Reproductive biology of wild and domesticated *Ensete ventricosum*: Further evidence for maintenance of sexual reproductive capacity in a vegetatively propagated perennial crop

Solomon Tamrat[1,2*], James S. Borrell[3*†], Eleni Shiferaw[4], Tigist Wondimu[1], Simon Kallow[5,6], Rachael M. Davies[5], John B. Dickie[5], Gizachew W. Nuraga[1], Oliver White[3], Feleke Woldeyes[4], Sebsebe Demissew[1], Paul Wilkin[3]

*Denotes joint first authors

[†]Corresponding author: j.borrell@kew.org

[1]Department of Plant Biology and Biodiversity Management, Addis Ababa University, Addis Ababa, P.O. Box 3293,5 Ethiopia.

[2]Department of Biology, Dilla University, Dilla, P. O. Box-419, Southern Ethiopia.

[3]Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK.

[4]Ethiopian Biodiversity Institute, Addis Ababa, P.O.Box: 30726, Ethiopia.

[5]Royal Botanic Gardens Kew, Millennium Seed Bank, Wakehurst, Ardingly, Sussex, RH17 6TN, UK.

[6]Katholieke Universiteit Leuven, Department of Biosystems, Willem de Croylaan 42, 3001, Leuven, Belgium.

Abstract

- Loss of sexual reproductive capacity has been proposed as a syndrome of domestication in vegetatively propagated crops, but there are relatively few examples from agricultural systems. In this study we compare sexual reproductive capacity in wild (sexual) and domesticated (vegetative) populations of enset (*Ensete ventricosum* (Welw.) Cheesman), a tropical banana relative and Ethiopian food security crop.
- We examined floral and seed morphology and germination ecology across 35 wild and domesticated enset. We surveyed variation in floral and seed traits, including seed weight, viability and internal morphology, and germinated seeds across a range of constant and alternating temperature regimes to characterise optimum germination requirements.
- We report highly consistent floral allometry, seed viability, internal morphology and days to germination in wild and domesticated enset. However, seeds from domesticated plants responded to cooler temperatures with greater diurnal range. Shifts in germination behaviour appear concordant with a climatic envelope shift in the domesticated distribution.
- Our findings provide evidence that sexual reproductive capacity has been maintained despite long-term near-exclusive vegetative propagation in domesticated enset. Furthermore, certain traits such as germination behaviour and floral morphology, may be under continued selection, presumably through rare sexually reproductive events. Compared to sexually propagated crops banked as seeds, vegetative crop diversity is typically conserved in living collections that are more costly and insecure. Improved understanding of sexual propagation in vegetative crops may have applications in germplasm conservation and plant breeding.

Keywords: clonal reproduction; domestication; Ethiopia; germination biology; Seed banking; vegetative propagation.

50 # Introduction

51 The erosion of plant genetic resources poses a substantial threat to food security and the diverse
52 benefits we derive from useful plants (Borrell et al., 2019; Powell et al., 2018). To address this
53 challenge a significant effort has been made to conserve >2 million unique accessions, representing
54 >16,500 plant species in 1750 gene banks worldwide (Commission for genetic resources on food and
55 agriculture 2010; Fu 2017). However an emphasis on conventional seed crops may overlook the
56 majority of perennial fruit crops for which vegetative propagation is the predominant means of
57 replication and seed generation may be rare or absent (Miller and Gross 2011; Castañeda-álvarez *et*
58 *al.* 2016; Migicovsky and Myles 2017). Vegetatively propagated crops are especially important in the
59 tropics (Denham *et al.* 2020), where *ex situ* or *in vitro* germplasm collections are currently the only
60 viable approach for conserving genetic resources (Thormann and Dulloo 2006). Maintaining such
61 collections is often logistically challenging and prohibitively expensive, particularly for developing
62 countries (Dulloo *et al.* 2013). Improving our understanding of sexual reproduction in vegetatively
63 propagated perennial crops, for example through sexually reproducing wild progenitors (Miller and
64 Gross 2011), has potential to address these challenges by enabling a broader range of useful plants
65 to be banked as seed and used in breeding programmes, enhancing conservation and use of genetic
66 diversity (McKey *et al.* 2010; J. S. Borrell *et al.* 2019; Pironon *et al.* 2019; Denham *et al.* 2020).

67 Clonally propagated food crops encompass at least 34 families and a wide variety of morphological
68 diversity (McKey *et al.* 2010). This diversity has hindered attempts to define a domestication
69 syndrome (McKey *et al.* 2010; Miller and Gross 2011; Denham *et al.* 2020), but one commonality is
70 the hypothesis that prolonged vegetative reproduction can lead to the loss of sexual reproductive
71 capacity (Eckert 2002; McKey *et al.* 2010; Barrett 2015; Denham *et al.* 2020). For example,
72 domestication of Pineapple (*Annas*) which involved both sexual and asexual selection, resulted in
73 reduced seed production through lower fertility and self-incompatibility (Chen *et al.* 2019). Loss of
74 sexual reproductive capacity, if widespread across domesticated vegetative crops, could significantly
75 hinder future crop breeding programmes and integration of useful alleles from crop wild relatives

76 (Dempewolf *et al.* 2014; Migicovsky and Myles 2017). Importantly, sexual reproductive capacity does
77 not have to be completely lost – reduced fertility, viability, altered floral allometry or germination
78 behaviour, as a result of deleterious mutations and genetic drift in reproductive traits, could still
79 significantly hinder programmes that seek to recombine or conserve diversity as seeds (McClure *et*
80 *al.* 2014; Iriondo *et al.* 2017; Migicovsky and Myles 2017; Munguía-Rosas and Jácome-Flores 2020).
81 However, surprisingly few studies have attempted to survey the sexual reproductive capacity of
82 vegetatively propagated crops in agricultural systems (Elias *et al.* 2001; Scarcelli *et al.* 2006).

83 Here, we investigate the impact of domestication on the reproductive biology of the perennial food
84 security crop enset (*Ensete ventricosum* (Welw) Cheesman) (Fig. 1). Enset is a giant monocarpic herb,
85 in the sister genus to the more widely known bananas (*Musa* L.*),* which provides a staple starch
86 source for 20 million people of South and South West Ethiopia (Borrell, Biswas, *et al.* 2019). Enset is
87 a useful system in which to survey maintenance of sexual reproductive potential, because wild enset
88 reproduction is exclusively sexual, whilst partly sympatric domesticated enset is exclusively
89 propagated vegetatively (Borrell et al., 2020) (Fig. 1B, 1C). The latter is achieved through removal of
90 the apical meristem and meristematic tissue of a two to three year old corm, resulting in the
91 generation of adventitious buds (Karlsson, Dalbato, Tamado, & Mikias, 2014). Domesticated enset is
92 not currently banked as seed in international (or national) collections (Guzzon and Müller 2016), and
93 only a handful of local institutes maintain field germplasm collections (specifically; Areka, Yerefezy,
94 Angacha and Hawassa, situated in southern Ethiopia). With increasing pressures due to climate
95 change (Conway and Schipper 2011) and emerging pests and pathogens (Blomme *et al.* 2017), this
96 represents a significant risk for the future sustainability of enset agriculture.

97 Conserving domesticated enset diversity as seeds has been considered challenging for several
98 reasons. Firstly, enset is monocarpic and is harvested before flowering (unlike banana), to avoid
99 reallocation of resources from edible storage organs to the inedible inflorescence (Borrell et al.,
100 2020). This means that developed flowers and fruits are rarely encountered in cultivation. Second,

101 previous studies have found enset germination to be highly variable (0-90% success) (Tesfaye 1992;
102 Messele 1994; Diro *et al.* 2003). The most detailed study to date by Karlsson et al (2013) found 5-
103 55% germination depending on the accession. Third, recent genomic analysis concluded that
104 accumulation of mutations in genes associated with flower initiation and seed development may
105 have contributed to enset's domestication (Tesfamicael *et al.* 2020). Finally, unlike other tuberous
106 perennials, such as Yam (*Dioscorea* L.) (Mengesha *et al.* 2013) or Cassava (*Manihot* Mill.) (Rival and
107 Mckey 2008), wild or sexually reproduced volunteer seedlings are not (knowingly) incorporated into
108 cultivated populations (Borrell et al., 2020). Therefore, little to no indigenous knowledge pertains to
109 enset sexual reproduction or seed germination.

110 In this study, we hypothesise that if sexual reproductive capacity has been conserved through
111 domestication, we should observe consistent floral trait allometry and comparable seed
112 morphology, viability and germination behaviour between wild and domesticated enset plants (see
113 for example maintenance of silicon herbivore-defence through domestication in grasses, Simpson et
114 al., 2017), or potentially, moderately divergent selection for the domesticated environmental niche
115 (Meyer *et al.* 2012). An alternative hypothesis would be that, after being released from selection
116 pressure, enset reproductive traits would be susceptible to deleterious somatic mutations and
117 genetic drift. Therefore, traits would be expected to display higher variance in domesticated
118 individuals than wild plants. A third potential scenario entails a bottleneck (though these are likely
119 weaker in perennial vegetative crops (Gaut *et al.* 2015)) in which domesticated enset represents a
120 subset of wild diversity, and therefore a subset of the morphological or behavioural diversity in
121 reproductive traits. To address these scenarios, we frame our analysis with three questions; i) Is
122 there evidence for changes in floral trait allometry, seed morphology or viability in domesticated
123 compared to wild enset? ii) do domesticated enset seed and floral traits exhibit higher variance than
124 under neutral expectations? iii) Does seed germination behaviour differ between wild and
125 domesticated enset, and how do these germination requirements relate to the climate of wild and

domesticated enset distributions? Finally, we discuss implications for maintenance of enset germplasm resources.

Materials and Methods

Sample collection

Enset in Ethiopia is readily distinguished in the field as the only member of its genus and may be subdivided into i) domesticated clonal landraces with farmers' vernacular names, and ii) wild sexually reproducing populations. We made 20 seed collections from seven wild and 13 domesticated individuals, in Spring 2018 (Table 1). We also separately collected 15 complete inflorescences comprising six wild and nine domesticated individuals (Table 2). Domesticated accessions were collected with permission from farmers' fields, and wild collections were made in river valleys at least 1 km from settlements cultivating enset, to mitigate the risk of feral or recently introgressed individuals. We sought to minimise collection distance between accessions to minimise differences arising from the maternal climatic environment, but due to only partly overlapping wild and domesticated distributions and the rarity of flowering individuals at the appropriate stage of maturity we highlight that our disjunct sampling may introduce additional variance.

To ascertain whether propensity to flower differs across landraces (enset domestication is hypothesised to select for delayed maturation, Borrell *et al.* 2020), we recorded i) vernacular names of all observed and documented landraces during six field visits (2017-2020), and ii) those observed flowering. We ranked both lists by the frequency at which landraces were observed and compared ranks using a Wilcoxon rank sum test to ascertain whether landraces observed flowering are a random sample of all observed landraces. All analyses were conducted in R software (R Core Team 2017).

Floral trait variance and allometry

150 Five male flowers from each of five consecutive bracts, and where available, five female flowers,
151 were sampled from each inflorescence and preserved in 70% ethanol. We recorded the position of
152 flowers on the peduncle (i.e. the number of bracts and number of rows of flowers from the base of
153 the inflorescence). Samples were carefully dissected, and morphological characters recorded using
154 digital Vernier callipers, accurate to 0.01mm. For the female flowers, we recorded the length and
155 width of the pedicel, pistil outer and inner tepals, style and staminodes. For the male flowers, we
156 recorded the length of the styloid, pedicel, the five anthers and filaments and the length and width
157 of the outer and inner tepals. Where specific tissues were absent and we were certain that this was
158 not due to a damaged sample (for example, four instead of five filaments), this trait was recorded as
159 zero.

160 To investigate morphological variation, we first applied Spearman's rank nonparametric correlation
161 to assess pairwise relationships for floral traits within domesticated and wild enset. We report
162 pairwise tables of correlation coefficients. Second, we tested for a significant difference between
163 domesticated and wild trait values. We aggregated measurements by individual plant to mitigate
164 pseudo-replication, and then performed a Bartlett test for homogeneity of variances and an
165 unpaired t-test for each trait, applying a Holm correction for multiple tests. All analysis were
166 performed in R software v3.4.1 (R Core Team 2017). Finally to assess differentiation in overall floral
167 morphology between wild and domesticated enset we used redundancy analysis ('rda')
168 implemented in the R package Vegan (Oksanen *et al.* 2019). We plotted data aggregated by each
169 individual and applied permutational multivariate analysis of variation using distance matrices
170 (PERMANOVA) using the function 'adonis', to test the degree to which variance in floral traits can be
171 explained by individual or group (i.e. domesticated or wild plants).

172 **Seed morphology and viability**

173 Seeds were extracted from ripe fruits by hand and the pulp containing seeds was washed thoroughly
174 until all the flesh was removed. After extraction and cleaning, seeds were air dried at room
175 temperature (~20°C) for one week, packaged and transferred to the Millennium Seed Bank, UK

176 where they were stored at a constant 60% relative humidity for 1-2 weeks (approximate humidity of
177 collection region). In addition to field collected enset seed, we also used non-Ethiopian enset seeds
178 legally sourced from the online horticultural distributor Rare Palm Seeds
179 (https://www.rarepalmseeds.com/) which are available in large quantities (hereafter RPS),
180 permitting us to screen a wider range of experimental conditions.

181 For each accession, 20-50 seeds were weighed individually, using a balance accurate to 0.001g. Seed
182 size (diameter on an x, y, and z axis) was recorded for 10-20 seeds per accession using a digital
183 caliper accurate to 0.01mm. Due to the highly non-uniform shape of enset seeds, with no consistent
184 long or short axis, seed volume (estimated as the cube of the three measured axes) was used for
185 subsequent analyses. We used a Faxitron Ultrafocus x-ray (Faxitron Bioptics, LLC), to measure total
186 seed area (cross section), endosperm area and testa thickness (outer integument) in five seeds per
187 accession (Figure S1). Seeds were positioned with the proximal end facing upwards, and results
188 averaged by accession. We tested each dataset for homogeneity of variances and normality before
189 applying an unpaired t-test to evaluate differences in population means between domesticated and
190 wild seeds.

191 Concurrently, tetrazolium tests were used to detect living tissue and viable seeds following the
192 standard protocol of Leist *et al.* (2003). Briefly, 7-60 seeds per accession (mean=55) were imbibed
193 for 24 hours, chipped to expose the endosperm and placed in 1% buffered 2,3,5-triphenyl
194 tetrazolium chloride for two days in the dark at 30°C. Subsequently, seeds were carefully dissected,
195 and the embryo staining pattern recorded. Germination proportions were corrected for viability
196 during subsequent analysis.

197 **Germination trials**

198 We performed four germination experiments, with temperature ranges selected based on
199 WorldClimV2 values for the region (Fick and Hijmans 2017). We exclude treatments involving
200 sulphuric acid, sodium hydroxide, ammonium nitrate, sodium hypochlorite or hot water as Karlsson

201 *et al.* (2013) reported that these had no significant positive effect on germination; and indeed,
202 scarification and 70% ethanol had significant negative effects. To mimic natural conditions we draw
203 on germination ecology in *Musa* (Laliberté 2016), where alternating temperature regimes are known
204 to be a key requirement in *M. balbisiana* due to exposure of the seeds on the soil surface to
205 alternating day and night temperatures (Stotzky *et al.* 1962; Kallow *et al.* 2021) and similar
206 behaviour reported by Tesfaye (1992) in enset.

207 i) Exp 1: First, using RPS seed we screened a range of 15 constant and alternating
208 temperature regimes to guide our experimental design for subsequent germination
209 tests. Constant conditions comprised six regimes: 10°C, 15°C, 20°C, 25°C, 30°C and 40°C.
210 Alternating conditions included 10-20°C of diurnal variation (12h:12h) to simulate larger
211 temperature shifts, comprising an additional nine regimes: 20/10°C, 25/10°C, 25/15°C,
212 30/10°C, 30/15°C, 30/20°C, 35/20°C, 40/20°C, 40/25°C.

213 ii) Exp 2: Based on results from the initial screening, seeds from seven wild accessions and
214 seven domesticated accessions (those with sufficient seeds available for full trials) were
215 exposed to a refined range of 11 temperature regimes: 10°C, 15°C, 20°C, 25°C, 30°C,
216 20/10°C, 25/10°C, 25/15°C, 30/10°C, 30/15°C, 30/20°C.

217 iii) Exp 3: To evaluate the relative importance of an absolute shift in ambient temperature
218 (e.g. warming due to disturbance) and regular diurnal temperature shifts (i.e. our
219 alternating temperature regimes) we exposed a subset of a subset of seven
220 domesticated accessions plus RPS, to five constant temperatures for three months
221 (10°C, 15°C, 20°C, 25°C, 30°C), and then moved them to the 'optimum' alternating
222 temperature (25/10°C) identified in earlier tests. Available wild seed was prioritised for
223 Exps 1/2.

224 iv) Exp 4: To evaluate the extent and influence of dormancy, a subset of seven
225 domesticated accessions plus RPS, was stratified at 10°C for three months, and then

226 transferred to five other temperature regimes: 25°C, 25/10°C, 25/15°C, 30/20°C,
227 30/15°C. We compare these to aggregated data from Exp2 that was not exposed to a
228 period of stratification.

229 In all germination tests seeds were placed on moist sand (300g sand, 42ml de-ionised water) and
230 sealed in clear plastic boxes (120 x 180 mm). Boxes were then sealed in plastic bags to minimise
231 moisture loss and contamination. Each box contained 60 seeds (except where specified), with a total
232 of 235 unique condition x accession combinations evaluated in this study. Seed boxes were placed in
233 the corresponding incubators with either constant temperature or 12 hour alternating temperature
234 cycles. All treatments included a 12-hour photoperiod. Germination was defined by radicle
235 emergence ≥2 mm, and tests were scored every 3-18 days, depending on activity, for 120 days. We
236 calculated the number of days required for 50% of the final germination count in each experimental
237 replicate. We aggregated these data by accession, excluding any replicate with zero germination.

238 To test for a significant difference between wild and domesticated germination behaviour (EXP2),
239 we fitted polynomial regression models for the logit transformed proportion germinated against a)
240 the daily temperature change and b) the mean temperature the replicate was exposed to. For each
241 variable we fitted two models, the first with all accessions and the second with an additional variable
242 grouping the data by type (wild v domesticated). We then used ANOVA to test whether grouping
243 produced a significantly better model fit. To evaluate the role of stratification and dormancy (EXP3),
244 we plotted the absolute temperature change between the first temperature and the mean of the
245 second temperature of the treatments, against germination proportion and applied linear
246 regression. The effect of a 10°C stratification treatment was compared to non-stratification using an
247 unpaired t-test (EXP4).

248 To understand whether differences in germination behaviour were concordant with differences in
249 local climate, bioclimatic data for Ethiopia was sourced from WorldClim v2 (Fick and Hijmans 2017)
250 at 2.5 arc minute resolution (~10 km). In the first instance, we extracted climate values for our study

accessions, and tested for significant differences in Annual Mean Temperature and Mean Diurnal Range using unpaired t-tests. We then collated 472 enset localities from GBIF (GBIF.org, 2018), publications (Borrell et al., 2019; Pironon et al., 2019) and field observations, and subsampled these to a 10 km grid consistent with the environmental data layers, retaining 94 unique domesticated records and 19 unique wild records. We extracted climate data for these cells and aggregated them for domesticated and wild enset separately.

Results

Observations, collection, processing and storage

Summary information for the 15 floral and 20 seed accessions are reported in Tables 1 and 2. Overall we surveyed 375 male flowers and 45 female flowers and harvested >8000 seeds. During field surveys, we documented 1864 observations of 453 named landraces from across the enset growing region. In addition, we recorded 39 flowering individuals of 26 landraces. After ranking by frequency of observation we found no significant difference in rank order ($W = 6251.5$, $p = 0.60$).

Floral trait allometry

Of 120 pairwise comparisons for male flowers, 100 were significantly correlated in domesticated and 72 significant in wild. Traits such as anther length and filament length were highly correlated with each other in both wild and domesticated flowers, whereas sepal length and stylode length were highly correlated with anther length in wild flowers, but only weakly correlated in domesticated flowers. Of 78 pairwise comparisons for female domesticated flowers, 34 were significant. Overall, we found relatively weak correlation for pedicel length with other traits, whereas Ovary length and Outer whorl fused tepal width were highly correlated with other traits. Pairwise correlation coefficients for male and female flower morphological traits are reported in Tables S1 and S2 respectively. Comparison of trait variance found little differences between wild and domesticated plants (Table 3). The only significant differentiation in trait means was for Sepal width, which was significantly wider in domesticated flowers ($t = -7.7$, $df = 12.4$, $p < 0.001$). With no wild female

277 flowers, it was not possible to test for significant differences in female floral trait morphology, thus
278 tissue means are reported in Table S3.

279 A redundancy analysis for male floral traits is plotted in Figure 2. Domesticated enset tend to vary on
280 the first axis, with Sepal width the most important contributing variable. The majority of wild
281 variation is on the second axis, with Filament length traits the most important contributing variables.
282 PERMANOVA showed that the distinction between wild and domesticated origin explained a
283 significant proportion of variation, whereas grouping samples by landrace did not (Table 4).

284 **Seed morphological and viability**

285 Seed volume ranged from 1.26 - 6.29 cm^3 and seed weight from 0.20 g - 2.85 g in domesticated
286 accessions, and 1.15 - 5.05 cm^3 and 0.78 g - 2.66 g respectively in wild accessions. After correction
287 for multiple tests we found no significant difference in seed morphology traits between wild and
288 domesticated seeds. These patterns of (non)significance were consistent even if poorly germinating
289 accessions are removed (*i.e.* Deri'ea, Suitiya landraces). Full morphological data are available in
290 Tables S4 and S5. Tetrazolium tests showed high variation in viability across accessions, with both
291 wild and domesticated accessions ranging from 0-100% viability. Mean viability was 55% and 49.5%
292 for wild and domesticated respectively with no significant difference ($t = 0.12$, $df = 12.9$, $p = 0.84$)
293 (Table 1).

294 **Germination trials**

295 Mean time to 50% germination (T50) was 36 days (sd = 15.7) for domesticated enset and 35 days (sd
296 = 8.6) for wild enset, with no significant difference detected ($t = 0.51$, $df = 11.89$, $p = 0.62$). In Exp 1.
297 alternating temperature regimes outperformed constant temperatures, with the exception of
298 constant 25°C (Figure 3). Based on these data we reduced our suite of temperature regimes in
299 subsequent experiments. ANOVA analysis of polynomial regression models for germination
300 behaviour in domesticated and wild enset were significantly different (i.e. grouping by accession
301 type resulted in significantly better model fit) for both the alternating temperature range ($F_{136,132}$ =

302 4.32, p = 0.003) and the mean experimental temperature ($F_{136,132}$ = 2.71, p = 0.033) (Fig. 4A).
303 Specifically, we found that domesticated accessions had an improved germination response in cooler
304 mean temperatures with higher alternating temperature amplitude. Comparison of germination
305 requirements to regional climatic conditions for wild and domesticated enset found that
306 domesticated enset is found in local climates that have significantly cooler Annual Mean
307 Temperatures (AMT) (t = -5.52, df = 31, p = <0.001), with significantly greater Mean Diurnal Range
308 (MDR) (t = 3.42, df = 19.2, p = 0.003), than wild enset (Fig. 4B). Importantly, there was no significant
309 difference in the AMT (t = -1.77, df = 12.56, p = 0.10) or MDR (t = -1.63, df = 13.96, p = 0.13) of our
310 collected accession sites, therefore this is unlikely to be solely a maternal effect.

311 Analysis of Exp 3. found a significant positive relationship between germination proportion and the
312 absolute temperature change from a constant to alternating temperature regime ($F_{1,38}$ = 6.37, p =
313 0.0159). Analysis of Exp 4. found that a three-month period of cold stratification at 10°C prior to an
314 experimental treatment also significantly improved germination compared to seeds immediately
315 exposed to the experimental treatment (t = 5.22, df = 26.4, p = <0.001) (Fig. 5). Full germination data
316 are available in Tables S6 and S7.

317 Discussion

318 It has frequently been suggested that prolonged vegetative reproduction during domestication can
319 lead to the loss of sexual reproductive capacity (Eckert 2002; Barrett 2015; Denham *et al.* 2020;
320 Tesfamicael *et al.* 2020). In this study we show evidence that the indigenous Ethiopian vegetative
321 crop enset has retained viable sexual reproductive potential through domestication. Currently, the
322 duration over which enset has been domesticated, and the temporal advent of vegetative
323 reproduction is unclear (Borrell *et al.* 2019). However, if we consider the extensive accumulation of
324 indigenous knowledge associated with enset cultivation (Garedew *et al.* 2017), and its origins in the
325 Ethiopian center of crop domestication (Harlan 1971), it is reasonable to conclude that enset was

326 not recently domesticated. Therefore, we suggest that the potential for sexual reproduction has
327 been maintained despite a prolonged period of vegetative propagation.

328 In our surveys, we found no evidence that certain landraces have lost the propensity to flower. Wild
329 and domesticated floral morphology was significantly differentiated (figure 2), but we did not find
330 evidence for increased variance in domesticated enset, or for loss of allometry which could be
331 indicative of inhibited function. The exception to this pattern was sepal width which was significantly
332 larger in domesticated flowers, even after correcting for multiple tests. It is difficult to ascertain
333 whether this is functional, or more likely developmentally linked to another gene under selection.
334 We found no evidence of differing seed viability rates between wild and domesticated enset, though
335 we did find high variability in seed viability across accessions, consistent with previous reports in
336 both enset (Karlsson *et al.* 2013) and *Musa* (Kallow *et al.* 2021). Internal and external seed
337 morphology was also consistent between wild and domesticated accessions.

338 Our germination trials indicate that surprisingly, the optimum germination requirements significantly
339 differ between domesticated and wild enset (Fig. 5). Domesticated enset has an increased
340 germination response in cooler mean temperatures (~22°C) and with an increased amplitude of
341 alternating temperature (Fig. 4A) compared to wild enset. Naturally, wild enset occupies consistently
342 warm moist tropical forest in Western Ethiopia, whereas the predominant region of contemporary
343 enset cultivation is a region with lower Annual Mean Temperature and higher Mean Diurnal Range
344 (Fig. 4B). This suggests, that wild and domesticated enset are potentially locally adapted to their
345 respective environments. Importantly, there was no significant difference between the Annual Mean
346 Temperature and Mean Diurnal Range of the wild and domesticated seed collection sites surveyed
347 here, despite being approximately 170km apart, suggesting that this observation is unlikely to be a
348 strong maternal effect.

349 When we consider enset floral morphology and seed germination behaviour together, there are
350 several evolutionary explanations for these observations. First, a scenario where selection pressure

351 has been relaxed as a result of domestication would be expected to show increased variance in
352 domesticated traits, perhaps as a result of deleterious somatic mutations and genetic drift, but we
353 do not observe this pattern in these data. If pervasive genetic drift is indeed in progress, then
354 potentially insufficient time or generations have passed for it to become apparent. Alternatively, in a
355 bottleneck scenario where sexual reproductive potential was not selected for, seed and floral
356 morphology might be expected to show reduced variance, and display a subset of wild diversity. Our
357 data do not provide strong evidence for this either.

358 Seeking a more parsimonious scenario, we suggest that despite virtually exclusive clonal propagation
359 in cultivation, it is possible that a small number of escaped or neglected domesticated plants are
360 continuing to reproduce sexually. Whilst deliberate sexual propagation is not reported by enset
361 farmers, agricultural practices that enable sexual progeny has been recorded through ennoblement
362 in yam (*Dioscorea*) (Cornet *et al.* 2010; Mengesha *et al.* 2013) and tolerance of volunteer manioc
363 seedlings in Cassava (*Manihot esculenta*) (Rival and Mckey 2008). Where this has occurred in a
364 novel agricultural environment, it is possible key functional traits have remained under balancing
365 selection, whilst germination traits have been subjected to some degree of directional selection. This
366 explanation is supported by both significantly different germination behaviour concordant with local
367 environment, and lack of differences in trait means or variance. Previous work has shown that a
368 comparatively low rate of sexual reproduction would be sufficient to maintain this balancing
369 selection (Rice and Chippindale 2001; Cutter 2019).

370 More broadly, we note that enset germination requirements appear consistent with reports from
371 *Musa* (Stotzky *et al.* 1962; Kallow *et al.* 2021). Specifically, in enset, alternating temperatures elicit a
372 stronger germination response than constant temperatures, though in *Musa* this is virtually an
373 absolute requirement. Using alternating temperatures as an environmental cue is hypothesised to
374 be a strategy for detecting disturbance and canopy gaps whereby solar radiation warms the seeds in
375 the day followed by a cooler ambient temperature at night. Enset is also reported to colonise

376 disturbed areas (Stotzky *et al.* 1962), and thus this trait appears to be conserved across the two
377 major branches of the Musaceae. Surprisingly, the magnitude of the transition from constant to
378 alternating temperature was also significantly associated with germination. A possible mechanism
379 may involve alternating temperatures reducing the ratio of Abscisic acid to Gibberellic acid (GA),
380 reducing water potential and initiating elongation and cell growth. This suggests that future
381 approaches involving application of GA to seeds or the growing medium may provide another
382 mechanism for initiating germination. In addition, increased germination is also observed where
383 seeds were stratified at a constant temperature prior to germination. Climate data indicates that the
384 domesticated distribution of enset may reach a minimum of 8.2°C in the coolest month, which
385 coincides with higher rainfall. Whilst no seasonality has been reported in enset flowering, this may
386 be an additional, putatively conserved, mechanism for optimising germination timing. We anticipate
387 that numerous factors such as fruit maturity, length and type of storage, epigenetic and other
388 factors may influence variability, though we do not have sufficient power to resolve these in this
389 study.

390 In conclusion, demonstrating the continued function of sexual reproduction in enset provides a
391 counter example to the perceived loss of sexual capacity in vegetatively propagated crops (Eckert
392 2002; McKey *et al.* 2010; Barrett 2015; Denham *et al.* 2020). Development of enset seed germination
393 protocols underpins the utility of diverse seed collections and is a compelling strategy to safeguard
394 the genetic diversity of a food security crop, with a lower risk of provenance information loss than
395 living plants in a germplasm collection (Thormann and Dulloo 2006). Furthermore, we provide initial
396 evidence that very low levels of sexual reproduction may be facilitating the local adaptation of enset
397 germination biology, influencing our interpretation of contemporary landrace diversification. An
398 improved understanding of enset germination biology is a useful prerequisite for future crop
399 development through sexual recombination of existing landraces, developing mapping populations
400 and the breeding of novel genotypes, with significant reciprocal potential in bananas (*Musa*), a
401 closely related and globally important group of clonally produced crops. In conclusion, we advocate

402 for a broader effort in developing germination protocols for vegetatively propagated crops to
403 provide an important alternative germplasm conservation strategy that is likely to
404 disproportionately benefit tropical species and developing country agriculture in the global south.
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422

Acknowledgements

The authors thank the Ethiopian Biodiversity Institute for facilitating the export of enset seeds for germination studies. We thank John Adams and Pablo Barreiro at the Millennium Seed Bank for lab support and Dr Yann Dussert, Prof Richard Nichols, Prof Pat J.S. Heslop-Harrison and Dr Manosh Biswas for fruitful discussions on the evolutionary ecology of vegetative reproduction. We also thank Robert Kessler for permitting publication of enset photographs. We thank Mr Tobias Spanner from Rare Palm Seeds for helpful information on seed provenance and morphological variation. Finally, we thank an anonymous reviewer for helpful comments on an earlier version of this manuscript. This work was supported by the GCRF Foundation Awards for Global Agricultural and Food Systems Research, entitled, 'Modelling and genomics resources to enhance exploitation of the sustainable and diverse Ethiopian starch crop enset and support livelihoods' [Grant No. BB/P02307X/1]. In addition, part of this work was funded as a subgrant from the University of Queensland from the Bill & Melinda Gates Foundation project 'BBTV mitigation: Community management in Nigeria, and screening wild banana progenitors for resistance' [OPP1130226].

447 # References

448 **Barrett SCH**. **2015**. Influences of clonality on plant sexual reproduction. *Proceedings of the National*
449 *Academy of Sciences* **112**: 8859–8866.

450 **Blomme G, Dita M, Jacobsen KS, *et al.* 2017**. Bacterial Diseases of Bananas and Enset: Current State
451 of Knowledge and Integrated Approaches Toward Sustainable Management. *Frontiers in Plant*
452 *Science* **8**: 1–25.

453 **Borrell J S, Biswas MK, Goodwin M, *et al.* 2019**. Enset in Ethiopia: a poorly characterised but
454 resilient starch staple. *Annals of Botany* **123 (5)**: 747–766.

455 **Borrell James S., Biswas MK, Goodwin M, *et al.* 2019**. Enset in Ethiopia: A poorly characterized but
456 resilient starch staple. *Annals of Botany* **123**: 747–766.

457 **Borrell J. S., Dodsworth S, Forest F, *et al.* 2019**. The climatic challenge: Which plants will people use
458 in the next century? *Environmental and Experimental Botany* **170**.

459 **Borrell JS, Goodwin M, Blomme G, *et al.* 2020**. Enset based agri-systems in Ethiopia: A systematic
460 review of production trends, agronomy, processing and the wider food security applications of a
461 neglected banana relative. *Plants, People, Planet* **2**: 212–228.

462 **Castañeda-álvarez NP, Khoury CK, Achicanoy HA, *et al.* 2016**. Global conservation priorities for
463 cropwild relatives. *Nature Plants* **2**: 1–6.

464 **Chen L, Vanburen R, Paris M, *et al.* 2019**. The bracteatus pineapple genome and domestication of
465 clonally propagated crops. *Nature Genetics* **51**: 1549–1558.

466 **Commission for genetic resources on food and agriculture**. **2010**. *The Second Report on the state of*
467 *the world's plant genetic resources for food and agriculture*. Rome.

468 **Conway D, Schipper ELF**. **2011**. Adaptation to climate change in Africa: Challenges and opportunities
469 identified from Ethiopia. *Global Environmental Change* **21**: 227–237.

470 **Cornet D, Deu M, Baco MN, Agbangla A, Duval MF, Noyer JL**. **2010**. Impact of farmer selection on
471 yam genetic diversity. *Conservation Genetics* **11**: 2255–2265.

472 **Cutter AD**. **2019**. Reproductive transitions in plants and animals : selfing syndrome , sexual selection
473 and speciation. *New Phytologist* **224**: 1080–1094.

474 **Dempewolf H, Eastwood RJ, Guarino L, Khoury CK, Müller J V., Toll J**. **2014**. Adapting Agriculture to
475 Climate Change: A Global Initiative to Collect, Conserve, and Use Crop Wild Relatives. *Agroecology*
476 *and Sustainable Food Systems* **38**: 369–377.

477 **Denham T, Barton H, Castillo C, *et al.* 2020**. The domestication syndrome in vegetatively propagated
478 field crops. *Annals of Botany* **125**: 581–597.

479 **Diro M, van Staden J, Bornman CH**. **2003**. In vitro regeneration of Ensete ventricosum from zygotic
480 embryos of stored seeds. *South African Journal of Botany* **69**: 364–369.

481 **Dulloo ME, Thormann I, Fiorino E, De Felice S, Rao VR, Snook L**. **2013**. Trends in Research using
482 Plant Genetic Resources from Germplasm Collections: From 1996 to 2006. *Crop Science* **53**: 1217–
483 1227.

484 **Eckert CG**. **2002**. The loss of sex in clonal plants In: Stuefer JF, Erschbamer B, Huber H, Suzuki J-I, eds.
485 *Ecology and Evolutionary Biology of Clonal Plants*. Dordrecht: Springer Netherlands, 279–298.

486 **Elias M, Penet L, Vindry P, McKey D, Panaud O, Robert T**. **2001**. Unmanaged sexual reproduction
487 and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (Manihot
488 esculenta Crantz), in a traditional farming system. *Molecular Ecology* **10**: 1895–1907.

489 **Fick SE, Hijmans RJ**. **2017**. WorldClim 2: new 1-km spatial resolution climate surfaces for global land
490 areas. *International Journal of Climatology* **37**: 4302–4315.

491 **Fu Y**. **2017**. The Vulnerability of Plant Genetic Resources Conserved Ex Situ. *Crop Science* **57**: 2314–
492 2328.

Garedew B, Ayiza A, Haile B, Kasaye H. **2017**. Indigenous Knowledge of Enset (Ensete ventricosum (Welw .) Cheesman) Cultivation and Management Practice by Shekicho People , Southwest Ethiopia. *Journal of Plant Sciences* **5**: 6–18.

Gaut BS, Díez CM, Morrell PL. **2015**. Genomics and the Contrasting Dynamics of Annual and Perennial Domestication. *Trends in Genetics* **31**: 709–719.

GBIF. **2018**. *GBIF.org (13th March 2018) GBIF Occurrence Download https://doi.org/10.15468/dl.8qg5ew*.

Guzzon F, Müller J V. 2016. Current availability of seed material of enset (Ensete ventricosum, Musaceae) and its Sub-Saharan wild relatives. *Genetic Resources and Crop Evolution* **63**: 185–191.

Harlan J. **1971**. Agricultural Origins: Centers and Noncenters. *Science* **239**: 17–18.

Iriondo JM, R. Milla SV, Casas RR de. **2017**. Reproductive traits and evolutionary divergence between Mediterranean crops and their wild relatives. *Plant Biology* **20**: 77–88.

Kallow S, Vandelook F, Mertens A, Panis B, Janssens SB. **2021**. Regulation of seed germination by diurnally alternating temperatures in disturbance- adapted banana crop wild relatives (Musa acuminata).

Karlsson LM, Dalbato AL, Tamado T, Mikias Y. **2015**. Effect of cultivar, traditional corm pre-treatment nd watering on sprouting and early growth of enset (Ensete ventricosum) suckers. *Experimental Agriculture* **51**: 232–243.

Karlsson LM, Tamado T, Dalbato AL, Mikias Y. **2013**. Seed morphology and germination of Ensete ventricosum (Musaceae). *Seed Science and Technology* **41**: 357–370.

Laliberté B. **2016**. *Global strategy for the conservation and use of Musa (banana) genetic resources: a consultative document prepared by the Global Musa Genetic Resources Network (MusaNet).* Rome: Bioversity International.

516 **Leist N, Krämer S, Jonitz A**. **2003**. ISTA working sheets on tetrazolium testing In: International Seed
517 Testing Association, .

518 **McClure KA, Sawler J, Gardner KM, Money D, Myles S**. **2014**. Genomics: A potential panacea for the
519 perennial problem. *American Journal of Botany* **101**: 1780–1790.

520 **McKey D, Elias M, Pujol ME, Duputié A**. **2010**. The evolutionary ecology of clonally propagated
521 domesticated plants. *New Phytologist* **186**: 318–332.

522 **Mengesha WA, Demissew S, Fay MF, Smith RJ, Nordal I, Wilkin P**. **2013**. Genetic diversity and
523 population structure of Guinea yams and their wild relatives in South and South West Ethiopia as
524 revealed by microsatellite markers. *Genetic Resources and Crop Evolution* **60**: 529–541.

525 **Messele T**. **1994**. Enset fruit and seed processing, germination and seedling development. *PGRC/E*
526 *Germplasm Newsletter (Ethiopia).* **2**.

527 **Meyer RS, Duval AE, Jensen HR**. **2012**. Patterns and processes in crop domestication: an historical
528 review and quantitative analysis of 203 global food crops. *New Phytologist* **196**: 29–48.

529 **Migicovsky Z, Myles S**. **2017**. Exploiting Wild Relatives for Genomics-assisted Breeding of Perennial
530 Crops. *Frontiers in Plant Science* **8**: 1–16.

531 **Miller AJ, Gross BL**. **2011**. From forest to field: Perennial fruit crop domestication. *American Journal*
532 *of Botany* **98**: 1389–1414.

533 **Munguía-Rosas MA, Jácome-Flores ME**. **2020**. Reproductive isolation between wild and
534 domesticated chaya (Cnidoscolus aconitifolius) in sympatry. *Plant Biology* **22**: 932–938.

535 **Oksanen J, Blanchet FG, Kindt R, *et al.* 2019**. Package “vegan”: Community ecology package. :
536 http://vegan.r-forge.r-project.org/.

537 **Pironon S, Etherington TR, Borrell JS, *et al.* 2019**. Potential adaptive strategies for 29 Sub-Saharan
538 crops under future climate change. *Nature Climate Change* **9**: 758–763.

539 **Powell W, Halewood M, Chiurugwi T, *et al.* 2018**. Tansley review Plant genetic resources for food
540 and agriculture : opportunities and challenges emerging from the science and information
541 technology revolution. *New Phytologist* **217**: 1407–1419.

542 **R Core Team**. **2017**. R: A language and environment for statistical computing. v3.4.1.

543 **Rice WR, Chippindale AK**. **2001**. Sexual Recombination and the Power of Natural Selection. *Science*
544 **294**: 555–559.

545 **Rival L, Mckey D**. **2008**. Domestication and Diversity in Manioc (Manihot esculenta Crantz ssp.
546 esculenta, Euphorbiaceae). *Current Anthropology* **49**: 1119–1128.

547 **Scarcelli N, Tostain S, Vigouroux Y, Agbangla C, Dainou O, Pham J -L. 2006**. Farmers' use of wild
548 relative and sexual reproduction in a vegetatively propagated crop . The case of yam in Benin.
549 *Molecular Ecology* **15**: 2421–2431.

550 **Simpson KJ, Wade RN, Hartley SE, Rees M, Osborne CP, Hartley SE**. **2017**. Still armed after
551 domestication? Impacts of domestication and agronomic selection on silicon defences in cereals.
552 *Functional Ecology* **31**: 2108–2117.

553 **Stotzky G, Cox EA, Goos RD**. **1962**. Seed Germination Studies in Musa. I. Scarification and Aseptic
554 Germination of Musa balbisiana. *American Journal of Botany* **49**: 515–520.

555 **Tesfamicael KG, Gebre E, March TJ, Sznajder B, Mather DE, Lopez CMR**. **2020**. Accumulation of
556 mutations in genes associated with sexual reproduction contributed to the domestication of a
557 vegetatively propagated staple crop, enset. *bioRxiv*: 1–31.

558 **Tesfaye M**. **1992**. The effect of soaking, temperature and other pretreatments on the germination of
559 "ensete" seed In: *Seed Science and Technology*.

560 **Thormann I, Dulloo ME**. **2006**. Techniques for ex Situ for Situ Plant Conservation In: RJ H, ed. *Plant*
561 *Conservation Genetics*. Haworth Press, .

Tables

Table 1. Wild and domesticated enset accessions sampled for seed morphology and germination analysis.

Origin	Landrace	Elevation (m a.s.l.)	Latitude (°N)	Longitude (°E)	Tetrazolium viability (%)	Annual Mean Temp. (°C)	Mean Diurnal Range (°C)	Seed count
Domesticated	Ganticho	1840	6.47	38.35	30	18.41	14.25	100
Domesticated	Midasho	2786	6.47	38.54	14	12.89	13.67	7
Domesticated	Kiticho	2787	6.48	38.54	50	12.89	13.67	16
Domesticated	Gefetano	1930	6.83	37.75	75	20.24	14.72	300
Domesticated	Wanadiya	2125	6.86	37.79	48	18.78	14.44	530
Domesticated	Gefetano	2125	6.86	37.79	46	18.78	14.44	540
Domesticated	Maze	2120	6.87	37.79	64	18.78	14.44	1265
Domesticated	Hala	2120	6.87	37.79	66	18.78	14.44	890
Domesticated	Suitiya	2120	6.87	37.79	0	18.78	14.44	75
Domesticated	Deri'ea	2700	7.93	37.9	0	15.37	12.97	600
Domesticated	Lemat	2053	8.45	38.03	67	17.97	13.49	100
Domesticated	Addis Ababa	2420	9.02	38.78	100	16.02	13.25	55
Domesticated	Addis Ababa	2430	9.03	38.76	84	16.02	13.25	1570
Wild	W1	1880	7.17	36.22	0	18.78	14.35	450
Wild	W2	1850	7.16	36.2	38	18.47	14.3	135
Wild	W3	1936	7.16	36.2	54	18.47	14.3	600
Wild	W4	1936	7.16	36.2	68	18.47	14.3	490
Wild	W5	1927	7.16	36.2	78	18.47	14.3	700
Wild	W6	1936	7.19	36.2	52	18.11	14.14	680
Wild	W7	1930	7.29	36.14	100	17.97	13.99	820
Wild (non-Ethiopian)	'Rare Palm Seeds'	-	-	-	94	-	-	1000

592

593 **Table 2.** Wild and domesticated enset accessions sampled for floral morphology analysis

Origin	Landrace	Elevation (m a.s.l.)	Latitude (°N)	Longitude (°E)
Domesticated	Ado	1846	6.46	38.36
Domesticated	Ganticho	1845	6.46	38.35
Domesticated	Ganticho	1856	6.46	38.35
Domesticated	Ganticho	1833	6.46	38.35
Domesticated	Midasho	1771	6.46	38.34
Domesticated	Midasho	1853	6.46	38.35
Domesticated	Midasho	1847	6.46	38.35
Domesticated	Ado	1826	6.47	38.35
Domesticated	Ado	1781	6.47	38.35
Wild	NA	1950	7.15	36.21
Wild	NA	1900	7.15	36.21
Wild	NA	1855	7.17	36.21
Wild	NA	1855	7.17	36.21
Wild	NA	2200	7.12	35.75
Wild	NA	2200	7.12	35.75

594

595

596

597

598

Table 3. Comparison of variance and means for enset floral and seed morphology traits.

Trait (mm)	Domesticated Trait mean (sd)	Wild Trait mean (sd)	Bartlett test K-squared	df	p-value	p-value (corrected)[1]	T-test T-value	df	p-value	p-value (corrected)
Floral morphology										
Pedicel (length)	8.18 (4.18)	12.62 (2.34)	1.69	1.00	0.19	0.97	2.63	12.77	0.02	0.17
Anther Length 1	30.18 (2.66)	29.56 (6.34)	4.34	1.00	0.04	0.59	-0.23	6.19	0.83	1.00
Anther Length 2	29.48 (2.66)	28.85 (6.18)	4.11	1.00	0.04	0.60	-0.23	6.25	0.82	1.00
Anther Length 3	28.69 (2.55)	28.25 (6.07)	4.34	1.00	0.04	0.59	-0.17	6.19	0.87	1.00
Anther Length 4	27.11 (2.52)	27.13 (5.72)	3.90	1.00	0.05	0.63	0.01	6.31	0.99	1.00
Anther Length 5	24 (3.32)	26.15 (5.6)	1.62	1.00	0.20	0.97	0.85	7.35	0.42	1.00
Filament Length 1	20.46 (6.24)	27.46 (2.96)	2.63	1.00	0.10	0.92	2.91	12.11	0.01	0.16
Filament Length 2	19.74 (6.11)	26.59 (3.01)	2.40	1.00	0.12	0.92	2.88	12.28	0.01	0.16
Filament Length 3	19.26 (6.1)	26 (2.88)	2.68	1.00	0.10	0.92	2.87	12.08	0.01	0.16
Filament Length 4	18.76 (6.05)	24.98 (2.62)	3.23	1.00	0.07	0.78	2.73	11.66	0.02	0.17
Filament Length 5	17.08 (6.07)	23.94 (2.52)	3.51	1.00	0.06	0.73	3.02	11.45	0.01	0.15
Sepal Length	55.36 (5.38)	54.85 (7.92)	0.88	1.00	0.35	1.00	-0.14	8.07	0.89	1.00
Sepal Width	13.39 (1.76)	8.06 (0.89)	2.26	1.00	0.13	0.92	-7.70	12.39	0.00	0.00
Petal Length Width	22.92 (3.95)	17.75 (2.75)	0.69	1.00	0.41	1.00	-2.99	12.93	0.01	0.15
Petal Width Width	16.97 (3.97)	12.27 (1.71)	3.25	1.00	0.07	0.78	-3.14	11.64	0.01	0.13
Stylode Length	24.69 (5.7)	18.16 (5.4)	0.02	1.00	0.90	1.00	-2.24	11.29	0.05	0.32
Seed morphology										
Volume (mm^3)	3156 (735)	3213 (569)	0.51	1.00	0.48	1.00	0.20	17.48	0.85	1.00
Weight (g)	1.29 (0.36)	1.74 (0.47)	0.56	1.00	0.45	1.00	2.25	12.63	0.04	0.21
Testa thickness	0.96 (0.21)	0.94 (0.13)	1.36	1.00	0.24	1.00	-0.29	11.79	0.78	1.00
Total area (mm^2)	173.2 (40.9)	200.1 (26.1)	1.29	1.00	0.26	1.00	1.57	11.87	0.14	0.43
Endosperm area (mm^2)	63.8 (12.7)	76.35 (14.5)	0.11	1.00	0.74	1.00	1.85	13.76	0.09	0.35

[1]Holm correction applied to p-value significance

610 **Table 4.** Permutational multivariate analysis of variation using distance matrices (PERMANOVA)

611 results for enset floral morphology with origin (domesticated vs wild) and landrace as explanatory

612 factors.

PERMANOVA	Df	Sums Of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)
Origin (wild vs domesticated)	1	49.22	49.22	3.43	0.23	0.01
Landrace	1	1.52	1.52	0.11	0.01	0.99
Both origin and landrace	1	1.36	1.36	0.09	0.01	0.99
Residuals	11	157.90	14.36		0.75	
Total	14	210			1	

613

614

615

616

617

618

619

620

621

622

623

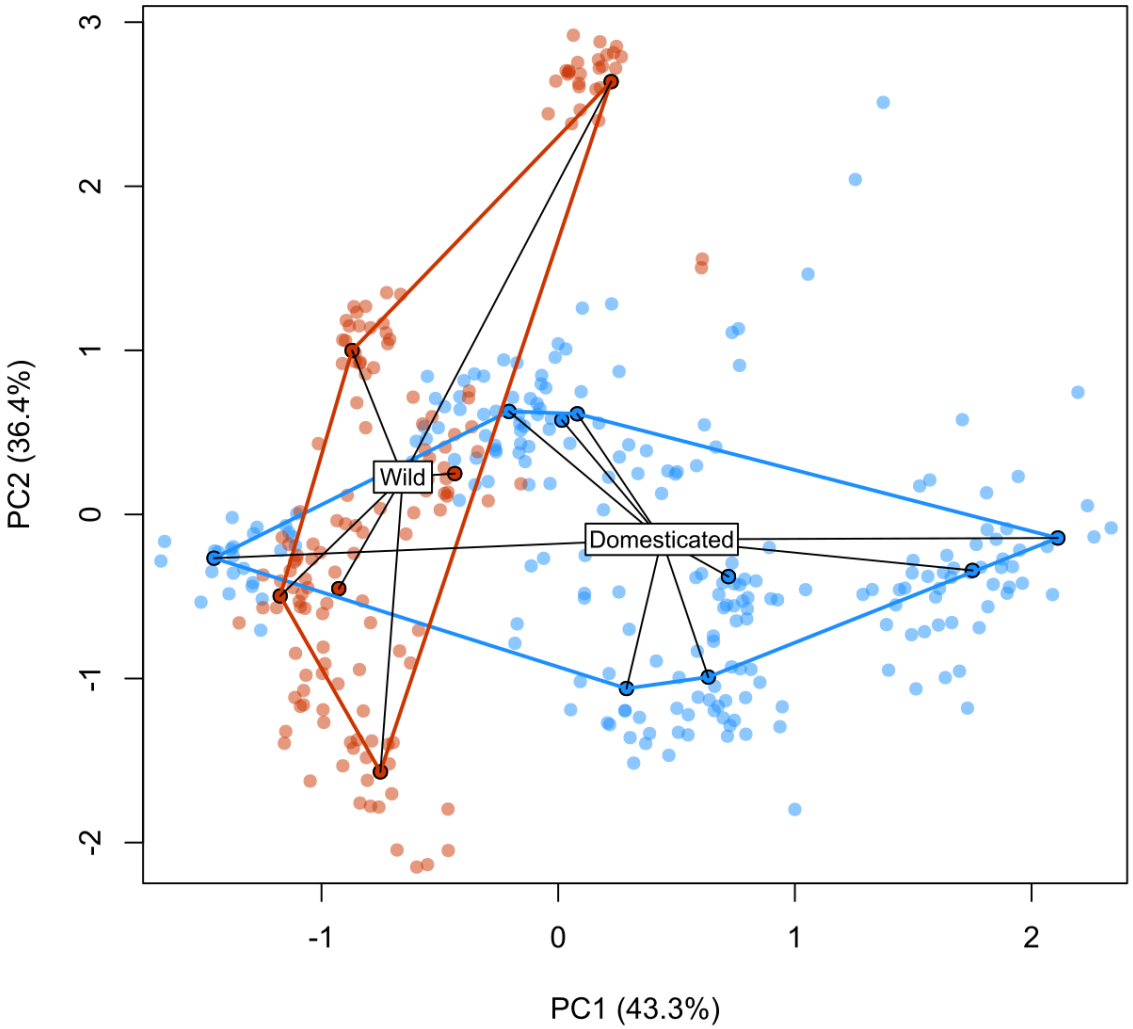
624

625

626

Figures

Fig. 1 Enset cultivation in Ethiopia. A) Mature enset flowering in a neglected field near Checha. B) A farmer removing the meristematic tissue of a two-year old enset, as part of processing for vegetative propagation. C) Numerous adventitious buds sprouting from prepared corm, near Bonga.



643

644 **Fig. 2** Redundancy analysis of male floral morphology in *Ensete ventricosum.* Solid points denote

645 means aggregated by sample, background points illustrate variation of floral traits.

646

647

648

649

650

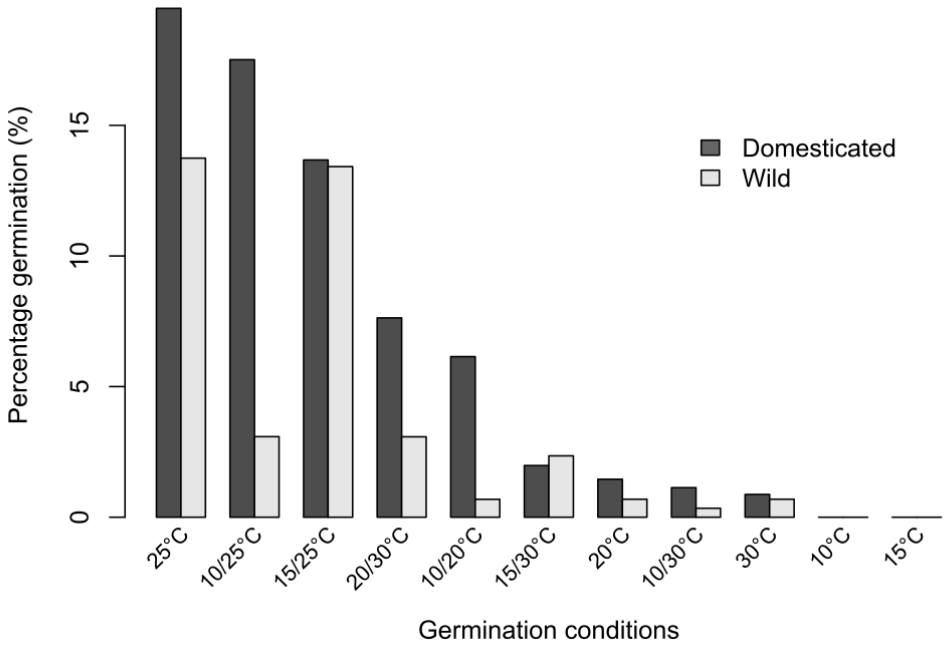


Fig. 3 Percentage germination across a range of environmental conditions for wild and domesticated enset (Exp 2), after 120 days. Data are corrected for variation in seed viability.

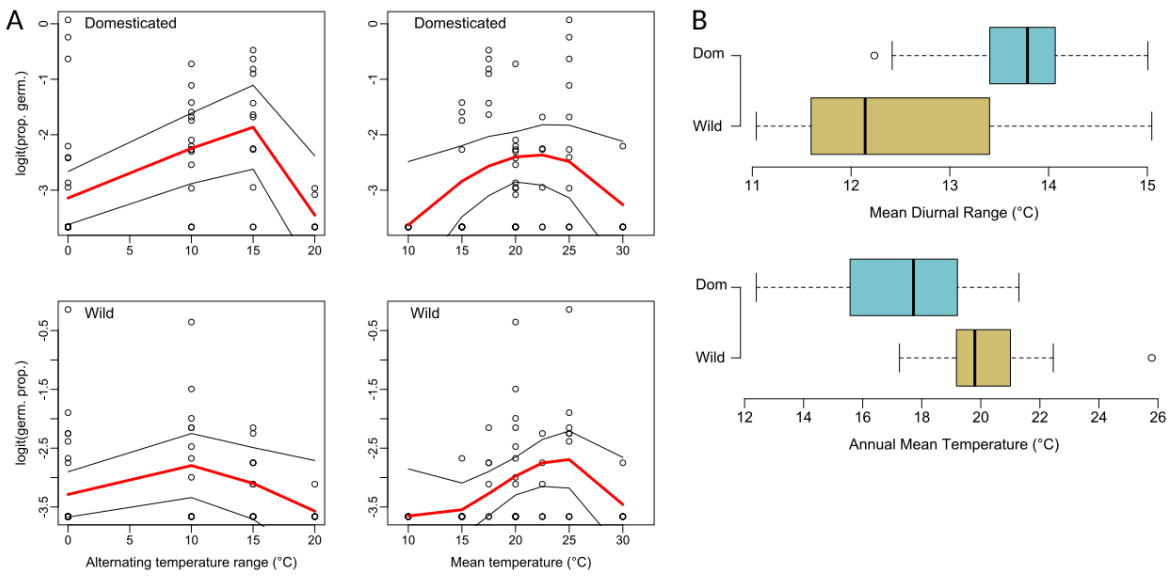


Fig. 4 Germination behaviour and regional climate variables for wild and domesticated enset in Ethiopia. A) Polynomial regression of logit transformed germination proportion in wild and domestic accessions under varying mean temperature and alternating temperature regimes. Each point denotes a single treatment, corrected to account for variation in seed viability. B) Boxplots of regional climate for domestic and wild enset records in Ethiopia.

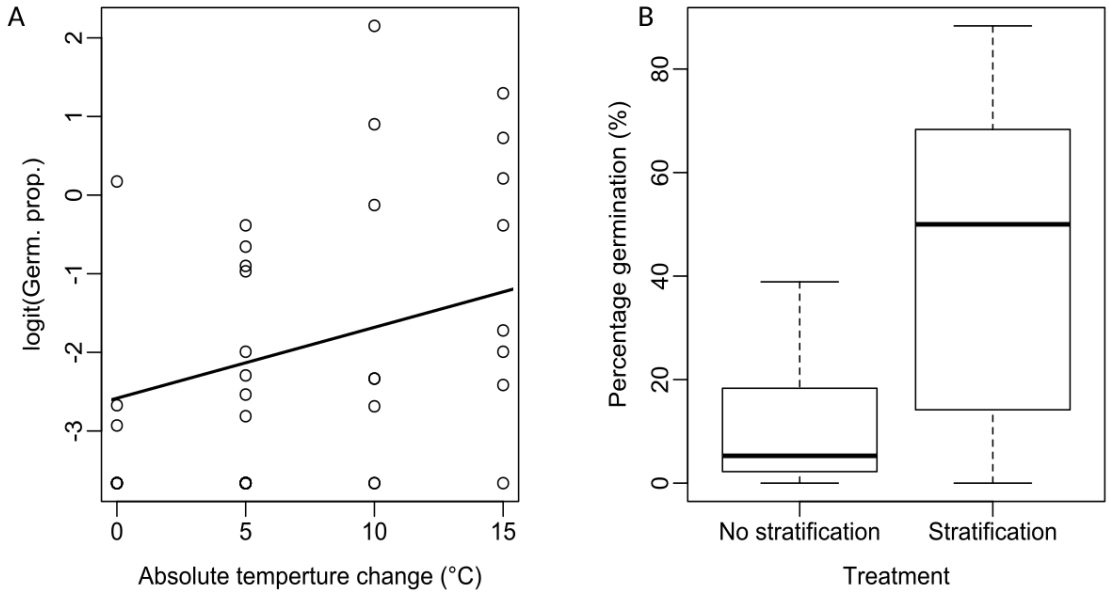


Fig. 5 Analysis of the influence of temperature shifts and dormancy on domesticated enset germination (EXPs 3 and 4). A) The influence of absolute temperature change from constant to alternating temperature, showing a positive relationship with increased germination response for larger temperature shifts. B) Comparison of germination response for stratified seeds (3 months at 10°C), versus no stratification (immediate exposure to experimental conditions).