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ORIGINAL RESEARCH

Evaluating trait variation and covariation in perennial, herbaceous crop candidate species using herbarium specimens

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Abstract
Contemporary agriculture in many parts of the world consists of annual plant species grown in monoculture, but recently interest has focused on developing perennial, herbaceous species for use in perennial polycultures that offer greater ecosystem services relative to annual monocultures. Because few perennial, herbaceous species were domesticated by early farmers, one of the initial steps in identifying wild, perennial, herbaceous species for potential crop development is understanding patterns of variation and covariation among reproductive traits that underpin yield and vegetative traits. Herbaria can serve as a valuable resource for taxonomically broad surveys of phenotypic variation and covariation for pre-breeding. In this study, we used herbarium specimens to investigate trait variation and covariation in five genera of the legume family (Fabaceae). We addressed two questions: (1) do vegetative and reproductive traits vary as a function of life span (annual vs. perennial species), and are these differences consistent across genera?; and (2) how do vegetative and reproductive traits correlate with one another across lifespan and/or within genera? Reproductive traits and vegetative traits were measured on 535 herbarium specimens representing annual and perennial species of *Astragalus*, *Lupinus*, *Phaseolus*, *Strophostyles*, and *Vigna*. While species exhibited intra- and interspecific variation, few morphological differences were observed among annual and perennial species. Correlations among vegetative traits and among reproductive traits were mostly positive when significant, and perennial species generally exhibited more positive correlations between vegetative and reproductive traits than annual species. These data have potential relevance for perennial crop development, and provide further support that herbaria represent a rich, relatively untapped resource of information about plant trait variation and covariation.

Keywords Agriculture · trait correlations

Introduction

Agriculture uses 38% of global land, one-third of which is dedicated to monocultures of annual plant species that have high reproductive outputs and short lifespans (FAO, 2021). While this system is highly efficient in producing large yields of single crops, there are concerns about ecological impacts including soil erosion and use of fertilizers and other inputs (Glover & Reganold, 2010; FAO, 2016; FAO, 2019). Recent research focuses on the development of new crops that advance ecological intensification (LaCanne & Lundgren, 2018; Kleijn et al., 2019), an approach which endeavors to maximize agricultural production while simultaneously providing positive ecosystem services (Bommarco et al., 2013).

Perennial polyculture agriculture, one promising mechanism of ecological intensification, involves two or more

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perennial, herbaceous species grown together. Like annual crops, perennials produce edible seeds and fruits that can be mechanically harvested; in addition, perennials live for multiple years, and through their deep, persistent root systems, they reduce erosion, allow for greater carbon storage, and absorption of water and nutrients (Glover & Reganold, 2010; Vico & Brunsell, 2018). When different perennial crops are grown in mixtures, they form an agricultural system that resembles natural ecosystems in diversity, perenniality, and sustainability (Kleijn et al., 2019).

Although many wild perennial, herbaceous species exist in nature, perennial herbaceous species were not domesticated by early farmers (Van Tassel et al., 2010), and have only recently emerged as a focus of plant breeding programs (Cox et al., 2006; DeHaan et al., 2016; DeHaan et al., 2023). Why perennial, herbaceous species were passed over by early farmers is the subject of ongoing discussion (Van Tassel et al., 2010). One school of thought posits that lifespan is negatively correlated with reproductive output in a given year, and that perennials produce relatively fewer reproductive structures (seeds) than annual species (Vico et al., 2016; Friedman, 2020). Another idea is that cultivated annuals produce significantly larger seeds than cultivated perennial herbaceous species (Vico et al., 2016); however, Herron et al. (2020) reported that cultivated perennial, herbaceous *Phaseolus* species had greater seed sizes than their wild progenitors, suggesting evolution in response to artificial selection is possible, but perhaps has not had enough time under domestication to achieve yields equivalent to annuals. Importantly, a recent study comparing domesticated rice with a newly developed perennial rice showed comparable yields in the annual (replanted each year) and the perennial rice over four years (Zhang et al., 2022).

Because of the high demand for cereals and legumes, which account for the largest portion of human caloric consumption across the globe (FAO, 2021), breeders are focusing on the development of perennial, herbaceous grain crops for use in perennial polycultures (DeHaan & Van Tassel, 2014; Krug & Tesdell, 2021). Correlations between reproductive and vegetative traits in emerging perennial grain crops are important to consider because the goal of domestication in perennial, herbaceous species is to increase yield while at the same time retaining perennating structures that provide critical ecosystem services (e.g., above- or below-ground meristems that overwinter, storage organs, etc.) (González-Paleo et al. 2016). Comparative analyses of domesticated plants (primarily annual species) and their wild progenitors demonstrate increases in seed size, plant size, leaf size, and seedling growth rate (Gómez-Fernández & Milla, 2022), and an overall reduction in coordinated trait evolution over the course of domestication and crop improvement (Milla et al., 2014). In looking at seeds, in nature there is a clear seed mass-seed output trade-off (small number of large seeds vs. large number of small seeds); however domestication has favored high seed mass and output, suggesting that crops, especially those grown for seeds, deviate from the classic seed-mass seed-output trade-off (Martin, 2021), and perhaps other trade-offs.

Patterns of trait variation and covariation in wild plant species with agricultural potential can be studied using living or preserved plant materials, and there are advantages and drawbacks to both. Living collections are ideal for plant phenotyping; however, there are significant financial and labor costs associated with the establishment and maintenance of large living collections of diverse perennial, herbaceous species. Further, it is sometimes difficult to study species native to different geographic areas under common conditions due to challenges with seed acquisition and/or viability. One alternative to using living plants to evaluate morphological variation is to use herbarium specimens—dried, mounted, and well-documented collections of plant materials. Herbaria were developed to record plant diversity around the world and to serve as vouchers for taxonomic and other studies; beyond this, herbaria have been used for a wide variety of research purposes and continue to be critical resources of diverse information about many aspects plant biology (Besnard et al., 2018; Park et al., 2018; Heberling et al., 2019; Lopez & Sassone, 2019; Micke & Parsons, 2023).

Herbarium specimens offer an invaluable resource for studying a wide range of species, at different developmental stages, with observations (collections) from across broad geographic areas. It is possible to extract information from herbarium specimens about both reproductive traits (e.g., flowering time, inflorescence size, number of flowers/inflorescences, seed size and shape) and vegetative traits (e.g., leaf and leaflet size, petiole/petiole length and thickness, stem thickness, internode length) (Herberling, 2022). Recent studies have used herbaria and botanical libraries to identify wild species that might be candidates for *de novo* domestication (Ciotir et al., 2019; Tesdell et al., 2020), and to inform selection of suitable forages (Micke & Parsons, 2023).

The goal of this study was to advance understanding of trait variation and covariation within wild perennial, herbaceous species in the economically and agriculturally important legume family (Fabaceae). Using trait values for reproductive and vegetative structures derived from herbarium specimens of perennial and annual herbaceous species, the following questions were addressed: (1) how do vegetative and reproductive traits differ morphologically across annual and perennial species, and are these differences consistent across genera; and (2) how do vegetative and reproductive traits correlate with one another, does this differ across lifespan and/or genera, and what does that mean for domestication of perennial, herbaceous species in agriculture? Results presented here contribute to the broader understanding of how traits vary and covary in annual and perennial plant species, important considerations in the development of selection pipelines for emerging perennial crops. In addition, this study provides further support that herbaria represent an immense resource of information about plant trait variation and covariation that are relevant for crop development.

Methods

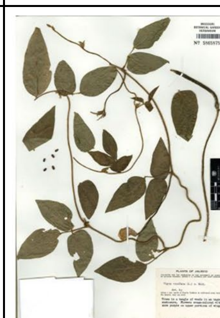
Study Systems

This study focused on genera within the large, economically and ecologically important plant family Fabaceae. We targeted genera that included contemporary crops, emerging crops, and/or species of ecological interest that had a history of human use, that have come to the attention of breeders as potential candidate species for domestication, and that were well represented in herbaria. We surveyed variation in annual and perennial members of five genera of Fabaceae: *Astragalus* L. (two annual species, two perennial species), *Lupinus* L. (two annuals, six perennials), *Phaseolus* L. (two annuals, two perennials), *Strophostyles* Elliot (one annual, one perennial), and *Vigna* Savi (three annuals, three perennials) (Table 1, Supplemental Table 1).

Trait Selection & Data Collection

This work used specimens housed in the Missouri Botanical Garden herbarium, one of the world's largest herbaria with more than seven million specimens collected primarily from wild populations. To study patterns of trait variation and covariation, we surveyed herbarium sheets for vegetative and reproductive traits that could be measured consistently on many different sheets for each species, and that could be observed in multiple species per genus. Target traits included size and shape measurements, and in some cases counts of flowers, fruits, seeds, stems, leaflets and leaves. Individual sheets were selected for inclusion in the study if they displayed both reproductive and vegetative structures that could be measured accurately (intact and undamaged specimens, structures of interest were present, and the same structures could be observed on multiple sheets). Reproductive traits selected for this study included

Table 1. An overview of the genera examined in this study. The group row correlates to which genera had the same measurements taken because the same data was not collected for every genus. Images displayed here were found through the Tropicos® database and photographed by MBG.

Genus	*Lupinus*	*Phaseolus*	*Strophostyles*	*Astragalus*		*Vigna*	
Example herbarium specimens*							
Annual species (# specimens)	2 species (20 specimens)	2 species (60 specimens)	1 species (40 specimens)	2 species (39 specimens)		3 species (59 specimens)	
Perennial species (# specimens)	6 species (124 specimens)	2 species (54 specimens)	1 species (42 specimens)	2 species (34 specimens)		3 species (63 specimens)	
Total specimens	144	104	82	73		122	
Traits measured	**Traits measured for Group 1 (*Lupinus, Phaseolus,* and *Strophostyles*)**			**Traits measured for Group 2 (*Astragalus*)**		**Traits measured for Group 3 (*Vigna*)**	
	Reproductive Traits	Vegetative traits		R	V	R	V
	Pod length Pod width	Terminal leaflet length Terminal leaflet width Stem width		Pod length Pod width Flower number Inflorescence length	Stem width Stem length Leaflet number per leaf	Pod length Seed/pod Seed area Flower length	Stem width Leaflet area

pod length and width, flower number, flower length, inflorescence length, and seed area. Vegetative traits included leaflet number per leaf, leaflet area, terminal leaflet length and width, stem length and width. All width measurements were made at the widest point on the structure. Leaflet area was calculated from scanned images of the terminal leaflet blade without the petiole using *ImageJ* (Schneider et al., 2012). In total, data were collected from 535 herbarium specimens representing 10 annual species and 14 perennial species (Table 1, Supplemental Table 1). Data were collected by undergraduate researchers that participated in the National Science Foundation (NSF) Research Experiences for Undergraduates (REU) program at the Missouri Botanical Garden, as well as Saint Louis University undergraduate researchers from 2016 to 2021.

Morphological features studied here were consistent in three of the five genera studied (*Lupinus*, *Phaseolus*, and *Strophostyles)* but were slightly different for *Astragalus* and *Vigna* (Table 1). Because the same data were not collected for every genus, data were analyzed in three groups based on which genera had the same trait measurements: *Lupinus*, *Phaseolus*, and *Strophostyles* (Group 1), *Astragalus* (Group 2), and *Vigna* (Group 3) (Table 1). Vegetative traits measured for Group 1 were leaf size and stem width. Stem length, stem width, and leaflet number per leaf were measured for Group 2 specimens. Vegetative traits measured for Group 3 were leaflet area and stem width. Reproductive traits measured on herbarium specimens for Group 1 included pod length and width. In addition to pod length and width, inflorescence length and flower number per inflorescence were also measured for Group 2. Work in Group 3 further expanded the number of traits measured, adding flower length (base of flower to longest petal edge), pod length, number of seeds per pod, and seed area. Measurements were made using digital calipers except for leaflet area, flower length, and seed area, which were captured from specimen scans using *ImageJ* (Schneider et al., 2012). One to ten structures (e.g. leaves or seeds) were measured for each specimen and individual trait measurements were averaged per sheet. The complete dataset can be found in Supplemental Table 2.

Data Analysis

Two-way ANOVAs tested the significance of lifespan, genus, and their interaction as fixed effects while controlling for species and specimen as random effects for traits within Group 1, and one-way ANOVAs tested the significance of lifespan as a fixed effect while controlling for the random effects of species and specimen in Groups 2 and 3 using *LME4* (v1.1–27.1, Bates et al., 2015). Post-hoc Tukey tests (using package *EMMEANS*, v1.7.0) were used to compare genus means in Group 1 when there was a significant effect of genus (Lenth et al., 2022). All statistical calculations were conducted in *R* v4.1.0 (R Core Team, 2021). Boxplots were generated using *GGPLOT2* (v3.3.5; Wickham, 2016) to visualize patterns of morphological variation to illustrate how traits differed among genera and within genera among annual and perennial species. Pearson's rank-order correlations among reproductive and vegetative traits within genera and lifespan resource allocation were computed with *CORRPLOT* (v0.92, Wei & Simko, 2021). Annual and perennial correlation matrices within each genus were compared using *CORTEST.JENNRICH* from package *PSYCH* (Revelle, 2021).

Results

Trait Variation: Effect of Lifespan, Genus, and Species on Vegetative and Reproductive Traits

Lifespan was not significant for vegetative or reproductive traits measured in *Lupinus, Phaseolus,* and *Strophostyles* (Table 2; Fig. 1A and B) and *Astragalus* (Table 3; Fig. 1C and D). We found a significant effect of lifespan for one vegetative and one reproductive trait measured within *Vigna* (Group 3): annual *Vigna* species exhibited slightly larger values for stem width than perennial *Vigna* species on average ($t = 2.98$, $P = 0.04$; Table 4; Fig. 1E). Perennial *Vigna* species exhibited longer flower lengths than annual *Vigna* species ($t = -3.15$, $P = 0.035$; Table 4; Fig. 1F).

Table 2. *Lupinus*, *Phaseolus*, and *Strophostyles* (Group 1). Type III analysis of variance (ANOVA) with Satterthwaite's method.

Trait class	Trait	Genus	Lifespan	Genus x lifespan	Species / Genus	Specimen ID / Species / Genus
Vegetative	Leaf length	F = 3.18	F = 0.13	F = 1.58	LRT = 134.53***	LRT = 692.48***
	Leaf width	F = 7.48*	F = 0.01	F = 0.92	LRT = 232.22***	LRT = 435.61***
	Stem width	F = 4.35	F = 0.95	F = 0.96	LRT = 77.58***	LRT = 1.1
Reproductive	Pod length	F = 0.87	F = 1.07	F = 0.48	LRT = 35.4***	LRT = 505.84***
	Pod width	F = 2.63	F = 1.00	F = 0.38	LRT = 34.73***	LRT = 470.45***

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

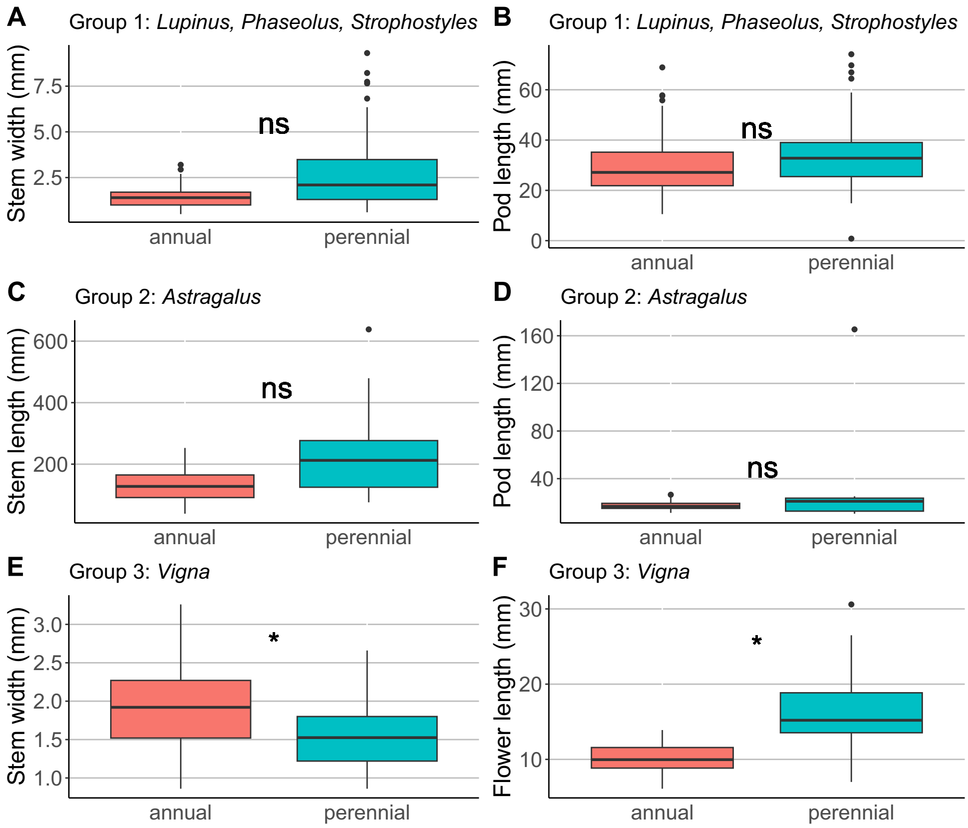


Fig. 1. Boxplots representing the effect lifespan in all groups. **A.** Stem width in *Lupinus, Phaseolus,* and *Strophostyles* (Group 1). **B.** Pod length for *Lupinus, Phaseolus,* and *Strophostyles* (Group 1). **C.** Stem length in Astragalus (Group 2); **D.** Pod length in *Astragalus* (Group 2). **E.** Stem width differences in *Vigna* (Group 3). **F.** Flower length differences within *Vigna* (Group 3). Different letters denote significant ($P < 0.05$) differences between groups.

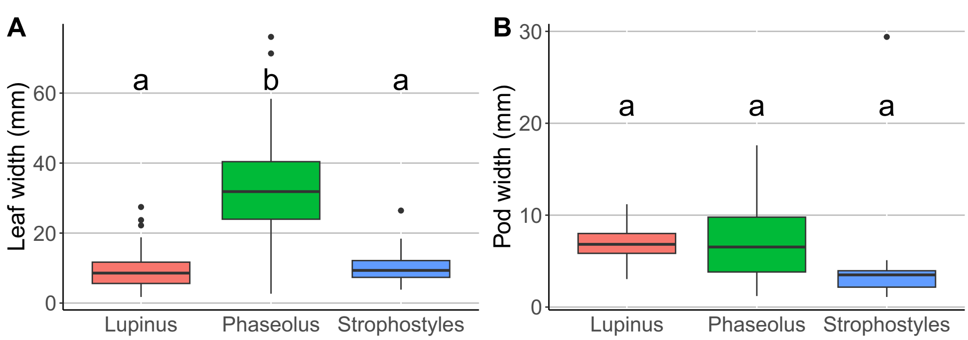


Fig. 2. Boxplots representing the effect genus in Group 1: *Lupinus, Phaseolus,* and *Strophostyles*. **A.** Differences in leaf width across genus. **B.** Differences in pod width across genus. Significant differences ($P < 0.05$) between annual and perennial groups are illustrated with an asterisk (*) and non-significant differences in the means are marked as "ns".

For the three genera where the same traits were measured (*Lupinus, Phaseolus,* and *Strophostyles* (Group 1)), the effects of genus on vegetative and reproductive trait variation were compared. A significant effect of genus was detected for one vegetative trait (leaf width; Fig. 2A): *Lupinus* and *Phaseolus* were significantly different for leaf width ($t = -3.79$, $P = 0.01$). There were no detected effects of genus on reproductive traits (Fig. 2B; Table 2). In contrast, variation at the species level was detected for all six traits measured in *Lupinus, Phaseolus,* and *Strophostyles* (Group 1), six out of seven traits for *Astragalus* (Group 2), and four out of six traits for *Vigna* (Group 3; Tables 2, 3, and 4).

Table 3. *Astragalus* (Group 2). Type III analysis of variance (ANOVA) with Satterthwaite's method.

Trait Class	Trait	Lifespan	Species / Lifespan	Specimen ID / Species / Lifespan
Vegetative	Leaflets per leaf	F = 5.69	LRT = 21.18***	LRT = 114.95***
	Stem length	F = 2.41	LRT = 11.09***	LRT = 104.5***
	Stem width	F = 2.72	LRT = 28.49***	LRT = 164.62***
Reproductive	Inflorescence length	F = 1.83	LRT = 49.01***	LRT = 38.88***
	Flowers per inflorescence	F = 1.35	LRT = 21.28***	LRT = 256.56***
	Pod length	F = 2.32	LRT = 0	LRT = 270.19***
	Pod width	F = 3.12	LRT = 20.59***	LRT = 386.92***

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

Table 4. *Vigna* (Group 3). Type III analysis of variance (ANOVA) with Satterthwaite's method.

Trait Class	Trait	Lifespan	Species / Lifespan	Specimen ID / Species / Lifespan
Vegetative	Leaflet area	F = 6.86	LRT = 3.29	LRT = 405.9***
	Stem width	F = 9.08*	LRT = 0.65	LRT = 117.12***
Reproductive	Flower length	F = 9.93*	LRT=32.09***	LRT = 9.14**
	Pod length	F = 0.16	LRT=59.73***	LRT = 175.52***
	Seeds per pod	F = 0.01	LRT=80.55***	LRT = 48.95***
	Seed area	F = 0.12	LRT=15.45***	LRT = 599.32***

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

Trait Covariation: Correlations Within and Across Lifespan

Correlation matrices were significantly different among annual and perennial species for the five genera examined (Fig. 3; Supplemental Figs. 1–5; see below for statistical test results). For annual species, 18 of the 60 total correlations across genera were positive, 3 of 60 correlations were negative, and 39 of the 60 correlations were non-significant (Fig. 3A, C, E, G, I; Supplemental Figs. 1–5). For perennial species, 25 of 60 correlations across all five genera were positive, zero of 60 trait correlations were negative, and 35 of 60 correlations were not significant (Fig. 3B, D, F, H, J; Supplemental Figs. 1–5). Patterns of trait covariation for each genus are described in detail below.

Lupinus (Group 1)

The overall correlation matrices of annual and perennial *Lupinus* species were significantly different ($X^2 = 94.74$, $P < 0.001$; Fig. 3A, B). Most pairwise trait correlations for annual *Lupinus* species were non-significant, except for a negative correlation between leaf width and pod length (r = -0.65), and positive correlations between leaf length and leaf width (r = 0.53), and leaf width and pod width (r = 0.64; Fig. 3A; Supplemental Fig. 1). For perennial *Lupinus* species, vegetative traits (leaf length, leaf width, stem width) were positively correlated with one another (correlation coefficients ranged from 0.59 to 0.85 for pairs of traits), and reproductive traits were positively correlated with one another (pod length and pod width, r = 0.66). There were no significant relationships between leaf and pod size traits, but stem width was positively correlated with pod length and pod width (r = 0.36 and 0.28, respectively; Fig. 3B; Supplemental Fig. 1).

Phaseolus (Group 1)

Phaseolus annual and perennial species also differed significantly in their patterns of trait correlations ($X^2 = 33.94$, $P < 0.001$; Fig. 3C, D). In annual *Phaseolus* species, leaf length and width were positively correlated (r = 0.80, Fig. 3C; Supplemental Fig. 2), and pod length and width were positively correlated (r = 0.60; Fig. 3C; Supplemental Fig. 2). Leaf width and pod width were negatively correlated (r = -0.38, Fig. 3C; Supplemental Fig. 2). In perennial *Phaseolus* species, leaf length and width were positively correlated (r = 0.77, Fig. 3D; Supplemental Fig. 2) and pod length and width were positively correlated (r = 0.61; Fig. 3D; Supplemental Fig. 2). Pod length was positively correlated with both pod leaf length (r = 0.39) and leaf width (r = 0.25; Fig. 3D; Supplemental Fig. 2) in perennial *Phaseolus* species.

Strophostyles (Group 1)a

The pattern of trait covariation differed significantly between *Strophostyles* annual and perennial species ($X^2 = 28.51$, $P < 0.01$; Fig. 3E, F). In annual *Strophostyles* species, leaf length and width were positively correlated (r = 0.56, Fig. 3E; Supplemental Fig. 3), and pod length and width were positively correlated (r = 0.53; Fig. 3E; Supplemental Fig. 3). Additionally, leaf width and pod length were positively correlated in annual species (r = 0.36; Fig. 3E; Supplemental Fig. 3). In contrast, the *Strophostyles* perennial species exhibited no significant pairwise trait correlations (Fig. 3F; Supplemental Fig. 3).

Astragalus (Group 2)

Relationships among pairs of traits differed between *Astragalus* annuals and perennials ($X^2 = 617.50$, $P < 0.001$; Fig. 3G, H). Among the annual species, the number of leaflets per leaf correlated positively with all reproductive traits (pod length, r = 0.68 and width, r = 0.70; inflorescence length, r = 0.57; and number of flowers per inflorescence, r = 0.52; Fig. 3G; Supplemental Fig. 4). Pod length and width (r = 0.72), pod width and inflorescence length (r = 0.71), and inflorescence length and flowers per inflorescence (r = 0.86; Fig. 3G) were also positively correlated.

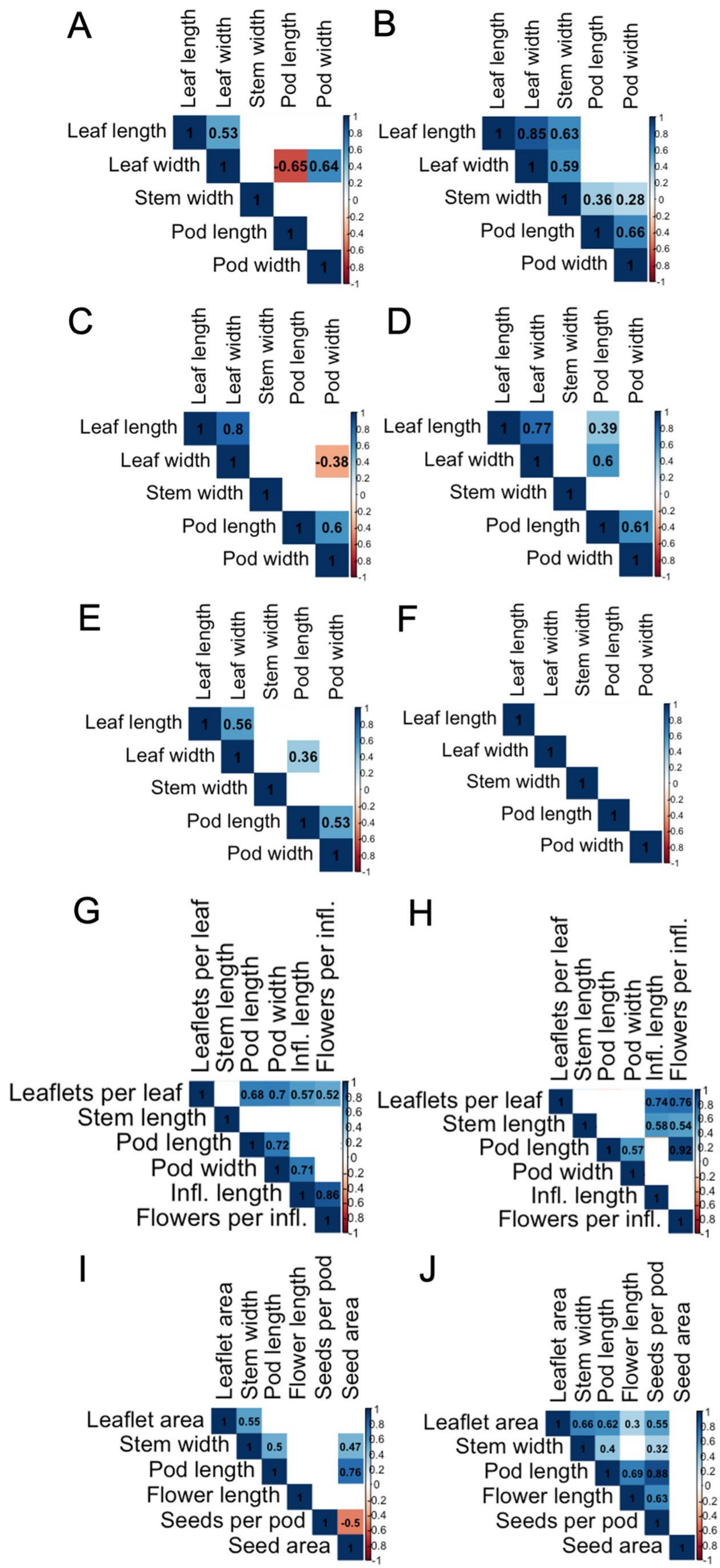


Fig. 3. Correlation matrices showing correlations within genus and within lifespan per genus. **A.** *Lupinus* annuals. **B.** *Lupinus* perennials. **C.** *Phaseolus* annuals. **D.** *Phaseolus* perennials. **E.** *Strophostyles* annuals. **F.** *Strophostyles* perennials. **G.** *Astragalus* annuals. **H.** *Astragalus* perennials. **I.** *Vigna* annuals. **J.** *Vigna* perennials. Positive correlations are shown in blue and negative correlations are shown in red, with more intense colors indicating a stronger correlation coefficient. Non-significant ($P < 0.05$) pairwise correlations are shown in white.

In perennials, positive correlations occurred between leaflets per leaf and inflorescence length (r = 0.74), leaflets per leaf and flowers per inflorescence (r = 0.76), stem length and inflorescence length (r = 0.58), and stem length and flowers per inflorescence (r = 0.54; Fig. 3H; Supplemental Fig. 4). Two additional correlations were significant among pairs of reproductive traits: pod length with pod width (r = 0.57) and pod length with number of flowers per inflorescence (r = 0.92); Fig. 3H; Supplemental Fig. 4).

Vigna (Group 3)

Vigna annuals and perennials were significantly different from each other with respect to trait correlations ($X^2 = 239.37$, $P < 0.001$; Fig. 3A, B). In annual *Vigna* species, seeds per pod was negatively correlated with seed area (r = -0.50), while stem width was positively correlated with leaflet area (r = 0.55), pod length (r = 0.50), and seed area (r = 0.47; Fig. 3I; Supplemental Fig. 5). Additionally, pod length was positively correlated with seed area (r = 0.76; Fig. 3I; Supplemental Fig. 5). Perennial *Vigna* species showed positive correlations for most traits with the exception of seed area which was not significantly correlated with any of the vegetative or reproductive traits measured (Fig. 3J; Supplemental Fig. 5).

Discussion

Herbarium specimens were used to assess vegetative and reproductive trait variation and covariation in annual and perennial members of five genera of Fabaceae. Trait variation was observed at the species level, with subtle differences stemming from life history strategy. Patterns of trait covariation, largely positive, differed among annual and perennial species, with perennial species displaying more positive trait correlations than annual species. These data contribute to a growing understanding of trait variation and covariation in wild, perennial, herbaceous species, and how relationships among structures differ with annual congeners. Together with other recent studies, this work offers valuable insights that support the potential of wild, herbaceous species for domestication and applications in perennial polyculture agriculture.

Intra- and Interspecific Variation in Vegetative and Reproductive Traits

Variation is the foundation of evolution and the required raw material for *de novo* domestication and breeding programs. In this study, both vegetative (leaflet length, width, and area; stem width and length) and reproductive traits (inflorescence length, flower number, pod length and width) showed high intraspecific variation as well as among-species differences. Intraspecific variation is garnering increasing attention (Siefert et al. 2015; Moran et al. 2016; Yang et al. 2020) and has been reported for several traits including trichomes in tomato (*Solanum lycopersicum*; Kaur et al., 2023), leaf variation in winegrapes (*Vitis vinifera*; Macklin et al., 2022); and nutrient concentrations of common beans and wild relatives (*Phaseolus* spp.; Schier et al., 2019), among others. Recent work has shown that intraspecific trait variation varies across scales (Martin et al., 2017) and plant age (Henn & Damschen, 2021). Additionally, intraspecific trait variation is recognized for its role in breeding crops for future climates (Litrico & Violle, 2015; Martin & Isaac, 2015) and ecological processes including community diversity and competition, among others (Crawford et al., 2019; Westerband et al., 2021; Holdridge & Vasseur, 2022). Data presented here further highlight abundant intraspecific variation in perennial herbaceous species, an important resource for future agricultural innovation.

Because the vast majority of herbaceous crops domesticated to date are annuals, there is a persistent question of how wild annual and perennial species differ (beyond lifespan), and whether differences among them might impact effects of directional selection for increased yield during the domestication process. Significant differences among annual and perennial species studied here were recovered in only one genus (*Vigna*), in which stem length and flower length were greater in perennial species (Fig. 1E, 1F). Data pointed to differences in stem width in Group 1 genera (*Lupinus, Phaseolus* and *Strophostyles*) and stem length in *Astragalus* as well, but differences were not significant (Figs. 1A, E). The lack of significant variation between lifespans for the expression of reproductive traits (i.e. pod length and width, and seed mass) is consistent with findings from Mazer (1989) and Herron et al. (2021a, b), where both found no difference in seed traits across lifespans. However, Herron et al. (2021a, b) reported a significant effect of lifespan on vegetative traits expressed at the seedling stage, including leaf number and height, observations consistent with other studies (Vico et al., 2016; Friedman, 2020). It is difficult to compare traits of annual plants with traits expressed in the first year of a multi-year existence in perennial species. Some studies have begun to compare growth of annual plants with growth of perennial species measured across multiple years; notably Zhang et al. (2022) compared domesticated annual rice with a perennial rice cultivar measured over five years. This landmark study demonstrated comparable yields of annual rice and the perennial rice domesticate over four of the five years, and relatively higher soil organic carbon in plots with perennial rice. Future work might expand this to investigate patterns of resource allocation in wild annual and perennial species over multiple years where both above- and below-ground traits are measured over multiple years under field conditions.

Correlations Among Traits in Perennial and Annual Species

The nature of trait covariation among reproductive and vegetative features is a fundamental question required for understanding evolutionary history and future evolutionary trajectories (Stearns 1989). It is particularly relevant for domestication of perennial grains, where strong directional selection for increased yield may impact vegetative traits, including perennating structures (those that contribute to perennial growth), and traits important for below-ground ecosystem services. In this study, correlations between vegetative and reproductive traits were overwhelmingly positive (Fig. 3) and perennial species exhibited more numerous positive correlations between traits than annual species. Positive relationships seen here may indicate a correlation in growth factors between vegetative and reproductive traits to better provide for the plant. For example, it is possible that larger leaves increase photosynthesis and food production, which provides more resources for greater pod development. For annuals, this study showed few positive correlations between vegetative and reproductive traits (Figs. 3E, G, I). The low number of positive correlations within annuals, as described earlier, could indicate the "Y" model of allocation (de Jong & van Noordwijk, 1992), a trade-off between traits. Data generated in this study were collected on mature plants; a previous study based on juvenile plants reported that correlations between vegetative and reproductive traits were stronger for annuals than perennials (Herron et al., 2021a, b). Differences among these two studies may indicate that patterns of trait correlations change over the development of plants, with annuals exhibiting greater trait covariation than perennials at the pre-reproductive stage, but not at later stages of maturity.

We observed only three negative correlations between reproductive and vegetative traits; in both cases, the significant negative correlation was between leaf width and either pod length or width. A negative correlation may be interpreted as a trade-off in resource allocation (de Jong & van Noordwijk, 1992); however, correlation does not always equal causation, and a negative correlation between two traits might reflect some other underlying processes (e.g., physiology) not directly measured here. More research focused on plant chemistry and resource allocation within specific species is needed to understand the proposed lack of correlation based on function. Further, we saw very few negative correlations among traits within the same structure type. For the sole example, *Vigna* annuals correlated negatively between seeds per pod and seed area (Fig. 3I). The relative paucity of negative correlations could indicate a lack of trade-offs between vegetative and reproductive traits, which supports the potential to select for increased reproductive traits without concomitant decreases in vegetative structures. Additionally, this study did not include traits directly related to perennation that may contribute to survival over multiple years and therefore could not access the potential tradeoffs between reproductive and survival traits.

Utility of Herbaria in Research Related to Future Agricultural Systems

As concerns mount about needs for more productive agricultural systems that mitigate, rather than exacerbate, environmental issues, there is increasing interest in developing agricultural systems that mimic nature in their perenniality and diversity (Glover & Reganold, 2010; DeHaan et al., 2023). Herbaria and the institutions that house them maintain large, diverse plant collections for research, conservation, and education (Miller et al., 2015; Marsico et al., 2020; Heberling, 2022). Globally, over 3,000 herbaria collectively house nearly 400 million specimens across 178 countries (Thiers, 2020), presenting an important opportunity to describe natural variation using specimens collected over the course of decades, even centuries, and across broad geographic areas (McGraw, 2001; Leger, 2013). This study contributes to a growing body of literature demonstrating wide applications of herbarium specimens in research focused on major contemporary issues (Loiselle et al., 2008; Davis et al., 2015; Yeates et al., 2016; Munson & Long, 2017; Monroe et al., 2019; Park et al., 2018; Heberling, 2022; Williams et al., 2021; Micke & Parsons, 2023), including future agricultural systems.

Here, we used herbarium specimens to test hypotheses about patterns of trait covariation in annual and perennial legume species for the purposes of understanding how perennial plants might change in response to selection for increased size of reproductive structures (i.e., pod size in legumes). These analyses illustrate the utility of herbarium specimens in characterizing natural variation in wild species, even with the constraint of using traits available per preserved specimen (e.g. leaf size and pod length). Comparative analyses like those conducted here offer an important window into one aspect of plant trait covariation, but there are important limitations. For example, herbarium specimens are typically of only a single individual from a population and there may be bias in the selection of that individual. Additionally, herbarium specimens preserve only a portion of an individual, not the entire plant: it is difficult if not impossible to measure total numbers of a given trait (i.e., flowers per plant, seeds per plant). Further, herbarium specimens often capture just a portion of the above-ground part of the individual, and root systems are rarely represented in herbarium specimens. It is difficult to determine to what extent the patterns we observed were due to the plants being grown and collected in different environments. Another consideration is the developmental stage (for example, some structures may not be fully formed at time of collection leading to underestimation of trait values) and year of growth in which the specimens were collected was certainly variable, which may have an impact on trait measurements. The drying of herbarium specimens may affect the sizes of structures overall or may differentially affect the morphology of structures and their measurements potentially altering correlations between traits relative to measurements of fresh samples. We recognize that these limitations may have biased the data generated here; however, we expect that any effects would not be species or lifespan specific, and therefore would not have influenced conclusions drawn. To address the limitations of preserved specimens, future studies might consider adding a complimentary common garden experiment, where perennial and annual species are grown in the same location under the same conditions (Herron et al., 2021a, b). Comparative analyses of herbarium specimens offer a valuable first pass at characterizing trait variation and covariation in vegetative and reproductive traits, and may guide subsequent analyses of living plants that incorporate comprehensive analyses of yield.

Conclusions

This study evaluated trait variation and covariation in perennial, herbaceous crop candidate species using measurements made on herbarium specimens. Results demonstrate high levels of variation within species, indicating that these wild, perennial, herbaceous species have potential for evolution under artificial selection. We observed positive correlations among many vegetative and reproductive traits, more so in perennial species than in annuals. Differences among patterns of trait covariation in annual and perennial species suggest potentially unique trait relationships in perennials; this may be particularly important for future breeding efforts in perennial herbaceous species that target both reproductive traits related to yield as well as vegetative traits, and hint that selection for one may not necessarily result in the reduction of the other. This and other studies provide an important foundation for nascent domestication programs in perennial, herbaceous species, and suggest that the evolution of reproductive traits in perennial species undergoing directional selection may differ from patterns observed previously in annual species. Results presented here were based on observations made on herbarium specimens and underscores the value of herbaria in studies aimed at supporting agricultural innovation, and in particular, the potential development of perennial, herbaceous crop candidate species.

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Author Contributions

AR curated and contributed to writing, EB, MS, SS, and SS collected data and contributed to writing; CC and SH provided supervision and contributed to writing; MJR curated and analyzed data, provided supervision and contributed to writing; AJM conceived the study, managed funding, provided supervision and contributed writing.

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Declarations

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

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