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GENETIC AND PHENOTYPIC CONSEQUENCES OF FARMER MANAGEMENT OF FERAL *Brassica rapa* IN MEXICO

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ABSTRACT

Understanding the process of reciprocal genetic and cultural change involved in domestication has long been a focus of archaeology, genetics, ethnobiology, and a variety of other fields. Unfortunately, it is difficult to make inferences about the complex ecological, cultural, and evolutionary factors involved in domestication processes that happened thousands of years ago. Cases of ongoing anthropogenic selection on managed wild plants can provide unique insights into these processes. Spontaneously occurring populations of feral field mustard (*Brassica rapa* L.) in Northwest Mexico present an opportunity to understand the dynamics of human selection on wild annual plants and the dynamics involved in domestication. We collected field mustard samples from populations cultivated by Rarámuri farmers and unmanaged populations in Chihuahua, Mexico, for phenotypic and population genotyping-by-sequencing analysis. We found significant differences in flowering time between managed and unmanaged populations and genetic differentiation of two managed populations from unmanaged populations in the same communities. We present rare evidence of the ongoing “redomestication” of a feral crop. This genomically-enabled short-generation plant could provide a powerful study system in the future for further understanding the ecological and cultural aspects of domestication.

KEYWORDS: contemporary evolution, domestication, incipient domestication, traditional resource management.

ABSTRACT

El comprender los procesos recíprocos de cambios genéticos y culturales implicados en la domesticación ha sido durante mucho tiempo uno de los enfoques de la arqueología, la genética y otros campos. Desafortunadamente, resulta difícil inferir los factores ecológicos, culturales y evolutivos complejos involucrados en los procesos de

domesticación que ocurrieron hace miles de años. Los casos de selección antropogénica continua en plantas silvestres manejadas pueden proporcionar conocimiento único sobre estos procesos. Las poblaciones de nabo feral (*Brassica rapa* L.) que ocurren espontáneamente en el noroeste de México, brindan una oportunidad para comprender la dinámica de la selección humana en plantas silvestres anuales y los aspectos involucrados en la domesticación. Se recolectaron muestras de mostaza de campo de poblaciones cultivadas por agricultores *rarámuri* y poblaciones no manejadas en Chihuahua, México, para el análisis fenotípico y de *genotyping-by-sequencing* de la población. Se encontraron diferencias significativas en el tiempo de floración entre las poblaciones manejadas y no manejadas, así como una diferenciación genética de dos poblaciones manejadas en comparación con las poblaciones no manejadas en las mismas comunidades. En este estudio, se presenta evidencia poco común sobre la “re-domesticación” continua de un cultivo feral. Esta planta de generación corta y con habilitación genómica, podría proporcionar un estudio de caso para futuras investigaciones que se dediquen a comprender más ampliamente los aspectos ecológicos y culturales de la domesticación.

PALABRAS CLAVE: domesticación, domesticación incipiente, evolución contemporánea, manejo tradicional de recursos.

INTRODUCTION

Domestication is driven by a complex combination of ecological, biological, and cultural factors (Price *et al.*, 2011; Gepts *et al.*, 2012). Understanding the process of reciprocal genetic and cultural change involved in the domestication process has long been a focus of archaeology, genetics, linguistics, and other disciplines (Meyer and Purugganan, 2013; Zeder, 2015) and provides insight into the nature of both contemporary crop genetic resources (Harris, 2012; Zeder, 2015) and evolutionary processes in general (*e.g.*, Darwin, 1868; Andersson and Georges, 2004; Ross-Ibarra *et al.*, 2007; Meyer and Purugganan, 2013). Research on domestication has focused on selection processes often taking place thousands of years ago (Larson *et al.*, 2014), in cases where it has been difficult to empirically characterize the ecological contexts and human practices involved (Parker *et al.*, 2014; Zeder, 2015).

To circumvent this difficulty, some researchers have turned to contemporary study systems in which humans manage otherwise wild plant populations by weeding, saving and sowing seeds, transplanting, or other activities (Gade, 1972; Bye, 1979; Casas *et al.*, 2007; Elias *et al.*, 2007; Hughes *et al.*, 2007; Blanckaert, 2011). Many of these studies have focused on non-model and long-lived

organisms such as cacti (*e.g.*, Casas *et al.*, 1997, 1999; Rodríguez-Arévalo 2006; Parra, 2008) and leguminous trees (Zárate *et al.*, 2005). Insights from annual plants with well-studied genomes could open up opportunities to understand evolution and domestication. Most of the past research has also focused on native plant species which have coexisted with local cultures for an unknown period of time (*e.g.*, Casas *et al.*, 2007; Aguirre-Dugua *et al.*, 2012), making the time depth of management unclear. Investigating anthropogenic selection on introduced weedy plants with known earliest dates of introduction, presents an opportunity to measure change to populations over a more limited time period.

Field mustard (*Brassica rapa* L.) managed by Indigenous farmers presents a potentially powerful study system to understand evolution under human management (Gade, 1972; Bye, 1979). In addition to a relatively short life-cycle that enables transplant experiments (Williams and Hill, 1986), *B. rapa* is equipped with a well-annotated genome (Wang *et al.*, 2011) and benefits from its close relationship to the model organism *Arabidopsis thaliana* (Yang *et al.*, 2005, Mun *et al.*, 2010). Field mustard is native to Eurasia, where it was originally domesticated and selected for morphologically diverse crop forms (*e.g.*, turnips, pak choi, napa cabbage, and oilseed crops), making it a model for extreme phenotypic divergence

under domestication (Gómez-Campo and Prakash, 1999; Zhao et al., 2005; Guo et al., 2014; McAlvay et al., 2021). Weedy forms of field mustard have spread to temperate areas worldwide (Hall, 2005; McAlvay, 2018). Weedy *B. rapa* may have reached the Americas as early as the 16th century (Gade, 1972; Bye, 1979; McAlvay, 2018) and now inhabits disturbed areas of coastal and highland regions from Canada to Argentina (McAlvay et al., 2017). Genetic evidence suggests that these populations are feral escapes derived from European or North African turnips potentially pre-adapting them to anthropogenic environments and/or human preferences (McAlvay et al., 2021).

Since its introduction, weedy *B. rapa* has been adopted as food, medicine, and fodder by human cultures ranging from northwestern Mexico to Patagonia (Gade, 1972; Berlin et al., 1974; Bye, 1979; Ladio, 2001; Vieyra-Odilon and Vibrans, 2001; McAlvay, 2018). Several cultures sow seeds in fertilized plots (Bye, 1979; Blancas et al., 2013; Solís and Estrada, 2014), intentionally spare plants when weeding fields (Gade, 1972; Weismantel, 1989; Vieyra-Odilon and Vibrans, 2001), and/or transplant individuals with desirable phenotypes (McAlvay, 2018). In the 1930s and 1940s, weedy field mustard was cultivated in Argentina as an oilseed crop when low prices for grains and high prices for the wild *aceite de colza* made it profitable (Tenenbaum 1937; Pascale 1976) These types of management activities may have evolutionary consequences for the targeted plant populations (Casas et al., 2007; Parra; 2010; Blanckaert et al. 2013).

Members of Rarámuri communities in northwestern Mexico manage field mustard in an especially intensive manner that may have consequences for diversity and selection in local *B. rapa* populations (Bye, 1979; McAlvay 2018;). The Rarámuri are a Uto-Aztecan speaking group (Dakin, 2004), inhabiting the northern Sierra Madre Occidental mountains of Chihuahua. Rarámuri people living in homesteads throughout the mountains pursue traditional milpa agriculture and diverse cash-economy livelihood practices (Wyndham, 2009). Many Rarámuri farming families take advantage of *quelites*—edible wild or weedy plants often harvested for their leafy greens—

growing spontaneously in crop fields and margins (Bye, 1979; Bye, 1981; LaRoche and Berkes, 2003). Among these *quelites*, *B. rapa* (known as **mekuásari** in the Rarámuri language) is prominent in the diet as a boiled leafy green (Bye, 1973, 1981; LaRoche and Berkes, 2003). Self-sown field mustard emerges spontaneously in the summer and quickly enters its reproductive stage, diverting biomass to stems and flowers and becoming fibrous and bitter, making it less desirable as a food resource (Bye, 1979). To extend the vegetative stage of field mustard, Rarámuri people sow seeds in tilled plots during early fall so that emerging plants will not be triggered to flower by the long day length of summer (Bye, 1979; McAlvay 2018). These plants are typically sown in plots where livestock corrals have been stationed and droppings have been deposited (Bye, 1979; LaRoche and Berkes, 2003). Rarámuri cultivation of *B. rapa* is recorded as early as 1776 (Bye, 1979). Humans are able to induce rapid evolutionary changes in plants and animals even on decadal time scales (Palumbi, 2001; Bone and Farres, 2001), and Rarámuri cultivation may be influencing the evolution of these field mustard populations.

Phenological and morphological traits may be under selection by Rarámuri farmers as life-history characteristics play an important role in many domestication processes (de Wet and Harlan, 1975). For example, Rarámuri management and preference for an extended vegetative stage in field mustard may be selecting for delayed flowering time. Franks and Weis (2009) demonstrated that the flowering time trait in *B. rapa* is capable of rapid adaptation, suggesting that this trait is susceptible to rapid shifts under artificial selection. During interviews we carried out for a previous study (approved by the University of Wisconsin-Madison's Social and Behavioral Science Institutional Review Board [#2014-0828 #IRB00022321; #2015-0666-CP001], and conducted in alignment with the International Society for Ethnobiology Code of Ethics, including informed consent), Rarámuri farmers expressed preference for larger plants and intentionally gathered seed from those plants (McAlvay, 2018). The preferences of plant managers have been demonstrated to drive selection in other studies of

ongoing domestication (Johns and Keen, 1986, Elias *et al.*, 2007, Blanckaert *et al.*, 2013). These changes are in line with the domestication syndromes (*sensu de Wet and Harlan, 1975*) observed in similar crops.

Past studies of ongoing domestication-like processes in Mexico have shown a variety of population genetic trends when comparing managed and unmanaged populations. While a bottleneck in diversity is predicted under domestication due to small founder populations (Doebley, 1992; Tanksley and McCouch, 1997), some studies of ongoing domestication have found higher genetic diversity in managed populations due to introgression from wild populations, and humans transporting propagules from other areas (Tinoco *et al.*, 2005; Zárate *et al.*, 2005). In other parts of Mexico gene flow between managed crops and local conspecifics or congeners is seen as a beneficial source of novel diversity (Wilkes, 1977; Nabhan, 1984). Pollinating insects are known to visit *B. rapa*, especially bees and flies (Warwick *et al.*, 2003; Rader *et al.*, 2009, 2013), and unplanted *B. rapa* in field margins may cross with *B. rapa* sown in fertilized plots, but as the bulk of unmanaged field mustard emerge with the first rains in the summer, a temporal reproductive isolation may exist (Bye, 1979). While Rarámuri farmers often sow seeds saved from previously planted parcels of field mustard, they occasionally collect seeds from spontaneously occurring individuals which might prevent a signal of reduced diversity (McAlvay, 2018). Increased genetic differentiation from local wild populations is also predicted due to prolonged isolation and selection (Parra *et al.*, 2008), but the genetic structuring of managed populations may also be influenced by seed exchange networks (Fuentes *et al.*, 2012; Jensen *et al.*, 2013) and gene flow with sympatric unmanaged populations (Sukopp *et al.*, 2005). Many Rarámuri regularly engage in informal exchange of seeds when visiting each other's houses and share seeds with those in need, exercising the principle of *kórima* (Ezequiel and Guadalupe, 2014). The Rarámuri word *kórima* encompasses a concept of mutual aid and cooperation (Rubio and Rodríguez 2014). For example, if one family has a crop failure, a neighbor may step in to provide them with part of their harvest. This aid may be returned in the future. This may lead

to increased exchange of seeds, with potential impacts on gene-flow. In many studies of ongoing domestication, levels of gene flow are high between managed and wild populations (Casas *et al.*, 2007).

To understand these dynamics, this study analyzed the genetic and phenotypic differences between unmanaged and Rarámuri-managed populations of field mustard. Specifically, we compared managed and unmanaged populations using single nucleotide polymorphisms (SNPs) derived from genotyping-by-sequencing (GBS) (Elshire *et al.*, 2011) to assess genetic diversity and differentiation. We also carried out a common garden experiment to investigate phenology and morphology. We hypothesized that managed populations would have no significant difference in nucleotide diversity compared to wild populations due to regular introgression with wild populations. We also hypothesized higher genetic differentiation between nearby unmanaged populations than between managed and unmanaged populations in the same area due to reproductive isolation. We hypothesized that managed populations would also have more rapid germination, a longer vegetative stage, and greater height due to selection by farmers.

MATERIALS AND METHODS.

Population sampling. This study was conducted in the Rarámuri communities of Bahuinocachi, Rancho Blanco, Norogachi, Gumisachi, Chogueta, Cocherare, Panalachi, Rejogochi, and San Ignacio, all in Chihuahua, Mexico. Unmanaged populations were identified through application of three criteria: (1) no local knowledge of past management; (2) greater than 3 km from dwellings and managed plots as most bee foraging activity happens within less than 3km (Kohl *et al.*, 2020); and (3) no evidence of management (tilling or weeding).

Managed populations were identified as those sown in manured plots from seed collected from previously sown *B. rapa* (Figure 1). For population genetic sampling, we collected young leaf material from an average of eight samples, each from eight unmanaged and five managed populations (Figure 2, see numbers per population in Table



Figure 1. *Mekuásari* (*Brassica rapa*) growing in Rarámuri territory in Chihuahua, Mexico.

1), and dried and stored it in silica gel. For the common garden experiment, seeds were collected from seven wild and four managed populations (Figure 2, Table 1) because mature siliques were not available for two populations.

Plants were sampled by walking a straight line across the patch and collecting the nearest plant at 1 meter intervals. We obtained seed and leaf samples through fieldwork conducted over three trips (a total of six weeks), with plants sampled under Robert Bye's permit for the project "*Conservación de la agrobiodiversidad de la Milpa Tarahumara, Chihuahua.*" Herbarium specimens were collected for each population and deposited at the Wisconsin State Herbarium (WIS) and National Autonomous University of Mexico Herbarium (MEXU). In order to ensure that the project was community-engaged, the authors returned results from the study to Rarámuri community members in a workshop setting in the summer of 2016.

Population genetic analyses. Genotyping-by-sequencing and SNP calling was conducted as in McAlvay *et al.* (2021). Briefly, DNA was extracted using CTAB (Doyle and Doyle, 1987), libraries constructed using the restriction enzyme ApeKI, and fragments sequenced using an Illumina HiSeq 2000 (Illumina Inc. San Diego, CA, United States) at the University of Wisconsin Biotechnology Center (UWBC). We used the GBS 2 pipeline in Tassel 5 (Glaubitz *et al.*, 2014) to process reads and call single nucleotide polymorphisms (SNPs) and Burrows-Wheeler Alignment (Li and Durbin, 2009) to align reads to a reference genome (Wang *et al.*, 2011).

We filtered SNPs using VCFtools (Danecek *et al.*, 2011). We set a minimum mean read depth of 3 to ensure that each SNP was reliably represented in the sequencing data. Only biallelic loci were included to allow for clearer interpretations of genetic variation. We required a minimum of 90% of genotypes to be scored per site, ensuring that each SNP had sufficient representation

Table 1. Populations of *B. rapa* sampled in *Rarámuri* communities in Chihuahua, Mexico. The number of samples per population takes into account the one individual removed from CHO_M2 and one individual removed from REJ_W7 due to low coverage (see SNP and taxon filtering section). Asterisk (*) indicates populations sampled for population genetic analyses but not for the common garden experiment.

POPULATION	COMMUNITY	MANAGED / UNMANAGED	NUMBER OF INDIVIDUALS SAMPLED
RAN_W3	Rancho Blanco	Unmanaged	10
NOR_W2*	Norogachi	Unmanaged	4
BAW_W1	Bahuinocachi	Unmanaged	4
PAN_W6	Panalachi	Unmanaged	11
SAN_W8	San Ignacio	Unmanaged	9
GUM_W5	Gumisachi	Unmanaged	5
REJ_W7	Rejogochi	Unmanaged	9
COC_W4	Cocherare	Unmanaged	8
SAN_M4	San Ignacio	Managed	5
COC_M5	Cocherare	Managed	10
GUM_M1	Gumisachi	Managed	10
CHO_M2	Choguita	Managed	6
PAN_M3	Panalachi	Managed	14

in the dataset (100% was used for PCA to maximize information). A minimum minor allele frequency of 1% was set to filter out rare alleles that may not contribute significantly to the overall population structure.

We then used Tassel 5 (Glaubitz *et al.*, 2014) to exclude sites with more than 50% heterozygosity, as high heterozygosity can indicate potential errors in SNP calling or mixed ancestry. We also removed individuals that had less than 50% of loci scored to ensure that we only included samples with sufficient data. After this filtering process, we retained a total of 33,360 SNPs.

We used Tassel 5 (Glaubitz *et al.*, 2014) to characterize nucleotide diversity (Nei and Li, 1979) and ANOVA to compare differences in nucleotide diversity across groups of populations (managed and unmanaged) with an alpha threshold of 0.05. To assess the genetic structure of managed and unmanaged populations of *B. rapa*, we used fastSTRUCTURE 1.0 (Raj *et al.*, 2014). We tested different group numbers (K) between 1 and 15 with ten replicates at each value. To determine which K value maximized marginal likelihood, we used the ChooseK.py utility included in the fastSTRUCTURE package. fastSTRUCTURE plots were visualized through STRUCTURE PLOT 2.0 (Ramasamy

et al., 2014). To further investigate genetic structure, we used Principal Component Analysis (PCA) in Plink (Purcell *et al.*, 2007) and visualized the resulting ordination using Genesis (Buchmann and Hazelhurst, 2014).

We also evaluated fixation indices (F_{ST}) (Weir and Cockerham, 1984) to investigate genetic differentiation between each pair of populations using Arlequin 3.5 (Excoffier *et al.*, 2005). To assess patterns of population structure across groups of populations (managed and unmanaged) we implemented Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992) in Arlequin 3.5 (Excoffier *et al.*, 2000). To determine genetic distances between populations included in the study, we generated a coalescent tree using SVDquartets (Chifman and Kubatko, 2014) implemented in Paup (Swofford, 2003). SVDquartets is a coalescent-based approach which evaluates quartets of taxa and combines them to infer the most likely tree. We evaluated all possible quartets and produced 100 bootstrap replicates.

Phenotypic analyses. To assess phenotypic differences across populations, we conducted a common garden experiment. To minimize maternal effects due to differing environmental conditions of each population, in the winter of 2016/17 we grew seeds bulked from all maternal plants in each population at the Walnut Street Greenhouses (WSG) at University of Wisconsin–Madison in 6” square plastic pots with Promix HP potting medium (Premier Tech, Rivière-du-Loup, Québec). Supplemental light was provided for 16 hours per day. Pollination bags were used to keep individuals from each population reproductively isolated. Seeds harvested from this initial round of reproduction were used for the common garden experiment. In September 2017, we set up a common garden experiment with the same location, medium, pots, and conditions, but with a randomized block design. We used six blocks, each containing nine flats, which in turn each contained thirteen pots. Into each of the thirteen pots of each flat, we randomly allocated a seed descended from one of the thirteen populations sampled, providing a total of 54 individual plants from each population across the entire experiment.

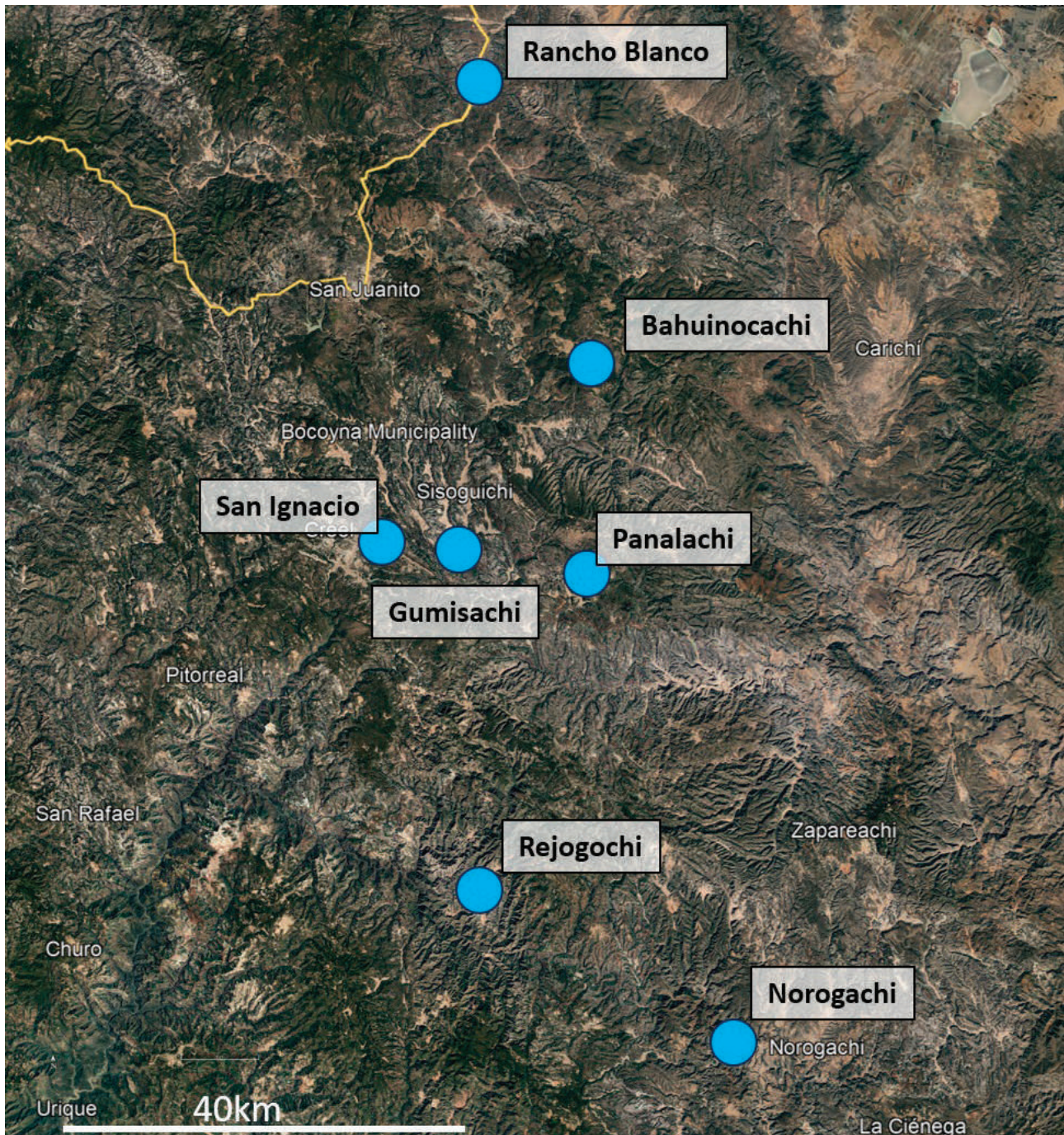


Figure 2. Locations of Rarámuri communities where field mustard populations were sampled for genetic analyses and the common garden experiment. Asterisks indicate populations that were sampled for genetic analyses but not the common garden experiment. The area depicted is bounded by 28.242383, -108.163675 to the northwest and 27.191312, -106.471295 to the southeast. Map data: 2018 Google, INEGI.

To compare the phenology and morphology of managed and unmanaged populations, we measured two phenotypic characters: height from ground to first open flower (cm) along the stem and days to flowering. Height and flowering time were selected as farmers mentioned preference for taller plants with delayed flowering in our

interviews (described in McAlvay, 2018). Measurements of phenology and morphology were analyzed using ANOVA to assess pairwise differences between populations in the same communities and between all managed and unmanaged populations.

RESULTS AND DISCUSSION

Population genetic analyses. After filtering, 333,55 SNPs and 103 individuals remained. One sample from CHO_M2 and one sample from REJ_W7 were removed due to low coverage (<50% of the loci scored). Nucleotide diversity (Table 2) did not follow a consistent pattern of higher diversity in managed or lower diversity in unmanaged populations. In pairwise comparisons between managed and unmanaged populations in the same communities, the results indicated that GUM_M1, PAN_M3, SAN_W3, and COC_W4 had higher diversity than their counterparts. ANOVA demonstrated that differences in nucleotide diversity among managed and unmanaged populations as a whole were not significant ($p = 0.39$).

The fastSTRUCTURE ChooseK function suggested that $K=1$ maximized marginal likelihood and a $K=3$ best explained the structure of the data. At all levels of K , admixture was evident among most populations (Figure 3).

PCA showed patterns like those observed with fastSTRUCTURE. PC1 separated unmanaged samples from Norogachi (NOR_W2), Bahuinocachi (BAW_W1), and Rancho Blanco (RAN_W3) and Cocherare (GUM_M1) from other samples (Fig. 4). PC2 separated managed samples from San Ignacio (SAN_M4) and managed samples from Cocherare (COC_M5) into their own clusters, the latter not overlapping other clusters. Some wild samples from San Ignacio (SAN_W8) were associated with San Ignacio managed samples (SAN_M4).

F_{ST} values (Table 3) ranged from 0.034 between managed and unmanaged populations in Panalachi (PAN_M3 and PAN_W6) to 0.399 between the managed San Ignacio population (SAN_M4) and the unmanaged Gumisachi population (GUM_W5). Significant levels of differentiation were detected between all but one (San Ignacio) pair of managed and unmanaged populations from the same communities.

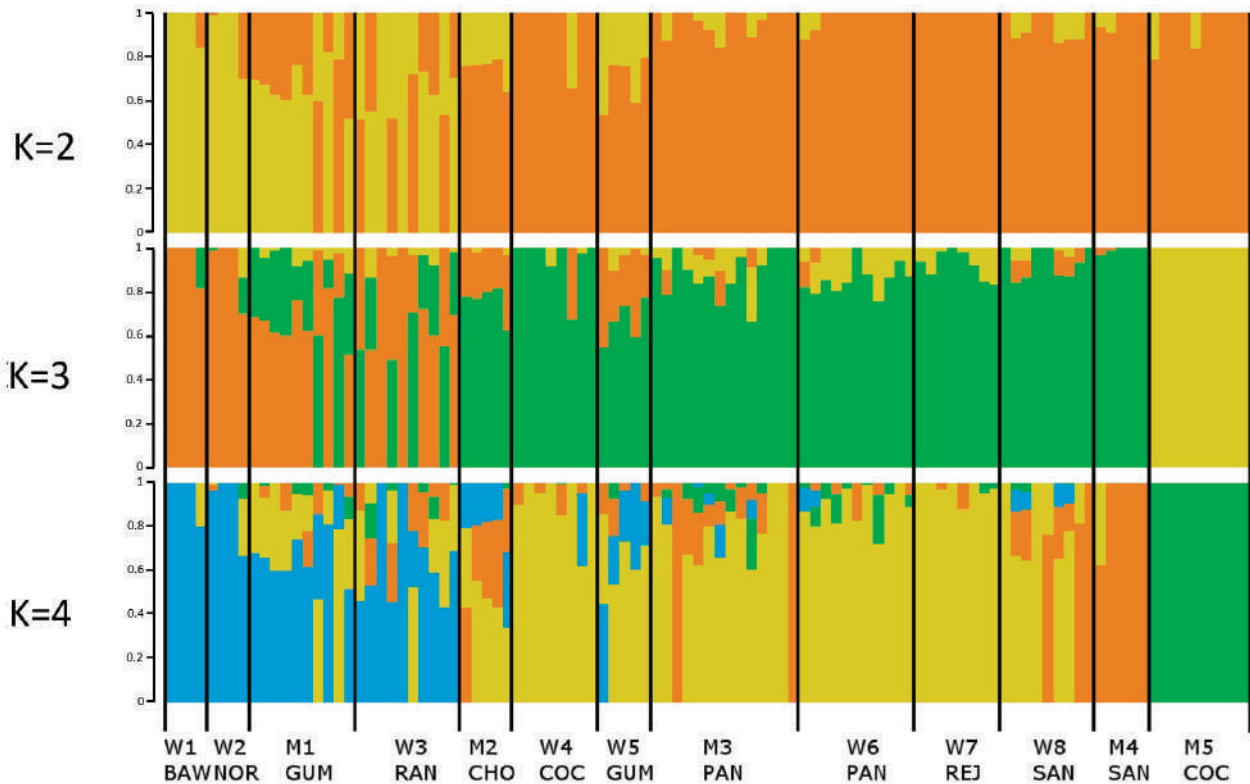


Figure 3. fastSTRUCTURE plot of populations of *B. rapa* from Chihuahua for values of K between 2-4. Each individual sample is represented by a single column, and each population delineated by vertical black lines.

Table 2. Nucleotide diversity of individual populations of field mustard (*Brassica rapa*) in Chihuahua and total nucleotide diversity of all managed versus all unmanaged populations.

POPULATION	π
RAN_W3	0.281
NOR_W2	0.279
GUM_M1	0.276
BAW_W1	0.274
CHO_M2	0.264
PAN_M3	0.259
PAN_W6	0.259
SAN_W8	0.258
GUM_W5	0.257
REJ_W7	0.249
COC_W4	0.247
COC_M5	0.241
SAN_M4	0.24
Group	π
Unmanaged	0.273
Managed	0.267

The SVDquartets analysis (Figure 5) was largely consistent with the PCA and fastSTRUCTURE analysis and clustered unmanaged populations from Bahuinocachi (BAW_W1), Norogachi (NOR_W2), and Rancho Blanco (RAN_W3) and the managed population from Gumisachi (GUM_M1) together. Pairs of managed and unmanaged

populations from Panalchi (PAN_W6 and PAN_M3) and San Ignacio (SAN_M4 and SAN_W8) clustered together whereas other pairs of managed and unmanaged populations (i.e., those from Cocherare and Gumisachi) were not most similar to each other.

Several populations consistently clustered together in our fastSTRUCTURE, SVDquartets, and PCA results. For example, the managed population from Gumisachi consistently groups with RAN_W3, NOR_2, BAW_W1. The managed population from Gumisachi also had high diversity compared to the local unmanaged population (Table 1). These findings are consistent with our interviews (Mcalvay, 2018) with the farmer managing this population, who had a personal seed bank and frequently exchanged *B. rapa* seed with farmers in different communities.

Managed populations from San Ignacio and Cocherare were differentiated from all other groups in fastSTRUCTURE and PCA and had the lowest levels of nucleotide diversity of any populations, suggesting potential effects of anthropogenic isolation or selection. Across all managed and unmanaged populations, there was not a clear trend in nucleotide diversity, which is consistent with the findings of Otero-Arnaiz *et al.* (2005) and Parra *et al.* (2008, 2010) with traditionally managed cacti.

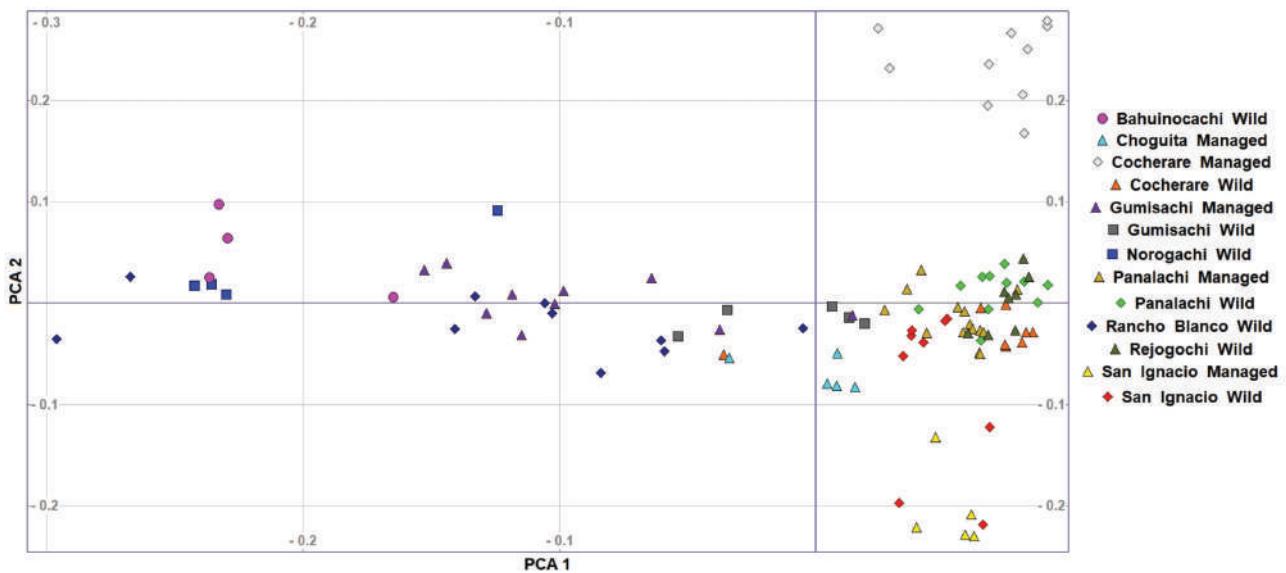


Figure 4. PCA of SNP data for samples of *B. rapa* populations collected in Chihuahua, Mexico.

Table 3. Pairwise F_{ST} values of populations of managed and unmanaged *B. rapa* in Chihuahua, Mexico. Asterisks indicate comparisons in which non-significant p-value (at alpha of 0.05) were recovered (table of p-values not displayed).

	BAW_ W1	CHO_ M2	COC_ W4	COC_ M5	GUM_M1	GUM_ W5	NOR_W2	PAN_ M3	PAN_ W6	RAN_ W3	REJ_ W7	SAN_M4	SAN_ W8
BAW_W1													
CHO_M2	0.190												
COC_W4	0.238	0.129											
COC_M5	0.242	0.175	0.197										
GUM_M1	0.067	0.059	0.132	0.175									
GUM_W5	0.301	0.173	0.179	0.224	0.119								
NOR_W2	0.049	0.142	0.205	0.216	0.035	0.246							
PAN_M3	0.191	0.078	0.082	0.158	0.085	0.135	0.162						
PAN_W6	0.226	0.104	0.101	0.166	0.105	0.182	0.197	0.034					
RAN_W3	0.052	0.047	0.132	0.174	0.0075*	0.119	0.02904*	0.087	0.106				
REJ_W7	0.262	0.127	0.086	0.179	0.120	0.196	0.223	0.070	0.087	0.128			
SAN_M4	0.383	0.190	0.221	0.255	0.177	0.399	0.336	0.160	0.201	0.171	0.248		
SAN_W8	0.193	0.055	0.105	0.179	0.077	0.151	0.174	0.065	0.076	0.075	0.098	0.06605*	

While reductions in diversity are often associated with domestication (Doebley, 1992), management of otherwise wild plants may involve relatively low levels of selection and/or human assisted migration of germplasm from other areas (Parra *et al.*, 2008).

Phenotypic analyses. The mean time from germination to flowering of all populations was 26.61 days ($s = 5.33$). Managed populations (mean = 27.93) had an average of roughly two and a half more days to flowering compared to unmanaged populations (mean = 25.36) at $\alpha = 0.05$ ($p < 0.0001$). Days until flowering (Figure 6) in pairwise comparisons of populations within the same community were significant between PAN_M3 (mean 31.74) and PAN_W6 (mean 24.76) ($p < .0001$) at $\alpha = 0.05$ but not between COC_W4 and COC_M5 ($p = 0.16$). In the case of the populations from Panalachi, the progeny of the managed population flowered a mean of 6.98 days later than unmanaged counterparts ($p < .0001$). The transition from vegetative to reproductive life stages in plants can be an important trait for domestication and artificial selection (Cockram *et al.*, 2007).

The mean height of all populations was 29.51cm ($s = 10.60$). The height of managed and unmanaged populations was not significantly different at $\alpha = 0.05$ ($p = 0.79$). Height (Figure 7) in pairwise comparisons of populations within the same community were not

significant between PAN_M3 and PAN_W6 ($p = .052$) or COC_W4 and COC_M5 ($p = 0.15$). Since height was measured using the standard “height to first open flower”, and Rarámuri interviewees generally expressed preferences for characteristics in the vegetative life stages, this measurement may have not captured farmer induced selection.

Our findings suggest that phenological shifts can be detectable even with relatively limited isolation and reductions in diversity in populations under human management. Since the leaf and stem material of *B. rapa* in its reproductive stage is fibrous and unpalatable to most Rarámuri farmers (Bye, 1979; McAlvay, 2018), and field mustard is capable of rapid bolting (Williams and Hill, 1984), it is not surprising to find evidence for relatively dramatic selection on this trait.

CONCLUSIONS

Phenotypic change with maintenance of high genetic diversity. Ongoing management of local plants should not be teleologically considered a stage in progression to more intensive selection or domestication (Turner *et al.*, 2011) and any ethnographic analogy should be cautiously applied (Currie, 2016). However, these findings provide insight into the domestication of crops, especially those that are thought to have originally

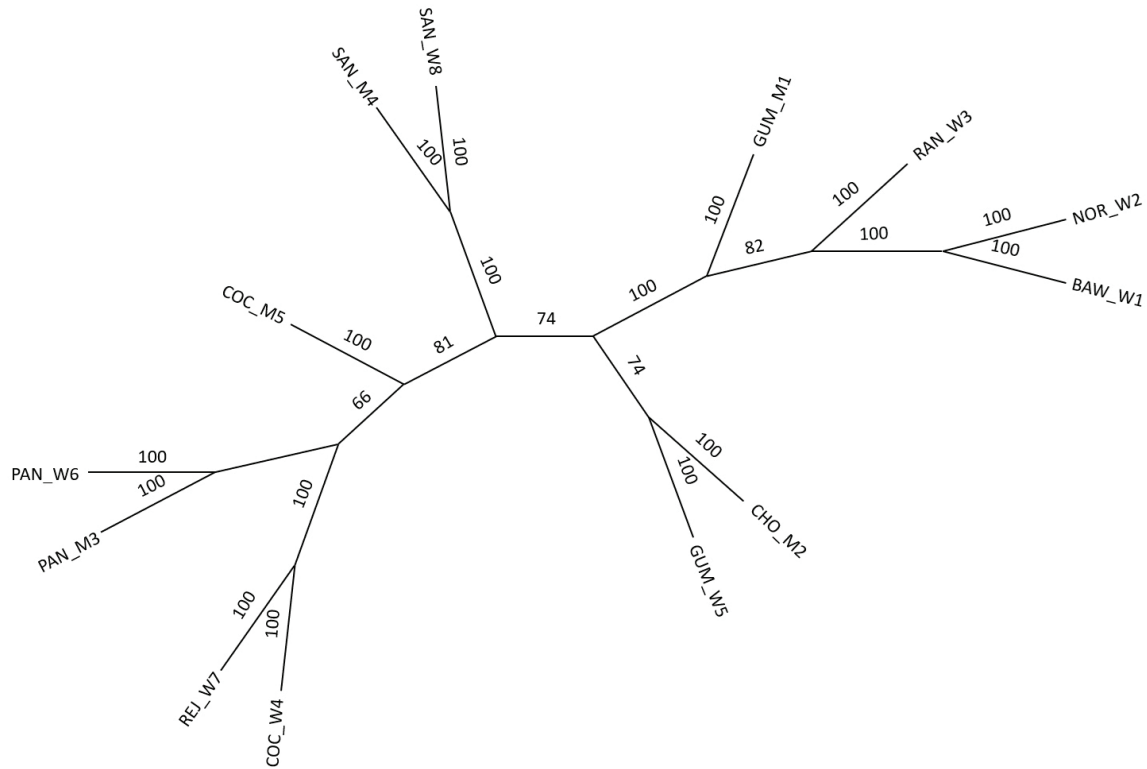


Figure 5. Unrooted Neighbor-joining phenogram of genetic distances between populations of *B. rapa* collected in Chihuahua Mexico recovered by SVDquartets.

existed as weeds in farmers' fields, including rice, sorghum, carrots (Harlan 1992), tomatoes (Gade, 1972), rye, oats (Vavilov, 1926), and lena camelina (Zohary and Hopf, 1994). In the early stages of domestication of carrots, tomatoes, and other weed-derived crops, as in other crops (Dempewolf *et al.*, 2012), substantial levels of gene-flow with sympatric unmanaged forms could have been prevalent, potentially weakening the strength of selection for desirable traits.

Many domestication processes, including *Brassica* crops (An *et al.*, 2019; Mabry *et al.*, 2021), likely occurred on a landscape scale and involved gene flow between populations under human selection, wild populations, congeners, and feral populations (Allaby *et al.*, 2022). The apparent evolution of flowering time differences observed in this study provides insights into a mechanism for the evolution of reproductive isolation in the early stages of domestication, with selection for an extended vegetative period, possibly having the direct consequence of reducing gene flow and facilitating

further selection. While farmers may have mitigated some undesirable gene-flow by growing propagules in isolated plots, the Rarámuri management of field mustard demonstrates that this may have also been accomplished by temporal isolation based on sowing time and phenology.

While this provides insights into historical domestication processes, we emphasize that there are risks in assuming the evolutionary trajectory of managed field mustard toward full reproductive isolation and further dependence on humans. Instead, the balancing of tradeoffs between desirable traits (delayed flowering time) and maintenance of genetic diversity could represent a locally-adapted strategy for management, which avoids the drawbacks of a narrow genetic base, while still benefiting from minor shifts toward preferred phenotypic characteristics. From these findings, we suggest that in certain cases, a “semi-domesticated” state may be seen not as a stage on the way to “full domestication” but instead a fully realized optimal balance.

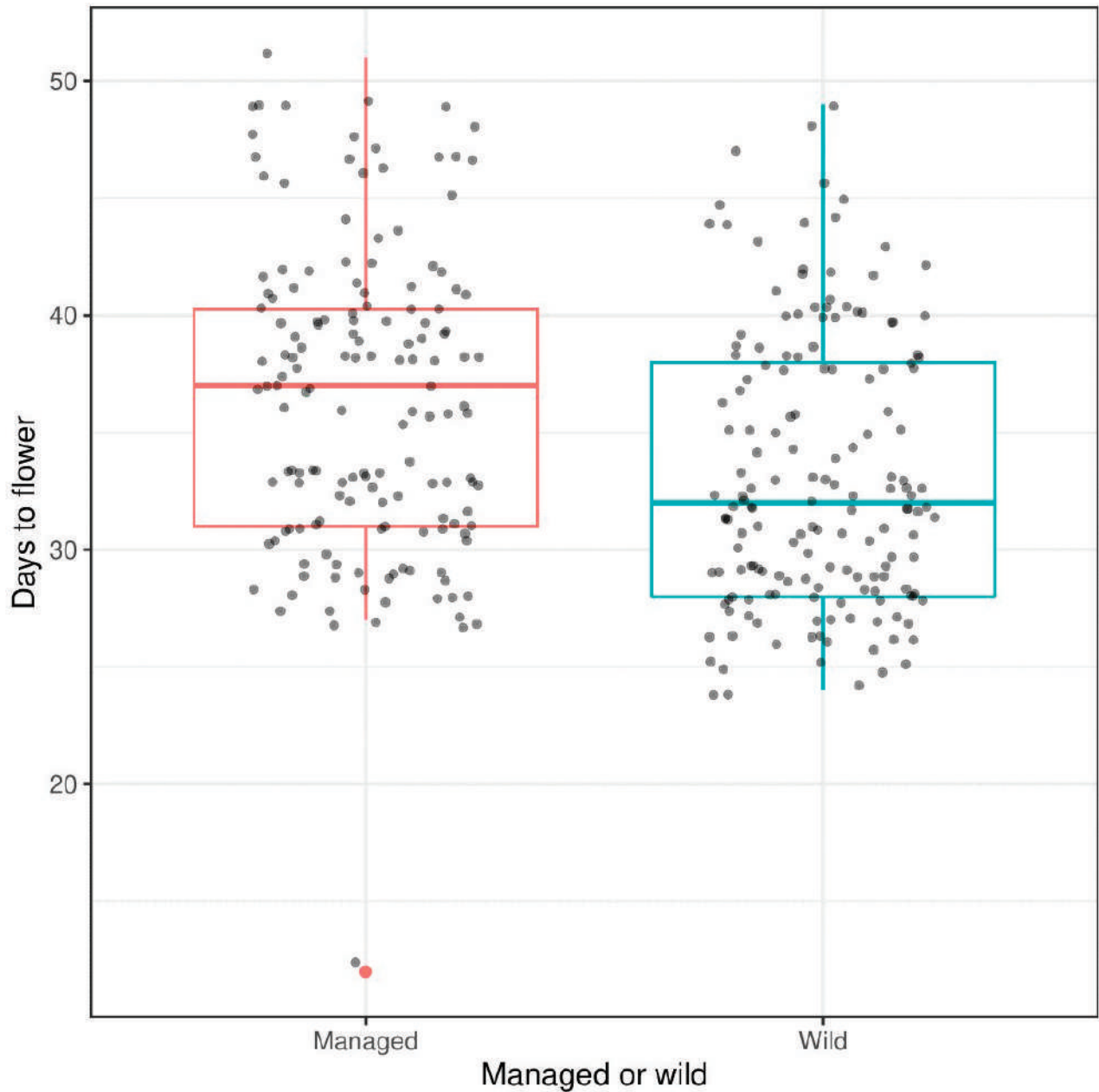


Figure 6. Days from germination of seeds to flowering from populations of *B. rapa* in Chihuahua. Black dots indicate measurements of individual plants.

Redomestication of an escaped crop. Our results raise the possibility of “redomestication” in feral crops. Feral crops may especially lend themselves to redomestication, given their genetic backgrounds, preadaptation to human managed agroecosystems, robustness, and local adaptation (Pisias *et al.*, 2022). This redomestication of feral plants has been proposed as a potential strategy for creating resilient crops in order to address global food security challenges, especially in the face

of climate change, but examples are rare (Pisias *et al.*, 2022; Mabry *et al.*, 2023). One future direction would be the investigation of relict populations of managed *B. rapa* in Argentina, where feral field mustard was briefly cultivated in the early 1900s (Tenenbaum, 1937; Pascale, 1976). These populations may still bear signatures of selection, and could represent an independent parallel “redomestication” for oilseeds from the same species managed by Rarámuri communities for leafy greens.

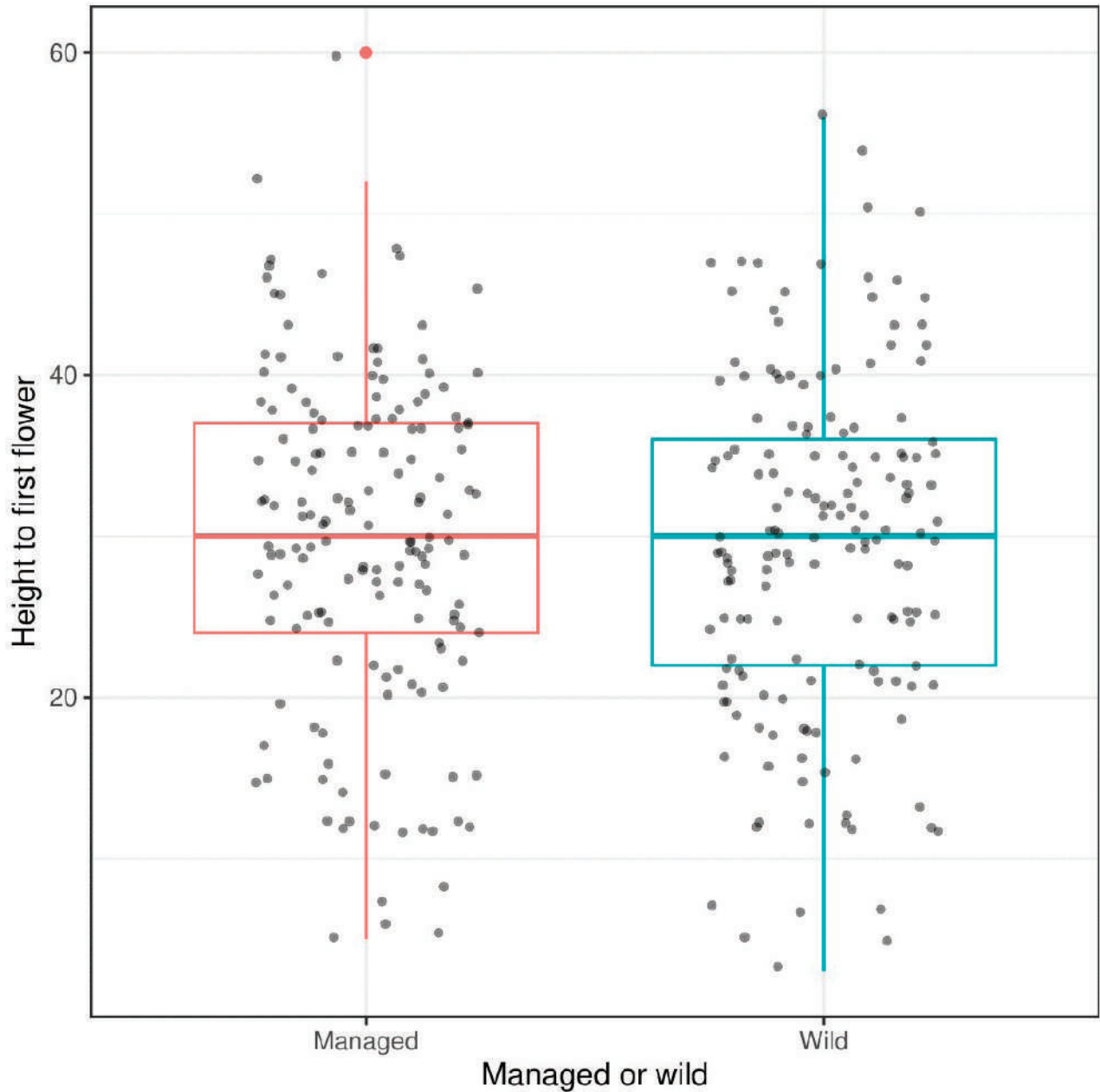


Figure 7. Height of plants to first flower in centimeters from populations of *B. rapa* in Chihuahua. Black dots indicate individual measurements of plants.

Similarly, feral *Brassica rapa* used in the Valley of Mexico for *vaina del canario* (McAlvay, 2018), may be subject to selection pressures for abundant and or large siliques. If selection is involved, the wide range of uses for different organs of *B. rapa* in different parts of Latin America may parallel the process of morphological diversification that occurred in Eurasia thousands of years ago, with selection on leaves, flowering tops, root-hypocotyls, and seeds resulting in dozens of distinct crops such as turnips, bok choy, Chinese cabbage, various oilseeds, and choy sum.

Biocultural heritage and adoption of an introduced plant. In many parts of highland Latin America *B. rapa* has become an important mainstay in local diets, especially in typically food-insecure seasons (Bye, 1979; Vieyra-Odilon and Vibrans, 2001; McAlvay, 2018). In some cases, communities have developed affective relationships with weedy field mustard, and it has become associated with cultural identity, spirituality, and poetry (McAlvay, 2018). If humans have exerted selection on populations of introduced field mustard,

this process represents a form of cultural heritage that is not currently protected by conservation efforts focused on crops or wild plants. Weedy field mustard is designated as an invasive or noxious weed in several countries as it is a weed in over 20 crops in more than 50 countries (Gulden *et al.*, 2018). This may lead policymakers, environmental groups, or others to overlook its importance for those who depend on it for food and encourage it, and thus it may possibly put these groups into conflict with one another (McAlvay, 2018). This issue may become more salient with the spread of aggressively invasive new populations of transgenic herbicide resistant field mustard that have rapidly spread throughout Argentina, and may reach other areas of Latin America. Since its introduction to Argentina in 2012, the transgenic invasive *B. rapa* has spread over more than one million hectares (AAPRESID, 2019).

Our findings suggest that introduced *B. rapa* represents a distinct piece of Rarámuri biocultural heritage. For conservation purposes, the selected populations should be considered in a similar category as traditional crop landraces, or culturally important wild edible plants, which may be included in conservation initiatives like useful plant conservation areas (Kor and Diazgranados, 2023), agroecosystem diversification (Ebel *et al.*, 2024), or germplasm preservation.

Future directions. There are a number of controversies in the study of domestication including the rate (Fuller *et al.*, 2014), intentionality, and drivers of evolution involved (Larson *et al.*, 2014). Despite the availability of numerous techniques to explore contemporary evolution (Merila and Hendry, 2014), only a handful have been applied to ongoing domestication processes. The basic understanding of genetic diversity and structure provided by this study lays the groundwork for future research using this inferentially powerful study system to test hypotheses about evolution under domestication. The genomic resources and short life-cycle of *B. rapa* enable the use of additional techniques and the ongoing nature of management in the area could allow longitudinal studies that address the strength of selection and rate of evolution.

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