table S5.** List of N=86 saponin compounds used in this study. See supplemental methods and materials

576 for details of compound identification.

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ID	Integration method	m/z	Adducts	Predicted formula	Neutral mass	RT	RMD
1	QL	943.5255	[M+H]+	C48H78O18	942.5177	4.39	556.95368
2	PG	1121.5739	[M+H]+	C54H88O24	1120.5661	3.43	508.83997
3	QL	959.4839	[M+H]+	C47H74O20	958.4761	3.46	504.33363
4	PG	1564.7015	[M+NH4]+	C69H110O38	1546.6675	3.56	448.32832
5	PG	1696.7433	[M+NH4]+	C74H118O42	1678.7093	3.56	438.07452
6	PG	1680.7473	[M+NH4]+	C74H118O41	1662.7133	3.58	444.62365
7	PG	1386.6191	[M+NH4]+	C62H96O33	1368.5851	3.59	446.40958
8	PG	1254.575	[M+NH4]+	C57H88O29	1236.541	3.59	458.32254
9	PG	1432.6592	[M+NH4]+	C64H102O34	1414.6252	3.6	460.12338
10	PG	989.4944	[M+H]+	C48H76O21	988.4866	3.64	499.64911
11	QL	1122.533	[M+NH4]+	C52H80O25	1104.499	3.64	474.819
12	PG	1240.5955	[M+NH4]+	C57H90O28	1222.5615	3.65	480.01141
13	PG	850.5154	[M+NH4]+	C42H72O16	832.4814	3.66	605.9855
14	PG	1372.6372	[M+NH4]+	C62H98O32	1354.6032	3.668	464.21589
15	QL	843.4363	[M+H]+	C42H66O17	842.4285	3.67	517.28862
16	PG	1108.552	[M+NH4]+	C52H82O24	1090.518	3.68	497.94687
17	PG	700.4287	[M+NH4]+	C36H58O12	682.3947	3.69	612.05373
18	PG	1680.7478	[M+NH4]+	C74H118O41	1662.7138	3.67	444.921
19	PG	1370.6246	[M+NH4]+	C62H96O32	1352.5906	3.73	455.70465
20	PG	1284.6232	[M+NH4]+	C59H94O29	1266.5892	3.71	485.1228
21	QL	1122.5686	[M+NH4]+	C53H84O24	1104.5346	3.79	506.51693
22	QL	1386.6535	[M+NH4]+	C63H100O32	1368.6195	3.76	471.27851
23	QL	1106.5372	[M+NH4]+	C52H80O24	1088.5032	3.79	485.47848
24	PG	1002.49	[M+NH4]+	C48H72O21	984.456	3.8	488.58352
25	QL	1092.5579	[M+NH4]+	C52H82O23	1074.5239	3.83	510.63655
26	PG	834.5207	[M+NH4]+	C42H72O15	816.4867	3.85	623.95097
27	PG	846.4849	[M+NH4]+	C42H68O16	828.4509	3.9	572.83952
28	PG	971.4843	[M+H]+	C48H74O20	970.4765	3.95	498.51552
29	PG	1076.5639	[M+NH4]+	C52H82O22	1058.5299	3.95	521.93933

30	QL	825.4274	[M+H]+	C42H64O16	824.4196	4	517.79236
31	PG	846.4849	[M+NH4]+	C42H68O16	828.4509	4.02	572.83952
32	PG	930.5058	[M+NH4]+	C46H72O18	912.4718	4.18	536.2714
33	PG	672.4684	[M+NH4]+	C36H62O10	654.4344	4.26	696.53831
34	PG	930.5418	[M+NH4]+	C47H76O17	912.5078	4.36	582.24144
35	PG	1059.567	[M+H]+	C53H86O21	1058.5592	4.4	535.12425
36	PG	652.4415	[M+NH4]+	C36H58O9	634.4075	4.36	676.68902
37	PG	1029.5268	[M+H]+	C51H80O21	1028.519	4.48	511.69139
38	PG	830.4896	[M+NH4]+	C42H68O15	812.4556	4.5	589.53177
39	PG	797.4678	[M+H]+	C42H68O14	796.46	4.53	586.60676
40	PG	816.5107	[M+NH4]+	C42H70O14	798.4767	4.56	625.46639
41	PG	670.4519	[M+NH4]+	C36H60O10	652.4179	4.59	674.023
42	PG	941.5033	[M+H]+	C48H76O18	940.4955	4.7	534.57062
43	PG	1056.5736	[M+NH4]+	C53H82O20	1038.5396	4.72	542.88693
44	PG	1069.5588	[M+H]+	C54H84O21	1068.551	4.74	522.45842
45	PG	652.4419	[M+NH4]+	C36H58O9	634.4079	4.97	677.30169
46	PG	654.4585	[M+NH4]+	C36H60O9	636.4245	5.3	700.57918
47	PG	1238.5797	[M+NH4]+	C57H88O28	1220.5457	3.76	469.40799
48	QL	1105.5431	[M+H]+	C53H84O24	1104.5353	3.44	491.25177
49	QL	1091.5636	[M+H]+	C53H86O23	1090.5558	3.49	516.32356
50	QL	1105.5789	[M+H]+	C54H88O23	1104.5711	3.54	523.61708
51	QL	1324.5802	[M+NH4]+	C60H90O31	1306.5462	3.69	438.02557
52	QL	1061.5161	[M+H]+	C51H80O23	1060.5083	3.69	486.1914
53	QL	1416.6571	[M+NH4]+	C64H102O33	1398.6231	3.7	463.83843
54	QL	1548.707	[M+NH4]+	C69H110O37	1530.673	3.71	456.50985
55	QL	1254.6108	[M+NH4]+	C58H92O28	1236.5768	3.73	486.84421
56	QL	973.501	[M+H]+	C48H76O20	972.4932	3.75	514.63738
57	QL	1254.6107	[M+NH4]+	C58H92O28	1236.5767	3.79	487.87988
58	QL	944.448	[M+NH4]+	C45H66O20	926.414	3.81	474.35116
59	QL	960.5162	[M+NH4]+	C47H74O19	942.4822	3.86	537.41936
60	QL	1090.5428	[M+NH4]+	C52H80O23	1072.5088	3.87	497.73379
61	QL	832.505	[M+NH4]+	C42H70O15	814.471	3.95	606.60296
62	QL	1087.568	[M+H]+	C54H86O22	1086.5602	4.06	513.25989
63	QL	832.505	[M+NH4]+	C42H70O15	814.471	4.07	606.60296

64	QL	959.52	[M+H]+	C48H78O19	958.5122	3.55	543.18758
65	QL	915.4567	[M+H]+	C45H70O19	914.4489	3.65	498.87668
66	QL	915.4581	[M+H]+	C45H70O19	914.4503	3.71	500.40521
67	QL	1386.6535	[M+NH4]+	C63H100O32	1368.6195	3.67	470.19728
68	QL	1076.4899	[M+NH4]+	C50H74O24	1058.4559	3.67	455.0902
69	QL	1062.5129	[M+NH4]+	C50H76O23	1044.4789	3.68	482.72355
70	QL	1048.5319	[M+NH4]+	C50H78O22	1030.4979	3.69	507.2807
71	QL	960.5157	[M+NH4]+	C47H74O19	942.4817	3.73	536.89908
72	QL	858.4452	[M+NH4]+	C42H64O17	840.4112	3.75	518.61202
73	QL	1224.6002	[M+NH4]+	C57H90O27	1206.5662	3.76	490.11914
74	QL	1092.5579	[M+NH4]+	C52H82O23	1074.5239	3.75	516.32356
75	QL	1192.5372	[M+NH4]+	C55H82O27	1174.5032	3.79	450.46813
76	QL	959.5209	[M+H]+	C48H78O19	958.5131	3.87	541.62515
77	QL	812.4423	[M+NH4]+	C41H62O15	794.4083	4.06	544.40789
78	QL	830.4894	[M+NH4]+	C42H68O15	812.4554	4.08	589.29109
79	QL	830.4901	[M+NH4]+	C42H68O15	812.4561	4.22	590.13346
80	QL	670.4518	[M+NH4]+	C36H60O10	652.4178	4.38	673.87395
81	QL	816.5099	[M+NH4]+	C42H70O14	798.4759	4.96	624.48722
82	PG	975.515	[M+H]+	C48H78O20	974.5072	3.66	527.92627
83	PG	827.442	[M+H]+	C42H66O16	826.4342	3.79	534.17641
84	QL	829.4568	[M+H]+	C42H68O16	828.449	3.65	550.7219
85	QL	974.495	[M+NH4]+	C47H72O20	956.461	3.83	507.9554
86	QL	1324.5802	[M+NH4]+	C60H90O31	1306.5462	3.79	438.02557