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Farmers' use of wild relative and sexual reproduction in a vegetatively propagated crop. The case of yam in Benin

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Abstract

The impact of traditional farmers' management on genetic diversity of vegetatively propagated crops is poorly documented. In this study, we analysed the impact of ennoblement of spontaneous yams, an original traditional farmers' practice, on the genetic diversity of yam (*Dioscorea* sp.) in Benin. We used 11 microsatellite markers on yam tubers from a small village in northern Benin and demonstrated that wild × cultivated hybrids are spontaneously formed. Many of the spontaneous yams collected by farmers from surrounding savannah areas for ennoblement were shown to be of wild and hybrid genotypes. Moreover, we demonstrated that some yam varieties have a wild or hybrid signature. Lastly, we performed a broader ranging genetic analysis on yam material from throughout Benin and showed that this practice is used in different ecological and ethno-linguistic regions. Through this practice, farmers create new varieties with new genetic combinations via sexual reproduction of wild and cultivated yams. This system, whereby a sexual cycle and asexual propagation are mixed, ensures potential large-scale cultivation of the best genotypes while preserving the potential for future adaptation.

Keywords: *Dioscorea* sp., farmers' practices, sexual reproduction, vegetatively propagated crop, wild crop relatives

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Introduction

Farmers in centres of crop diversity contribute to the 'evolutionary continuum linking the prehistoric predomesticates with the present-day cultivars' (Frankel *et al*. 1995). The actual genetic consequences of farmers' management of crop diversity are still subject to research and argument. It has been postulated that farmers' use of hybridization between traditional crops and their wild relatives increases the crops' genetic diversity (review in Jarvis & Hodgkin 1999). However, farmers' selection of new genetic combinations created by hybridization has not been clearly established, and has been studied principally for plants propagated by seeds (e.g. maize, Wilkes 1977; pearl millet, Robert & Sarr 1992; review in Jarvis & Hodgkin 1999). How farmers use the sexual reproduction and the diversity of wild relatives for vegetatively propagated crops is poorly documented. Here we propose to analyse farmers' use of sexual reproduction of yam — a vegetatively propagated crop — and its consequences for genetic diversity.

Yam (*Dioscorea* sp.) is a vegetatively propagated crop. Yam fields in traditional agroecosystems are seeded with tuber fragments from the previous harvest. In West Africa, the main cultivated species is *Dioscorea rotundata*. Although many cultivated varieties of this dioecious species produce seeds, no direct use of these seeds by farmers has ever been reported. In Benin, *D. rotundata* grows in sympatry with two wild relatives: *Dioscorea abyssinica* in northern Benin and *Dioscorea praehensilis* in the south (Terauchi *et al*. 1992; Hamon *et al*. 1995). Wild yam species reproduce sexually (Coursey 1976).

In West Africa, sociological studies have documented a unique farmer practice (Dumont & Vernier 2000; Vernier *et al*. 2003). Following Mignouna & Dansi (2003), we will call this practice 'ennoblement'. Farmers collect tubers of wild yams and plant them in their fields. They select tubers for their likeness to cultivated varieties, e.g. in northern Benin, they look for plants with large green stems, with large tubers and white flesh and lacking spines. Plants

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selected by farmers and going through the ennoblement process will be named 'pre-ennobled' yams in this paper. According to farmers, some of these plants develop — after 3–6 years of special cultivation practices — a tuber that is morphologically close to those of cultivated varieties. The tubers are then multiplied and cultivated if farmers are satisfied with their morphology. The biological processes underlying the change in tuber morphology and its maintenance over generations are unknown. In an experimental attempt to cultivate the wild species *D. praehensilis*, Chikwendu & Okezie (1989) observed changes in plant and tuber morphology over 8 years of vegetative multiplication.

No studies have monitored the entire yam ennoblement cycle over several years to assess the success of this process. Such monitoring is difficult because of the length of the process (3–6 years). As a direct analysis was not feasible, Scarcelli *et al*. (2006) analysed the diversity of cultivated genotypes in order to detect products of ennoblement. They obtained the first molecular evidence that pre-ennobled yams include not only wild plants, as initially assumed, but also intermediate genotypes, which could originate from interspecific hybridization. Controlled interspecific hybridizations have shown that wild and cultivated species are interfertile (Akoroda 1985) but, to our knowledge, no evidence of natural hybridization has ever been reported.

The present study aimed to conduct an in-depth analysis of the ennoblement process. First, we investigated *in situ* interspecific hybridization between wild and cultivated species. Then, we studied the genetic nature of the pre-ennobled yams, i.e. wild, hybrid or cultivated genotypes. We first conducted a case study in a village in northern Benin, and then performed a country-wide survey to extend our conclusions.

Materials and methods

Plant material

Village-level analysis. We collected materials to analyse the genetic origin of pre-ennobled yams in the village of Gorobani, in northern Benin. In this region, crop fields are set up in reclaimed wooded savannah. *Dioscorea abyssinica*, a wild related species, grows in sympatry with *Dioscorea rotundata*, the cultivated species, i.e. grows in the savannah area surrounding the fields of cultivated yams. About 5% of the 350 farmers of the village practiced ennoblement. Farmers cultivated 5–22 different yam varieties in their fields. Here, all tubers with the same name were considered to be a variety. This suggests that a variety could contain identical genotypes through vegetative propagation along with different genotypes if farmers do not note any morphological differences among them. A previous study (Baco *et al*. 2004) has shown that farmers of this village use the same morphological characteristics to define their named varieties. Farmers claimed that two varieties, 'Ourou Yessingué' and 'Dompikou', were the result of ennoblement. They described the variety 'Ourou Yessingué' as a cultivated variety that had been mixed with ennobled wild tubers. Farmers described the variety 'Dompikou' as the result of the ennoblement of a single tuber of wild yam (*D. abyssinica*).

For this study, we have selected five farmers that practice ennoblement. In the fields of these five farmers, we have collected a total of nine tubers of pre-ennobled yams. We also have collected one tuber per cultivated variety, representing 46 cultivated samples (*D. rotundata*) and including samples of varieties 'Ourou Yessingué' and 'Dompikou'. We then collected wild (*D. abyssinica*) samples in the savannah area surrounding the five selected farmers' fields. A total of 105 wild plants were collected. We studied hybridizations between wild and cultivated yam species by performing a paternity analysis on progenies of wild and cultivated plants. In the five selected farmers' fields, we collected 93 seeds from seven cultivated plants and 102 seeds from seven wild plants in the surrounding savannah.

Large-scale analysis. To get a wider picture of the impact of the yam ennoblement practice, we also performed a large-scale study throughout Benin. We selected eight villages (Fig. 1) where yam cultivation is important and ennoblement is practiced. The village sample encompassed a broad range of situations with respect to geography, ethnicity and ecology. In southern Benin, the two sampled villages, i.e. Gounoukouin and Amakpa, are inhabited by the Fon ethnic group. In this region, the vegetation is principally rainforest, in which grows another wild relatives, *Dioscorea praehensilis*. Assaba and Djagballo, two villages of the Nagot ethnic group, were sampled in central Benin. The two wild species are parapatric in this region, as it is covered by a mosaic of forest and wooded savannah, which are the habitats of *D. praehensilis* and *D. abyssinica*, respectively. In northern Benin, four villages were sampled, i.e. Gorobani, Guessou Bani, Wari and Yarra. In each village, we found the two ethnic groups named Bariba and Gando. *D. abyssinica* grows on wooded savannah, the locally predominant vegetation type. We collected 30 tubers of pre-ennobled yams in the eight villages (location of each sample is reported in Table S1, Supplementary material). Fifty-six cultivated tubers of *D. rotundata* were collected in the eight villages and were representative of the most frequent varieties in Benin (Dansi *et al*. 1999). Among these samples, farmers claimed that the variety 'Gban' was the result of the ennoblement of *D. praehensilis*. We sampled 104 wild plants, corresponding to 71 *D. abyssinica* plants and 33 *D. praehensilis* plants, throughout the distribution area of the two wild species in Benin (Hamon *et al*. 1995).

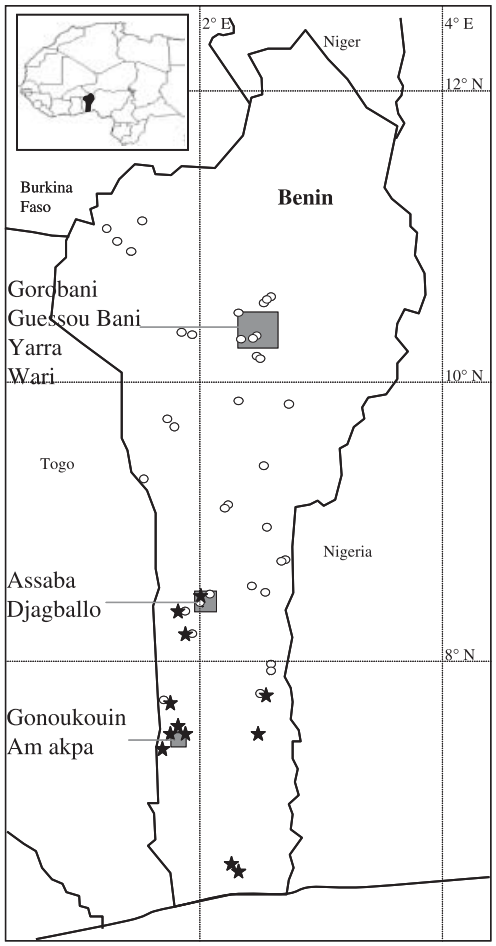

Fig. 1 Geographical origin of analysed samples. For the large-scale analysis, cultivated and pre-ennobled samples were collected in the eight villages located on the map, and wild samples (*Dioscorea abyssinica* and *Dioscorea praehensilis*) were collected within the entire distribution range of these species. For the village-level analysis, samples were collected in the village of Gorobani in northern Benin.

DNA extraction and microsatellite analysis

Tubers were collected in the field and then grown in a greenhouse. DNA extraction from stem apices was done following Scarcelli *et al*. (2005). For the paternity analysis, extractions were done from nongerminated seeds using the same protocol. For the genetic analysis, 11 microsatellite markers (2D06, 2D08, 2E07, 1A01 3F04, 3G04, 1C12, 2C05, 3B12, 2E09 and 1F08, Tostain *et al*., 2006) were amplified following Scarcelli *et al*. (2005). Migration was carried out using an automatic sequencer ABI PRISM™. 3100 (Applied Biosystems). Microsatellite alleles were scored using GENESCAN and GENOTYPER software packages (Applied Biosystems).

Data analyses

D. rotundata, *D. abyssinica* and *D. praehensilis* have 40 chromosomes and were long considered to be tetraploid species. We have previously shown that *D. rotundata* is diploid (Scarcelli *et al*. 2005) and suggested that *D. abyssinica* and *D. praehensilis* are also diploid. The genotyping data were consistent with these conclusions, as we observed only one or two alleles per sample for each microsatellite locus. Our genetic analyses were thus appropriate for diploid species.

We calculated observed heterozygosity (H_O), gene diversity (H_E) and allelic richness (measure of the number of alleles per locus independent of sample size) using FSTAT 2.9.3.2 (Goudet 1995). To test for deviation from Hardy–Weinberg proportion, F_{IS} was calculated and tested (exact test, 500 batches and 5000 iterations per batch) using GENEPOP 3.4 (Raymond & Rousset 1995). The F_{ST} differentiation parameter between species was calculated and tested (G test, 10 000 randomizations) with FSTAT. Pre-ennobled yams were assigned to genetic groups using a Bayesian approach via the STRUCTURE 2.0 software package (Pritchard *et al*. 2000). For the village-level analysis, the number K of assumed populations was considered to be $K = 2$, corresponding to the cultivated and wild groups. We used the admixture model with a burn-in period of 30 000 steps and 10^6 MCMC replicates. For each individual, the proportion q of its genome derived from each group, and its 95% confidence interval, were calculated. Five independent runs were performed without any previous information on the plant origins. We also calculated, for each individual, its probability of having an ancestry (first and second generation) in each group. We also performed the analysis for different numbers of assumed populations ($K = 2$ to $K = 8$) in order to test the reproducibility of the assignment regardless of K. We have then calculated ΔK (Evanno *et al*. 2005), a statistics based on the rate of change in the log probability of data between successive K values, to have an estimation of the real number of clusters. The large-scale analysis was made in the same way. The number of populations was set at $K = 3$ populations, corresponding to two wild groups (*D. abyssinica* and *D. praehensilis*) and a cultivated group.

Hybridization study

To assess the extent of hybridization between wild and cultivated yams, we performed a paternity analysis on progenies of wild and cultivated maternal parents. The objective was to determine whether these progenies resulted from fertilization by a male originating from the

local wild or the cultivated yam species, which we did by developing a paternity analysis method (a complete description of this method is given in the Supplementary material). Briefly, we designed an algorithm that reconstructs the genotype of the male gamete using the genotype of an offspring and that of its maternal parent. A log-likelihood ratio (LOD score) is calculated as the probability that the male gamete comes from the wild (*D. abyssinica*) population divided by the probability that the male gamete comes from the cultivated (*D. rotundata*) population. We used allele frequencies of the wild and the cultivated populations to calculate this LOD score. We need to evaluate if a given value of LOD score statistically supports the origin of the gamete in each population. We used a population simulation to construct two LOD score distributions for each maternal parent, assuming either that male gametes came from the wild population or that male gametes came from the cultivated population. These simulated offspring genotypes were constructed with alleles randomly chosen from the maternal parent and from a randomly chosen paternal parent of the wild or the cultivated population. We then compared the LOD score obtained for genotyped offspring to that obtained by simulation. For each population we calculated the proportion P of simulated offspring with a lower LOD score ratio than that of the genotyped offspring and that originated from either a cultivated or a wild male parent. A male gamete was assigned to one population if the proportion P obtained was $0.025 < P < 0.975$. If the LOD score was outside this interval ($P < 0.025$ or $P > 0.975$), we assumed that the male gamete is not derived from this population. If the male gamete was derived from neither population, it was considered nonassigned.

As this analysis was made only on nongerminated seeds, we seeded the progeny of the wild and cultivated maternal plants used in the paternity analysis and monitored germination for 3 weeks. We also seeded the progeny of a control *D. rotundata* × *D. rotundata* cross. We planted a total of 16 seeds for each maternal plant, corresponding to 112 seeds for wild maternal plants, 112 seeds for cultivated maternal plants and 16 seeds for the control cross.

Results

Village-level analysis

Genetic diversity. Eleven loci were amplified in a total of 160 samples (105 *Dioscorea abyssinica*, 46 *Dioscorea rotundata*, nine pre-ennobled yams), revealing 168 alleles. Eleven percent of these alleles were specific to the cultivated sample (*D. rotundata*) and 51% to the wild sample (*D. abyssinica*). The mean number of alleles per locus was 7.4 for *D. rotundata* and 11.4 for *D. abyssinica*. For *D. rotundata* and *D. abyssinica*, respectively, the expected heterozygosity ranged from 0.64 and 0.69 and the observed heterozygosity from 0.63 to 0.58. We found significant differentiation ($P < 0.001$) between the two species ($F_{ST} = 0.25$). Overall, the wild species showed a slight heterozygote deficit ($F_{IS} = 0.04$, $P < 0.001$), while the cultivated species presented a heterozygote excess ($F_{IS} = -0.13$, $P < 0.001$). Locus 3G04 showed a strong heterozygote deficit in both species ($F_{IS} = 0.32$ for wild species and $F_{IS} = 0.22$ for cultivated species, $P < 0.001$).

Assignment of pre-ennobled yams. The nine pre-ennobled tubers were assigned using the STRUCTURE program (q values are reported in Table S2, Supplementary material). A stable ancestry estimate value (q) was obtained in five independent runs. The two groups obtained were closely consistent with the taxonomic classification (Fig. 2).

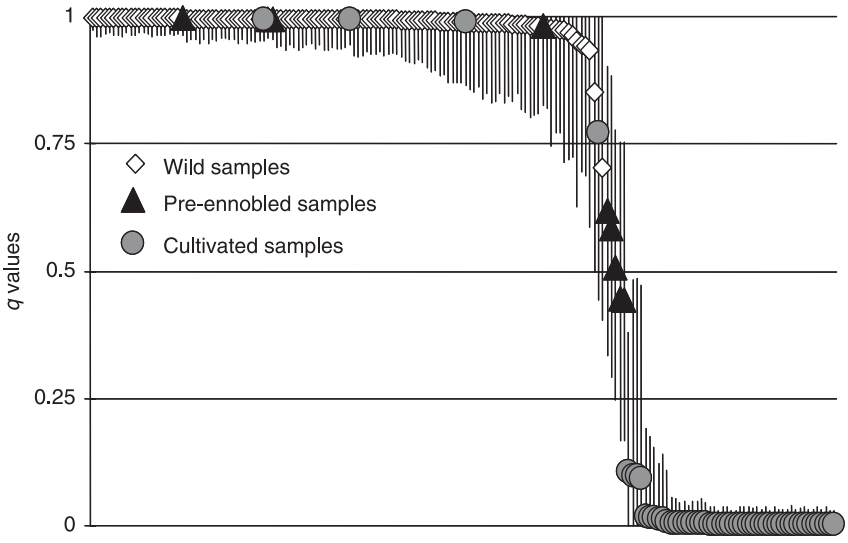

Fig. 2 Assignment of yam samples to wild and cultivated species. For each individual, q values were calculated with STRUCTURE, assuming $K = 2$. The 95% confidence intervals were calculated by assuming a migration rate of 0.01. With a threshold of 0.8, two groups appeared: the *Dioscorea rotundata* group ($0 < q < 0.2$) in which only cultivated yams clustered, and the *Dioscorea abyssinica* group ($0.8 < q < 1$) where all the wild yams clustered.

Table 1 Ancestry analysis of samples with an admixed genotype

Individual	Assumed population	*q* value	Alternative population	Probability to cluster in the alternative population		
				Generation 0	Generation 1	Generation 2
Pre-ennobled samples						
D652	Wild	0.51	Cultivated	0.00	0.97	0.03
D671	Wild	0.44	Cultivated	0.00	0.94	0.06
D674	Wild	0.58	Cultivated	0.00	0.29	0.58
D675	Wild	0.62	Cultivated	0.00	0.30	0.58
D676	Wild	0.45	Cultivated	0.00	0.94	0.06
Wild sample						
A549	Wild	0.70	Cultivated	0.00	0.001	0.40
Cultivated sample						
CR654	Cultivated	0.23	Wild	0.28	0.28	0.07

This analysis was done with STRUCTURE, for $K = 2$ and using a migration rate of 0.01. For each sample, *q* values gave the proportion of its genome derived from the assumed population. The probability of coming from the alternative population was calculated at generation 0 (direct immigrant), 1 (one parent) and 2 (one grandparent). For example, the pre-ennobled sample D652 was assigned in the wild population with $q = 0.51$. This individual had a probability $P = 0.00$ of coming from the cultivated population. The probabilities were 0.97 and 0.03 that this individual had a single parent or grandparent, respectively, in the cultivated population.

Ninety-nine per cent of the wild samples clustered in group 1 and 86% of cultivated samples clustered in group 2. We considered that groups 1 and 2 represented *D. abyssinica* and *D. rotundata*, respectively. Three cultivated plants were assigned to the wild group (*D. abyssinica*). One cultivated plant and one wild plant had admixed ancestry in the two groups (Table 1).

Out of the nine pre-ennobled yams, four presented strong ancestries in the wild group and were interpreted as wild yams. Five pre-ennobled yams had admixed ancestry in the two wild and cultivated groups. The ancestry analysis (Table 1) showed that these five samples had a high probability that one of their parents and/or grandparents came from the cultivated species. This analysis suggested that these samples are *D. abyssinica* × *D. rotundata* hybrids.

To confirm that our methodology (sample size, number of loci, Bayesian assignment method) was powerful enough to reliably detect hybrid genotypes, we created one hundred hybrid genotypes from two plants randomly drawn from the wild and cultivated yam populations. We assigned these simulated hybrids using STRUCTURE. All the simulated hybrids were detected as having admixed ancestry (mean $q = 0.52$ with a 95% confidence interval = 0.36–0.69). We also confirmed that there was a high probability that the simulated hybrid genotypes had a parent in each population (mean $P = 0.85$), and there was a low probability that they were direct migrants (mean $P = 0.01$).

The assignment of three cultivated plants to the wild *D. abyssinica* group means that *D. abyssinica* genotypes were present in the cultivated varieties. This suggested the successful ennoblement of *D. abyssinica* individuals. One of these individuals was the 'Dompikou' variety. The farmer claimed that this variety was ennobled from a *D. abyssinica* tuber, and this was confirmed by the genetic analysis. The farmer described the variety 'Ourou Yessingué' as a cultivated variety that had been mixed with ennobled wild tubers but no wild genotype was found in this variety, possibly because of the small sample size.

We tested the reproducibility of these results by performing the assignment for $K = 2$ to $K = 8$, with five repetitions. The likelihood slightly increased, with *K* ranging from $K = 2$–8, showing a plateau effect (Pritchard *et al*. 2000), but the assignment to wild and cultivated species did not significantly change. Using ΔK (Evanno *et al*. 2005), the best estimation of group number that we found was for $K = 2$. These groups corresponded to the two yam species and thus could be explained by their biological features. We also conducted five repetitions for $K = 2$, removing the 3G04 locus that presented a high heterozygote deficit. The assignment of pre-ennobled plant did not change. Finally, we tested the reproducibility of these results regarding the Hardy–Weinberg assumption by using an assignment method based on genetic distance that does not assume Hardy–Weinberg equilibrium (Cornuet *et al*. 1999). Results obtained were consistent with those obtained using STRUCTURE (data not shown).

Study of hybridization. We performed a paternity analysis since the admixed ancestry observed for some plants in the above analysis suggested that there was some interspecific hybridization. Progenies of seven *D. abyssinica* maternal parents (102 seeds) and seven cultivated maternal parents (93 seeds) were genotyped at 11 microsatellite loci. Each maternal parent was also genotyped (see Table S1, Supplementary material).

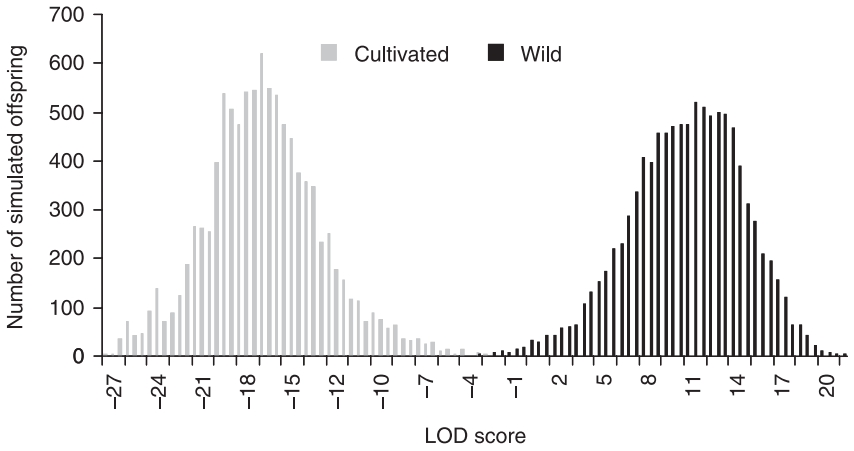

Fig. 3 LOD score distribution obtained by simulation. The LOD score corresponds to the log likelihood ratio that the male gamete comes from the wild (*Dioscorea abyssinica*) or the cultivated (*Dioscorea rotundata*) population. In this example, the maternal parent is from the cultivated population. Offspring were created using a randomly selected male parent in the wild population (black bars, 10 000 offspring) or in the cultivated population (grey bars, 10 000 offspring).

Table 2 Paternal origin of progeny of sympatric wild and cultivated yams

Offspring whose male parent was assigned to	Maternal parent	
	wild	cultivated
Wild population	95	68
Cultivated population	0	3
Not assigned	7	22
Total	102	93

Ninety-three seeds of the progeny of seven cultivated yams (*Dioscorea rotundata*) and 102 seeds of the progeny of seven wild yams (*Dioscorea abyssinica*) were analysed.

We assessed the population of origin of the male gamete of each offspring. The two reference populations of the wild and cultivated species were almost the same as those used in the previous study. We only removed two plants that showed a hybrid genotype and four cultivated varieties clustering with the wild species *D. abyssinica*. The two reference populations consisted of 104 *D. abyssinica* and 42 *D. rotundata* genotypes (see Table S1, Supplementary material). For each maternal parent, LOD score distributions obtained from simulated offspring created from *D. abyssinica* and from *D. rotundata* male gamete plants did not overlap (Fig. 3). This allowed a good assessment of the origin of male gametes.

The assignment of male gametes to *D. abyssinica* or *D. rotundata* for each genotyped offspring was performed (Table S3, Supplementary material). For progeny of wild maternal parents, 93.6% of the male gametes were assigned to the wild population and no male gamete was assigned to the cultivated population (Table 2). For progeny of cultivated maternal parents, 3.2% of the male gametes were assigned to the cultivated population and 77.4% were assigned to the wild population (Table 2). The latter result showed that spontaneous interspecific hybridizations had occurred between wild and cultivated yams.

For the hybridization analysis, a male gamete was not assigned to one population if the proportion P obtained was $0.025 < P < 0.975$. Then, it could be expected that, by chance alone, 5% of gametes would not be assigned. For the progenies of wild maternal parents, we found 6.4% nonassigned gametes, and this result was not significantly different from what was expected by chance (G-test, $P = 0.413$). For the progenies of cultivated maternal parents, 22% of the gametes were not assigned, which is a higher percentage than would be expected by chance (G-test, $P < 0.001$). The observed LOD scores of these gametes were between the two LOD score distributions (male gametes of cultivated and wild origin). One possible explanation for this pattern is that cultivated maternal parents had hybridized with cultivated × wild hybrids. This hypothesis will need further analysis to be clearly established.

This analysis was performed on nongerminated seed. However, in other experiments, we have monitored the germination of the progeny of each maternal plant and of a control cross. Considering our previous results, we expected only wild × wild individuals in the progeny of wild maternal plant, 78% of wild × cultivated hybrids in the progeny of cultivated maternal plant, and only cultivated × cultivated individual in the control cross. We observed germination of 88% of the wild plant progeny, 68% of the cultivated plant progeny and 56% of the controlled cross progeny. This suggested that cultivated and wild seeds, but also hybrid seeds, could germinate.

Large-scale analysis

Genetic diversity of wild and cultivated yams. Eleven microsatellite loci were amplified in a total of 160 samples (71 *D. abyssinica*, 33 *Dioscorea praehensilis* and 56 *D. rotundata*) collected in different regions of Benin, revealing 196 alleles. Four percent of these alleles were specific to *D. rotundata*, 11% to *D. praehensilis* and 34% to *D. abyssinica*. The mean number of alleles per locus ranged from 5.9 to 11.4,

Table 3 Diversity analysis of the cultivated species (*Dioscorea rotundata*) and the two wild species (*Dioscorea abyssinica* and *Dioscorea praehensilis*) in the large-scale analysis

	D. abyssinica	*D. praehensilis*	*D. rotundata*
Analysed samples	71	33	56
Allelic richness	11.4	9.6	5.9
H_E	0.71	0.74	0.53
H_O	0.63	0.67	0.51

Allelic richness (measure of the number of alleles per locus independent on sample size; calculation based on a sample size of 32 individuals); genetic diversity (H_E) and observed heterozygosity (H_O) were calculated for each species.

depending on the species (Table 3). This result showed that cultivated yams are less genetically diverse than wild species. The expected heterozygosity ranged among species from 0.53 to 0.71 and the observed heterozygosity from 0.51 to 0.67. F_{ST} values were calculated per locus among the three species and between pairs of species. The global differentiation was high and highly significant (F_{ST} = 0.18, $P < 0.001$). There was significant differentiation between *D. rotundata* and *D. abyssinica* (F_{ST} = 0.24, $P < 0.001$), between *D. rotundata* and *D. praehensilis* (F_{ST} = 0.18, $P < 0.001$) and between *D. abyssinica* and *D. praehensilis* (F_{ST} = 0.10, $P < 0.001$). All species, *D. abyssinica*, *D. praehensilis* and *D. rotundata*, showed a heterozygote deficit (F_{IS} = 0.12, 0.11 and 0.05, respectively, $P < 0.001$). Two loci (2E07 and 3G04) showed a strong heterozygote deficit in the three species ($F_{IS} \geq 0.2$ for 2E07 and $F_{IS} \geq 0.33$ for 3G04).

Assignment of pre-ennobled yams. The 30 pre-ennobled tubers were assigned in one of the three species, *D. abyssinica*, *D. praehensilis* and *D. rotundata*, using STRUCTURE (*q* values are reported in Table S4, Supplementary material). A stable ancestry estimate value (*q*) was obtained with five independent runs. The three groups obtained were highly consistent with the botanical classification (Fig. 4A). Ninety-seven per cent of the *D. abyssinica* samples clustered in group 1, 82% of the *D. praehensilis* samples clustered in group 2, and 93% of the *D. rotundata* samples clustered in group 3. Since the groups were extremely homogeneous with respect to species, we considered that groups 1, 2 and 3 represented *D. abyssinica*, *D. praehensilis* and *D. rotundata*, respectively. A discrepancy between the taxonomic classification (made at collection time) and the genetic classification was noted for two samples collected as *D. praehensilis* but which clustered with the *D. rotundata* group, and for one sample of the cultivated variety that clustered with the wild group of *D. praehensilis*. Nine individuals collected in the wild and cultivated populations had admixed ancestry in two groups. Five could be considered as *D. abyssinica* × *D. rotundata* hybrids, three as *D. praehensilis* × *D. rotundata* hybrids and one as a *D. abyssinica* × *D. praehensilis* hybrid.

Out of the 30 pre-ennobled yams, four (13%) were assigned to the *D. abyssinica* group and four (13%) to the *D. praehensilis* group (Fig. 4b). These samples were interpreted as wild yams. Eleven (37%) pre-ennobled yams had admixed ancestry and were considered to be hybrids. Out of these 11 samples, four corresponded to *D. abyssinica* × *D. rotundata* hybrids and five to *D. praehensilis* × *D. rotundata* hybrids. Two samples showed ancestry in both of the

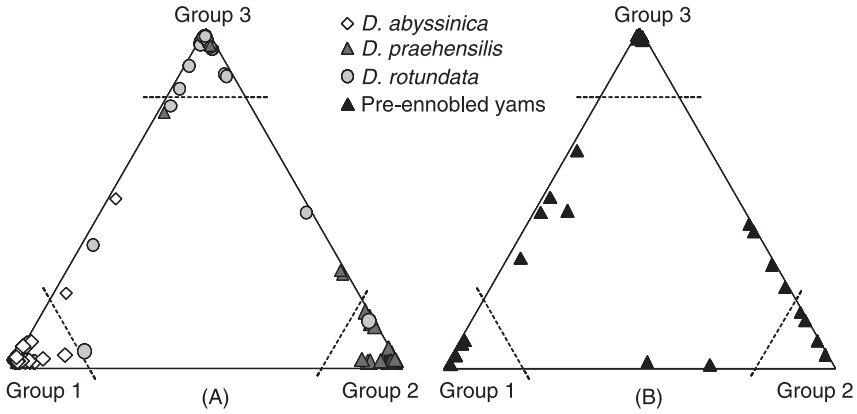

Fig. 4 Assignment of pre-ennobled yams from the large-scale analysis. For each individual, *q* values were calculated with STRUCTURE, assuming $K = 3$. The analysis was performed using no previous information on the plant origins and with all the samples (56 cultivated plants, 33 *Dioscorea praehensilis* plants, 71 *Dioscorea abyssinica* plants and 30 pre-ennobled plants). For legibility, we separately plotted the two wild groups and the cultivated group (A) and the pre-ennobled yams (B). Considering a threshold of 0.8 (dashed line), we defined three groups corresponding to *D. abyssinica* (wild group 1), *D. praehensilis* (wild group 2) and *Dioscorea rotundata* (cultivated group 3). The origin of the pre-ennobled yams (B) was diverse; some originated from one of the two wild groups and the cultivated group, others had an admixed origin.

wild species. Finally, 37% of the pre-ennobled yams were assigned to the *D. rotundata* group. As in the village-scale analysis, the ancestry analysis confirmed the wild, cultivated or hybrid origin of these pre-ennobled samples (data not shown). Pre-ennobled yams that presented a wild or a hybrid genotype were found in the four ethnic groups analysed.

The assignment of one cultivated variety to the wild group *D. praehensilis* means that the *D. praehensilis* genotype was present in the cultivated varieties. This suggested the successful ennoblement of *D. praehensilis* individuals. This individual was the 'Gban' variety. Farmers presented this variety as ennobled from *D. praehensilis*. The genetic analysis confirmed the farmers' statement. To conduct a more in-depth analysis of this variety, we sampled nine tubers of the 'Gban' variety in six different villages (see Table S1, Supplementary material). Three samples were identical to the first analysed sample with respect to the 11 analysed loci, and were collected in three different villages. The other six individuals were genetically different from each other. We assigned these individuals using the same procedure as used for pre-ennobled yams. Seven samples were assigned to the *D. praehensilis* group and two could be considered as *D. praehensilis* × *D. rotundata* hybrids. These results indicated that the 'Gban' variety is a mixture of several independent ennoblements. On the other hand, the presence of the same genotype in different villages suggested that ennoblement products could have circulated between villages.

To test the reproducibility of these results, we performed the assignment for $K = 3$ to $K = 8$ with five repetitions for each K. As in the village-scale analysis, the likelihood slightly increased with K, from $K = 3–8$, but the assignment of pre-ennobled samples did not significantly change. Using ΔK (Evanno *et al*. 2005), we found that the best estimation of group number was for $K = 3$. These groups corresponded to the three yam species and thus could be explained by their biological features. We also performed five repetitions for $K = 3$, while removing loci 2E07 and 3G04, which presented a high heterozygote deficit. The pre-ennobled plant assignment did not change. Finally, we tested the reproducibility of these results regarding the Hardy–Weinberg assumption by using an assignment method based on genetic distance that does not assume Hardy–Weinberg equilibrium (Cornuet *et al*. 1999). Results obtained were consistent with those obtained using STRUCTURE (data not shown).

Discussion

Sexual reproduction and ennoblement

We analysed the ennoblement process in the village of Gorobani. In this village, we collected wild, cultivated and pre-ennobled yams. We characterized the diversity of local varieties and local wild populations in which farmers selected yams for ennoblement. We showed that farmers voluntarily introduce yams in their field that have a signature of wild and wild × cultivated hybrid origin. This raised the question as to whether any interspecific hybridization had occurred. We performed a paternity analysis to answer this question. We obtained genetic evidence of spontaneous hybridizations between *Dioscorea abyssinica* and cultivated yams. Maternal plants of cultivated yams are mostly pollinated by wild males, which leads to hybrid seeds. However, maternal plants of wild yams are not pollinated by cultivated males. Several different explanations could explain why such an asymmetry in crosses is observed: a higher number of pollinating wild plants, lower quality and efficiency of pollen in the vegetatively cultivated species or nonsynchronization of flowering between male and female in the cultivated species *Dioscorea rotundata* (Segnou *et al*. 1992). As DNA was extracted from nongerminated seeds only, we have no data on the viability of these hybrids. However, the germination tests suggested that hybrids could germinate. Moreover, as some hybrid genotypes were found in pre-ennobled yams, this gives indirect support for their ability to germinate and to survive in the savannah.

To assess if the selection of wild or hybrid genotypes for ennoblement was specific to the village of Gorobani, we performed the same analysis at a large scale. We considered eight different villages, corresponding to four different ethnic groups. For this analysis, we did not consider any potential geographical differentiation. Indeed, such differentiations were considered for the STRUCTURE analysis ($K = 4$ to $K = 8$), but as the assignment did not change, we concluded that any differentiation that may exist did not influence our results. Results obtained in the village-level analysis were confirmed in the large-scale analysis. In the eight studied villages, farmers also select wild and hybrid yams for ennoblement.

Since ennoblement is a very long process, we could not monitor pre-ennobled tubers from the time they are selected until they are cultivated. To assess if ennoblement successfully introduces wild and hybrid genotypes into the cultivated pool, we have analysed a large sample of cultivated yams. In the village-level and the large-scale analysis, we found that nine of the 46 cultivated samples analysed showed a signature of wild (*D. abyssinica* or *Dioscorea praehensilis*) or hybrid (*D. abyssinica* × *D. rotundata* or *D. praehensilis* × *D. rotundata*) origin. The geographical location of these samples was consistent with the ecological distribution of the wild species from which each presumably originated. Indeed, cultivated accessions showing a *D. praehensilis* or *D. rotundata* × *D. praehensilis* hybrid genotype were found in southern Benin. Cultivated accessions showing a *D. abyssinica* or *D. abyssinica* × *D. rotundata*

hybrid genotype were found in northern and central Benin. This suggests that the wild and hybrid genotypes detected in the cultivated pool were locally ennobled from wild relatives or hybrids with these relatives.

Based on these results, we conclude that the ennoblement process actually succeeds and leads to the integration of new genotypes in cultivated varieties. We showed that it occurs in different ecological and ethno-linguistic regions. This practice involves two wild species (*D. abyssinica* and *D. praehensilis*) with different geographical distributions, and leads farmers to integrate, in the cultivated yam pool, wild genotypes and hybrids between wild and cultivated species.

Our results suggested that cultivated genotypes could survive in a wild environment and could then be selected for ennoblement by farmers. Indeed, a large share of the pre-ennobled tubers (37%) had cultivated genotypes and two accessions (P413 and P431) sampled as wild plants were found to have ancestry in the cultivated group. These cultivated genotypes could be volunteers (i.e. genotypes identical to existing cultivated varieties) or progenies of cultivated varieties (i.e. new genotypes produced by genetic recombination). Indeed, in the paternity analysis, we observed three *D. rotundata* × *D. rotundata* spontaneous hybridizations ($0.42 < P < 0.86$). Such crosses lead to new genetic combinations within the cultivated pool. As it was not possible to discriminate between volunteers and recombinant genotypes, we could not determine whether farmers selected new genotypes or only existing genotypes for ennoblement. Moreover, we were unable to determine whether *D. rotundata* genotypes had been introduced through ennoblement in the cultivated yam pool since we could not discriminate between a *D. rotundata* genotype introduced by ennoblement and all the other cultivated *D. rotundata* genotypes. We thus cannot conclude that ennoblement results in the introduction of some cultivated genotypes in the cultivated pool. This hypothesis does, however, seem reasonable. Since farmers succeed in ennobling wild yams, it is likely that they could succeed in ennobling cultivated yams.

In Benin, ennoblement is practiced by fewer than 5% of yam farmers (Dumont & Vernier 2000). However, ennoblement has an impact beyond the fields of farmers who practice it, especially because of the substantial flow of yam tubers between farmers and villages (Baco *et al*. 2004). The results of our analysis of polymorphism in the 'Gban' variety revealed that one clone, ennobled from the wild species *D. praehensilis*, was also present in the neighbouring villages. Overall, 8.9% of the cultivated samples analysed in the large-scale analysis had a wild or hybrid genotype. This demonstrates that ennoblement has had a sizeable impact, even though we might have underestimated it because it was not possible to assess the ennoblement origin of *D. rotundata* genotypes.

Genetic consequences of farmers' use of sexual reproduction in yam

Wild yam ennoblement appears to be the only farmers' practice that spans two rarely documented farmers' practice categories: (a) practices involving the manipulation of wild crop relatives; and (b) those that use sexual reproduction in vegetatively propagated crops.

(a) In a stimulating review, Wood & Lenné (1997) mentioned 'the largely anecdotal nature of the evidence to support the direct involvement of farmers in the manipulation of wild relatives', and these authors were even critical of the most documented case, i.e. the use of teosinte by Mexican farmers (Wilkes 1977). Few studies have documented the introduction of wild relatives by farmers in the cultivated pool (review in Jarvis & Hodgkin 1999), and most of these studies have never provided any genetic evidence. In our study, we give genetic evidence that yam farmers select wild and hybrid yams, and that some cultivated plants clearly present a wild or hybrid signature.

(b) Farmers' use of sexual reproduction was previously reported in other vegetatively propagated crops. In cassava (Elias *et al*. 2000), Amerindian tribes intentionally incorporated, in the cultivated pool, volunteers arising from sexual reproduction of cultivated plants. Similar use of seedlings by Ghanaian cassava farmers was also reported (Manu-Aduening *et al*. 2005). In potato, Quiros *et al*. (1992) and Johns & Keen (1986) noticed that some Andean farmers collected fruits on cultivated potatoes and planted the seeds. This is not common but Johns & Keen (1986) also suggested that volunteers originating from seeds can grow in the fields and then could be selected and introduced in the cultivated pool by farmers. In yam, we showed that yam farmers introduced products of the sexual reproduction of wild and cultivated yams in the cultivated pool. The use of sexual reproduction of wild yams will increase the genetic diversity of cultivated yams by introducing new alleles and new combinations.

Sexuality produces new genetic combinations by recombination during meiosis and by combining genes from both parents. We will not discuss the relative advantages of sexuality vs. asexuality (for a review, see Barton & Charlesworth 1998). However, most theoretical explanations in favour of sexuality imply that there is a relative long-term advantage: the potential for future adaptation will be preserved thanks to diversity created by recombination. In contrast, asexuality has an immediate advantage as a suitable set of phenotypic/genetic characteristics is preserved.

The example of yam shows that through ennoblement, farmers' management combines both sexual and asexual reproduction of vegetatively cultivated crops. Farmers could thus test and select new genotypes from the pool of new combinations produced by sexual reproduction. The fact that farmers combine the agronomic advantages

of clonal propagation with the diversity introduced by sex has already been developed for the case of cassava (e.g. Elias *et al*. 2001). In particular, Pujol *et al*. (2005) have shown the selection by farmers for the most vigorous genotypes produced by sexual recombination. In these two cases, farmers could then benefit from the long-term advantage of sexuality that may preserve future adaptations to different environments and farmers' pressures. They could also benefit from the immediate advantage of asexuality by preserving their best genotypes from recombination.

Our study demonstrates, for a vegetatively propagated crop, that traditional farmers' practices preserve evolutionary processes and enhance genetic diversity (Brush 2000). The agronomical, social and cultural factors that could explain the maintenance of the ennoblement practice in some farming communities remain largely unknown. This would be an essential question to address given the role of this practice in nurturing yam diversity, and this would help define the role farmers could play in germplasm conservation and improvement.

Indeed, Brown & Brubaker (2002) identified 'security of preserving farmers' knowledge' as one of the indicators for sustainable management of plant genetic resources *in situ*. Preserving and encouraging local knowledge on yam ennoblement would certainly be a key point in raising awareness of local populations about the value of wild yam diversity. This would then contribute to the *in situ* conservation of wild populations and, in turn, to the on-farm conservation of cultivated yam diversity.

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Supplementary material

The supplementary material is available from http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2958/MEC2958sm.htm

Appendix S1 Hybridization analysis.

Table S1 Characteristics of each analysed sample. Each individual was identified by its name, the species under which it was classified in the field survey and the sampling coordinates (longitude and latitude). *Dioscorea abyssinica* and *Dioscorea praehensilis* were wild species and *Dioscorea rotundata* was the cultivated species. We also mentioned if the sample was (1) or not (0) used for the village-level, the large-scale or the paternity analysis. The paternity analysis was carried out with the wild (*D. abyssinica*) and cultivated (*D. rotundata*) samples from the village-level analysis, except for samples that were not classified under the species mentioned here by the assignment analysis (see Table S3). 'Female' = maternal parents used in the paternity analysis. We also sampled nine tubers of the 'Gban' variety. For each sample of this variety, all samples identified with a * were identical for the 11 loci.

Table S2 Classification of each sample of the village-level analysis according to the assignment analysis. The analysis was conducted using STRUCTURE with $K = 2$. For each individual, its assumed species, q values obtained for each group and the assignment results are shown. The assignment was conducted with a threshold of 0.8.

Table S3 Assignment of male gametes. For each offspring, we give its name, its mother's name and species, the proportion P of simulated offspring with a lower LOD score than its and that originated from either a wild *Dioscorea abyssinica* (Pw) or a cultivated *Dioscorea rotundata* (Pc) male parent. A male gamete was assigned to a population only if the proportion P obtained was $0.025 < P < 0.975$. If a male gamete was derived from neither population, it was considered nonassigned.

Table S4 Classification of each sample of the large-scale analysis according to the assignment analysis. The analysis was performed using STRUCTURE with $K = 3$. For each individual, its assumed species, q values obtained for each group and the assignment results are shown. The assignment was performed with a threshold of 0.8.

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The research of Jean-Louis Pharn's IRD team focuses on plant genetic diversity, domestication and evolution of crop species complexes in tropical agroecosystems and the genetic consequences of farmers' management of diversity. Yves Vigouroux, a junior scientist, is interested in population genetics, domestication and genetic basis of plant adaptation. Serge Tostain studies the genetic diversity of yams and the use of wild yams by farmers in West Africa and Madagascar. A valuable partnership on yam genetics has been developed with Clément Agbangla and Ogoubi Daïnou's team in Benin, which has a strong interest in diversity studies of plant genetic resources and Mendelian genetics. This work was a part of Nora Scarcelli's PhD research on the dynamics of yam diversity in Benin. Nora completed her PhD in November 2005 and has moved to a postdoctoral position in Paula Kover's lab in Manchester, UK, to work on the genetics of adaptation of *Arabidopsis thaliana*.
