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ORIGINS OF DOMESTICATION AND POLYPLOIDY IN OCA (*OXALIS TUBEROSA*; OXALIDACEAE). 3. AFLP DATA OF OCA AND FOUR WILD, TUBER-BEARING TAXA[1]

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Many crops are polyploids, and it can be challenging to untangle the often complicated history of their origins of domestication and origins of polyploidy. To complement other studies of the origins of polyploidy of the octoploid tuber crop oca (*Oxalis tuberosa*) that used DNA sequence data and phylogenetic methods, we here compared AFLP data for oca with four wild, tuber-bearing *Oxalis* taxa found in different regions of the central Andes. Results confirmed the divergence of two use-categories of cultivated oca that indigenous farmers use for different purposes, suggesting the possibility that they might have had separate origins of domestication. Despite previous results with nuclear-encoded, chloroplast-expressed glutamine synthetase suggesting that *O. picchensis* might be a progenitor of oca, AFLP data of this species, as well as different populations of wild, tuber-bearing *Oxalis* found in Lima Department, Peru, were relatively divergent from *O. tuberosa*. Results from all analytical methods suggested that the unnamed wild, tuber-bearing *Oxalis* found in Bolivia and *O. chicligastensis* in NW Argentina are the best candidates as the genome donors for polyploid *O. tuberosa*, but the results were somewhat equivocal about which of these two taxa is the more strongly supported as oca's progenitor.

Key words: AFLP; Andean crops; domestication; neighbor-joining; nonmetric multidimensional scaling; Oxalidaceae; *Oxalis tuberosa*; polyploidy.

Polyploidy has played an important role in plant evolution in general and has been especially important in crop species. In addition to the challenges of untangling the often complicated history of polyploid origins, the study of the origins of some polyploids involves molecular comparisons of closely related species, among which there may be little variation in DNA sequences. As an example, we here present results of a study comparing AFLP data of an understudied domesticated crop with four potential progenitor candidates.

Oxalis tuberosa Molina, commonly known as oca, is primarily cultivated in the central Andes. Like many other "underutilized" crops, it plays an important role in the food security of rural communities (Pastor, et al., 2008). It originated from within a clade informally known as the "*Oxalis tuberosa* alliance," a group of several dozen morphologically similar species found through the central and northern Andes (Emshwiller, 2002a). Cultivated oca was found to be octoploid in most studies, but the majority of the wild species in the alliance clade are diploid, with only a few wild polyploids (reviewed in Emshwiller, 2002b). Those alliance species for which there are published chromosome counts share a base chromosome number rare in *Oxalis* ($x = 8$) (e.g., de Azkue and Martínez, 1990). The majority of the alliance species do not form tubers, but wild, tuber-bearing populations have been found in four geographic areas from central Peru to northwestern Argentina (Fig. 1), and these seem to comprise four species (as discussed later). However, only two of the four taxa have ever been described as species (see Discussion section), i.e., *O. picchensis* R. Knuth from southern Peru and *O. chicligastensis* R. Knuth from northwestern Argentina (Figs. 1 and 2). The other two wild, tuber-bearing *Oxalis* taxa, which are as yet unnamed, will simply be referred to in this paper by shorthand designators: LimaW/T for the wild, tuber-bearing *Oxalis* populations found in the western slopes of the Andes in Lima Department, Peru, and BolW/T for the wild, tuber-bearing *Oxalis* populations found on the eastern side of the Andes in Bolivia (Figs. 1 and 2).

Although generally considered to be a single species, oca may comprise two molecularly distinct groups. Ethnobotanical studies (E. Emshwiller, unpublished data) in the district of Pisac in Cusco Department, Peru, in 1997 found that traditional Quechua-speaking farmers in that area distinguished two use-categories of oca: (1) the several sweeter folk cultivars of the use-category wayk'u were usually cooked fresh after a few days to sweeten in the sun, and (2) the sour khaya use-category, with only the folk cultivar P'osqo, was cultivated separately and used exclusively for processing into khaya, dried tubers that can be preserved for years. Preliminary AFLP data using a single primer combination found that these two use-categories formed separate clusters in the results of neighbor-joining analysis (Emshwiller, 2006a), suggesting that their evolutionary

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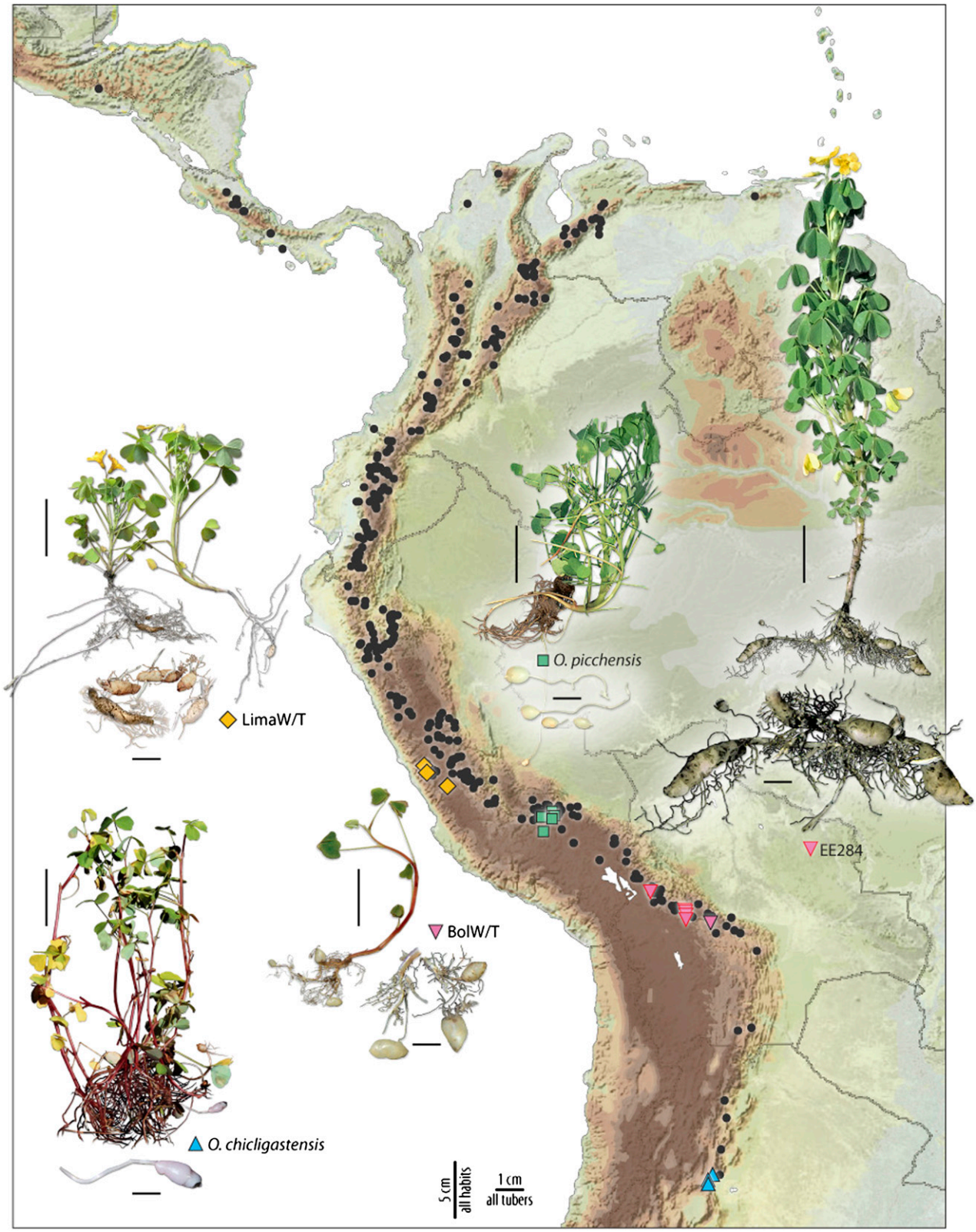

Fig. 1. Map of known distributions of the four wild, tuber-bearing *Oxalis* taxa included in this study, within the range of the *Oxalis tuberosa* alliance. More localities are indicated than were sampled in this study. Images of plant habit are at the same scale, and all images of the tubers are at another scale. Yellow diamonds: localities of the wild, tuber-bearing taxon of Lima Department, Peru (LimaW/T); green squares: *O. picchensis*; pink triangles: wild, tuber-bearing taxon of Bolivia (BolW/T), including specimen EE284; blue triangles: *O. chicligastensis*; black circles: other wild species (nontuber-bearing) in the *Oxalis tuberosa* alliance clade.

histories may differ, perhaps in their origins of polyploidy and/or origins of domestication.

Among the two loci used in previous studies aimed at understanding the origins of polyploidy and domestication of oca, DNA sequence data of chloroplast-expressed (but nuclear-encoded) glutamine synthetase (ncpGS) provided more variation than did the internal transcribed spacer of nuclear ribosomal DNA (ITS) (Emshwiller and Doyle, 2002). The different sequence classes of ncpGS within individual plants of oca and the Bolivian wild, tuber-bearing *Oxalis* (BolW/T) were separated by molecular cloning for use in phylogenetic analyses. Fixed heterozygosity and separate placement of oca's sequence classes on the ncpGS gene tree suggested that these classes represent homeologous loci and that oca is an allopolyploid and possibly an autoallopolyploid. The ncpGS results identified both wild, tuber-bearing taxa included in that study, *O. picchensis* and BolW/T, as progenitor candidates, leading to the working hypothesis that these two *Oxalis* taxa may have hybridized to form cultivated oca (Emshwiller and Doyle, 2002; Emshwiller, 2006b; Zeder et al., 2006).

Two observations suggested that oca's origins might be more complex than the simple hybridization scenario just presented. First, the *O. picchensis*-like ncpGS sequence was absent from one of the nine individual plants sampled, which might have had any of several explanations, including the possibilities of introgression, separate origins of polyploidy, or loss of that sequence type after polyploidization, among others (discussed in Emshwiller and Doyle, 2002, p. 1054). Second, preliminary AFLP data of *O. picchensis* showed few markers that were shared with all oca samples, contrary to expectations for the progenitor of a polyploid (E. Emshwiller, unpublished data). In addition, although the two wild, tuber-bearing taxa sampled in the prior studies with ITS and ncpGS (i.e., *O. picchensis* and BolW/T), were the only such tuber-bearing taxa known at the time, that is no longer the case. Populations of two different wild, tuber-bearing *Oxalis* taxa have since been found in Tucumán, Argentina (*O. chicligastensis*), and in Lima Department, Peru (LimaW/T). The preliminary AFLP data and the finding of additional tuber-bearing, wild *Oxalis* populations both indicated the need to revisit the story of oca's origins, using a combination of different sources of data.

Polymorphic markers that have been used in evolutionary studies of *Oxalis* have included AFLP (Tosto and Hopp, 2000; Emshwiller, 2006a) and ISSR (Malice et al., 2007; Pissard et al., 2006, 2008a, b) in *O. tuberosa*, and microsatellites in *O. alpina* (Tsyusko et al., 2007). AFLP data have been used in studies of variation among oca and some wild diploid species (Tosto and Hopp, 2000), in a comparison between AFLP data and the folk taxonomy of oca traditional cultivars (Emshwiller, 2006a), as well as in studies of intraspecific variation within cultivated oca (Adrianzén, 2006; Biondi, 2006; Zorilla, 2006; Schibli, 2007; K. Vivanco, Universidad Nacional Agraria La Molina, unpublished manuscript, W. Cruz, Universidad Nacional Federico Villarreal, unpublished manuscript). An ongoing international collaborative project is using AFLP data of a much larger sampling of cultivated oca from throughout the Peruvian Andes to study the distributions of clonal genotypes of cultivated oca and the effect of exchange networks among farmers on the genetic structure of the crop.

Here we apply AFLP data to compare the four known wild, tuber-bearing *Oxalis* taxa in the *O. tuberosa* alliance with a selection of cultivated oca samples, to clarify whether they are distinct from each other and whether they had a role in the origin of polyploidy of *O. tuberosa*. Specifically, this study uses analyses of a single AFLP data set to: (1) determine whether the wild, tuber-bearing *Oxalis* populations from four geographic areas are separate taxa and whether the two use-categories of oca are molecularly distinct groups; (2) assess repeatability when comparing AFLP data with morphotypes recognized in folk taxonomy, when using a different, but overlapping, sample of individual oca plants and an increase in AFLP primer combinations compared to the previous study (Emshwiller, 2006a); and (3) assess the evidence that each of the wild taxa might have been a genome donor of octoploid oca.

MATERIALS AND METHODS

Sampling strategy—AFLP data were generated for 3–5 individuals of each of the four wild, tuber-bearing *Oxalis* taxa (Table 1), and 57 cultivated *O. tuberosa* from Cusco Department (Table 2). In some cases, the populations of the wild, tuber-bearing *Oxalis* sampled are the only ones that are known (e.g., LimaW/T). The samples of BolW/T included typical wild plants as well as an individual, EE284, that was found near an area where cultivated oca was grown, and which was more robust than the others. The sample of cultivated oca included both use-categories: 10 were P'osqo, the traditional cultivar used exclusively for khaya, and 47 were other folk cultivars of the wayk'u use-category. To assess repeatability, we resampled 20 of the tubers that had been included in the previous study, all of them from Pisac District in Calca Province of Cusco Department, Peru (Emshwiller, 2006a). Additional samples were selected from two nearby areas in Cusco Department (Table 2), which were each less than 50 km straight line distance from the communities that had been collected in 1997, close enough that they would share some of the same cultivars. Some of these newer samples appeared similar enough that they probably belong to the same clonal genotype as those in the prior study, so these samples provided further tests of congruence of AFLP and morphological data.

Molecular methods—Fluorescent amplified fragment length polymorphism (AFLP) data were generated for seven primer combinations following Myburg et al. (2001) with minor modifications as follows. DNA isolations used either DNeasy columns (Qiagen, Carlsbad, California, USA) or CTAB extractions (Doyle and Doyle, 1990), that were subsequently cleaned using a modification of the Alexander et al. (2007) protocol after it was determined that the CTAB extractions did not yield DNA of sufficient purity for AFLP. Genomic DNA was digested for 2 h at 37°C in a 5-μL reaction with approximately 84 ng DNA, 0.05 μL of 100 ng/100 μL BSA, 5 U of EcoRI, and 5 U of MseI (both New England BioLabs, Beverly, Massachusetts, USA). Adapters and primers (made by Integrated DNA Technologies, Coralville, Iowa, USA) used sequences of Vos, et al. (1995). Adapters were ligated to the ends of the digested DNA fragments immediately after digestion. The ligation reactions were incubated at 16°C for 14 h, and included the following reagents in each 10-μL volume: 5 μL digestion product, 3.6 μL of double-distilled (dd) H_2O, 1 μL of 10× ligase buffer, 0.19 μL of 50 μM double-stranded EcoRI adapter, 0.19 μL of 50 μM double-stranded MseI adapter, and 40 U of T4 ligase (New England BioLabs).

Ligation products were diluted with ddH_2O (1 : 5) and then used in the first round of amplification. These preselective amplifications included the following reagents in a 25-μL volume: 2.5 μL of 10× buffer, 1.5 μL of 25 mM $MgCl_2$, 2 μL of 2.5 mM (each) dNTP, 0.38 μL of 20 μM of each primer, which had only one selective base (C on the MseI primer) or none (EcoRI primer), 1.25 U *Taq* polymerase, and 5 μL diluted ligation product. The thermal cycling protocol included an initial incubation at 72°C for 60 s; 20 cycles of 94°C for 50 s, 56°C for 60 s, 72°C for 120 s; and a final 72°C for 120 s. The resultant product was diluted in ddH_2O (1 : 19) to prepare for the final, selective amplification step. The seven selective primer combinations had two or three selective bases as follows (indicated by EcoRI selective bases/MseI selective bases): AC/CAC, TC/CTG, AG/CTG, GC/CGA, AGC/CAT, GC/CTC, ATT/CGA (the first combination is same as the single AFLP primer pair used in Emshwiller, 2006a). Selective amplifications included the following in a 25-μL reaction: 9.25 μL of ddH_2O, 2.5 μL of 10× buffer, 1.5 μL of 25 mM $MgCl_2$, 3.0 μL of 2.5 mM (each) dNTP, 0.5 μL of deionized formamide (Hi-Di, Applied Biosystems, Foster City, California), 2.5 μL of 10 μM MseI primer, 0.5 μL of 10 μM EcoRI primer (labeled with 5′6-FAM fluorescent tag), 1.25 U *Taq* polymerase, and 5 μL of diluted preselective amplification product. Thermal cycling protocol included 9 cycles

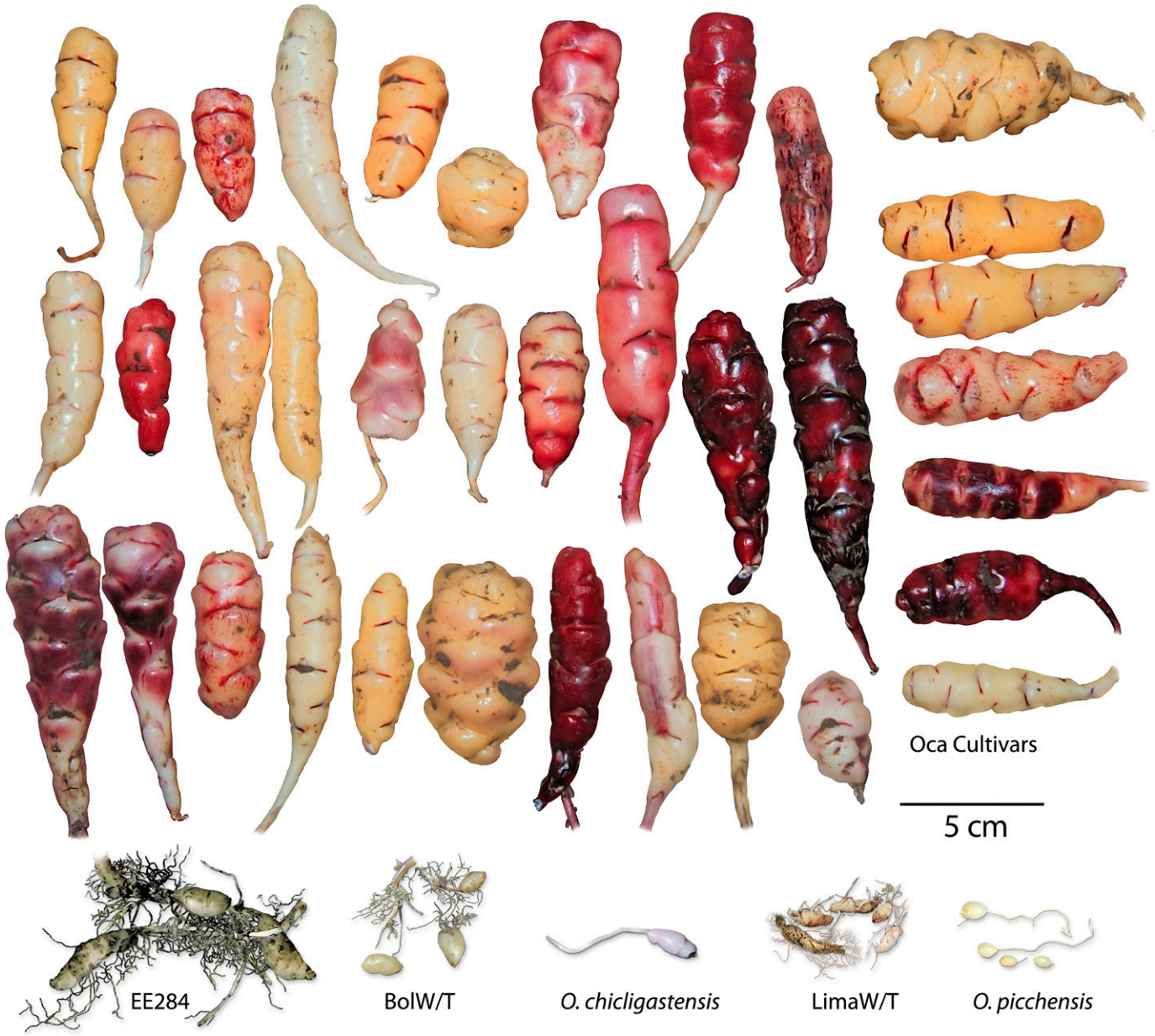

Fig. 2. Comparative size of tubers of domesticated and wild *Oxalis*. The cultivated oca tubers shown here were a particularly diverse sample of tubers grown by one family in Huánuco Department, Peru. On the bottom row are tubers of each of the wild, tuber-bearing *Oxalis* taxa studied here. Because of our as-yet-limited sampling of some of these taxa, we make no claim that the tubers shown here are typical in size for the taxon.

of 94°C for 50 s, 65°C for 60 s (decrease 1°C per cycle), 72°C for 120 s; followed by 20 cycles of 94°C for 50 s, 56°C for 60 s, 72°C for 120 s; and a final incubation at 72°C for 10 min. Amplification products were run on an ABI 3730XL capillary sequencer at the University of Wisconsin Biotechnology Center with fluorescently labeled lane standard (500ROX, Applied Biosystems). Chromatograms were visualized using GeneMarker (Softgenetics, State College, Pennsylvania, USA) and scored for presence/absence of fragments.

Data analyses—The single binary data set representing presence or absence of 399 AFLP peaks in each sampled individual was analyzed by (1) nonmetric multidimensional scaling (NMDS) analyses, based on Jaccard distances, performed using the software package VEGAN for R (Dixon, 2003); and (2) neighbor-joining analysis (N-J), based on Nei and Li (1979) distances, performed in the program PAUP* version 4.0b10 (Swofford, 2002). We also evaluated AFLP peaks looking for those that were shared as either fixed or polymorphic among the sampled oca and wild, tuber-bearing *Oxalis* populations (data not shown). We hoped to find peaks diagnostic for each of the four wild, tuber-bearing *Oxalis* taxa (i.e., that were fixed in that particular taxon and absent from the other three wild taxa), which were also fixed in one or both use-categories of oca (our "gold standard"). However, very few gold-standard peaks were found, and expansion of the criteria to include polymorphic peaks also yielded equivocal results, so the peak sharing evaluation results will only be discussed briefly.

RESULTS

Nonmetric multidimensional scaling—Each of the wild, tuber-bearing *Oxalis* populations from different geographic areas separated from each other in the plot of the first two

TABLE 1. Voucher information for specimens of wild, tuber-bearing *Oxalis* taxa sampled for AFLP data.

Collection no.	Taxon	Country	1st political div.	2nd political div.
EE259	*Oxalis* sp. (BolW/T)	Bolivia	Cochabamba	Ayopaya
EE260	*Oxalis* sp. (BolW/T)	Bolivia	Cochabamba	Ayopaya
EE262	*Oxalis* sp. (BolW/T)	Bolivia	Cochabamba	Ayopaya
EE284	*Oxalis* sp. (BolW/T)	Bolivia	Cochabamba	Chapare
EE500	*O. picchensis*	Peru	Cusco	Cusco
EE531	*O. picchensis*	Peru	Cusco	Calca
EE1152	*O. picchensis*	Peru	Apurimac	Cotabambas
EE1001	*O. chicligastensis*	Argentina	Tucumán	Chicligasta
EE1002	*O. chicligastensis*	Argentina	Tucumán	Chicligasta
EE1003	*O. chicligastensis*	Argentina	Tucumán	Chicligasta
EE1004a	*O. chicligastensis*	Argentina	Tucumán	Chicligasta
EE1004b	*O. chicligastensis*	Argentina	Tucumán	Chicligasta
EE1110	*Oxalis* sp. (LimaW/T)	Peru	Lima	Huarochirí
EE1169	*Oxalis* sp. (LimaW/T)	Peru	Lima	Canta
EE1185	*Oxalis* sp. (LimaW/T)	Peru	Lima	Canta

dimensions in the results of nonmetric multidimensional scaling analysis (Fig. 3). The samples of the populations from southern and central Peru, *O. picchensis* and LimaW/T, were particularly well separated from each other and from any of the other sampled populations. In comparison with those two taxa, the samples of *O. chicligastensis* and those of BolW/T are not as distantly separated from each other. Nonetheless, the sampled accessions of the Bolivian and Argentinean populations do not overlap with each other or with cultivated oca under current sampling. There is also no overlap between the sampled plants of the two use categories of cultivated oca, confirming the previous observation of a distinct separation among the khaya and the wayk'u categories with only one primer combination and a smaller sample of cultivated oca accessions (Emshwiller, 2006a).

Neighbor-joining analysis—Similarly to the NMDS results, the neighbor-joining results also show that the two Peruvian wild taxa, *O. picchensis* and LimaW/T, are the most distantly separated in their AFLP data among the sample groups included here (Fig. 4). There is less separation among cultivated oca, BolW/T, and *O. chicligastensis*. Among these wild taxa of Bolivia and Argentina, most of the sampled plants from Bolivia formed a cluster close to the cultivated oca accessions, with the *O. chicligastensis* samples forming another cluster somewhat more distantly from oca. However, one accession from Bolivia, EE284, joined loosely with the *O. chicligastensis* samples in this analysis. This particular plant was collected from an area very close to fields of cultivated oca. Because of its proximity to oca and the observation that it was more robust and had larger tubers than most wild *Oxalis*, it was suspected to be either a hybrid or an escape from cultivation. In the process of adding AFLP data from more primer combinations, this sample resolved differently in different analyses, joining alternatively with BolW/T, *O. chicligastensis*, or the P'osqo oca samples in different analyses, but always linked only distantly from any of these clusters (results not shown). We do not know the reason for these changes in its position in the results, but one possibility might be that this plant was indeed of hybrid origin, although not necessarily a hybrid with cultivated oca. Nonetheless, as noted before, the Bolivian and Argentinean samples are nonoverlapping with each other or with cultivated oca in the NMDS results.

TABLE 2. Collection information for specimens of cultivated oca, *Oxalis tuberosa* Molina, sampled for AFLP data.

Collection no.	Country	Dept.	Province	District	Use-category
97: 02–13	Peru	Cusco	Calca	Pisac	Khaya
97: 11–07	Peru	Cusco	Calca	Pisac	Khaya
97: 46–14	Peru	Cusco	Calca	Pisac	Khaya
97: 46–15 [a]	Peru	Cusco	Calca	Pisac	Khaya[a]
97: 47–13	Peru	Cusco	Calca	Pisac	Khaya
05: 26–03–14	Peru	Cusco	Urubamba	Ollantaytambo	Khaya
05: 26–08–12	Peru	Cusco	Urubamba	Ollantaytambo	Khaya
05: 26–09–03	Peru	Cusco	Urubamba	Ollantaytambo	Khaya
05: 26–09–04	Peru	Cusco	Urubamba	Ollantaytambo	Khaya
05: 33–03–05	Peru	Cusco	Paucartambo	Colquepata	Khaya
05: 33–03–06	Peru	Cusco	Paucartambo	Colquepata	Khaya
97: 02–05	Peru	Cusco	Calca	Pisac	Wayk'u
97: 11–03	Peru	Cusco	Calca	Pisac	Wayk'u
97: 11–01	Peru	Cusco	Calca	Pisac	Wayk'u
97: 12–05	Peru	Cusco	Calca	Pisac	Wayk'u
97: 14–07	Peru	Cusco	Calca	Pisac	Wayk'u
97: 19–01	Peru	Cusco	Calca	Pisac	Wayk'u
97: 21–06	Peru	Cusco	Calca	Pisac	Wayk'u
97: 22–06	Peru	Cusco	Calca	Pisac	Wayk'u
97: 31–08	Peru	Cusco	Calca	Pisac	Wayk'u
97: 35–04	Peru	Cusco	Calca	Pisac	Wayk'u
97: 40–02	Peru	Cusco	Calca	Pisac	Wayk'u
97: 46–05	Peru	Cusco	Calca	Pisac	Wayk'u
97: 47–06	Peru	Cusco	Calca	Pisac	Wayk'u
97: 48–02	Peru	Cusco	Calca	Pisac	Wayk'u
97: 50–02	Peru	Cusco	Calca	Pisac	Wayk'u
05: 17–06–01	Peru	Ayacucho	Huanta	Luricocha-Huayllay	Wayk'u
05: 26–01–10	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–01–09	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–01–19	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–02–02	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–02–12	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–05–03	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–05–13	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–06–12	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–06–24	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–06–39	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–06–39 [b]	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–06–41	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–06–41 [b]	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–06–47	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–06–50	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–07–04	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–07–46	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–07–47	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–08–13	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–08–42	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–09–01	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–09–02	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–10–20	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–10–28	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–10–35	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–12–30	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–10–42	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–10–43	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–11–07	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u
05: 26–11–14	Peru	Cusco	Urubamba	Ollantaytambo	Wayk'u

[a] This might have been a mixed-up tube. The plant was recorded as khaya-type in the field and it clustered with other khaya samples in the previous results of N-J analysis with a single primer combination (Emshwiller, 2006a). However, in the current N-J results, it grouped with the wayk'u cluster.

[b] These were replicate samples.

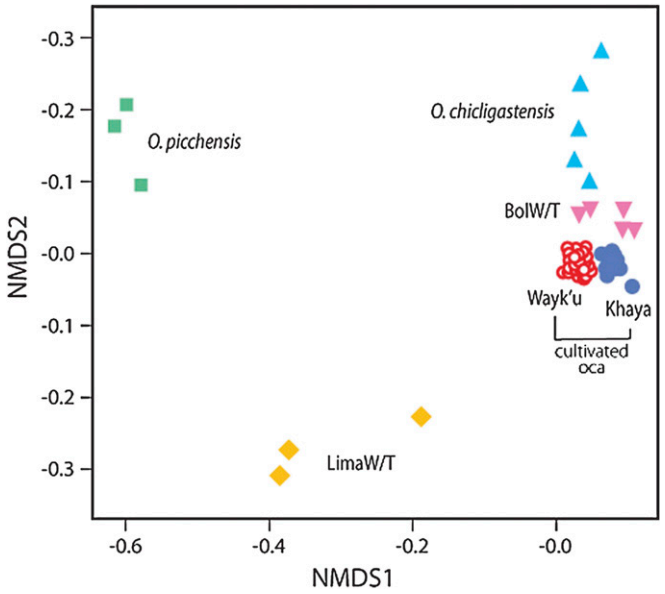

Fig. 3. Results of nonmetric multidimensional scaling (NMDS) analysis of AFLP data from all four wild, tuber-bearing taxa and both use-categories (wayk'u and khaya) of cultivated oca. Yellow diamonds: wild, tuber-bearing taxon of Lima Department, Peru (LimaW/T); green squares: *O. picchensis*; pink triangles: wild, tuber-bearing taxon of Bolivia (BolW/T); turquoise-blue triangles: *O. chicligastensis*; open red circles: wayk'u oca cultivars; dark blue circles: the khaya oca cultivar P'osqo.

In agreement with the NMDS results, the two use-categories that were recognized by the farming households in the district of Pisac formed separate groups in distance analyses of AFLP data. Not only did four P'osqo accessions that were resampled from the Pisac sample of 1997 separate from the wayk'u varieties, but additional tubers that appeared morphologically similar to P'osqo tubers, that had been collected from areas not far from Pisac District, also joined the same cluster with the Pisac P'osqo tubers in the N-J results. One exception (no. 46-15) was probably a mix-up of DNA tubes, based on its morphology and also its having joined the P'osqo cluster previously (Emshwiller, 2006a).

A secondary goal of this study was to test repeatability of the method by comparing the current results with seven AFLP primer combinations with previous results using a single primer combination and somewhat different laboratory methods (Emshwiller, 2006a). In addition to testing whether the two use-categories would still resolve as distinct clusters as more data were added, we tested whether samples of each of several wayk'u folk cultivars would cluster together. Repeatability was good. The morphotypes sampled here, from Pisac and from other provinces in Cusco Department, separated into similar clusters as had previously been found with a single AFLP primer combination (Emshwiller, 2006a) and were congruent with the morphotypes distinguished by the local farmers, with very few exceptions. Samples from Pisac joined with morphologically similar samples as previously, although the arrangements within clusters were not necessarily identical, and they also clustered with morphologically similar tubers from nearby provinces. There were a few cases of dissimilar tubers joining the same cluster, for which we do not know whether or not they might involve mix-ups of samples in the field or laboratory.

DISCUSSION

Comparison of AFLP profiles among domesticated *O. tuberosa* of two use-categories and four wild, tuber-bearing *Oxalis* taxa provides evidence regarding the distinctness of each of these entities. In partial contrast with previous studies using ncpGS, the AFLP results suggest that the wild taxa that are most closely related to domesticated oca are those of Bolivia or possibly Argentina, rather than those of Peru. This study provides an example of how different sources of data may lead to different insights.

Insights from AFLP data regarding distinctness and taxonomy of wild, tuber-bearing* Oxalis *taxa—Two of the four wild, tuber-bearing *Oxalis* taxa studied here were given species-level names, *O. picchensis* R. Knuth and *O. chicligastensis* R. Knuth, whereas the populations in Lima Department, Peru, and those of Bolivia have never been named as distinct taxa. None of the four taxa were considered distinct species in the treatment of *Oxalis* subgenus *Oxalis* by Lourteig (2000), but our current results have implications for their distinctiveness.

Lourteig (2000) considered the name *O. picchensis* to be a synonym of *O. petrophila* R. Knuth, but these taxa differ in both morphological and molecular data (Emshwiller, 2002a, 2006b). The type specimen of *O. picchensis* was lost in the destruction of the Berlin herbarium, and the populations from which it was collected were probably extirpated because the type locality, Cerro Piccho, is now within a built-up area of the expanding city of Cusco. However, this taxon can still be found near Cusco city and elsewhere in Cusco and Apurimac departments. Despite the fact that Lourteig (2000) did not recognize *O. picchensis* as a distinct species, she described the new species *O. apurimacensis* Lourteig, for which she did not mention underground structures. Examination of some of the specimens listed in the protologue for this species suggested that she included material that might actually be *O. picchensis* (E. Emshwiller, personal observations).

The samples of *O. chicligastensis* used in this study were collected near the type locality in Chicligasta Department in Tucumán Province, Argentina. Although Lourteig (2000) considered this name to be a synonym of *O. tuberosa*, all the sampled individuals of this taxon separate from all oca samples tested here in the NMDS and N-J results, suggesting that under current sampling this taxon appears to be distinct from oca.

The AFLP data presented here also suggest that the two unnamed wild, tuber-bearing *Oxalis* taxa are also distinct. AFLP data suggest that the LimaW/T populations are clearly distinct from *O. tuberosa* and should probably be considered a new species. The molecular separation of the BolW/T populations from cultivated oca is not as great, but under current sampling they do not overlap in the NMDS ordination results.

Thus, our current results suggest that each of these four wild, tuber-bearing *Oxalis* taxa are separate entities from each other and from cultivated oca. The separation of *O. chicligastensis* and the Bolivian taxon from each other and from cultivated oca still needs to be further confirmed, as a single, possibly hybrid, individual (EE284) violated the separation of all these taxa in the N-J results. Pending further study of species delimitations, we provisionally retain both names, *O. chicligastensis* and *O. picchensis*, as distinct species and also suggest that LimaW/T and BolW/T are probably separate species as well. However, the geographic sampling of the current study is limited. Samples

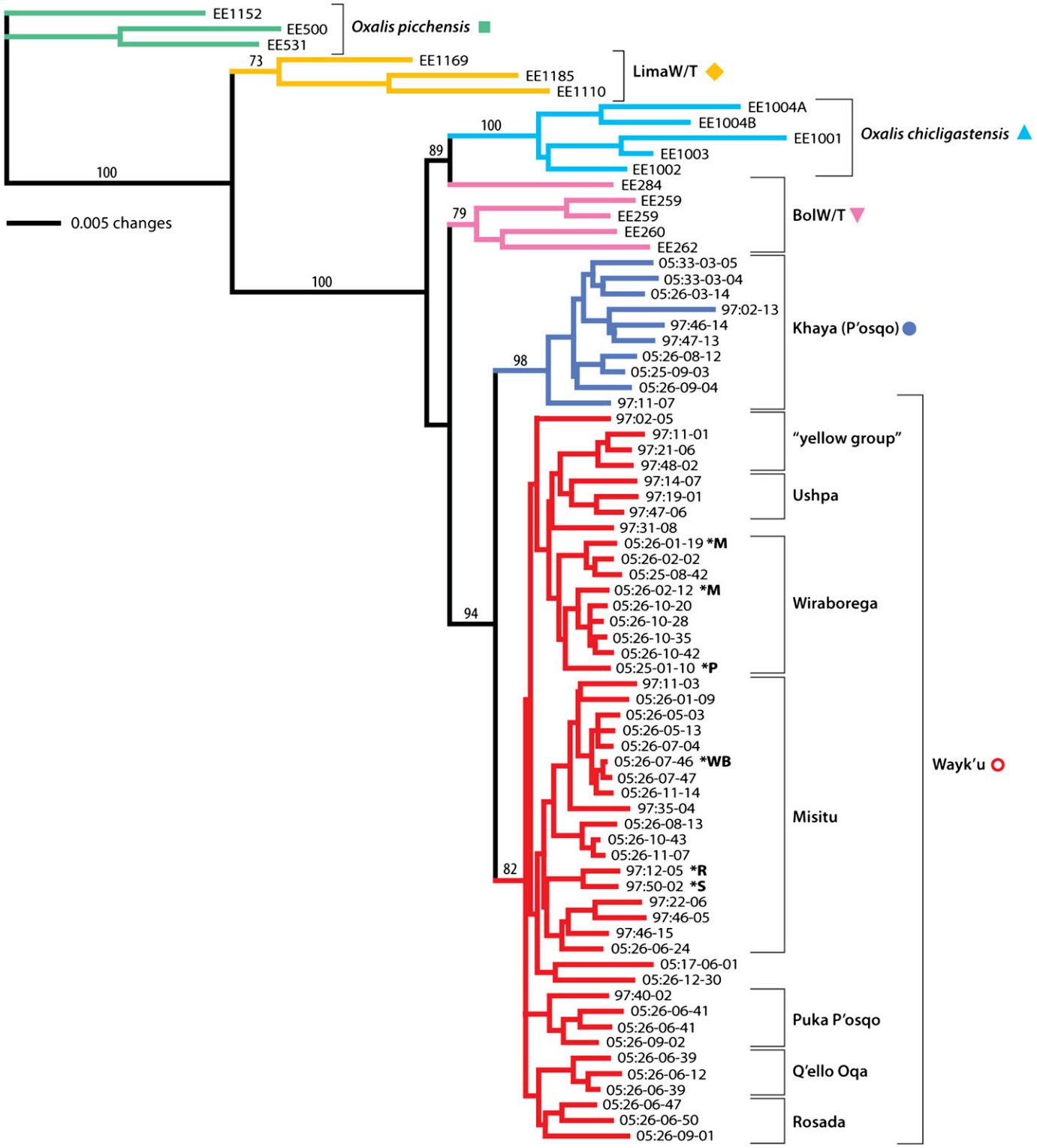

Fig. 4. Results of neighbor-joining (N-J) analysis of AFLP data from all four wild, tuber-bearing taxa and both use-categories (wayk'u and khaya) of cultivated oca. Green branches: *O. picchensis*; yellow branches: wild, tuber-bearing taxon of Lima Department, Peru (LimaW/T); pink branches: wild, tuber-bearing taxon of Bolivia (BolW/T); turquoise-blue branches: *O. chicligastensis*; red branches: wayk'u oca cultivars; darker blue branches: khaya oca cultivar P'osqo. Bootstrap support from 10 000 replicates is shown only on the branches leading to these groups, not within them.

of LimaW/T are as yet known from only three populations, and those of *O. chicligastensis* were collected within a very limited area. Although the wild, tuber-bearing populations of Peru and Bolivia appear to be geographically separated from each other (E. Emshwiller, personal observations), additional exploration is needed to determine whether those of Bolivia (BolW/T) and northwestern Argentina (*O. chicligastensis*) are geographically separated and to clarify whether these taxa are truly distinct from each other and from cultivated oca.

Distinctness of wayk'u and khaya use-categories—Cultivated oca samples were selected to permit a re-examination of some of the samples used in the previous study (Emshwiller, 2006a). Also included were oca tubers collected from other communities in Cusco Department, that were suspected, based on tuber morphology, of being examples of the same oca clones as had been included in that study (see Table 2). The sampled oca accessions formed two distinct and non-overlapping groups in the NMDS results and joined two clusters in the N-J results, corresponding to the two use-categories that are recognized by traditional farmers in the district of Pisac in Cusco Department, Peru. That is, the P'osqo cultivar used exclusively for khaya always separated from the wayk'u cultivars in both NMDS and N-J analyses, with the exception of one possible mix-up of tubes. This separation reinforces the previous AFLP results of an overlapping sample (Emshwiller, 2006a), suggesting that these use-categories are molecularly distinct. This molecular divergence has important implications for the study of the origins of oca, because it suggests that the use-categories have some as-yet-unknown difference in their evolutionary histories, which might possibly represent separate origins of domestication and/or separate origins of polyploidy.

Quechua and Aymara-speaking farmers in other areas of the Andes also distinguish "sweet" oca folk cultivars from "sour" or "bitter" varieties, much as they do with potatoes. However, we do not yet know whether these categories correspond to the wayk'u and khaya categories from the communities in Cusco Department that were studied here and that were so divergent from each other in our results. Ongoing studies will confirm whether this separation of use-categories is upheld in a sampling of oca that has been collected from throughout the Peruvian Andes. Additional expeditions to collect more samples of the wild taxa should help to clarify whether the divergence among the use-categories indicates multiple domestications. Nonetheless, the finding of molecular divergence among these two use-categories is an example of how ethnobotanical information can reveal evolutionary differences that might have been overlooked otherwise.

With respect to the groupings of the wayk'u cultivars in the N-J results, with the exception of a few accessions that joined the "wrong" cluster, these results were quite congruent with morphological groups of tubers from these three districts and also with previous results (Emshwiller, 2006a) using a single primer combination and a different, but overlapping, set of samples. These results indicate that these seven AFLP combinations are sufficient to distinguish clones of oca and that the results are reliable and reasonably repeatable across different datasets.

***Origins of polyploidy in* Oxalis tuberosa**—*State of knowledge of ploidy levels of the sampled taxa*—Information on ploidy levels is important for the interpretation of the current results, but it is incomplete for the wild, tuber-bearing *Oxalis* taxa included here. Most cultivated oca are octoploid (reviewed in Emshwiller, 2002b), but as of yet, there are no reports of octoploid wild, tuber-bearing *Oxalis*. Flow cytometry data indicated that *O. picchensis* is tetraploid (Emshwiller, 2002b), and *O. chicligastensis* is probably tetraploid as well, based on a report by Brücher (1969) that probably represents that taxon (reviewed in Emshwiller, 2006b). The ploidy level of the Bolivian, wild, tuber-bearing populations is unknown, but fixed heterozygosity for ncpGS suggests that they too are probably polyploid (Emshwiller and Doyle, 2002). Ongoing flow cytometry studies at the International Potato Center by Kelly Vivanco (unpublished data) have tentatively found the unnamed populations from Lima Department to be hexaploid, and some sampled plants of P'osqo oca to be tetraploid, but those observations still need to be confirmed.

Expectations for polyploid origins—Although most cultivated oca is octoploid, and the wild, tuber-bearing *Oxalis* studied here are probably of lower ploidy levels, we do not think that oca must necessarily be a hybrid between two of the taxa studied here. There exist several other possibilities. One possibility is that oca may have originated from autopolyploidization of a wild allotetraploid, possibly one of the taxa studied here. Another possibility is that one of the wild, tuber-bearing *Oxalis* studied here hybridized with another taxon, which would not itself have to be another tuber-bearing taxon and could even be a diploid species. Testing all the 2*x* species in the alliance with AFLP was beyond the scope of this paper, but additional tuberless, diploid species in the alliance are being tested in work with single-copy nuclear loci.

Implications of AFLP results for origins of octoploid O. tuberosa—AFLP data provide a broader sampling of the genome than previous studies with ITS and ncpGS data, and the results with AFLP led to different conclusions than the previous studies based on ncpGS data (Emshwiller and Doyle, 2002). These previous results suggested that *O. picchensis* of southern Peru might be one of the genome donors of octoploid oca, because the ncpGS sequence of that taxon was identical to one of the sequence classes of oca. In contrast, the current results with AFLP, regardless of analytical method, suggested that *O. picchensis* does not fit the pattern expected for a genome donor of octoploid *O. tuberosa*. AFLP data of *O. picchensis* are the least similar to oca (of either use-category) of the four wild, tuber-bearing *Oxalis*. However, the possibility still exists that *O. picchensis* might have had some role in the evolution of the octoploid cultigen, perhaps through introgressive hybridization. The LimaW/T populations are quite divergent from *O. picchensis* and should probably be described as a new species. They are likewise divergent enough from cultivated oca that it is unlikely that they were a progenitor of the crop. Nonetheless, we will continue to include both these taxa in studies that will use single-copy nuclear loci to confirm the origins of oca.

Compared to the two Peruvian wild taxa, the other two wild, tuber-bearing *Oxalis* taxa, found in Bolivia and Argentina, are more strongly supported by AFLP data as being progenitor candidates for octoploid oca, regardless of the analytical methods, which all concur on this point. Although the latter two wild, tuber-bearing taxa, BolW/T and *O. chicligastensis,* are relatively close to each other and to *O. tuberosa* in both the N-J and the NMDS results, they are non-overlapping in the ordination results. In results of both these distance-based analyses, the samples of the Bolivian populations are closer (more similar) to cultivated oca than the *O. chicligastensis* samples. On the other

hand, only *O. chicligastensis* had any of the "gold standard" diagnostic markers that it shared, as fixed, with cultivated oca, in the peak-sharing evaluation (data not shown), so its possible role in the origin of oca should not be dismissed. Despite the fact that many peaks were shared, as fixed, between each of the wild taxa and oca, these shared, fixed peaks were usually present in more than one wild, tuber-bearing *Oxalis* taxon (they were not diagnostic). Only *O. chicligastensis* had diagnostic peaks that were absent in all the other wild, tuber-bearing *Oxalis* taxa: one peak that it shared with all oca samples and another one that it shared with all wayk'u oca samples. Although the default expectation for polyploids is additivity (e.g., Pires et al., 2004), i.e., that markers that are fixed in the progenitors will be fixed in the polyploid derivative, this default expectation is often violated in many polyploids studied to date (Wendel, 2000; Liu and Wendel, 2002; Pikaard, 2001; Lukens et al., 2004), and several processes may lead to "loss" of bands. This process of loss of molecular markers in polyploids may be the explanation for the low number of "gold standard" peaks observed here. On the other hand, the pattern may be complicated because the progenitor candidates are closely related, so that fixed markers are shared among multiple taxa.

The neighbor-joining results must also be interpreted with caution, because having a shorter distance between clusters in the N-J results is not definitive by itself to identify crop progenitors. The results indicate that AFLP data of BolW/T are more similar to those of both use-categories of cultivated oca than are those of *O. chicligastensis*, but this should not necessarily be interpreted to mean that the Bolivian populations sampled are necessarily the progenitors of oca. The fact that neighbor-joining is a distance-based method means that its results do not necessarily reflect phylogenetic relationships among species. When used in studies within species, the potential for gene flow and meiotic recombination among populations with separate origins can lead to results that appear to suggest "monophyletic" origins when in fact there were multiple origins (Allaby and Brown, 2003; Allaby et al., 2008). Polyploidy adds additional complexity and can make the N-J results even more difficult to interpret, especially when such analyses are undertaken with samples of different ploidy levels in the same analysis. The representation of N-J results as a tree-like diagram tempts many to interpret the results as a hierarchical phylogeny, which is inappropriate if the true history includes hybrid origins, as is often the case for polyploids. In addition, it is possible that the dominant nature of AFLP markers would result in clustering of taxa that have the same ploidy level, regardless of their true relationships, because with dominant markers only nulliplex (completely homozygous recessive) individuals will not have a particular marker, whereas one indicator of allopolyploidy is fixed heterozygosity. Thus, individuals of several different homeologous genotypes might have the same AFLP phenotype for a particular marker or group of markers, because gene dosage is not considered, only the presence and absence.

The incongruence between the AFLP results presented here and the previous results with ncpGS (Emshwiller and Doyle, 2002), especially with respect to the role of *O. picchensis* in the origins of *O. tuberosa*, serves to underscore the complexity of polyploid evolution. Nonetheless, the currently available AFLP data support the idea that the wild, tuber-bearing *Oxalis* populations from Bolivia (BolW/T) and *O. chicligastensis* of Argentina are the better candidates as genome donors of oca. Future studies are planned to increase sampling of individuals and populations to improve the geographic representation of these two taxa, and will also include other sources of data. These future studies will assess whether these taxa are indeed separate, distinct taxa and further test whether they were genome donors of octoploid oca, and will also investigate whether the two use-categories of cultivated oca might represent separate origins of domestication.

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