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No disruption of rhizobial symbiosis during early stages of cowpea domestication

Gabriel S. Ortiz-Barbosa,[1] Lorena Torres-Martínez,[2] Angela Manci,[1] Sierra Neal,[2] Tarek Soubra,[2] Fizzah Khairi,[2] Jerry Trinh,[2] Paola Cardenas,[2] and Joel L. Sachs[1,2,3,4]

[1]*Department of Microbiology and Plant Pathology, University of California, Riverside, Riverside, California 92521*

[2]*Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, Riverside, California 92521*

[3]*Institute of Integrative Genome Biology, University of California, Riverside, Riverside, California 92521*

[4]*E-mail: joels@ucr.edu*

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Modern agriculture intensely selects aboveground plant structures, while often neglecting belowground features, and evolutionary tradeoffs between these traits are predicted to disrupt host control over microbiota. Moreover, drift, inbreeding, and relaxed selection for symbiosis in crops might degrade plant mechanisms that support beneficial microbes. We studied the impact of domestication on the nitrogen-fixing symbiosis between cowpea and root-nodulating *Bradyrhizobium*. We combined genome-wide analyses with a greenhouse inoculation study to investigate genomic diversity, heritability, and symbiosis trait variation among wild and early-domesticated cowpea genotypes. Cowpeas experienced modest decreases in genome-wide diversity during early domestication. Nonetheless, domesticated cowpeas responded efficiently to variation in symbiotic effectiveness, by forming more root nodules with nitrogen-fixing rhizobia and sanctioning nonfixing strains. Domesticated populations invested a larger proportion of host tissues into root nodules than wild cowpeas. Unlike soybean and wheat, cowpea showed no compelling evidence for degradation of symbiosis during domestication. Domesticated cowpeas experienced a less severe bottleneck than these crops and the low nutrient conditions in Africa where cowpea landraces were developed likely favored plant genotypes that gain substantial benefits from symbiosis. Breeders have largely neglected symbiosis traits, but artificial selection for improved plant responses to microbiota could increase plant performance and sustainability.

KEY WORDS: Africa, bioinoculant, *Bradyrhizobium*, breeding, cowpea, domestication.

Modern agricultural practices and intense selection for yield can degrade plant-microbial symbioses (Porter and Sachs 2020). Breeding practices select aboveground traits, while neglecting belowground plant features, and evolutionary tradeoffs between these traits can disrupt host control over microbiota (Denison 2015). Moreover, the small effective population sizes of domesticated plants, the increased inbreeding, and relaxed selection for traits that are not critical to agriculture (Renaut and Rieseberg 2015; Moyers et al. 2017; Gaut et al. 2018; Marques et al. 2020) can each lead to the degradation of host mechanisms that regulate microbiota (Porter and Sachs 2020). Seminal data from staple crops, such as soybean and wheat, show that root-associated microbiota provide less benefit to modern cultivars when compared to their wild or less-domesticated varieties (Hetrick et al. 1992; Kiers et al. 2007). Differences between crops and their wild relatives can sometimes be directly tied to traits that were favored under artificial selection, such as in maize, where selection for earlier flowering time reduced colonization by arbuscular mycorrhizal fungi (Sawers et al. 2018). In other cases, effects of artificial selection vary with the soil environment. Inoculation of diverse herbaceous crops under phosphorus-rich conditions showed that wild plants are often more responsive to soil mutualists compared to domesticated relatives (Martin-Robles et al. 2018). For legumes, evidence suggests that high soil nitrogen concentrations might reduce the net benefits that host plants receive from symbiosis with nitrogen-fixing rhizobia (Weese et al. 2015).

Legume crops are unique among crops in their capacity to obtain substantial amounts of nitrogen by associating with

rhizobia (West et al. 2002; Gordon et al. 2016). Biological nitrogen fixation (BNF) by rhizobia offers an attractive alternative to chemical-nitrogen fertilization as it comes without fossil fuel costs or polluting by-products. However, the optimization of BNF can be difficult to attain in practice. The main challenge is that legumes encounter a diversity of rhizobial strains that vary in the degree of compatibility and benefits they provide for the host, including ineffective rhizobia that instigate nodule formation but offer little or no fixed nitrogen (Yates et al. 2011; Sachs et al. 2018). To maximize fitness, legumes must invest in rhizobia that provide benefits to the host and defend against ineffective or incompatible strains (Denison 2000; West et al. 2002). Legumes can select some rhizobia during nodule formation, by responding to strain-specific genetic signals (Masson-Boivin and Sachs 2018; Wang et al. 2018). Additionally, plants can choose partners based on signals that indicate qualities of the potential partner (i.e., Partner choice; Simms and Taylor 2002). After nodulation has occurred, legumes can reduce within-nodule proliferation rates of ineffective rhizobia relative to beneficial strains (i.e., postinfection sanctions) (Denison 2000; Kiers et al. 2003; Oono et al. 2011; Regus et al. 2017). However, the prevalence of ineffective rhizobia, both in natural and agronomic soils, suggests either that host mechanisms are unable to extirpate uncooperative genotypes from their local environment or that hosts are encountering strains that are compatible with different host species and are ineffective on the focal host species (Sachs et al. 2018; Gano-Cohen et al. 2020).

Cowpeas (*Vigna unguiculata* L. Walp.) are versatile legumes, grown for their high nutritional value, protein-dense seeds, drought tolerance, and capacity to fix nitrogen with diverse rhizobia (Foyer et al. 2016). Wild cowpeas, categorized as *Vigna unguiculata* subsp. *dekindtiana*, are native to Africa (Ali et al. 2015) and are the progenitor of domesticated cowpea (Coulibaly et al. 2002). Modern cowpea cultivars evolved from two populations of early-domesticated landraces arising in northern and southern regions of Africa, referred to as Genepool 1 and Genepool 2 populations, which are each most closely related to wild cowpeas from the same geographic region (Huynh et al. 2013). These cowpea landraces are consistent with stage two of the four proposed stages of crop domestication (Gaut et al. 2018). During stage two, plants increase the frequency of domesticated alleles through a domestication bottleneck that occurs when cultivation separates domesticated from wild genotypes. However, only in later domestication stages is there geographic radiation of plants into multiple environments (stage three) and expansion of human practices (that might include fertilization, inoculation, etc.), or intensive breeding to maximize yield among locally adapted varieties (stage four) (Meyer and Purugganan 2013; Gaut et al. 2018). Relative to wild cowpeas, these landraces have shifted from outbreeding to self-compatibility, lost seed dormancy and pod dehiscence, flower earlier, and have enhanced seed number and pod size (Pasquet 1996; Singh et al. 1997). Domesticated cowpeas predominantly form nodules with *Bradyrhizobium* and occasionally *Rhizobium* strains (Shamseldin et al. 2017), but no work that we are aware of has examined rhizobial symbiosis in wild cowpeas and it is unknown whether cowpeas can sanction ineffective rhizobia, as has been demonstrated for soybeans (Kiers et al. 2003). Field inoculation of domesticated cowpeas mostly employs *Bradyrhizobium* spp., which can increase shoot biomass, grain yield, percent of nitrogen derived from the atmosphere (%Ndfa), and nodulation, but effects vary widely among experiments (Martins et al. 2003; Zilli et al. 2009; Ulzen et al. 2016; Boddey et al. 2017; Kyei-Boahen et al. 2017; Ulzen et al. 2019; Woliy et al. 2019). Symbiosis traits in crops, that is, host traits that regulate colonization, infection, and fitness gains from microbiota, might be key factors that drive variation in plant performance (Porter and Sachs 2020). To date, breeding programs in cowpea and other legumes have neglected symbiosis traits when selecting parental material.

Here, we investigated how domestication has influenced symbiosis traits in cowpeas. Using eight wild cowpea genotypes and twelve early-domesticated landrace genotypes, we quantified changes in mean trait values and genetic variance associated with clonal and mixed strain inoculation of *Bradyrhizobium diazoefficiens* as well as whole soil inoculation. The 20 cowpea genotypes were selected from a set of 438 cowpea accessions reported in Huynh et al. (2013) and were further genotyped for a genome-wide set of single nucleotide polymorphic sites (SNPs) to test whether the patterns of genetic divergence could predict differences in segregating variation in symbiosis traits between wild and domesticated cowpeas. In a clonal strain inoculation experiment, we used the *B. diazoefficiens* type strain USDA110-ARS and an ineffective mutant on cowpea that was derived from it, USDA110-LI. In a parallel experiment, we inoculated plants with soil rinsates from a California field site where a multiparent intercross population of cowpea genotypes have been propagated for multiple seasons (Huynh et al. 2018). We estimated components of genetic variation and heritability of symbiosis traits when cowpeas are exposed to different inoculation treatments. Our goals were to (i) quantify and compare genetic diversity of wild and domesticated cowpeas, (ii) examine whether symbiosis traits, in particular sanctions or partner choice mechanisms of nonfixing rhizobia, became degraded during the process of domestication, and (iii) measure the heritability of symbiosis traits and their potential to be selected upon in agronomic settings.

Materials and Methods

GENOME-WIDE VARIATION OF COWPEA ACCESSIONS

To examine genetic variation and admixture between wild and cultivated cowpea, we performed a combined analysis of 380 landraces and 58 wild cowpea accessions reported in Huynh et al. (2013) using the 1536-SNP GoldenGate genotyping assay. Huynh et al. (2013) analyzed wild and domesticated genotypes separately, with a focus on geographic origin. To maintain consistency with Huynh et al. (2013), SNPs with a minimum allele frequency (MAF) <0.05 and with a call rate <0.90 were discarded, for a final filtered set of 920 SNPs. Genetic differentiation was evaluated using a principal component analysis (PCA) with the package *adegenet* (Jombart 2008). Admixture and structure were examined using the R package LEA (Frichot et al. 2014; Frichot and François 2015). One to 10 ancestral populations (i.e., entropy criterion; $K = 1$–10) were assumed using 100 repetitions. To test if patterns of genetic diversity differed among populations, a generalized mixed model analysis using SNP loci as our random factor was implemented (Kamvar et al. 2016; Costa et al. 2021). The GLMM with a Beta distribution and a logit link function was modeled using the package glmmTMB (Brooks et al. 2017; Douma and Weedon 2019). Post hoc comparisons based on the model were performed with the R package emmeans (Searle et al. 2012). Population statistics were estimated with the R package *hierfstat* (Goudet 2005).

To have a more robust estimation of the genomic-level variation and relationships among the 20 focal cowpea lines, we further genotyped the wild accessions using the Illumina Cowpea iSelect Consortium Array, screening 51,128 SNPs across the cowpea genome. Domesticated accessions were previously genotyped with the same array (Muñoz-Amatriaín et al. 2017). SNPs with an MAF <0.1 and with a call rate <0.95 were discarded using the R package *snpReady* (Granato et al. 2018), for a final filtered set of 34,762 SNPs. Pairwise genetic distances were estimated with the R package *adegenet* (Jombart 2008) and neighbor-joining was used to reconstruct phylogenetic relationships. Branch support values were evaluated by a bootstrap analysis where SNPs were sampled with replacement 100 times using the *phylo.boot* function of the package *ape* (Paradis and Schliep 2018).

COWPEA GENOTYPES

The eight wild cowpea accessions originate from Botswana (PI632890), Tanzania (PI632876, PI632892), Zimbabwe (PI632891), and Niger (PI632882, PI632879, PI632880, PI632881). The twelve domesticated cowpeas include a population that is largely restricted to northern Africa, with genotypes from Egypt (Tvu-9492), Senegal (Tvu-14346), Benin (Tvu-8834), Nigeria (Tvu-3804), and Niger (Tvu-15591, Tvu-14971; hereafter Genepool 1) and a population from southern Africa, with genotypes from Mozambique (NamuesseD, Nhacoongo-3, Muinana–Lawe), Tanzania (Tvu−1280), Malawi (INIA34), and Zambia (Tvu-13305; Genepool 2; Huynh et al. 2013). Domesticated accessions were only selected from germplasm collections made before 1975. After this year, transfer of cowpea germplasm began between different African breeding programs, causing admixture among accessions (Huynh et al. 2013). Moreover, only landraces with an admixture score <0.01 were selected based on analyses reported in Huynh et al. (2013) to minimize effects of introgression. This threshold was not imposed in the wild genotypes to maintain a full spectrum of the genetic variation segregating within wild populations. Seeds were obtained from the USDA germplasm collection (Griffin, GA).

Bradyrhizobium STRAINS

USDA110 was isolated from soybean in the United States (Kaneko et al. 2002) and is a broadly used inoculant for legume crops (Keyser et al. 1982; Chamber and Iruthayathas 1988; Urtz and Elkan 1996; Musiyiwa et al. 2005). Strains related to USDA110 are found to nodulate cowpea in Africa (Pule-Meulenberg et al. 2010). Most cowpea cultivars respond positively to USDA110 inoculation (Keyser et al. 1982), and it provides substantial nitrogen fixation to cowpeas compared with other rhizobial strains (Yelton et al. 1983; Chamber and Iruthayathas 1988). USDA110-ARS (hereafter, Fix+) is a spontaneous mutant of USDA110 arising from antibiotic selection on azide (10 μg mL^{-1}), rifampicin (500 μg mL^{-1}), and streptomycin (1000 μg mL^{-1}; Kuykendall and Weber 1978) that was confirmed to efficiently fix nitrogen on six genotypes of soybeans (Kiers et al. 2007). USDA110-LI (hereafter, Fix-) was also a spontaneous mutant of USDA110 originally isolated from soybean nodules based on colony morphology with white, opaque mucoid colonies formed on modified yeast mannitol medium (YM) and a five- to 10-fold reduced efficiency at fixing nitrogen measured by acetylene reduction assay (Kuykendall and Elkan 1976). Strains were obtained from the USDA National Rhizobium Germplasm Resource Collection (Beltsville, MD).

INOCULATION EXPERIMENTS

Seeds were surface sterilized in bleach (5% sodium hypochlorite), rinsed in sterile ddH_2O, scarified, and planted in bleach-sterilized 1-gallon plastic pots containing an autoclave-sterilized 50:50 mix of silica sand and limestone flour silica sand, which contains negligible nutrients to support plant growth (Regus et al. 2015). Three seeds were planted per pot from June 13, 2018 to June 15, 2018. On June 21, 2018, seedlings were thinned to one plant per pot to size match the remaining seedlings among plant lines. One day later, rhizobial inoculation followed. Greenhouse

temperatures averaged 86°F ± 14°F (standard error, SE) and relative humidity was 55% ± 20%.

For the clonal strain experiment, Fix+ and Fix– strains were plated on a modified arabinose gluconate medium (MAG; Sachs et al. 2009) and a single colony per strain was spread onto 8-10 plates to generate dense lawns. After 7 days of growth, the cells were washed from the plates into liquid MAG media and cell concentrations were quantified by colorimetry. Liquid cultures were centrifuged at ∼750 × *g*, spent media was removed, and the cells were resuspended in sterile ddH_2O at a concentration of 1×10^8 cells mL^{-1}. Plants were inoculated with either 5 mL of the Fix+ or Fix– clonal *Bradyrhizobium* cells (single inoculation, 5×10^8 cells), 5 mL of a mixture comprising equal concentrations of both strains (co-inoculation, 2.5×10^8 cells of each strain), or 5 mL sterile ddH_2O as a control.

To investigate variation in symbiosis traits when hosts were exposed to an intact microbial community, we performed a soil inoculation experiment. Field soil was sampled from the University of California Riverside Agricultural Experiment Station at four sites within a 5-acre field where diverse cowpeas are propagated (coordinates: 33.967, −117.339; Huynh et al. 2018). The field has a history of cultivating cowpea during odd-numbered years, starting in 2003. Additionally, the field is intercropped with barley and occasionally with other legume crops such as soybean and pigeonpea. The field has not been inoculated with any rhizobia. Soil was passed through a sterilized 2-mm sieve (6 L per site), and apportioned into aliquots of 400 g. From each sample, 400 mL of sterile water was added, the sieved soil was shaken vigorously, filtered twice through eight layers of sterile cheesecloth, and the filtered supernatants were pooled into sterile flasks, which were allowed to settle overnight at room temperature. This method allows us to inoculate plants with a diverse community of microbes from the supernatant, and to avoid adding sediments to the inoculated plants that could change the soil texture and chemical makeup (Unkovich and Pate 1998). The supernatant from each flask was divided into two equal portions, one of which was autoclaved and allowed to cool to serve as a negative control, whereas the other was reserved at room temperature and used for inoculation. Seedlings were inoculated with 10 mL of each microbial inoculum (alive or dead) and each one was separately plated (100 μL) in MAG and incubated at 29°C for 8 days to confirm high densities of slow growing bacteria such as *Bradyrhizobium*.

In both experiments, plants were fertilized weekly by applying 10 mL of Jensen's solution with 1 g L^{-1} $K^{15}NO_3$ (2% ^{15}N by weight), which includes all the necessary micronutrients (Somasegaran and Hoben 1985) and a minimal concentration of nitrogen to support cowpea growth. Plant genotypes and inoculation treatments were randomly arranged within blocks in the greenhouse with five plant replicates per inoculation treatment × plant genotype combination, except for controls that had three replicates. The clonal strain experiment had 360 plants, including 300 that were inoculated (20 lines × 3 inoculation treatments × 5 replicates) and 60 control plants (20 lines × 3 replicates). The soil inoculation experiment had 160 plants, including 100 that received the live inoculum (20 lines × 5 replicates) and 60 that received the autoclaved control (20 lines × 3 replicates).

PLANT HARVEST AND NODULE CULTURING

Harvest occurred from July 30, 2018 to August 3, 2018 and from August 13, 2018 to August 23, 2018 because of the time needed to carefully wash roots, and dissect and culture nodules, as described below. Plants were removed from pots, washed free of sand, and dissected into root, shoot, and nodule portions. Nodules were counted and photographed. Rhizobia were sub-cultured from nodules of co-inoculated plants to differentiate Fix+ and Fix– strains. Nodules were surface sterilized and subsequently crushed and streaked on solid MAG media. Isolated colonies were subcultured on MAG with rifampicin (500 μg mL^{-1}) and streptomycin (1000 μg mL^{-1}), selecting for Fix+, and YM media, on which Fix– exhibits fast growth and slimy appearance. Five nodules each from three co-inoculated plants per genotype were randomly picked and assessed (∼15 nodules per genotype, 268 total). From each nodule, ∼50 colonies were counted to estimate the proportion of Fix+ to Fix– strains (11,586 colonies in total).

Leaf ^{15}N "atom percent difference", a measure of nitrogen fixation (Regus et al. 2014), was estimated as the percentage of ^{15}N atoms over total nitrogen in each sample (Unkovich et al. 2008). The $\delta^{15}N$ of each sample was calculated by comparing ^{15}N abundance expressed as parts per thousand relative to atmospheric N_2; these values were used to compare among plants inoculated with Fix+ and Fix– strains following the formula:

$$\delta^{15}N\% = \frac{\text{sample atom}\%^{15}N - 0.3663}{0.3663} \times 1000.$$

To calculate these values, individual leaves of each plant were oven dried, powdered using steel bead beaters at 14,000 rpm, and 4 mg per plant was transferred into individual tin capsules, including four replicates per genotype for the Fix+, Fix–, and two replicates for control inoculation treatments (178 samples total). Isotopic analyses were performed at the UC Davis Stable Isotope Facility.

TRAIT DATA ANALYSIS

Size comparisons among wild and domesticated populations were performed by calculating scale free measurements to minimize effects of initial seedling size. Investment into symbiosis was calculated by dividing the dry nodule biomass of each plant

over the total biomass. Host growth response was calculated by subtracting the mean biomass values (i.e., shoot, root, and nodules) of the control plants within a population from the inoculated plants belonging to the same group, dividing by the control value, and multiplying the quotient by 100 (Regus et al. 2015). Means per population were calculated for plants harvested during the same week to account for variation in days post inoculation.

$$\text{Host growth response}\% = \frac{\text{Total biomass inoculated plant}_i - \text{Mean biomass controls}_j}{\text{Mean biomass controls}_j} \times 100, \quad (1)$$

where i indicates plant replicate and j indicates population mean value.

Dry nodule biomass values of co-inoculated plants (where a subset of nodules was used for subculturing) were inferred by generating a wet-to-dry nodule weight linear regression (per genotype). To test for postinfection sanctions, a binomial test was used to evaluate whether nodule occupancy of Fix+ deviated from the null expectation of 50% given that the strains were inoculated in equal proportions. Results were analyzed independently for each genotype tested.

Linear mixed models (LMMs) were used to analyze differences in symbiosis traits among the three populations defined by Huynh et al. (2013), that is, Genepool 1, Genepool 2, and wild cowpeas (three-population analysis). However, because landraces of Genepools 1 and 2 are each most closely related to wild cowpeas from the same region (Huynh et al. 2013), we also analyzed comparisons that divided the wild cowpeas into southern Africa populations (PI632890, PI632876, PI632892, PI632891; i.e., Wild-1) and northern Africa populations (PI632882, PI632879, PI632880, PI632881; i.e., Wild-2, four-population analysis). Inoculation treatment and population were treated as fixed effects, cowpea genotype and genotype × treatment interactions were treated as random effects, and days postinoculation was used as a covariate. Response variables were transformed if necessary to improve normality. Analyses were performed using The R project for Statistical Computing version 3.6.1 (R Core Team 2020).

COMPONENTS OF TRAIT VARIATION

Independent LMMs were constructed to estimate the components of variation in each symbiosis trait under the clonal inoculation treatments, where genotypic effects could be best isolated. Models of variance-covariance structure were used to test whether the expression of additive genetic variance (σ^2_a) in each symbiosis trait varied among treatments, or among the wild and domesticated populations (three-population analysis), and if the expression of σ^2_a in populations varied among treatments. Because of limited sampling of plant genotypes, it was not practical to conduct this specific analysis using the four-population approach. The variance-covariance matrix for the genotype effect known as the additive relationship matrix was estimated from the SNP data with the *A.mat* function in *sommer* (Covarrubias-Pazaran 2016). To test if the additive genetic variance in the trait of interest varies among the levels of the factor of interest (treatment, population, population × treatment), a model where the among-genotype variance was constrained to be the same across levels was compared with a heterogeneous variance structure model (Table S1). Differences in the expression of genetic variance were assessed using log-likelihood tests among models (Shaw 1991). Breeding values of each genotype were estimated by best linear unbiased prediction (BLUP) (Bauer et al. 2006; Liu et al. 2008; Piepho et al. 2008), taking into account the additive relationship matrix among genotypes (genomic BLUPs or GBLUPs). Narrow-sense heritability (h^2) was estimated as the proportion of additive variance of two alleles at a locus over the phenotypic variance ($h^2 = V_A/V_P$) (Bernardo 2020). Analyses were performed in the R package *sommer* (Covarrubias-Pazaran 2016).

Genetic correlations among traits were estimated following Falconer and Mckay (1996) and implemented by Etterson and Shaw (2001) and Saxton (2004), where the correlation between any pair of traits i and j, r_{Aij}, was estimated as follows, where COV_{Aij} is the covariance between an individual's breeding value for one trait and its breeding value for the other trait:

$$r_{Aij} = \frac{COV_{Aij}}{\sqrt{V_{Ai} - V_{Aj}}},$$

where V_{Ai} is the genetic variance of trait i and V_{Aj} is the genetic variance of trait j. To estimate the genetic correlation between traits, we performed multi-trait and multi-environment LMMs (Covarrubias-Pazaran 2016) with treatment, population, and days since inoculation as fixed factors, and cowpea genotype as random effect.

Results

GENOME-WIDE VARIATION IN WILD AND DOMESTICATED COWPEA POPULATIONS

Both the three- and four-population analyses (i.e., genetic clusters) were supported by the entropy criterion in LEA (i.e., $k = 3$, $k = 4$; 1536-SNP assay; Figs. 1 and S1). Many domesticated accessions maintain substantial ancestry from wild cowpeas (i.e., admixed cowpeas); however, domesticated accessions from either of the two Genepools defined by Huynh et al. (2013) exhibit less evidence of admixture with wild cowpeas (Fig. 1), consistent with breeding under crop production (Gaut et al. 2018). Genepools 1 and 2 were more divergent between them ($F_{ST} = 0.18$ [0.17-0.19]) than with the wild population (Genepool 1 vs. wild: $F_{ST} = 0.13$

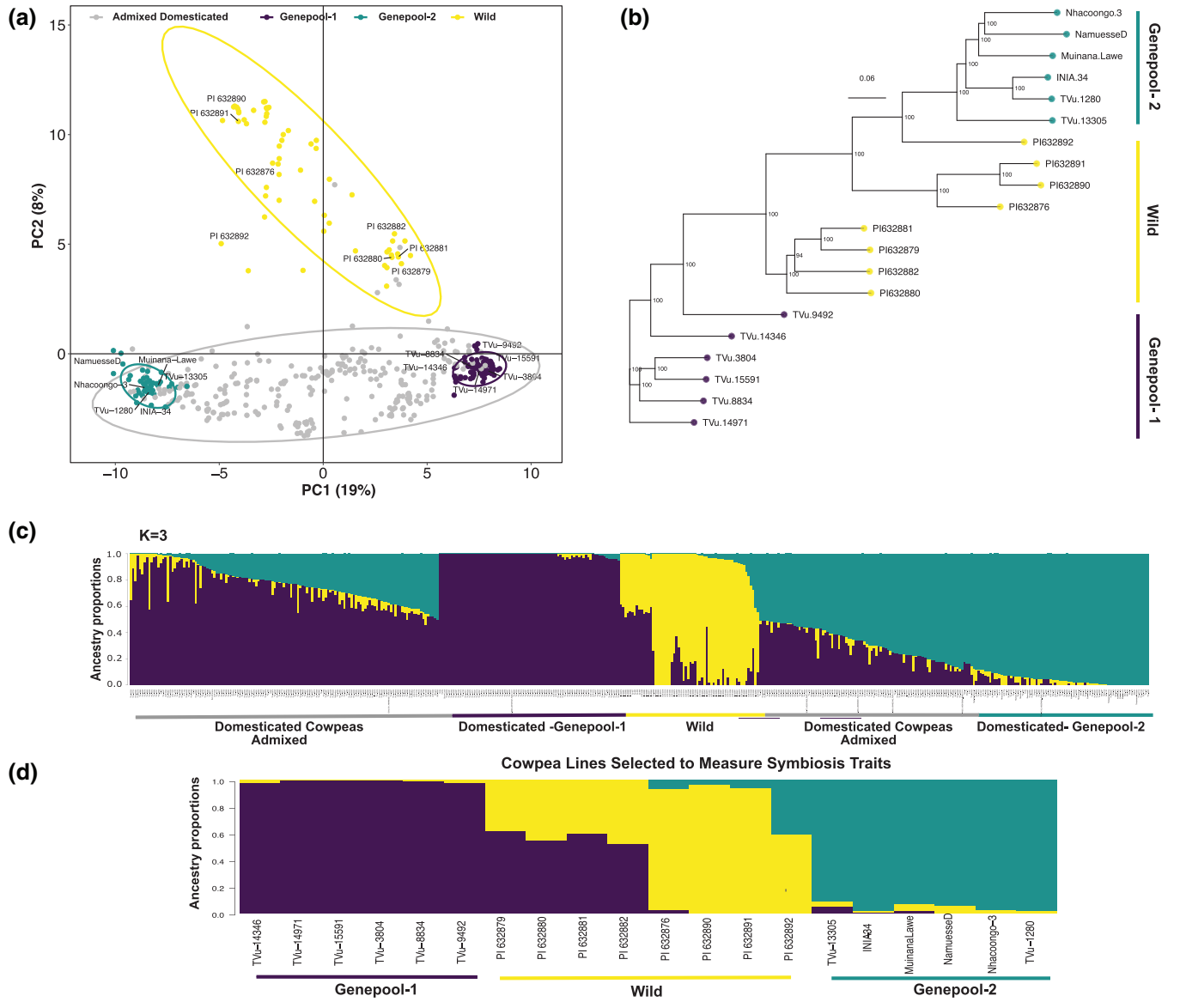

Figure 1. **Patterns of genetic differentiation in wild and domesticated Cowpeas. (a) Principal component analysis (PCA) showing patterns of genetic clustering among domesticated and wild cowpea genotypes sampled by Huynh et al. (2013) and from which 20 genotypes were selected for analysis of symbiosis traits (dots with labels; see Supporting Information for details). Purple and green dots represent accessions that were defined as representatives of Genepool 1 and Genepool 2, respectively, based on low admixture (<0.01; Huynh et al. 2013), and the remainder genotypes are gray. (b) Unrooted neighbor-joining tree of the 20 selected cowpea genotypes, indicating that Genepools 1 and 2 taxa are each most closely related to wild cowpeas from the same geographic region. (c, d) Ancestry proportions of cowpea accessions derived from sparse nonnegative matrix factorization algorithm (sNMF) using the cowpea genotypes and SNP genotyping sampled by Huynh et al. (2013) (see Supporting Information; Fig. S1). Results are presented when $k = 3$ to indicate the three populations presented by Huynh et al. (2013) (c) and for the 20 selected genotypes (d). Most landraces maintain substantial ancestry from wild cowpeas (i.e., admixed cowpeas), whereas landraces from either of the two defined Genepools exhibit less evidence of admixture with wild cowpeas.**

[0.13-0.14]; Genepool 2 vs. wild: $F_{ST} = 0.12$ [0.10-0.12]), supporting previous findings that suggested two separate domestication events and the maintenance of allelic variation from wild cultivars in these two distinct pools of domesticated accessions (Huynh et al. 2013; Muñoz-Amatriaín et al. 2017). Phylogenetic analysis of the twenty accessions genotyped with a larger set of SNPs (51,128-SNP assay; Fig. 1) supported the hypothesis that Genepools 1 and 2 are each most closely related to wild cowpeas from northern Africa (PI632882, PI632879, PI632880, PI632881) and southern Africa (PI632890, PI632876, PI632892, PI632891), respectively. These data are consistent with divergent subsets of wild germplasm being carried to

northern and southern regions of Africa during waves of human migration, with modest degrees of gene flow between them (Huynh et al. 2013; Muñoz-Amatriaín et al. 2017).

The domesticated populations experienced a modest but significant reduction in gene diversity (H_s; ~6.25%) relative to the wild cowpeas (i.e., three-population analysis; H_s: $\chi_3{}^2 = 12{,}636$, $P < 0.01$). H_s was significantly different among all three populations (Table S2), whereas heterozygosity (H_o) was only significantly different between Genepool 2 and the wild cowpeas ($t = 1.56$, $P < 0.01$; Table S2). When the wild cowpeas were separated in two distinct groups (i.e., four-population analysis), H_o was not significantly different between the wild population and the two domesticated populations (Table S3), whereas H_s was significantly different among most populations except between Genepool 1 and the wild population from southern Africa ($t = -1.389$, $P = 0.5063$; Table S3).

GENOTYPIC VARIATION IN SYMBIOSIS TRAITS

Nodulation of cowpea genotypes

The domesticated cowpea populations were more responsive to inoculation, forming more nodules and varying more between treatments (Fig. 2). In the clonal strain experiment, the wild genotype PI632891 formed nodules in only ~50% of inoculated plants, whereas the wild genotype PI632890 did not form any nodules in any treatment. All other genotypes formed nodules in at least 70% of inoculated replicates (mean = 95.2% ± 2.79%; Table S4). None of the control plants formed any nodules. Moreover, both domesticated populations formed significantly more nodules than the wild cowpeas (mean nodule counts: wild, 8.55 ± 0.82; Genepool 1, 119.7 ± 12.72; Genepool 2; 142.8 ± 11.52; Table 1), but there was no difference between the domesticated populations. The same trend was observed for the soil inoculation experiment (wild, 18.87 ± 2.07; Genepool 1, 119.38 ± 9.19; Genepool 2, 140.6 ± 8.86; $t_{17} = 5.77$; $P \leq 0.001$; Fig. 2; Table S5).

Domesticated cowpea populations formed more nodules in the Fix+ treatment relative to Fix–. For Genepool 1, both the Fix+ and the co-inoculation treatments formed significantly more nodules than the Fix– treatment (Fix+, 135.6 ± 17.1; co-inoculation, 179.8 ± 23.2; Fix–, 39.26 ± 9.25; Table S6). For Genepool 2, the same pattern was found (Fix+, 167.48 ± 19.04; co-inoculation, 182.8 ± 23.02; Fix–, 79.03 ± 10.54; Table S6). For the wild cowpea genotypes, there was no significant differences in the number of nodules formed when comparing Fix+ and Fix– inoculations (Table S6).

Investment

In the clonal strain experiment, domesticated cowpea populations invested a higher proportion of plant biomass into nodules than the wild cowpeas (wild cowpeas, 0.007 ± 0.0008; Genepool 1, 0.02 ± 0.001; Genepool 2, 0.02 ± 0.001), but there was no difference between the domesticated populations (Fig 2; Table S5). These differences were not seen in the soil inoculation experiment (wild cowpeas, 0.0341 ± 0.003; Genepool 1, 0.0303 ± 0.001; Genepool 2, 0.0362 ± 0.003; Table S5).

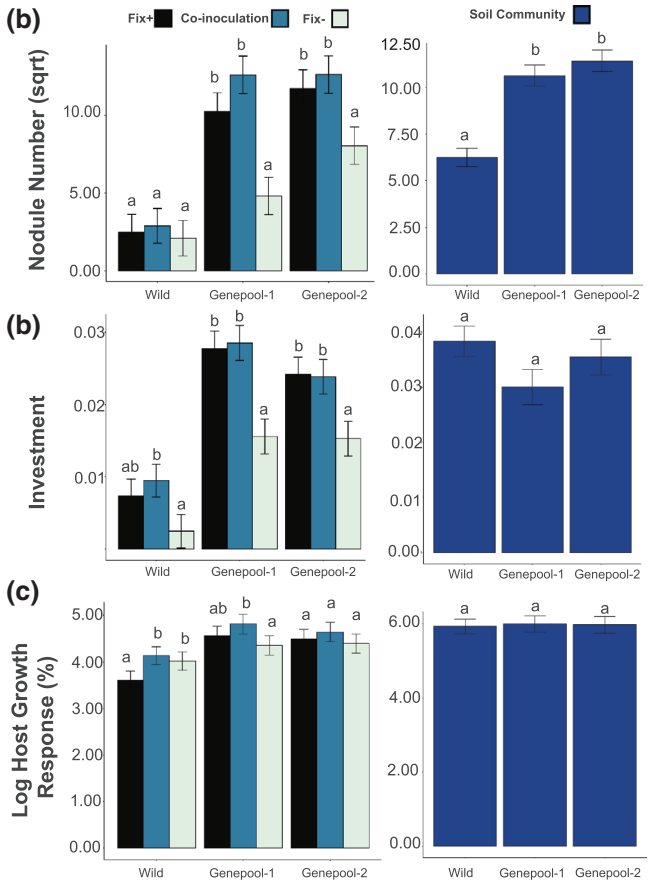

Figure 2. Least-square means of symbiosis trait values of wild and domesticated cowpeas under different inoculation treatments. (a) Least-square mean of transformed nodule counts, (b) least-square mean of investment, and (c) least-square mean of the logarithm of host growth response (%). The black bars represent plants that were inoculated with the Fix+ strain, blue bars represent plants Co-inoculated with the Fix+ and the Fix– strains, and light green bars represent plants inoculated with the Fix– strain. Dark blue bars represent a separate experiment testing soil community inoculum. Standard errors above and below the means are indicated for each group. Connecting letters report statistically significant differences among treatments within each of the Genepools using Tukey's post hoc tests.

Mean nodule biomass

In the clonal strain experiment, wild cowpeas formed nodules that were 1.4 ± 0.3 mg on average, whereas Genepools 1 and 2 produced higher and lower values, respectively (1.8 ± 0.2 mg; 0.9 ± 0.1 mg), but no significant differences for mean nodule

Table 1. LMMs testing the differences on plant traits among wild and landrace populations of cowpea genotypes inoculated with USDAI-110 ARS (Fix+) and USDA110 L1(Fix–), co-inoculated with an equal proportion of both and a soil community experiment. * P < 0.05, ** P < 0.01, *** P < 0.001.

	Sqrt number of nodules			Log_{10} dry nodule biomass		Investment		Log10 Host growth response (%)		δ15N			Log10 Mean nodule weight	
Single inoculation														
Fixed effects	χ^2	df	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	df	*P*	χ^2	*P*
Harvest day	14.31	1	0.0001	65.39	<0.001***	1.32	0.2493	69.7	<0.001***	8.45	1	0.003**	5.32	0.021*
Population	38.1	2	<0.001***	32.75	<0.001***	60.06	<0.001***	8.18	0.016*	3.57	2	0.16	6.83	0.03*
Treatment	133.18	2	<0.001***	52.26	<0.001***	61.29	<0.001***	14.3	<0.001***	33.22	1	<0.001***	0.17	0.67
Population × Treatment	60.8	4	<0.001***	13.88	0.007**	5.81	0.21	9.81	0.04*	0.92	2	0.63	10.32	0.005**
Random effects														
Line	117.73	1	<0.001***	24.95	<0.001***	13.57	0.0002***	46.8	<0.001***	6.22	1	0.012*	0.29	0.5842
Treatment:Line	29.33	5	<0.001***	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Soil community														
Fixed effects	χ^2	Df	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	Df	*P*	χ^2	*P*
Harvest day	0.072	1	0.7873	9.31	0.002**	11.55	0.0006***	17.2	<0.001***	NA	NA	NA	6.07	0.01*
Population	56.21	2	<0.001***	24.14	<0.001***	3.88	0.14	0.05	0.97	NA	NA	NA	8.78	0.01*
Random effects														
Line	9.82	1	0.001**	1.84	0.173	0	0.99	4.07	0.04*	NA	NA	NA	19.5	<0.001***

Table 2. Components of variation and estimates of heritability for three symbiosis traits under the three inoculation treatments.

Trait	Treatment	V_A	SE	V_P	SE	h^2	SE
Host growth response	Fix+	0.20	0.04	0.88	0.15	0.24	0.04
	Co-inoculation	0.09	0.03	0.50	0.09	0.19	0.05
	Fix–	0.03	0.02	0.34	0.08	0.09	0.05
Number of nodules	Fix+	15.94	5.93	77.90	25.78	0.32	0.04
	Co-inoculation	22.10	8.12	57.92	16.79	0.38	0.04
	Fix–	4.77	2.07	42.61	11.58	0.11	0.04
Investment	Fix+	0.0000	0.0001	0.0010	0.0002	0.00	0.12
	Co-inoculation	0.0000	0.0001	0.0010	0.0002	0.00	0.11
	Fix–	0.0007	0.0004	0.0019	0.0004	0.37	0.13

biomass were found among the three populations (Table S5). Only the wild cowpeas had significant differences between Fix+ and Fix– treatments, with Fix+ inoculated plants producing nodules that were almost twice the mean mass (∼2.1 mg) of those on Fix- plants (∼1.3 mg; $t_{41} = 2.189$, $P = 0.034$; Table S7). Under the Fix+ treatment, wild genotypes formed bigger nodules on average than Genepool 2 (Table S5). Under the Fix– treatment, Genepool 1 formed bigger nodules than wild genotypes and Genepool 2 (Table S5). In the soil community experiment, there were no significant differences among the cowpea populations for mean nodule biomass (Table S5).

Host growth response and nitrogen fixation

In the clonal strain experiment, growth response to inoculation varied significantly between wild and domesticated cowpea populations (Table 1). The domesticated populations showed consistently higher values for host growth response to inoculation when the Fix+ strain was present (Fix+ and co-inoculation), whereas wild cowpeas showed the lowest host growth response values for single inoculation with the Fix+ strain (Table S7). In the soil inoculation experiment, there was no significant difference in host growth response values between wild cowpeas and the domesticated populations (Table 1). There were significant treatment effects of the Fix+ versus Fix– treatments on nitrogen fixation ($\delta^{15}N$; $\chi^2_{(2)} = 33.22$, $P \leq 0.001$; Table 1). Under the Fix+ treatment, wild cowpeas had $\delta^{15}N$ values of 833.81 ± 54.23, Genepool 1 obtained 641 ± 64.21, and Genepool 2 had 643.17 ± 62.65, whereas for the Fix– the values were higher in all cases (i.e., *less* nitrogen fixation), consistent with a significant reduction of nitrogen fixation with the Fix– strain (wild, 1052.33 ± 71.15; Genepool 1, 960.38 ± 62.67; Genepool 2, 887.94 ± 53.73; Table S7).

Four-population analysis

There were no significant differences among the wild cowpeas from northern and southern Africa for nodule number, investment into symbiosis, and nodule biomass (Tables 1 and S8). Among the traits measured, we only found differences in the mean nodule biomass values for the soil community, where nodule size for the Wild-1 population was significantly different from both domesticated populations ($t_{16} = -3.4$, $P = 0.01$; Table S9), but it was not different among domesticated and Wild-2. Previously reported differences and patterns among wild and domesticated populations were consistent with the three-population analysis for all other traits (Figs. S3 and S4).

HERITABILITY AND POTENTIAL FOR SELECTION

A significant genetic variation component was observed for some of the symbiosis traits tested (Table 2). Moderate levels of heritability were observed for the number of nodules ($h^2 = 0.32 \pm 0.12$) and host growth response ($h^2 = 0.23 \pm 0.09$); however, heritability was very low for investment ($h^2 = 0.09 \pm 0.07$).

Heritability for host growth and the number of nodules varied among inoculation treatments (Table 2) and between the wild cowpeas and domesticated populations (Table 3). For host growth, the expression of additive genetic variation (σ^2_a) was highest in the Fix+ treatment ($\chi^2 = 9.428$, $P < 0.01$; Table S1), whereas for the number of nodules it was highest under the co-inoculation treatment ($\chi^2 = 24.20$, $P < 0.01$; Table S1), suggesting that selection could shape both nodulation and symbiotic benefits. Higher σ^2_a value for host growth response was observed in the wild cowpeas, relative to the domesticated Genepools ($\chi^2 = 19.62$, $P < 0.01$; Tables 3 and S1), whereas for the number of nodules σ^2_a was higher in the domesticated Genepools ($\chi^2 = 41.69$, $P < 0.01$; Tables 3 and S1), suggesting that domestication has affected these symbiosis traits in opposing ways. The expression of σ^2_a in host growth and number of nodules also varied among cowpea populations depending on the inoculation treatment imposed ($\chi^2 = 51.37$, $P < 0.01$; $\chi^2 = 70.74$, $P < 0.01$; Tables 4 and S1). The additive genetic variation in

Table 3. Components of variation and estimates of heritability for three symbiosis traits for the three populations tested.

Trait	Population	V_A	SE	V_P	SE	h^2	SE
Host growth response	Genepool 1	0.06	0.05	0.33	0.06	0.18	0.12
	Genepool 2	0.1	0.07	0.42	0.09	0.23	0.13
	Wild	0.15	0.11	0.86	0.16	0.17	0.11
Number of nodules	Genepool 1	3.12	2.55	21.04	3.74	0.15	0.11
	Genepool 2	6.3	4.34	17.32	4.64	0.36	0.16
	Wild	0.14	0.12	1.38	0.23	0.1	0.08
Investment	Genepool 1	0.0001	0.0001	0.0015	0.0003	0.03	0.07
	Genepool 2	0.0004	0.0002	0.0014	0.0003	0.25	0.12
	Wild	0.0001	0.0001	0.0013	0.0002	0.06	0.08

Table 4. Components of variation and estimates of heritability observed for the three populations under the different inoculation treatments for two symbiosis traits where an interaction between population and treatment was found.

Trait	Population	Treatment	V_A	SE	V_P	SE	h^2	SE
Host growth response	Genepool 1	Fix+	0.05	0.07	0.77	0.18	0.07	0.08
	Genepool 1	Co-inoculation	0.16	0.13	0.82	0.23	0.19	0.11
	Genepool 1	Fix–	0.10	0.10	0.91	0.23	0.11	0.09
	Genepool 2	Fix+	0.26	0.21	1.36	0.41	0.19	0.11
	Genepool 2	Co-inoculation	0.01	0.06	1.03	0.23	0.01	0.06
	Genepool 2	Fix–	0.05	0.05	0.72	0.18	0.07	0.06
	Wild	Fix+	1.29	0.90	3.88	1.63	0.33	0.11
	Wild	Co-inoculation	0.37	0.27	2.12	0.89	0.17	0.06
	Wild	Fix–	0.09	0.13	1.62	0.59	0.05	0.06
Number of nodules	Genepool 1	Fix+	5.00	3.85	13.28	4.41	0.38	0.20
	Genepool 1	Co-inoculation	9.25	6.24	17.57	6.58	0.53	0.19
	Genepool 1	Fix–	6.72	4.53	11.63	4.70	0.58	0.18
	Genepool 2	Fix+	8.55	5.59	14.03	5.75	0.61	0.17
	Genepool 2	Co-inoculation	8.53	5.86	17.82	6.30	0.48	0.19
	Genepool 2	Fix–	3.32	2.79	11.58	3.45	0.29	0.19
	Wild	Fix+	0.00	0.06	1.05	0.29	0.00	0.06
	Wild	Co-inoculation	0.09	0.14	1.39	0.37	0.09	0.14
	Wild	Fix–	0.45	0.35	1.15	0.40	0.39	0.21

investment was very low; the addition of the relationship matrix did not provide an increase of the model fit, so components of variation were estimated without it. The expression of σ^2_a in investment differed among the Fix+, Fix–, and co-inoculation treatments ($\chi^2 = 10.15$, $P = 0.04$; Table S1), with the highest variance observed in the Fix– (Table 4; Fig. 3). No differences in σ^2_a were observed among populations and there was no dependency of these values on the inoculation treatment imposed ($\chi^2 = 2.37$, $P = 0.31$; Table S1).

Genetic correlations among the different symbiosis traits, including host growth response, nodule number, and investment, were positive in all cases (Table 5). However, the only significant correlation was observed between investment and the nodule number ($r_A = 0.98$, $P < 0.01$), indicating that selection on either of these traits can influence the other. Cowpea population was an important predictor of the genetic correlation between traits ($\chi^2_{12} = 35.25$, $P < 0.01$), indicating that correlated responses to selection would vary among these populations.

Table 5. Genetic correlations between traits estimated across treatments and populations.

Multi-trait model	r_A	SE	P
Investment–Host growth response	0.24	0.19	0.59
Nodule number–Host growth response	0.43	0.24	0.08
Investment–Nodule number	0.98	0.03	<0.01

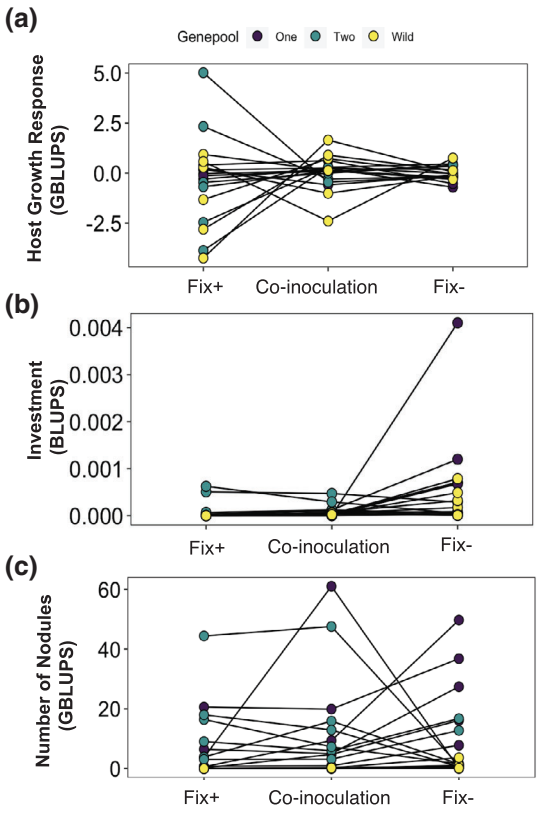

Figure 3. Additive genetic variation of symbiosis traits in domesticated and wild cowpeas in response to three different inoculation treatments. Symbiosis traits included (a) host growth response, (b) investment, and (c) number of nodules. Dots represent the breeding values for each genotype estimated from the best linear unbiased prediction (BLUPs) from a model where the genetic variance was allowed to differ among populations and rhizobial treatments. Colors indicate the population of each genotype. The dispersion among the dots represents genetic variation in the trait (V_A).

POSTINFECTION SANCTIONS AGAINST INEFFECTIVE RHIZOBIA

There was no evidence that postinfection sanctions varied among the cowpea genotypes. The Fix+ strain dominated the nodules of co-inoculated plants in all tested host genotypes, and in every case the Fix+ strain was found in nodules more often than expected by chance ($P < 0.001$). Of the 11,586 colonies scored from nodules, 98.94% belonged to the Fix+ strain and 1.06% were identified as Fix–. The Fix– strain was only recovered from two wild and one domesticated genotypes and only four nodules were found to be co-infected by both strains.

Discussion

We uncovered little evidence for degradation of symbiosis associated with cowpea domestication, despite marked differences among the cowpea populations. The decline in genetic diversity during the early stages of cowpea domestication was modest (∼6%; Table S2) in comparison to wheat and soybean, both of which show a substantial degradation in symbiosis traits (Hetrick et al. 1992; Kiers et al. 2007). In the case of wheat, diversity loss from wild *Triticum tauschii* to landrace cultivars was approximately three times more severe than cowpea (Reif et al. 2005). For soybean, bottlenecks reduced genetic diversity to over 50% compared to *Glycine soja*, but this was mainly due to an unusually low level of genetic diversity in the wild progenitor followed by a loss of diversity during the domestication bottleneck (Hyten et al. 2006; Guo et al. 2010). Conversely, we found that the populations of domesticated cowpeas (i.e., Genepools 1 and 2) exhibit more genetic divergence among them than either one of them compared to the wild cowpeas, suggesting that these two populations recently diverged from their wild progenitors, and supporting the presence of substantial genetic diversity that breeding could capitalize upon (Muñoz-Amatriaín et al. 2017). For the symbiosis traits we examined, heritability values were relatively low and varied with the rhizobial strain treatments. However, the presence of higher additive genetic variation in host growth and nodule number when cowpeas were exposed to an effective nitrogen-fixing strain indicates that there is breeding potential that could improve these symbiosis traits when a beneficial strain is present in the soil, thus enhancing the hosts capacity to regulate rhizobia.

Importantly, the reduction in genome-wide genetic variation among domesticated cowpea did not always indicate a loss of additive genetic variance of symbiosis traits. Although for host growth response, the component of additive genetic variance was modestly reduced in domesticated relative to wild cowpeas, for the number of nodules, additive genetic variance was substantially increased in the domesticated populations (Table 3). These differences in the components of genetic variation among traits can be due to different effects of selection in aboveground and belowground traits during domestication. Fisher (1930) predicted that as beneficial alleles become fixed due to selection, the additive genetic variance will become depleted. Traits that are intensely selected during domestication have experienced reductions in additive variation, such as root length in rice (Karavolias et al. 2020) and multiple fitness-related traits in maize (Yang et al. 2019). Therefore, it is possible that the reduction in additive variation in host growth response in the domesticated cowpeas is due to its positive correlation with an aboveground trait such as seed number or yield (Kyei-Boahen et al. 2017), which was selected for during domestication (Lo et al. 2018; Lonardi et al. 2019). Conversely, the number of nodules might have been affected by diversifying belowground selective processes during domestication as the different landraces likely encountered a broad

diversity of rhizobia across different growing regions in Africa (Pule-Meulenberg et al. 2010). Agricultural settings in Africa, where the cowpea landraces were developed, usually involve growing crops without external nutrient, microbial, or water inputs (Singh et al. 1997), and thus the cowpea landraces have been exposed to varied edaphic and environmental conditions across the continent. This edaphic diversity might have maintained additive variation in nodulation.

The trait of sanctions appeared to be unaffected during cowpea domestication, even though it was found to be degraded in more-domesticated soybeans (Kiers et al. 2007). We uncovered very little variation for sanctions capacity across all subcultured nodules from tested cowpeas, suggesting that this trait could be fixed in some legume species (Wendlandt et al. 2019). Conversely, we uncovered evidence for an evolutionary shift toward enhanced host investment into symbiosis in domesticated cowpea populations, indicated by a significant increase in the proportion of host biomass that supports nodules. Across domesticated populations, we saw higher investment into symbiosis in the Fix+ and co-inoculation treatments compared to the Fix–. Although this result might imply that increased investment was favored under artificial selection for yield, there was very low heritability for the investment trait, and we found no significant genetic correlation between host investment and host growth benefit from symbiosis. These results do not allow us to conclude that this trait shift in domesticated cowpeas improves benefits from symbiosis, but it might suggest that multiple traits are correlated with an increase in host biomass. Of all the traits that we examined, one which is consistent with the degradation hypothesis in domesticated populations is mean nodule size. For wild cowpeas, mean nodule size was larger in the presence of the Fix+ strain relative to Fix–, a trend that was not seen for domesticated populations. These data might suggest that the wild cowpeas have the capacity to adaptively regulate nodule size dependent on the amount of nitrogen fixed in each nodule, as has been shown for other legumes (Regus et al. 2015; Quides et al. 2017).

We uncovered no significant variation between the northern and southern populations of wild cowpeas in terms of symbiosis traits, despite their separate geographic distributions. Among the genotypes that consistently formed nodules, our results showed that wild cowpeas gained low or no growth benefit from both the Fix+ and Fix– strains compared to the benefits gained by the domesticated genotypes in single inoculations (Fig. 2). Similar patterns were uncovered with the $\delta^{15}N$ data for all populations (Table S7). No such differences were uncovered in the soil inoculation experiment, where soil slurries were used from a site where diverse cowpea lines were cultivated over multiple generations (Huynh et al. 2018). These results suggest that the domesticated genotypes have experienced relaxation of symbiont specificity, relative to the wild cowpeas that appear unable to gain benefits from USDA110. The number of nodules was also consistently smaller for wild cowpeas compared to domesticated populations in both settings. A potential target for the genetic basis of these changes is SNPs that link both domestication and nodulin genes (Muñoz-Amatriaín et al. 2017), as well as genomic regions associated with increased organ size during domestication, because they could prove to be fundamental in host regulation and response to symbionts (Lo et al. 2018; Lonardi et al. 2019). Further testing of nodulation and host growth with African *Bradyrhizobium* strains could provide fundamental insights into the evolution of host-symbiont specificity during the domestication process.

Low heritability values for some symbiosis traits suggest that environmental variation can play an important role in their phenotypic expression. For instance, low additive variation was observed for host investment, suggesting that the relative biomass a plant invests into nodules depends largely on the environmental context of the host plant. However, the higher additive genetic variance observed in host growth and the number of nodules indicates that there is potential to select on these traits to enhance benefits from symbiosis. Efforts to improve nitrogen fixation in legumes are focused largely on choosing beneficial rhizobia, but there is a need to provide a coordinated plant-bacteria breeding strategy (Sinclair and Nogueira 2018). Among the cowpeas studied here, Genepool 2 contains the best potential for further breeding, given that a higher heritability was observed among these cowpea genotypes for both the number of nodules and host growth. The fact that all of these genotypes are interfertile with modern domesticated cowpeas suggests that both wild cowpeas and landraces could be used as potential resources for introgression with domesticated varieties to increase genetic variation in breeding programs. Further screening for these traits could potentially allow growers to select for accessions that can improve their growth in the presence of compatible rhizobia.

Our work was focused on examining the early steps of domestication, and thus the conclusions that we can draw might not apply to modern cowpea cultivars. Given the basic conditions in which the cowpea landraces are propagated (Singh et al. 1997), they have probably not been exposed to heavy chemical fertilization or further reductions in genetic diversity, common in later stages of domestication with geographical expansion and intense breeding of the crop (Gaut et al. 2018), all factors that might be important in the disruption of symbiosis traits (Porter and Sachs 2020). Thus, it could be that degradation of symbiosis traits occurs more commonly with intense artificial selection during the latter stages of domestication, as was observed in soybeans (Kiers et al. 2007) and wheat (Hetrick et al. 1992). Symbiosis traits could be largely protected or even potentially enhanced under simple agricultural conditions that lack chemical fertilization, in particular if aboveground traits such as growth and yield

are correlated with the capacity to gain limiting nutrients from local microbiota. Our results also highlight potential breeding strategies that take symbiosis traits into account—such as nodulation counts and growth effects of inoculation—that could improve productivity of cowpea in the future by shedding light on how domestication has shaped symbiosis and how this knowledge can be used for sustainable crop improvement strategies.

AUTHOR CONTRIBUTIONS

GSO and JLS planned and designed the research. GSO, AM, and TS performed the experiment. GSO, TS, SN, FK, PC, JT, and AM collected the data. GSO, JLS, and LTM analyzed the data. GSO, LTM, and JLS wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

All trait data, SNP data, and R codes used for the project are available in Dryad under the following link: https://doi.org/10.5061/dryad.8kprr4xpt.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Patterns of admixture in 379 domesticated Cowpea accessions and 58 wild genotypes.
Figure S2. Mean symbiosis trait values of wild and domesticated cowpeas under different inoculation treatments.
Figure S3. Mean investment into symbiosis trait values of wild and domesticated cowpeas under different inoculation treatments. Analysis performed for the four cowpea populations studied
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