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► **To cite this version:**

Valérie Le Corre, Mathieu Siol, Yves Vigouroux, Maud Tenaillon, Christophe Délye. Adaptive introgression from maize has facilitated the establishment of teosinte as a noxious weed in Europe. Proceedings of the National Academy of Sciences of the United States of America, 2020, 117 (41), pp.25618-25627. 10.1073/pnas.2006633117. hal-03007689

HAL Id: hal-03007689

<https://hal.science/hal-03007689v1>

Submitted on 25 Nov 2020

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2 **Main Manuscript for**

3 **Adaptive introgression from maize has facilitated the establishment**
4 **of teosinte as a noxious weed in Europe**

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18

19 **Classification:** BIOLOGICAL SCIENCES, EVOLUTION

20 **Keywords:** plant invasion, rapid adaptation, genetic introgression, flowering time, herbicide
21 resistance.

22 **Author Contributions:** V.L.C, M.S, Y.V. M.I.T. and C.D. co-designed research; V.L.C., M.S. and C.D.
23 performed research and analyzed data; V.L.C., M.S., M.I.T., Y.V. and C.D. wrote the manuscript and
24 V.L.C. coordinated the overall study.

25

26 **Abstract**

27 Global trade has considerably accelerated biological invasions. The annual tropical teosintes, maize
28 closest wild relatives, were recently reported as new agricultural weeds in two European countries,
29 Spain and France. Their prompt settlement under climatic conditions differing drastically from that of
30 their native range indicates rapid genetic evolution. We performed a phenotypic comparison of French
31 and Mexican teosintes under European conditions, and showed that only the former could complete
32 their life cycle during maize cropping season. To test the hypothesis that crop-to-wild introgression
33 triggered such rapid adaptation, we used single nucleotide polymorphisms to characterize patterns of
34 genetic variation in French, Spanish and Mexican teosintes as well as in maize germplasm. We showed
35 that both Spanish and French teosintes originated from *Zea mays* ssp. *mexicana* race “Chalco”, a weedy
36 teosinte from the Mexican highlands. However, introduced teosintes differed markedly from their
37 Mexican source by elevated levels of genetic introgression from the high latitude Dent maize grown in
38 Europe. We identified a clear signature of divergent selection in a region of chromosome 8 introgressed
39 from maize and encompassing *ZCN8*, a major flowering time gene associated with adaptation to high
40 latitudes. Moreover, herbicide assays and sequencing revealed that French teosintes have acquired
41 herbicide resistance via the introgression of a mutant herbicide-target gene (*ACC1*) present in herbicide-
42 resistant maize cultivars. Altogether, our results demonstrate that adaptive crop-to-wild introgression
43 has triggered both rapid adaptation to a new climatic niche and acquisition of herbicide resistance,
44 thereby fostering the establishment of an emerging noxious weed.

45

46 **Significance Statement**

47 The emergence of noxious weeds poses serious threat to agricultural production. Understanding their
48 origin and evolution is therefore of major importance. Here we analyzed the intriguing case of teosinte,
49 a wild relative of maize originating from Mexico that recently emerged as an invasive weed in maize
50 fields in Europe. Patterns of genetic variation revealed extensive genetic introgression from maize
51 adapted to temperate latitudes into European teosintes. Introgressed genomic regions harbored a key
52 flowering time gene and an herbicide resistance gene. Our results exemplify how adaptive introgression
53 can foster the evolution of crop wild relatives into weeds difficult to control. Hybridization is an
54 evolutionary force that should not be underestimated when forecasting invasiveness risks.

55

56 **Main Text**

57

58 **Introduction**

59

60 Globalization of trade and transports has considerably accelerated the rate of dispersal of species
61 outside their native range (1). In Europe, the rate of introduction of alien plants has increased
62 exponentially during the last century (2). This rate is expected to further increase in all temperate regions
63 of the Northern Hemisphere due to climate changes (3). Alien plants are a serious threat to native wildlife
64 and its associated ecosystem services, and can have direct detrimental consequences on agriculture
65 production or human health (4). Understanding the origin and establishment of invasive plants is
66 therefore of major importance. This includes deciphering the dynamic of the genetic composition of
67 populations associated with founding events and geographical expansion and identifying the adaptive
68 genetic changes sustaining their habitat shifts (5,6). Such inferences are however challenging when
69 introductions are ancient, histories of invasion complex and when admixture between multiple source
70 populations has taken place (e.g. 7). It is therefore particularly valuable to access the very early
71 colonization steps in recently introduced species (*sensu* 8).

72 Among invasive species, those that colonize agricultural areas are interesting in several respects: they
73 have immediate consequences on crop production sustainability; they may spread rapidly via human-
74 mediated dispersal and farming activities (9); they display a suite of specific adaptive characteristics
75 also described as “the agricultural weed syndrome” (10). This syndrome includes traits such as seed
76 dormancy, short life-cycle and high fecundity. Two broad categories of agricultural weeds can be
77 distinguished: those that evolved from crop relatives and those that evolved from wild species unrelated
78 to any crop (11). Crop-related weeds display particular mechanisms of adaptation including adaptive
79 genetic introgression from the crops leading to the acquisition of crop-mimicry traits (12, 13). Many crop-
80 related wild species are among the most problematic weeds worldwide. Well-known examples are
81 weedy rice, wild sorghum species, and wild sunflower species (12, 13).

82 Here we focused on the extremely recent invasion of Europe by emerging noxious weeds related to
83 maize (*Zea mays* ssp. *mays*), i.e. the annual teosintes. The European Food Authority has officially
84 reported the presence of teosinte as weeds in maize production areas in Spain and France in 2016 (14).
85 In Spain, teosintes have invaded an area in the provinces of Aragon and Catalonia where they cause
86 important yield loss in maize fields (15). In France, teosintes are present in the north of the Nouvelle
87 Aquitaine region. According to a technical report, French teosintes were first observed in the early 1990's
88 (16). In their native range, teosintes most closely related to maize (i.e. from the *Zea mays* species)
89 encompass three annual subspecies: ssp. *huehuetenangensis* with a narrow distribution in western
90 Guatemala (17), ssp *parviglumis* (hereafter: *parviglumis*) and ssp *mexicana* (hereafter: *mexicana*) both
91 encountered in Mexico, the cradle of maize domestication. *Parviglumis* is considered as the ancestor of
92 maize (18, 19) and grows in the west coast lowlands of Mexico under warm and humid tropical
93 conditions. *Mexicana* grows in the central highlands of Mexico, at elevations up to 2800 meters, under

94 cooler and drier conditions (17). The geographical distributions of these two subspecies slightly overlap
95 and hybridization occurs (20). Interestingly, gene flow from mexicana to maize has contributed to
96 highland adaptation of maize landraces (21). Field observations in Mexico describe parviglumis as
97 forming large populations in natural and semi-natural habitats, whereas mexicana is mainly observed
98 as a weed within maize fields, where it can cause severe yield loss (22-24).

99 While genetic assessment of French weedy teosintes is currently lacking, two previous studies have
100 attempted to establish the origin of Spanish teosintes. Their genetic characterization through single
101 nucleotide polymorphism (SNP) genotyping combined with existing SNP datasets for maize and
102 Mexican teosinte populations has however failed to clearly group Spanish teosintes with either
103 parviglumis or mexicana. Instead, Spanish teosintes were found intermediate between maize and
104 mexicana (25). Microsatellite markers further confirmed the importance of maize contribution to the
105 genetic make-up of Spanish teosintes (26). Here, we collected French teosinte populations and describe
106 for the first time their genetic diversity. This new dataset was combined with previously published ones
107 in order to, (i) elucidate the taxonomic origin of French teosintes and identify the source populations, (ii)
108 assess their genetic similarity with Spanish teosintes, (iii) describe the extent of genetic admixture
109 between European teosintes and cultivated maize and (iv) identify genomic regions that have
110 contributed to the successful adaptation of teosintes as weeds in European maize fields.

111

112

113 **Results**

114

115 **Spanish and French teosintes both originate from *Zea mays* ssp. *mexicana***

116 Phenotypic data from a common garden experiment conducted in Dijon demonstrated that French
117 teosintes displayed two morphological characteristics, sheath pubescence (52% of plants) and red-
118 colored sheaths (75% of plants), that were observed also in some mexicana plants but not in
119 parviglumis, as previously described (20) (*SI Appendix*, Fig. S1). Genetic variation at 24,544 SNP data
120 was first investigated using Principal Component Analysis (PCA). A clear genetic structuring between
121 teosintes and maize appeared along the first axis that explained 8% of the variation (Fig. 1A). The
122 second axis representing 4.6% of the variation, separated parviglumis, mexicana and the European
123 teosintes into three non-overlapping groups (Fig. 1A). This second axis revealed a much closer proximity
124 of European teosintes to mexicana than to parviglumis.

125 Results from fastStructure (27) at the subspecies level ($K=3$, ssp. *parviglumis*, ssp. *mexicana*, ssp.
126 *mays*) further supported this observation, with European teosintes being of predominant mexicana
127 ancestry (Fig. 1B). Increasing the number of genetic groups to $K=11$ (Fig. 1B and *SI Appendix*, Dataset
128 S1) confirmed previous reports defining, in addition to the Spanish and French teosintes, nine reference
129 genetic groups (28-32): (i) parviglumis accessions clustered into four geographical genetic groups
130 (hereafter, PARV1, PARV2, PARV3 and PARV4) (Fig. 1B and *SI Appendix*, Fig. S2); (ii) some

131 parviglumis populations were highly admixed with mexicana (29); (iii) mexicana accessions grouped into
132 two genetic clusters (hereafter MEX1 and MEX2) corresponding to geographical races «Chalco» and
133 «Central Plateau», respectively (17, 29, Fig. 1B and *SI Appendix*, Fig. S2); (iv) in maize, the three
134 observed genetic clusters corresponded to three major germplasm pools (Fig. 1B), the tropical
135 landraces, the Dent inbred lines and the Flint inbred lines (hereafter TROP, DENT and FLINT), with
136 admixture among them (31, 32). In agreement with the PCA results, the results of FastStructure at $K =$
137 11 separated French and Spanish teosinte populations in two distinct genetic clusters, different from the
138 nine reference genetic groups found across parviglumis, mexicana and maize clusters.

139 Consistently with results at $K=3$, the average pairwise genetic differentiation F_{ST} between the
140 French/Spanish teosintes with mexicana accessions (0.138/0.195) was on average smaller than with
141 the parviglumis populations (0.195/0.213) (*SI Appendix*, Table S1). Pairwise F_{ST} between French and
142 Spanish teosinte populations was 0.237, a value greater than that observed between mexicana and
143 parviglumis populations (0.105). Genetic diversity within groups as measured by Nei's heterozygosity
144 was similar for Spanish (0.251) and French teosintes (0.221). These values stand within the range of
145 genetic diversity estimates both within mexicana (0.273 for MEX1 and 0.258 for MEX2) and within
146 parviglumis clusters (ranging from 0.131 to 0.305; *SI Appendix*, Table S1).

147 We further employed the f -statistics framework (33) to test histories of divergence among parviglumis,
148 mexicana, and the European teosintes, using *Zea luxurians* as an outgroup. Observed values of
149 f_4 (French or Spanish teosinte, *Z. luxurians*; mexicana, parviglumis) were consistently significantly
150 positive, again arguing in favor of a tree topology where both the French and the Spanish teosintes
151 populations are more closely related to mexicana than to parviglumis (Fig. 2A). The f_4 values observed
152 for the two mexicana genetic clusters, MEX1 and MEX2, were however similar, so that the origin of
153 European teosinte could not be more precisely refined using this statistic.

154 **Footprints of admixture from maize to the European teosintes**

155 The FastStructure analysis detected footprints of maize admixture within French and Spanish teosintes
156 (Fig. 1B). In order to examine admixture patterns in more details, we used TreeMix (34) to reconstruct
157 phylogenetic relationships among the nine reference genetic groups defined by fastStructure
158 (ancestry>0.8, *SI Appendix*, Dataset S1) and the European teosintes. Without migration, the topology
159 inferred was in agreement with the known relationships among subspecies (*SI Appendix*, Fig. S3A).
160 European teosintes were most closely related to mexicana. This topology explained 98.4% of the
161 observed covariance among populations. Adding five migration events increased the proportion of
162 variation explained (99.7%), with the likelihood reaching an asymptote (*SI Appendix*, Fig. S3C-D). In the
163 maximum-likelihood tree with five migration events (Fig. 2B), both French and Spanish teosintes were
164 closest to the mexicana reference group MEX1, the “Chalco” mexicana group.

165 Treemix analyses pinpointed migration between Dent and Tropical maize lines, likely reflecting the
166 admixed origin of Corn Belt Dents between Northern Flint ancestors and tropical material (31). There

167 was also evidence for admixture between maize (ancestral node or edge) and both parviglumis and
168 mexicana. Those events are well documented (18, 20-21). More importantly, both French and Spanish
169 teosintes displayed admixture from the Dent maize reference group (Fig. 2B). Note that the migration
170 event between Dent maize and Spanish teosintes was the most-supported with an estimated weight of
171 0.39, while the migration edge between Dent maize and French teosintes was added in third (*SI*
172 *Appendix*, Fig. S4, estimated weight=0.14).

173 Admixture between European teosintes and each of the three maize reference group was further tested
174 using a four-population test where each mexicana reference group was used as a sibling population:
175 f_4 (European teosinte group, mexicana reference group; maize reference group, parviglumis). We first
176 verified that none of the reference mexicana group was itself admixed with either maize or parviglumis
177 by estimating f_4 (MEX1, MEX2; maize, parviglumis), which was consistently not significantly different
178 from zero (*SI Appendix*, Table S2). The four-population tests for admixture in French or Spanish teosinte
179 instead were all significant (*SI Appendix*, Table S2). In agreement with estimated weights for migration
180 edges, Z-scores were greater for Spanish teosintes in comparison to French teosintes, and for the Dent
181 maize group in comparison to Tropical and Flint. The proportion of Dent maize ancestry estimated using
182 the f_4 ratio estimator was 0.122 (95% confidence interval 0.114 – 0.131) for French teosintes and 0.422
183 (95% confidence interval 0.411 – 0.434) for Spanish teosintes.

184 **Introgression from maize has contributed to the adaptation of teosintes in Europe**

185 As tropical, short-day plants, native teosintes flower very late or not at all at higher latitudes (35, 36). A
186 shift towards long-day flowering was therefore necessary for European teosintes to adapt to temperate
187 latitudes. To verify these predictions, we grew plants from all French and from 12 populations of native
188 teosintes in Dijon, France. French teosintes initiated their male flowering from the end of June to the
189 end of July (534 to 1059 growing degree-days after sowing). In contrast, we observed a much-delayed
190 transition to flowering in native teosintes. A single plant out of 24 Mexican mexicana and a majority of
191 Mexican parviglumis plants (17 out of 24) remained vegetative until the end of the experiment (at the
192 beginning of November). When occurring, flowering started at the beginning of September (1703
193 degree-days) in mexicana and at the end of October (2221 degree-days) in parviglumis (Fig. 3A). The
194 flowering of most French teosintes overlapped with the flowering of the three European maize varieties
195 used as controls (which flowered respectively at 782, 904 and 987 degree-days). Synchronous flowering
196 with maize was also observed in infested fields (Fig. 3B). Altogether, these results suggested that
197 flowering-time genes played a prominent role in teosinte adaptation to European day length.

198 We sought signatures of selection using our SNP data, paying specific attention to flowering time genes.
199 We first conducted a PCA using mexicana and European accessions. Second, we performed a genome
200 scan using pcadapt (37) based on squared loadings of the two first principal components (*SI Appendix*,
201 Fig. S5). The two first principal components together explained 15% of the variation. The first component
202 captured the differentiation between native mexicana and European teosintes (Fig. 4A), and we detected
203 45 outlier SNPs significantly associated with it at an FDR of 0.1% (Fig. 4B). The second component

204 mainly differentiated the French teosintes from the Mexicana and Spanish teosintes (Fig. 4A) and
205 revealed only 2 significant outlier SNPs (*SI Appendix*, Fig. S6). Twenty-five out of the 45 outliers
206 detected with component 1 (>55%) were found on chromosome 8 (Fig. 4B). Interestingly, we observed
207 elevated levels of genetic differentiation ($F_{ST} > 0.60$) of both French teosintes and Spanish teosintes
208 with native mexicana at a subset of 12 outlier SNPs all located on the same region of chromosome 8
209 (*SI Appendix*, Fig. S7).

210 We hypothesized that this pattern resulted from a recent adaptive introgression of a maize chromosomal
211 fragment into the European teosintes. Searching for maize introgression using the ELAI software indeed
212 revealed a peak of introgression from maize to the European teosintes on chromosome 8 (Fig. 5). A
213 cluster of 9 adjacent outlier SNPs distant by less than 10kb was located under this peak. Remarkably,
214 this cluster included the major maize flowering time gene *ZCN8* (38). Note that a second major flowering
215 time gene, *RAP2.7*, and its regulator, *VGT1* (39) were located in the introgressed region but not in close
216 vicinity to any of the outlier SNPs. Patterns of linkage disequilibrium (LD) in the region surrounding these
217 two candidate genes uncovered a signal of elevated LD around *ZCN8* in French teosintes and to a
218 lesser extent in Spanish teosintes (Fig. 5). We recovered no such pattern around *RAP2.7*. A detailed
219 examination of genotypes along chromosome 8 further confirmed the presence of a high frequency
220 extended haplotype at *ZCN8* in French teosintes. This same haplotype seems present in the Spanish
221 teosinte but is still segregating, suggesting weaker or partial selection at this position if any (*SI Appendix*,
222 Fig. S8).

223 **Acquisition by French teosintes of an herbicide resistance gene introgressed from maize**

224 In France, maize cultivars resistant to the herbicide cycloxydim carry an allele of the gene encoding
225 acetyl-CoA carboxylase 1 (*ACC1*) with a mutation that confers resistance to herbicides inhibiting this
226 enzyme (Duo System®, 40). Because French teosintes grow in the vicinity of such cultivars, we
227 assessed their sensitivity to cycloxydim. Out of 200 French teosinte seedlings assayed, 86 (43%) were
228 rated resistant to cycloxydim and 114 (57%) were rated sensitive. As controls, we used a sensitive (RGT
229 TetraXX) and a resistant (RGT CarmiDuo) maize cultivar. As expected, all plants from the former cultivar
230 were rated sensitive while all plants from the latter were rated resistant. To explore the possibility that
231 resistant alleles were transferred from maize to teosintes, we genotyped the *ACC1* gene in teosintes
232 and maize at all known codons involved in herbicide resistance. We detected one mutation at codon
233 1781 (Ile to Leu) in the resistant maize variety (RGT CarmiDuo) and in some of the French teosintes.
234 All CarmiDuo maize plants were homozygous mutant. All plant from the sensitive maize cultivar
235 (TetraXX) were homozygous wild-type. Among the French teosinte plants assayed for herbicide
236 sensitivity, all 86 herbicide-resistant plants were homozygous mutants at codon 1781. Among the 114
237 herbicide-sensitive teosinte plants, 78 were heterozygous and 36 were homozygous wild-type.

238 *ACC1* is located on chromosome 2, in a region where a peak of squared loadings was observed with
239 the second principal component of the PCA differentiating the French from the Spanish teosintes (*SI*
240 *Appendix*, Fig. S6). Although it did not pass the FDR at 0.1%, this region was associated with a large

241 divergence between French and Spanish teosintes (Fig. 4A). When examining the pattern of
242 introgression over the chromosome 2 using ELAI, it was clear that a large genomic region encompassing
243 *ACC1* and spanning more than 500 SNPs from our genotyping chip had been introgressed from maize
244 into homozygous *ACC1*-mutant teosinte plants, whereas wild-type plants showed no introgression and
245 heterozygotes showed the expected halved ancestry dosage (Fig. 6A). Sequencing the full genomic
246 DNA corresponding to the coding sequence of *ACC1* revealed that all mutant teosintes carried one
247 same *ACC1* allele that was exactly identical to the allele carried by herbicide-resistant maize cultivars,
248 whereas wild-type *ACC1* alleles carried by non-mutant French teosintes clustered with the alleles carried
249 by the mexicana accessions (Fig. 6B). Taken together, these results demonstrate that the mutant *ACC1*
250 allele present in French teosinte was introgressed from cultivated, herbicide-resistant Duo System
251 maize.

252

253

254 **Discussion**

255

256 We report here the first genetic description of the very recent settlement in France of teosintes, which
257 are recognized in their native tropical range as a major threat to agricultural production (41). We
258 addressed three main questions: Where do they originate? How did they adapt to Europe? To which
259 extent has introgression from maize facilitated their establishment?

260 Our results clearly assigned both French and Spanish teosintes (25) to *Zea mays* ssp. *mexicana*, and
261 suggested a single geographical origin for all invasive populations of teosintes reported to date in
262 Europe. We identified the source genetic group as the mexicana race “Chalco” (MEX1). This finding is
263 interesting in at least two respects: first, Chalco teosintes are located at elevations ~2300 m and higher
264 (29). They are therefore adapted to moderate rainfall and low temperatures (17), environmental
265 conditions that are closer to the European climate than the Mexican tropical lowlands. Second, Chalco
266 teosintes are the ones that hybridize the most frequently with maize (42, 43). Plants carrying hybrid-like
267 cobs have been reported frequently in Mexico for Chalco teosintes growing within or near maize fields
268 (23, 24). In fact, Chalco teosintes have consistently been described as weeds infesting cultivated maize
269 fields (22, 24, 41). This parallels the sites colonized in France and Spain, which are chiefly maize fields
270 (15).

271 However, the native and introduced ranges differ strongly in their latitude and hence their photoperiod,
272 short-days in the native range *versus* long-days in the introduced one. In Mexico, mexicana occurs at
273 latitudes comprised roughly between 18° North and 20° North. In Europe, invasive mexicana are
274 observed at latitudes comprised between 42° North (in Spain) and 46° North (in France), which would
275 correspond to an area north of Chicago in the US. Flowering is accelerated by short-days in native
276 teosintes (35). We indeed confirmed that native mexicana populations flowered very late in France and
277 were unable to produce seeds before maize harvest time. On the contrary, the flowering period of French

278 teosintes was much earlier, and overlapped that of maize. The establishment of mexicana in Europe
279 therefore most likely involved a substantial genetic shift in the control of flowering time.

280 Given the narrow time window for adaptation to occur from *de novo* mutations (two to three decades),
281 we combined outlier detection in European teosintes and the examination of introgression patterns from
282 maize to test whether pre-adapted local maize varieties had contributed to teosinte adaptation. In line
283 with this hypothesis, we detected introgression in both Spanish and French teosintes. Interestingly, we
284 observed a marked pattern of introgression in a genomic region that contains *ZCN8*, a gene that
285 underlies one of the largest maize flowering time quantitative trait locus (QTL). Consistently, this region
286 was enriched for outlier SNPs displaying high differentiation between native and European mexicana
287 populations. *ZCN8* is a key floral activator of the maize flowering time pathway also known to be involved
288 in photoperiod sensitivity (44). Guo et al. (38) have shown that two polymorphisms with an additive effect
289 in the promoter of *ZCN8* are associated with early flowering time under long days. These polymorphisms
290 exist as standing variation in mexicana. They have been under strong selection during early maize
291 domestication and contributed to latitudinal adaptation in this crop (31, 38). While introduction to Europe
292 of a pre-adapted, early-flowering mexicana population is a possibility, here we propose that introgression
293 from a maize early flowering variant at *ZCN8* opened up a new niche for weedy teosintes in Europe.
294 Indeed, a clear signal of selective sweep was observed around *ZCN8* in French teosintes, consistent
295 with a single event of adaptive introgression, i.e. hard sweep signature. Interestingly, a similar haplotype
296 was observed in Spanish teosintes, albeit with a greater level of heterozygosity, which suggests an
297 ongoing, incomplete selective sweep (*SI Appendix*, Figure S8). Genetic introgression was not limited to
298 this genomic region but was pervasive in all European teosintes (*SI Appendix*, Fig. S9). Given the
299 complexity and number of genes involved in the regulation of maize flowering time, we suspect several
300 genes other than *ZCN8* to contribute to the substantial shift in flowering time in European teosintes (see
301 *SI Appendix*, Fig. S9 and Dataset S2 for a proposed list of candidate genes). Our experimental design,
302 however, recovered no specific selection signal at any of the *a priori* flowering time candidate genes.

303 We pinpointed the Dent genetic group as the most likely donor of the introgressed segments. Because
304 modern maize varieties deriving from the Dent germplasm are widely cultivated in Europe but not in
305 Mexico, where tropical germplasm is dominant (32; 45), this suggests that hybridization has occurred
306 after the introduction of mexicana to Europe. In other words, our results are consistent with a scenario
307 where European maize varieties adapted to temperate latitudes have contributed to the establishment
308 of teosintes in Europe. Note that hybridization is seemingly still occurring, as plants carrying hybrid-like
309 cobs are regularly observed in infested maize fields in Spain (25, 26) as well as in France (*SI Appendix*,
310 Fig. S10). One surprising outcome of our study is the much lower global introgression rate in the French
311 as compared to the Spanish teosintes (*SI Appendix*, Fig. S9), despite the seemingly earlier French
312 introduction. Introgression segments can convey alleles that are deleterious for the maintenance of
313 weediness traits and eliminated over time by purifying selection (13), perhaps explaining subtler
314 introgression patterns in French teosintes. Along this line, we detected very little introgression from
315 maize to European teosintes on an extended portion of the short arm of chromosome 4 (*SI Appendix*,

316 Fig. S9). This region encompasses a large domestication QTL hotspot containing several loci involved
317 in the variation of domestication traits between maize and teosinte, as well as the incompatibility locus
318 *TCB1* (46, 47, see *SI Appendix*, Fig. S9 and Dataset S2). Our results, together with those of a previous
319 study (43) strongly suggest that introgression from maize to mexicana is counter-selected in this
320 genomic region. In European teosintes, such selection most likely contributes to preserve wild alleles
321 necessary for the maintenance of the weedy phenotype.

322 Last, but not least, we detected a second adaptive introgression specific to the French teosintes and
323 involving a large region of chromosome 2. This region encompasses an allele of the herbicide-target
324 gene *ACC1* carried by herbicide-resistant maize cultivars. Such cultivars have been authorized in
325 France since 2001 and are cultivated in the area where teosintes occur (16). Teosinte plants
326 homozygous for this mutant allele are herbicide-resistant, which is clearly an adaptive trait in agricultural
327 fields. Since the history of cultivation of resistant maize cultivars in France is quite recent, it follows that
328 the introgression of the *ACC1* region in French teosintes is also very recent, consistent with the fact that
329 a large region of chromosome 2 encompassing *ACC1* was introgressed.

330 In conclusion, while introgression has been proposed as a key source of adaptive genetic variation (48,
331 49), establishing it has been particularly challenging with only a handful of reported cases. Notorious
332 examples often illustrate the contribution of wild relatives to domesticated gene pools (50), more rarely
333 the reverse (but see 51-53). This is because crop-to-wild gene flow may not always be beneficial since
334 many characteristics making crops suitable to cultivated environments are detrimental for wild or weedy
335 forms (e.g., non-shattering seeds, lack of dormancy, bolting time) (10). In some instances, however, a
336 crop allele can provide a clear advantage to a wild or weedy form, e.g. by conferring a given resistance
337 or by allowing a niche shift. Here, we present two clear evidence of adaptive introgression from locally
338 adapted crop varieties to a wild relative. One introgression facilitated reproduction under temperate
339 latitudes, the other enabled plants to thrive in herbicide-treated fields. Together, those introgressions
340 contributed to the establishment of a new weed. Previous studies have reported a low rate of
341 spontaneous hybridization between mexicana and cultivated maize, less than 1% per generation (54).
342 However, first-generation hybrids display a great vigor, and are highly male fertile (55). We propose that
343 the rare first-generation hybrids served as a bridge for the transfer of maize genes into mexicana
344 populations, fostering their local adaptation. This result nicely parallels the contribution of mexicana
345 alleles to highland maize landraces adaptation (42). In sum, we demonstrate that crop-wild introgression
346 can be a two-way street, allowing the transfer of beneficial variants to both partners. Our work highlights
347 the importance of introgression in allowing large evolutionary shifts or even opening up new niches. In
348 the case of maize and teosinte, the common consensus was that given their ecology and biology, the
349 risk of seeing teosinte emerge as a problematic weed under a temperate climate was remote (17, 20).
350 Here we not only show that such risk exists, but more generally that crop-wild introgression should not
351 be underestimated when forecasting invasiveness risks.

352

353

354 **Materials and Methods**

355

356 **Plant material and genotyping**

357 Teosinte seeds were collected from eight cultivated fields in the region of Nouvelle Aquitaine, France,
358 in autumn 2017 (*SI Appendix*, Table S3). Geographic distances between fields varied from 0.25
359 kilometers to 11 kilometers. In spring 2018, seeds were germinated in growth chambers at 25°C and
360 16h day length. Leaf samples were harvested from a total of 70 French teosintes individuals (4 to 14
361 individuals per field population). Leaf fragments were ground in liquid nitrogen and DNA extracted using
362 kit NucleoSpin Plant II (Macherey-Nagel). Genotyping was performed by Eurofins Genomics using the
363 Illumina MaizeSNP50 BeadChip array (Illumina, San Diego, CA, USA). SNPs were called using the
364 GenomeStudio algorithm (Illumina). Out of 56,110 markers contained on the chip, 49,574 could be
365 successfully genotyped on all plants.

366 **Phenotypic assays**

367 A common garden experiment was conducted in 2018 at INRA in Dijon, France (47.32°N; 05.10°E) to
368 compare phenotypic variation in French and Mexican teosintes. Seed material from six populations of
369 the subspecies *mexicana* and six populations of the subspecies *parviglumis* were obtained from M.I.
370 Tenailon (collection described in 56, 57). This material was used as reference material in the common
371 garden (see below). The experiment included 48 plants from the eight populations of French teosinte
372 (six plants per population), 24 plants from the six mexicana populations (three to five plants per
373 population) and 24 plants from the six parviglumis populations (four plants per population). We included
374 three maize varieties commercialized in France: ES Gallery (36 plants), RGT CarmiDuo (12 plants) and
375 RGT TetraXX (12 plants). All seedlings were transplanted one week after sowing and arranged in a
376 semi-randomized single block design with alternate rows of teosinte and maize. The experiment was
377 set up on the 24th of May and ended on the 6th of November. We measured traits related to early growth
378 and architecture (plant height, number of leaves on the main tiller and number of primary tillers), leaf
379 shape (length, width and their ratio), the presence of trichomes on leaf sheaths, sheath color and
380 flowering time (time to emission of the tassel and time to silking). Due to the much-delayed transition to
381 reproduction in Mexican teosintes (see results), post-reproductive traits were not considered.

382 **Herbicide sensitivity bioassay**

383 French teosintes have almost exclusively been observed in maize-growing fields. Growers in the area
384 where teosintes are present have tried to control it using non-genetically modified, herbicide-resistant
385 maize cultivars (Duo System®, BASF) that withstand the application of the herbicide cycloxydim.
386 Bioassays were conducted to assess the herbicide sensitivity of French teosinte seedlings issued from
387 seeds from the eight populations collected in maize fields. Seedlings were grown in individual pots in a
388 glasshouse at 22/18 °C day/night with 14-h photoperiod. At the 2-leaf stage, cycloxydim was applied as

389 the commercial herbicide Stratos Ultra (BASF, 100 g/L cycloxydim) at the recommended French field
390 rate (200 g/ha cycloxydim) on 200 teosinte seedlings (25 per population) and on 25 seedlings from each
391 of one classical (RGT TetraXX) and one herbicide-resistant (RGT CarmiDuo) maize cultivar that were
392 included as herbicide-sensitive and herbicide-resistant controls, respectively. Twenty-five additional
393 French teosinte seedlings and 25 seedlings of each maize cultivar were sprayed with water to serve as
394 an untreated control. After 48 hours, the last 0.5 cm of the first leaf of every sprayed seedling was
395 collected for ACCase genotyping (see below). Plants phenotypes were rated three weeks after herbicide
396 application, when herbicide-sensitive control maize plants were clearly dead. Plants killed by the
397 herbicide were rated sensitive (S), while surviving plants were rated resistant (R).

398 **SNP array data**

399 Genotype data for the 70 French teosintes was combined with published and available data for the
400 following material: 40 accessions of Spanish teosintes (25), 314 accessions of parviglumis (28, 29), 332
401 accessions of mexicana (28, 29), 94 maize landraces from Meso- and Central-America (58) and 155
402 maize inbred lines from North-America and Europe (32). We only kept SNPs that were shared and
403 correctly scored among the different datasets, the final combined dataset consisted of 24,544 SNPs
404 genotyped on 1,005 accessions (<http://doi.org/10.5281/zenodo.3959138>). For analyses requiring an
405 outgroup, we included the SNPs data available for twelve accessions of *Zea luxurians* (25) using the
406 24,544 markers above.

407 **Acetyl-CoA carboxylase genotyping and sequencing**

408 Herbicide-resistant, Duo system maize cultivars grown in French fields to control teosinte populations
409 all carry an herbicide-resistant mutant allele of *ACC1*, one of the two maize acetyl-CoA carboxylases
410 (40). The mutation involved has not been published, but the major acetyl-CoA carboxylase codons
411 involved in herbicide resistance are known (codons 1781, 1999, 2027, 2041, 2078, 2088 and 2096 as
412 standardized in 59). Two herbicide-resistant maize cultivars (RGT CarmiDuo and RGT EXXplicit) and
413 the 200 French teosinte plants used in herbicide sensitivity bioassays were genotyped at these codons.
414 The sequences of the two maize acetyl-CoA carboxylases homeologs (Genbank accessions
415 XM_020548014 for *ACC1* and XM_008664827 for *ACC2*) were aligned and gene-specific primers were
416 designed for *ACC1*. Primers pairs AC1ZM3/AC1ZM3R and AC1ZM2/AC1ZM2R (*SI Appendix*, Table
417 S4) were used to amplify *ACC1* regions carrying codon 1781 and codons 1999 to 2096, respectively.
418 Mutations were sought in the amplicons obtained using previously described assays (59).

419 The *ACC1* protein-coding sequence of 12,002 nucleotide with its 32 introns was fully sequenced on both
420 strands in 14 individual plants: one plant from each of the two herbicide-resistant maize cultivars, three
421 French teosinte individuals homozygous mutant at *ACC1* and three homozygous wild-type at *ACC1* as
422 determined after genotyping, one parviglumis individual in each of two Mexican populations, one
423 mexicana individual in each of two Mexican populations, and two *Zea mays ssp. huehuetenangensis*
424 individuals that were used as an outgroup. PCR primers used for sequencing are in *SI Appendix*, Table

425 S4. All sequences were aligned with the maize reference *ACC1* sequence (genbank XM_020548014).
426 A phylogenetic tree was generated using the Neighbour-joining method as implemented in Mega 10.0.5
427 (60) with 1,000 bootstraps.

428 **Population genetic structure**

429 A principal component analysis (PCA) was conducted using the Adegenet R package (61). The
430 clustering program FastStructure (27) was run to evaluate ancestry proportions for K genetic groups,
431 with K varying from 1 to 12 with five replicates for each value of K and using the “simple prior” option
432 (flat beta-prior over allele frequencies). To evaluate the repeatability across runs, and rule out true
433 multimodality (as opposed to cluster labels switching), we ran the program CLUMPP v.1.1.2 using the
434 Greedy algorithm (62). Genetic diversity within each genetic group and pairwise genetic differentiation
435 (F_{ST}) values were calculated using the last version of the EggLib package (63).

436 **Origin of European teosintes**

437 In this analysis, we aimed at inferring the Mexican origin of European teosintes. We first defined Mexican
438 reference groups of parviglumis and mexicana. We considered the results from FastStructure at K=11,
439 as this was the value for which the observed genetic clustering for Mexican teosintes and maize was in
440 best agreement with previous studies (28-32). This clustering revealed six teosinte genetic groups (four
441 from parviglumis, two from Mexicana) as well as three maize genetic groups (tropical landraces, Dent
442 and Flint inbred lines). We retained individuals with an ancestry higher than 0.8 in each group. This set
443 of 628 individuals defined our nine reference groups (*SI Appendix*, Dataset S1).

444 To get a first insight on the proximity between each European teosinte population (Spanish and French)
445 and the reference groups of parviglumis and mexicana, we used the f -statistics first introduced by Reich
446 (64). F -statistics provide a measure of genetic drift between populations, based on the branch length
447 separating them on a simple phylogeny (65). The four-population $f4$ -statistics can be used to investigate
448 ancestry relationships and find the closest relative of a contemporary population by comparing different
449 tree topologies (33; 19). We used $f4$ (European teosinte, *Zea luxurians*; mexicana, parviglumis), where
450 mexicana and parviglumis are the two putative ancestors to European teosintes and *Zea luxurians* is an
451 outgroup. The value of this $f4$ statistics is expected to be positive if the European teosinte descends
452 from mexicana, negative if it descends from parviglumis and null in case of no ancestry relationship (see
453 19 for a similar analysis). Observed $f4$ values were calculated using the fourpop program in TreeMix
454 1.13 (34). Note that we make here the implicit assumption of no gene flow between reference groups.
455 We considered more complex scenarios in the following section.

456 **History of admixture among teosintes and maize**

457 We inferred the relationship between cultivated maize and teosintes using Treemix 1.13 (34) on the nine
458 reference genetic groups defined above. The analysis was based on SNPs allele frequencies in Spanish
459 teosintes, French teosintes and the nine reference genetic groups. Maximum likelihood trees were built

460 using 200 SNP-windows to account for linkage disequilibrium. We tested the addition of 0 to 10 migration
461 events, by building 10 replicate trees for each. We considered as the most meaningful number of
462 migration events the first value at which the mean likelihood of trees and the proportion of explained
463 covariance among groups stabilized towards their maximum asymptotic values.

464 As both the comparison of f -statistics for varying tree topologies and Treemix results assigned mexicana
465 as the most likely ancestor of European teosintes, we performed a four-population test (64, 65)
466 considering (test population, mexicana; maize, parviglumis). We estimated the f_4 statistics for all
467 combinations of the test population being either Spanish or French teosintes, and considering the two
468 mexicana reference groups (MEX1, MEX2), the three maize reference groups, and all parviglumis
469 reference groups grouped together. The expected value of this f_4 statistics is zero if (test population,
470 mexicana) and (maize, parviglumis) form two independently diverged clades. Significant deviation from
471 zero indicates admixture. Before implementing this test, we verified that the two reference mexicana
472 groups were not themselves admixed with either maize or parviglumis, which would confuse
473 interpretation. We did so by estimating f_4 (MEX1, MEX2; maize, parviglumis). Finally, under an
474 admixture scenario of the test population with maize, the admixture proportion in the test population was
475 estimated as the ratio of the two statistics f_4 (parviglumis, *Zea luxurians*; test population, mexicana) and
476 f_4 (parviglumis, *Zea luxurians*; maize, mexicana). Here, in line with the reasoning in Patterson et al. (65)
477 and Peter (33), we used *Zea perennis* as the outgroup, mexicana and maize as the two potential
478 contributors to the admixed test population, and parviglumis as a subspecies more closely related to
479 one of the contributors, here to maize. A 95% confidence interval for the admixture proportion was
480 obtained from a block jackknife procedure, where each block of 200 SNPs was removed in turn.

481 **Signatures of selection and genomic patterns of introgression**

482 A genome-wide scan for signature of positive selection in European teosintes was performed using a
483 principal component analysis over all European teosintes and mexicana populations as implemented in
484 pcadapt (64). In contrast to F_{ST} -based approaches, pcadapt does not require any *a priori* grouping of
485 individuals into populations. It is well suited to scenarios of population divergence and range expansion,
486 as principal components are able to discriminate successive divergence and selection events (37). We
487 performed the analysis for each principal component (component-wise method) and used the loadings
488 (correlation between each PC and each SNP) as the test statistic. Outlier SNPs were identified by
489 transforming the p-values into q-values with a cut-off value of 0.001, ensuring a false discovery rate
490 lower than 0.1% using the R package qvalue (67).

491 We investigated genome-wide patterns of introgression from cultivated maize using the ELAI software
492 (68). Parameters used were 2 upper-layer clusters and 10 lower-level clusters, 30 EM steps and 10
493 generations of admixture between the two source populations identified in the Treemix and f -statistics
494 analyses (non-admixed reference genetic groups as identified above, namely MEX1 and DENT). We
495 thus analyzed each French (Spanish, respectively) teosinte individual as resulting from the introgression
496 between the haplotypes of the two sources populations, MEX1 and Dent. We then plotted the average

497 ancestral allele dosage over all French (Spanish, respectively) teosinte individuals. ELAI analyses were
498 performed separately for each chromosome.

499

500

501 **Acknowledgments and funding sources**

502

503 We thank Bruno Chauvel (INRAE) for bringing to our attention the presence of teosintes in maize fields
504 in France. We thank Séverine Michel from INRAE for herbicide sensitivity bioassays and molecular
505 analysis of the ACC1 gene. We thank Delphine Madur from GQE-Le Moulon for handling DNA samples
506 used for SNP array genotyping. GQE-Le Moulon benefits from the support of Saclay Plant Sciences-
507 SPS (ANR-17-EUR-0007). M. I. T. and Y.V. are supported by an ANR grant (ANR-19-CE32-0009).

508

509 **References**

510

- 511 1. Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era
512 of globalization. *Journal of Applied Ecology* 46, 10-18. DOI: [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2664.2008.01600.x)
513 [2664.2008.01600.x](https://doi.org/10.1111/j.1365-2664.2008.01600.x)
- 514 2. Lambdon PW, Pyšek P, Basnou C, Hejda M, Arianoutsou M, Essl F, Jarošík V, Pergl J, Winter
515 M, Anastasiu P, Andriopoulos P, Bazos I, Brundu G, Celesti-Grappo L, Chassot P, Delipetrou
516 P, Josefsson M, Kark S, Klotz S, Kokkoris Y, Kühn I, Marchante H, Perglová I, Pino J, Vilà M,
517 Zikos A, Roy D, Hulme P E (2008) Alien flora of Europe: species diversity, temporal trends,
518 geographical patterns and research needs. *Preslia* 80, 101-149.
- 519 3. Seebens H, Essl F, Dawson W, Fuentes N, Moser D, Pergl J, Pyšek P, van Kleunen M, Weber
520 E, Winter M, Blasius B (2015) Global trade will accelerate plant invasions in emerging
521 economies under climate change. *Global Change Biology* **21**, 4128–4140. DOI:
522 <https://doi.org/10.1111/gcb.13021>
- 523 4. Vilà M, Hulme PE (2017) Impact of biological invasions on ecosystem services. Springer Nature,
524 359 pages. DOI: <https://doi.org/10.1007/978-3-319-45121-3>
- 525 5. Sax DF, Stachowicz JJ, Brown JH, Bruno JF, Dawson MN, Gaines SD, Grosberg RK, Hastings
526 A, Holt RD, Mayfield MM, O'Connor MI, Rice WR (2007) Ecological and evolutionary insights
527 from species invasions. *Trends in Ecology and Evolution* 22, 465-471. DOI:
528 <https://doi.org/10.1016/j.tree.2007.06.009>
- 529 6. Van Kleunen M, Bossdorf O, Dawson W (2018) The ecology and evolution of alien plants *Annual*
530 *Review of Ecology, Evolution, and Systematics* 49, 25–47. DOI:
531 <https://doi.org/10.1146/annurev-ecolsys-110617-062654>
- 532 7. Barker BS, Andonian K, Swope SM, Luster DG, Dlugosch KM (2017) Population genomic
533 analyses reveal a history of range expansion and trait evolution across the native and invaded
534 range of yellow starthistle (*Centaurea solstitialis*). *Molecular Ecology* 26, 1131-1147. DOI:
535 <https://doi.org/10.1111/mec.13998>
- 536 8. Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ (2000)
537 Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*
538 6, 93–107. DOI: <https://doi.org/10.1046/j.1472-4642.2000.00083.x>
- 539 9. Petit S, Alignier A, Colbach N, Joannon A, Le Couer D, Thenail C (2013) Weed dispersal by
540 farming at various spatial scales. A review. *Agronomy for Sustainable Development* 33, 205-
541 217. DOI: <https://doi.org/10.1007/s13593-012-0095-8>
- 542 10. Baker HG (1974) The evolution of weeds. *Annual Review of Ecology and Systematics* 5, 1-24.
543 DOI: <https://doi.org/10.1146/annurev.es.05.110174.000245>
- 544 11. Vigueira CC, Olsen KM, Caicedo AL (2013) The red queen in the corn: agricultural weeds as
545 models of rapid adaptive evolution. *Heredity* 110, 303–311. DOI:
546 <https://doi.org/10.1038/hdy.2012.104>

- 547 12. Guo L, Qiu J, Li, LF, Lu B, Olsen K, Fan L (2018) Genomic clues for crop-weed interactions and
548 evolution. Trends in Plant Science, 23, 1102-1115. DOI:
549 <https://doi.org/10.1016/j.tplants.2018.09.009>
- 550 13. Ellstrand NC, Merimans P, Rong J, Batsch D, Ghosh , de Jong TJ, Haccou P, Lu BR, Sbow
551 AA, Stewart Jr CN, Strasburg JL, van Tienderen PH, Vrieling K, Hooftman D (2013)
552 Introgression of crop alleles into wild or weedy populations. Annual Review of Ecology,
553 Evolution, and Systematics 44, 325–45. DOI: <https://doi.org/10.1146/annurev-ecolsys-110512-135840>
- 554
- 555 14. European Food Safety Authority (2016) Relevance of new scientific evidence on the occurrence
556 of teosinte in maize fields in Spain and France for previous environmental risk assessment
557 conclusions and risk management recommendations on the cultivation of maize events
558 MON810, Bt11, 1507 and GA21. EFSA supporting publication 2016:EN-1094. 13 pp. DOI:
559 <https://doi.org/10.2903/sp.efsa.2016.EN-1094>
- 560 15. Martínez Y, Cirujeda A, Gómez MI, Marí AY, Pardo G (2018) Bioeconomic model for optimal
561 control of the invasive weed *Zea mays* subsp (teosinte) in Spain. Agricultural Systems 165,
562 116–127. DOI: <https://doi.org/10.1016/j.agsy.2018.05.015>
- 563 16. Arvalis (2013) Téosinte: une adventice qui demande une vigilance toute particulière. 13/14
564 Service Communication Marketing Arvalis (Institut du vegetal), 4 pages.
- 565 17. Sánchez-González JdJ, Ruiz-Corral JA, Garcíá GM, Ojeda GR, Larios LDIC, Holland JB,
566 Miranda-Medrano R, García-Romero GE (2018) Ecogeography of teosinte. PLoS ONE 13,
567 e0192676. DOI: <https://doi.org/10.1371/journal.pone.0192676>
- 568 18. Matsuoka Y, Vigouroux Y, Goodman MM, Sanchez GJ, Buckler E, Doebley J (2002) A single
569 domestication for maize shown by multilocus microsatellite genotyping. Proceedings of the
570 National Academy of Sciences of the United States of America 99, 6080–6084. DOI:
571 <https://dx.doi.org/10.1073%2Fpnas.052125199>
- 572 19. Ramos-Madrigal J, Smith BD, Moreno-Mayar JV, Gopalakrishnan S, Ross-Ibarra J, Gilbert MT,
573 Wales N (2016) Genome sequence of a 5,310-year-old maize cob provides insights into the
574 early stages of maize domestication. Current Biology 26, 3195–3201 DOI:
575 <https://doi.org/10.1016/j.cub.2016.09.036>
- 576 20. Hufford M, Martinez-Meyer E, Gaut BS, Eguiarte LE, Tenailon MI (2012). Inferences from the
577 historical distribution of wild and domesticated maize provide ecological and evolutionary
578 insight. PLoS ONE. 7(11): e47659.
- 579 21. Gonzalez-Segovia E, Pérez-Limon S, Cíntora-Martínez GC, Guerrero-Zavala A, Janzen GM,
580 Hufford MB, Ross-Ibarra J, Sawers RJH (2019) Characterization of introgression from the
581 teosinte *Zea mays* ssp. mexicana to Mexican highland maize. PeerJ 7, e6815. DOI:
582 <http://dx.doi.org/10.7717/peerj.6815>
- 583 22. Collins GN (1921) Teosinte in Mexico. The Journal of Heredity 12, 339-350.

- 584 23. Wilkes HG (1977) Hybridization of maize and teosinte, in Mexico and Guatemala and the
585 improvement of maize. *Economic Botany* 31, 254-293. DOI:
586 <https://doi.org/10.1007/BF02866877>
- 587 24. Vibrans H, Estrada Flores JG (1998) Annual teosinte is a common weed in the valley of Toluca,
588 Mexico *Maydica* 43, 45-48.
- 589 25. Trtikova M, Lohn A, Binimelis R, Chapela I, Oehen B, Zemp N, Widmer A, Hilbeck A (2017)
590 Teosinte in Europe – searching for the origin of a novel weed. *Scientific Reports* 7, 1560. DOI:
591 <https://doi.org/10.1038/s41598-017-01478-w>
- 592 26. Díaz A, Taberner A, Vilaplana L (2020) The emergence of a new weed in maize plantations:
593 characterization and genetic structure using microsatellite markers. *Genetic Resources and*
594 *Crop evolution* 67, 225–239. DOI: <https://doi.org/10.1007/s10722-019-00828-z>
- 595 27. Raj A, Stephens M, Pritchard JK (2014) *fastSTRUCTURE*: Variational inference of population
596 structure in large SNP data sets. *Genetics* 197, 573-589. DOI:
597 <https://doi.org/10.1534/genetics.114.164350>
- 598 28. Aguirre-Liguori JA, Tenailon MI, Vásquez-Lobo A, Gaut BS, Jaramillo-Correa JP, Montes-
599 Hernandez S, Souza V, Eguiarte LE (2017) Connecting genomic patterns of local adaptation
600 and niche suitability in teosintes. *Molecular Ecology* 26, 4226-4240. DOI:
601 <https://doi.org/10.1111/mec.14203>
- 602 29. Pyhäjärvi T, Hufford MB, Mezouk S, Ross-Ibarra J (2013) Complex patterns of local
603 adaptation in teosinte. *Genome Biology and Evolution* 5, 1594–1609. DOI:
604 <https://doi.org/10.1093/gbe/evt109>
- 605 30. Fukunaga K, Hill J, Vigouroux Y, Matsuoka Y, Sánchez-González JJ, Liu K, Buckler ES,
606 Doebley J (2005) Genetic diversity and population structure of teosinte. *Genetics* 169, 2241–
607 2254. DOI: <https://doi.org/10.1534/genetics.104.031393>
- 608 31. Brandenburg J-T, Mary-Huard T, Rigail G, Hearne SJ, Corti H, Joets J, Vitte C, Charcosset A,
609 Nicolas SD, Tenailon MI (2017) Independent introductions and admixtures have contributed to
610 adaptation of European maize and its American counterparts. *PLoS Genet* 13, e1006666. DOI:
611 <https://doi.org/10.1371/journal.pgen.1006666>
- 612 32. Unterseer S, Pophaly SD, Peis R, Westermeier P, Mayer M, Seidel MA, Haberer G, Mayer KFX,
613 Ordas B, Pausch H, Tellier A, Bauer , Schön CC (2016) A comprehensive study of the genomic
614 differentiation between temperate Dent and Flint maize. *Genome Biology* 17, 137. DOI:
615 <https://doi.org/10.1186/s13059-016-1009-x>
- 616 33. Peter BM (2016) Admixture, population structure, and F-statistics. *Genetics* 202, 1485-1501.
617 DOI: <https://doi.org/10.1534/genetics.115.183913>
- 618 34. Pickrell JK, Pritchard JK (2012) Inference of population splits and mixtures from genome-wide
619 allele frequency data *PLoS Genetics* 8, e1002967. DOI:
620 <https://doi.org/10.1371/journal.pgen.1002967>
- 621 35. Emerson RA (1924) Control of flowering in teosinte: short-day treatment brings early flowers.
622 *Journal of Heredity* 15, 41-48. DOI: <https://doi.org/10.1093/oxfordjournals.jhered.a102386>

- 623 36. Minow MAA, Ávila LM, Turner K, Ponzoni E, Mascheretti I, Dussault FM, Lukens L, Rossi V,
624 Colasanti J (2018) Distinct gene networks modulate floral induction of autonomous maize and
625 photoperiod-dependent teosinte. *Journal of Experimental Botany* 69, 2937–2952. DOI:
626 <https://doi.org/10.1093/jxb/ery110>
- 627 37. Duforet-Frebourg N, Luu K, Laval G, Bazin E, Blum MGB (2016) Detecting genomic signatures
628 of natural selection with principal component analysis: application to the 1000 genomes data.
629 *Molecular Biology and Evolution* 33, 1082-1093. DOI: <https://doi.org/10.1093/molbev/msv334>
- 630 38. Guo L, Wang X, Huang C, Li C, Li D, Yang CJ, York AM, Xue W, Xu G, Liang T, Chen Q,
631 Doebley, Tian F (2018b) Stepwise *cis*-regulatory changes in *ZCN8* contribute to maize flowering
632 time adaptation. *Current Biology*, 28, 3005-3015. DOI:
633 <https://doi.org/10.1016/j.cub.2018.07.029>
- 634 39. Salvi S, Sponza G, Morgante M et al. (2007) Conserved noncoding genomic sequences
635 associated with a flowering-time quantitative trait locus in maize. *Proceedings of the National*
636 *Academy of Sciences of the United States of America* 104, 11376-11381. DOI:
637 <https://doi.org/10.1073/pnas.0704145104>
- 638 40. Gengenbach BG, VanDee KL, Egli MA Hildebrandt KM, Yun SJ, Lutz SM, Marshall LC, Wyse
639 DL, Somers DA (1999) Genetic relationships of alleles for tolerance to sethoxydim herbicide in
640 maize. *Crop Science* 39, 812-818. DOI:
641 <https://doi.org/10.2135/cropsci1999.0011183X003900030033x>
- 642 41. Balbuena-Melgarejo A, Rosales-Robles E, Valencia-Hilario JC, González-Huerta A, Pérez-
643 López DdJ, Sánchez-Nava S, Franco-Malvaíz AL, Vences-Contreras C (2011) Competencia
644 entre maíz y teocintle: efecto en el rendimiento y sus componentes. *Field corn and teocintle*
645 *competition: effect on grain yield and grain yield components. Centro Agrícola* 38, 5-12.
- 646 42. Doebley J, Stec A, Wendel J, Edwards M (1990) Genetic and morphological analysis of a maize-
647 teosinte F2 population: Implications for the origin of maize. *Proceedings of the National*
648 *Academy of Sciences of the United States of America* 87, 9888-9892. DOI:
649 <https://doi.org/10.1073/pnas.87.24.9888>
- 650 43. Hufford MB, Lubinsky P, Pyhäjärvi T, Devengenzo MT, Ellstrand NC, Ross-Ibarra J (2013) The
651 genomic signature of crop-wild introgression in maize. *PLoS Genetics* 9, e1003477. DOI:
652 <https://doi.org/10.1371/journal.pgen.1003477>
- 653 44. Meng X, Muszynski MG, Danilevskaya ON (2011) The FT-Like *ZCN8* Gene functions as a floral
654 activator and is involved in photoperiod sensitivity in maize. *The Plant Cell* 23, 942-60. DOI:
655 <https://doi.org/10.1105/tpc.110.081406>
- 656 45. Rojas-Barrera IC, Wegier A, Sánchez-González JdJ, Owens GL, Rieseberge LH, Piñero D
657 (2019) Contemporary evolution of maize landraces and their wild relatives influenced by gene
658 flow with modern maize varieties. *Proceedings of the National Academy of Sciences of the*
659 *United States of America* 116, 21302-31311. DOI: <https://doi.org/10.1073/pnas.1817664116>

- 660 46. Chen Q et al. (2019) TeoNAM: a nested association mapping population for domestication and
661 agronomic trait analysis in maize. *Genetics* 213, 1065-1078. DOI:
662 <https://doi.org/10.1534/genetics.119.302594>
- 663 47. Lu Y, Hokin SA, Kermicle JL, Hartwig T, Evans MMS (2019) A pistil-expressed pectin
664 methylesterase confers cross-incompatibility between strains of *Zea mays*. *Nature*
665 *Communications* 10, 2304. DOI: <https://doi.org/10.1038/s41467-019-10259-0>
- 666 48. Anderson E (1953) Introgressive hybridization. *Biological Reviews* 28, 280-307. DOI:
667 <https://doi.org/10.1111/j.1469-185X.1953.tb01379.x>
- 668 49. Stebbins GL (1971) Relationship between adaptive radiation, speciation and major evolutionary
669 trends. *Taxon* 20, 3-16. DOI: <https://doi.org/10.2307/1218529>
- 670 50. Burgarella C, Barnaud A, Kane NA, Jankowski F, Scarelli N, Billot C, Vigourous Y, Berthouly-
671 Salazar C (2019) Adaptive introgression: an untapped evolutionary mechanism for crop
672 adaptation. *Frontiers in Plant Sciences* 10, 4. DOI: <https://doi.org/10.3389/fpls.2019.00004>
- 673 51. Snow AA, Culley TM, Campbell LG, Sweeney PM, Hegde SG, Ellstrand NC (2010). Long-term
674 persistence of crop alleles in weedy populations of wild radish (*Raphanus raphanistrum*). *New*
675 *Phytologist*, 186, 537–548. <https://doi.org/10.1111/j.1469-8137.2009.03172.x>
- 676 52. Merotto AJr, Goulart ICGR, Nunes AL, Kalsing A, Markus C, Menezes VG, Wander AE (2016).
677 Evolutionary and social consequences of introgression of NON-transgenic herbicide resistance
678 from rice to weedy rice in Brazil. *Evolutionary Applications*, 9, 837–846.
679 <https://doi.org/10.1111/eva.12387>
- 680 53. Corbi J, Baack EJ, Dechaine JM, Seiler G, Burke JM (2017). Genome-wide analysis of allele
681 frequency change in sunflower wild-crop hybrid populations evolving under natural conditions.
682 *Molecular Ecology*, 27(1), 233–247. <https://doi.org/10.1111/mec.14202>
- 683 54. Ellstrand NC, Garnir LC, Hedge S, Guadagnuolo R, Blancas L (2007) Spontaneous
684 hybridization between maize and teosinte. *Journal of Heredity* 98, 183-187. DOI:
685 <https://doi.org/doi:10.1093/jhered/esm002>
- 686 55. Guadagnuolo R, Clegg J, Ellstrand NC. 2006. Relative fitness of transgenic vs. non-transgenic
687 maize teosinte hybrids, a field evaluation. *Ecological Applications* 16, 1967–1974. DOI:
688 <https://doi.org/10.1890/1051-0761>
- 689 56. Munoz Diez C, Gaut BS, Meca E, Scheinvar E, Montes-Hernandez S, Eguiarte L, Tenailon MI
690 (2013). Genome size variation in wild and cultivated maize along altitudinal gradients.
691 *NewPhytologist*. 199, 264-276. DOI: <https://doi.org/10.1111/nph.12247>
- 692 57. Fustier MA, Brandenburg JT, Lapeyronnie J, Eguiarte LE, Boitard S, Vigouroux Y, Manicacci D,
693 Tenailon MI (2017) Local adaptation of teosintes along altitudinal gradients using whole
694 genome sequencing of pooled samples. *Molecular Ecology* 26, 2738-2756. DOI:
695 <https://doi.org/10.1111/mec.14082>
- 696 58. Takuno S, Ralph P, Swarts K, Elshire RJ, Glaubitz JC, Buckler ES, Hufford MB, Ross-Ibarra J
697 (2015) Independent molecular basis of convergent highland adaptation in maize. *Genetics* 200,
698 1297–1312. DOI: <https://doi.org/10.1534/genetics.115.178327>

- 699 59. Délye C, Pernin F, Michel S (2011) 'Universal' PCR assays detecting mutations in acetyl-
700 coenzyme A carboxylase or acetolactate-synthase that endow herbicide resistance in grass
701 weeds. *Weed Research* 51, 353–362. DOI: <https://doi.org/10.1111/j.1365-3180.2011.00852.x>
- 702 60. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary
703 Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547-1549.
704 DOI: <https://doi.org/10.1093/molbev/msy096>
- 705 61. Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers.
706 *Bioinformatics* 24, 1403-1405. DOI: <https://doi.org/10.1093/bioinformatics/btn129>
- 707 62. Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for
708 dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*
709 23, 1801-1806. DOI: <https://doi.org/10.1093/bioinformatics/btm233>
- 710 63. De Mita S, Siol M (2012) EggLib: processing, analysis and simulation tools for population
711 genetics and genomics. *BMC Genetics* 13, 27. DOI: <https://doi.org/10.1186/1471-2156-13-27>
- 712 64. Reich D, Thangaraj K, Patterson N, Price AL, Singh L (2009) Reconstructing Indian population
713 history. *Nature* 461, 489-495. DOI: <https://doi.org/10.1038/nature08365>
- 714 65. Patterson N, Moorjani P, Luo Y, Mallick S, Rohland N, Zhan Y, Genschoreck T, Webster T,
715 Reich D (2012) Ancient admixture in human history. *Genetics* 192, 1065–1093. DOI:
716 <https://doi.org/10.1534/genetics.112.145037>
- 717 66. Luu K, Bazin E, Blum MGB (2017) Pcadapt: an R package to perform genome scans for
718 selection based on principal component analysis. *Molecular Ecology Resources* 17, 67-77. DOI:
719 <https://doi.org/10.1111/1755-0998.12592>
- 720 67. Storey JD, Bass AJ, Dabney A, Robinson D (2019). qvalue: Q-value estimation for false
721 discovery rate control. R package version 2.18.0, <http://github.com/jdstorey/qvalue>
- 722 68. Guan Y (2014) Detecting structure of haplotypes and local ancestry. *Genetics* 196, 625-642.
723 DOI: <https://doi.org/10.1534/genetics.113.160697>
- 724

725 **Figures legends**

726

727 **Figure 1.** Genetic structure based on SNP data for teosinte samples collected in France combined with
728 available SNP data for Spanish teosintes, wild teosintes populations from Mexico and cultivated maize
729 accessions (24 544 SNPs and 1005 individuals). (A) Principal Component Analysis with axes 1 and 2
730 (12.6 % of the variation explained). (B) Population structure and admixture patterns revealed by
731 fastStructure. Each color represents a genetic group and individuals (vertical lines) are partitioned into
732 segments whose length represents the admixture proportions from K genetic groups. Ancestry
733 proportions are shown for $K=3$ genetic groups (top) and $K=11$ genetic groups (bottom). At $K=11$, the
734 following nine reference genetic groups were identified in Mexican teosintes and maize: PARV1 (red),
735 PARV2 (dark red), PARV3 (light pink), PARV4 (brown), MEX1 (yellow), MEX2 (gold), TROP (blue),
736 DENT (light green), FLINT (dark green).

737

738 **Figure 2.** Origin of European teosintes and admixture with maize. (A) Genetic relationships between
739 European teosintes and their two putative ancestors, mexicana (H1) and parviglumis (H2), inferred using
740 the four-populations (f_4) test. Each of the reference populations inferred by FastStructure for mexicana
741 (two groups: MEX1 and MEX2) and parviglumis (4 groups : PARV1, PARV2, PARV3 and PARV4) were
742 tested for ancestry relationship. *Zea luxurians* (LUX) is used as the outgroup. Theoretical tree topologies
743 and the corresponding sign of the f_4 -statistics are shown at the top. Points indicate observed f_4 values
744 for each pair of mexicana and parviglumis reference populations, with horizontal bars showing 3.3
745 standard errors. Inference was made separately for French teosinte (TEO-FR, on the left) and Spanish
746 teosinte (TEO-SP, on the right). (B) Treemix analysis: maximum-likelihood tree showing the
747 relationships among French teosintes (TEO-FR), Spanish teosintes (TEO-SP) and the 9 reference
748 groups identified from the fastStructure analysis for parviglumis accessions (PARV1, PARV2, PARV3
749 and PARV4), mexicana accessions (MEX1 and MEX2) and cultivated maize: tropical landraces (TROP),
750 Dent inbred lines (DENT) and Flint inbred lines (FLINT). Five migration events were inferred and shown
751 on the tree as arrows connecting genetic groups. Yellow to red color indicates the intensity (weight) of
752 each migration event.

753

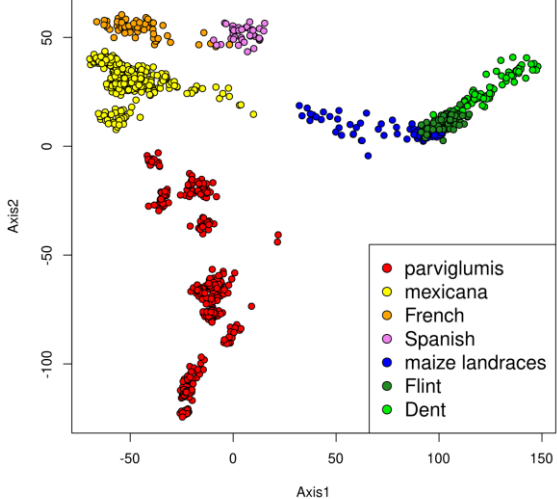
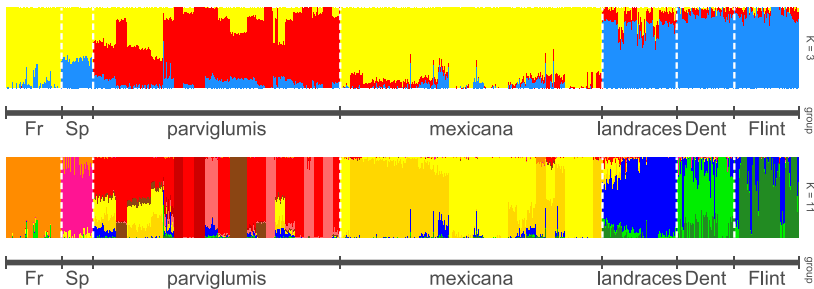
754 **Figure 3.** Flowering phenology in European teosintes. (A) Time to male flowering assessed from a
755 common garden experiment in Dijon, France. The histogram shows the number of degree-days from
756 sowing to tassel initiation in 48 plants of French teosintes, 24 Mexican teosintes of the subspecies
757 mexicana and 24 Mexican teosintes of the subspecies parviglumis. One mexicana plant and 17
758 parviglumis are not represented as they were still in a vegetative stage at the end of the experiment. (B)
759 A teosinte population within a maize field in France. Flowering is synchronous between teosintes and
760 maize.

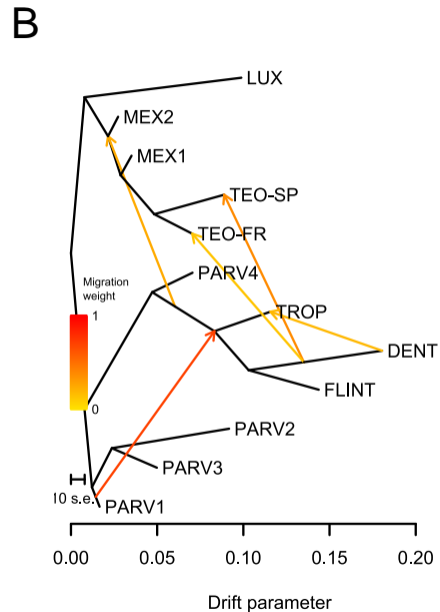
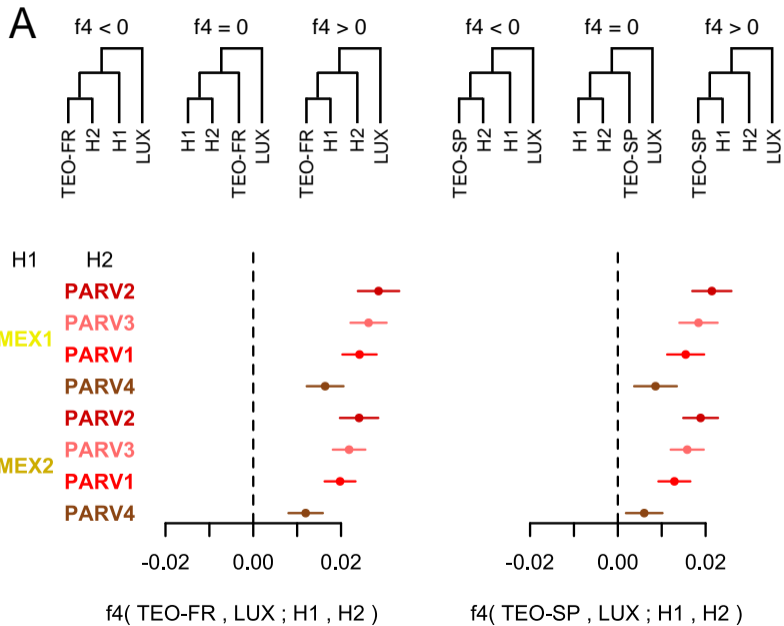
761

762 **Figure 4.** Detection of outlier SNPs along the 10 chromosomes of the genome based on a Principal
763 Component Analysis of mexicana accessions, French teosintes and Spanish teosintes using pcadapt.
764 (A) Projection of accessions onto axes 1 and 2 of the Principal Component Analysis. (B) Manhattan plot
765 of the *P*-values of SNPs with the first principal component of the PCA. The 45 top SNPs having *q*-values
766 less than 0.1% are displayed in red.

767
768 **Figure 5.** Patterns of local ancestry as inferred by ELAI along chromosome 8 and the matrix of linkage
769 disequilibrium (LD) in a region that includes the candidate genes *ZCN8* and *RAP2.7*, for (A) French
770 teosintes and, (B) Spanish teosintes. The ELAI plots show ancestry allele dosages (y-axis) for Dent
771 cultivated maize along the chromosome 8 (x-axis). Positions of the outlier SNPs identified with pcadapt
772 are marked with red dots. LD was estimated using the r^2 statistics for all pairs of SNPs in a region of
773 about 30 Mb starting 10 Mb upstream of *ZCN8* and ending 10 Mb downstream of *RAP2.7* (positions
774 116,880,531 to 146,012,084 on reference genome B73 v4)). SNP positions at which LD could not be
775 calculated (absence of polymorphism or missing data) are marked in white.

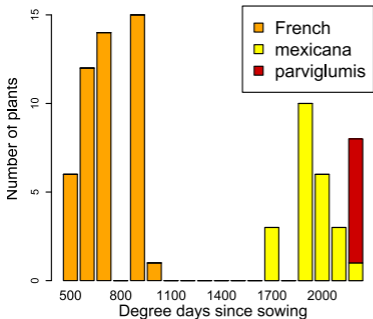
776
777 **Figure 6.** Introgression of a mutant *ACC1* gene from maize into French teosintes. (A) Pattern of local
778 ancestry in French teosintes along chromosome 2 as inferred by ELAI. The plot shows ancestry allele
779 dosages (y-axis) for Dent maize along the chromosome (x-axis). Individuals homozygous for the mutant
780 allele (RR) at *ACC1* are shown in blue, heterozygous (RS) in brown and homozygous for the non-mutant
781 (SS) allele are shown in green. The red arrow points to the SNP that is closest to the *ACC1* gene. (B)
782 Neighbour-joining tree showing the relationships among Acetyl-CoA carboxylase gene sequences in
783 two herbicide-tolerant maize varieties (EXXplicit and CarmiDuo), in the reference B73 maize inbred line,
784 in French teosinte accessions homozygous for the wild-type ACCase allele (SS) or homozygous for the
785 resistant mutant allele (RR) and in Mexican teosinte accessions (two mexicana, two parviglumis and
786 two *Zea mays* ssp. *huehuetenangensis*).

A**B**



A

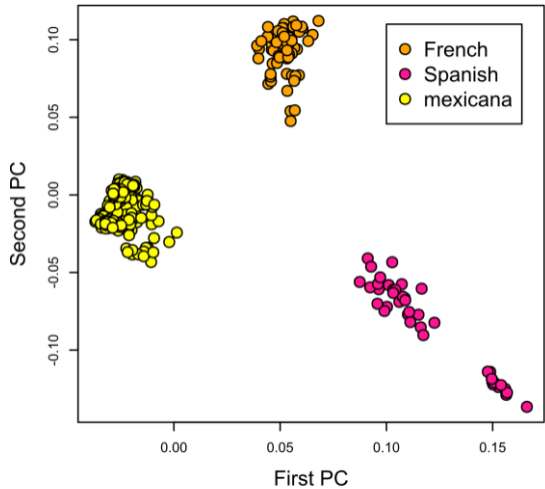
Male flowering time



B



A



B

