Evolution and conservation of clonally-propagated crops: insights from AFLP data and folk taxonomy of the Andean tuber “oca,” Oxalis tuberosa.

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Evolution and Conservation of Clonally Propagated Crops
Insights from AFLP Data and Folk Taxonomy of the Andean Tuber Oca (Oxalis tuberosa)

Vegetatively propagated crops play an enormous role in feeding the world. They include crops that are important worldwide, such as sugarcane, potato, cassava, sweet potato, banana, and plantain, as well as crops of local or regional importance, such as true yam, edible aroids, and several minor Andean roots and tubers. Many of these crops are grown primarily for subsistence, under traditional, non-industrialized farming systems, which still represent much of world agriculture. Thus they serve as an important safety net against starvation. These agroecosystems retain great diversity of potential use for future breeding efforts (Elias and McKey, 2000), yet studies of the dynamics of genetic diversity in these systems are few. We lack information about how evolutionary factors, such as selection and gene flow, differ between clonally propagated and seed-propagated crops. To understand the evolution and conservation needs of any crop, we need to learn about several aspects: the crop's origins and what wild species are closely related to it, how human influence has affected its evolution under domestication, how its diversity is distributed, and the factors that affect whether that diversity is maintained or lost. As the first effort in a research program aimed at understanding the dynamics of genetic diversity of cultigens and their wild relatives and the continuing human role in their evolution, this chapter discusses research on the Andean tuber crop oca (Oxalis tuberosa Molina).

Oca is considered second to potatoes among these minor tuber crops in the diet and farming system of millions of Quechua and Aymara peasant farmers in Ecuador, Peru, and Bolivia, and its potential in other parts of the world is demonstrated by its recent commercialization in New Zealand (National Research Council, 1989). Oca is primarily a starchy staple in Andean communities of subsistence farmers, providing some variety from potatoes in a largely tuber-based diet, but it is also rich in vitamins.

Oca Diversity

Oca tubers look like elongated potato tubers (figure 14.1), with their eyes (lateral buds) embedded in prominent transverse ridges, which may be colored differently from the rest of the tuber in some cultivars (cultivated varieties). Although it remains capable of sexual reproduction (Villenas Ramirez, 1997; Trognitz et al., 1998), oca is propagated exclusively vegetatively in traditional agriculture. Nonetheless, it still maintains phenotypic diversity. Pigmentation of the tuber is particularly variable, with colors ranging from nearly white to nearly black, with shades of pink, red, purple, yellow, and orange, with various patterns of distribution of colors on both the exterior
Origins of Polyploidy in Octoploid Oca

Like the potato and many other domesticated plants, oca is polyploid, in this case octoploid (with eight sets of chromosomes). Thus one aspect of understanding oca’s evolution involves determining its origin of polyploidy and its phylogenetic relationships with wild species. Specifically, we need to determine not only from what wild species oca was domesticated but also what species contributed genomes to the polyploid crop. Cultivated oca has been found to be octoploid in most studies (de Azkue and Martínez, 1990; Medina Hinostroza, 1994; Valladolid et al., 1994; Emshwiller, 2002b), although there are conflicting reports. The genus Oxalis comprises 500–800 species, most of them in South America and southern Africa, making the search for the origins of polyploidy in oca a challenge. Cytological studies revealed that oca was part of the O. tuberosa alliance, a group of morphologically similar species that share the same base chromosome number, $x = 8$ (de Azkue and Martínez, 1990). Other Oxalis species have base chromosome numbers from 5 to 12, with 7 most common. Current data suggest that the alliance includes more than the dozen species originally studied by de Azkue and Martínez (1990), probably several dozen species from throughout the central and northern Andes (Emshwiller, 2002a). Molecular studies investigating the origins of oca used DNA sequence data from two loci, the internal transcribed spacer (ITS) of nuclear ribosomal DNA and the chloroplast-expressed (but nuclear-encoded) isozyme of glutamine synthetase (ncPGS). The ITS data confirmed the monophyly of the O. tuberosa alliance and the origins of oca from within this group, but ITS had insufficient variation to identify oca’s progenitors (Emshwiller and Doyle, 1998). An intron-containing region of ncPGS, however, provided more informative variation than ITS (Emshwiller, 2002a; Emshwiller and Doyle, 2002). Three different sequence classes of ncPGS within an individual plant were separated by molecular cloning for use in phylogenetic analyses. Fixed heterozygosity and separate placement of the sequence classes on the ncPGS gene tree suggested that these three classes represent homeologous loci and that oca is of hybrid origin (allopolyploid) and probably autoallopolyploid (at least one genome is present in more than two copies).

Data from ncPGS identified two wild tuber-bearing taxa, O. picchensis of southern Peru and a yet-unnamed species from Bolivia, as progenitor candidates that may have hybridized to form cultivated oca (Emshwiller and Doyle, 2002). Flow cytometry data indicated that O. picchensis is tetraploid (Emshwiller, 2002b), and although the ploidy level of the Bolivian taxon...
is unknown, it is probably also polyploid, based on its fixed heterozygosity for ncpGs sequence classes among the sampled plants. Other sources of data are needed to test this working hypothesis and resolve unanswered questions about the origins of polyploidy in oca. That these origins might be complex is suggested by variation in ncpG sequences from different plants, especially the absence of the O. picicensis-like sequence from one of the nine individual O. tuberosa plants sampled. Alternative hypotheses to explain this absence include multiple origins of polyploidy, varying ploidy levels in cultivated oca, introgression of the O. picicensis-like sequence through wild-crop gene flow, or loss of this sequence class through chromosomal rearrangements after polyploidization (see reviews in Soltis and Soltis, 1999; Wendel, 2000; Liu and Wendel, 2003). In addition, another wild tuber-bearing taxon from northwestern Argentina, O. chilegastensis, appears to be another possible candidate as genome donor for oca, based on both morphology and DNA sequence data (unpublished data). Thus, despite recent progress in the identification of good candidates as the genome donors of polyploid oca, several alternative hypotheses are congruent with the current data. Future studies are planned to use amplified fragment length polymorphism (AFLP) as an independent source of data for examining these working hypotheses.

**Ethnotaxonomy and Clonal Crops**

The evolution of crops is affected by the management of folk cultivars in traditional agricultural systems, especially in the way in which humans act as agents of selection and dispersal. Thus ethnographic studies combined with genetic studies of crop diversity using molecular markers can elucidate the human influence on crop evolution. Conservation of crop genetic diversity often is said to be linked to knowledge and use; loss of knowledge goes hand in hand with loss of diversity (IPGRI, 2001). Therefore, if we are to understand crop evolution in traditional agriculture and plan for in situ conservation, it is vital to study folk taxonomy. Understanding how crop diversity is named and classified by farmers is key to “how this diversity is perceived and valued by farmers” (Elias et al., 2001a:156) and thus to “understanding behavioral patterns that affect crop evolution” (Quiros et al., 1990:256). Folk nomenclature has been studied in clonal crops such as potato (LaBarre, 1947; Jackson et al., 1980; Brush et al., 1981; Zimmerer, 1991b; Brush and Taylor, 1992), cassava (Boster, 1984, 1985, 1986; Salick et al., 1997; Elias et al., 2000a, 2000b, 2001a, 2001b), sweet potato (Prain et al., 1995; Nazarea, 1998; Prain and Campilan, 1999), and ensete (Shigeta, 1996), and research is ongoing in these and other crops (PLEC, 2001). Studies have compared folk nomenclature with molecular markers (DNA or allozyme) in potato (Quiros et al., 1990; Zimmerer and Douches, 1991; Brush et al., 1995; Zimmerer, 1998) and cassava (Elias et al., 2000a, 2001a, 2001b). However, the generalizability of these results is unknown, and there is a need to expand on these studies and provide comparison with other crops.

**Ethnobotanical and Ethnotaxonomic Studies in Pisac District, Southern Peru**

To identify factors that affect whether oca genetic diversity is being lost or maintained in traditional Andean agriculture, I conducted an ethnobotanical survey in three indigenous peasant communities (Viacha, Amaru, and Sacaca) in Pisac District, Cusco Department, in southern Peru in 1997 (Emshwiller, 1998). Semistructured interviews in Spanish and Quechua focused on the knowledge and management of the crop by traditional Andean farmers. Information was elicited about how traditional cultivars of oca are named, classified, recognized, acquired, selected, and managed. Some questions focused on how much of a family's harvest went for sale, seed, and home consumption; methods of storage, preparation, and cooking; whether some cultivars were disappearing; pest and disease management; and how propagation material is exchanged between families and between communities.

To study the folk nomenclature and taxonomy of oca variation I asked about the names and characteristics of the cultivars and their preferred uses. Farmers distinguish the culinary traits of tubers, describing them as sweet or sour and their texture as floury, watery, or firm. Similarly to the situation observed by Boster (1984) for cassava, farmers were knowledgeable about these culinary characteristics but did not distinguish between the cultivars in terms of agronomic traits or ecological needs.

This study revealed that oca, like potato, is classified into use categories (sensu Zimmerer, 1991a). Oca tubers are either cooked fresh or preserved in dried form. Sweet cultivars, called *wayk'u* (boiling) oca, usually are exposed to sunlight for a few days to sweeten them and then either boiled whole or roasted in *wafis* (temporary earth ovens made of clods of soil). In contrast, sour cultivars are preserved by processing into dried oca tubers called *khaya* (figure 14.1, table 14.1). *Khaya* is prepared by exposing tubers
### Table 14.1 Folk Cultivars of Pisac Communities Viacha, Amaru, and Sacaca

<table>
<thead>
<tr>
<th>Use Category</th>
<th>Folk Cultivars</th>
<th>Subcultivars</th>
<th>Exterior Color</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khaya</td>
<td>P'isqa</td>
<td>Kasipata</td>
<td>Pale yellow</td>
<td>Very sour, used exclusively for khaya</td>
</tr>
<tr>
<td>Wayku (sometimes</td>
<td></td>
<td></td>
<td>Magenta pink</td>
<td>Firm texture</td>
</tr>
<tr>
<td>grouped with khaya)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka panti</td>
<td>Misitu</td>
<td>Magenta pink</td>
<td></td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka kashira</td>
<td>Q'iso misitu</td>
<td>Orangish with brown streaks</td>
<td>Claviform</td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka kashira</td>
<td>Yana misitu</td>
<td>Nearly black</td>
<td>Claviform</td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka kashira</td>
<td>Q'iso misitu</td>
<td>Yellow with darker streaks</td>
<td>Ovoid</td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka kashira</td>
<td>Higos misitu</td>
<td>Orangish with brown streaks</td>
<td>Ovoid</td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka kashira</td>
<td>Tullu misitu</td>
<td>Orangish with brown streaks</td>
<td>Long cylindrical</td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka kashira</td>
<td>K'api misitu</td>
<td>Orangish with brown streaks</td>
<td>Long cylindrical</td>
</tr>
<tr>
<td>Wayku</td>
<td>Usapa</td>
<td>Yanu uspa</td>
<td>Pinkish white</td>
<td>Floury texture</td>
</tr>
<tr>
<td>Wayku</td>
<td>Usapa</td>
<td>P'uka uspa</td>
<td>Mottled red</td>
<td></td>
</tr>
<tr>
<td>Wayku</td>
<td>Usapa</td>
<td>Yana uspa</td>
<td>Purple-black</td>
<td></td>
</tr>
<tr>
<td>Wayku</td>
<td>Hana's q'iso,</td>
<td>Yanu uspa</td>
<td>Yellow at base grading to red apex</td>
<td>Clusters in “yellow group”</td>
</tr>
<tr>
<td>Wayku</td>
<td>waqankillay</td>
<td>P'uka uspa</td>
<td>Pale yellow</td>
<td>Clusters in “yellow group”</td>
</tr>
<tr>
<td>Wayku</td>
<td>Q'iso kashira</td>
<td>P'uka uspa</td>
<td>Yellow with red eyes</td>
<td>Clusters in “yellow group”</td>
</tr>
<tr>
<td>Wayku</td>
<td>K'api kashira</td>
<td>P'uka uspa</td>
<td>White with pale pink eyes</td>
<td></td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka q'iso</td>
<td>P'uka uspa</td>
<td>White with pale pink blotches</td>
<td>Chiliku is Quechua pronunciation</td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka q'iso</td>
<td>P'uka uspa</td>
<td>Red</td>
<td>of the Spanish chaleco = vest</td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka q'iso</td>
<td>P'uka uspa</td>
<td>Shiny red</td>
<td>Sour but grown with wayku</td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka q'iso</td>
<td>P'uka uspa</td>
<td>Orangish red</td>
<td></td>
</tr>
<tr>
<td>Wayku</td>
<td>P'uka q'iso</td>
<td>P'uka uspa</td>
<td>Orangish red</td>
<td></td>
</tr>
</tbody>
</table>

Very roughly, more common cultivars are listed toward the top, less common below. Some unsampled cultivars found in these communities are not listed.
and conversely, whether single genotypes bear several names; and how AFLP markers correspond with the morphologically heterogeneous complex cultivars that are distinguished by some but not all farmers. In the latter case of complex cultivars that include subcultivars, some alternative hypotheses include that these subcultivar groups are similar but distinguishable genotypes, dissimilar genotypes that have converged on similar morphological traits, or indistinguishable by AFLP (i.e., either the phenotypic differences have no genetic basis or they result from mutations that are not reflected in AFLP profiles).

Materials and Methods

Sampling

Tubers collected during the ethnotaxonomic survey were used in this AFLP study so that genotypes as distinguished by AFLP data could be compared with the ethnotaxonomy of oca folk cultivars. However, because of the variation between farmers in knowledge of oca varietal names and whether they were applied consistently, a comparison of AFLP data with the names given by each individual farmer would conflate potential genotypic variation within cultivars with inconsistency in the use of names. Therefore, the names supplied by each farmer were compared with a separate grouping based on morphological traits. In this chapter, I report on a comparison of the AFLP data with the tuber morphotypes based on my own visual assessment in which I grouped together tubers that looked similar enough that they might belong to the same clonal genotype. I then called each morphotype group by the name (or names, if recognized as synonyms) that was applied most often to that morphotype by knowledgeable farmers. In most but not all cases, the group to which I independently assigned the tuber agreed with the name given by the farmer (or a variant or synonym of that name). Future stages of this project will incorporate information from the cases of disagreement between the names to which the farmers and I assigned the tuber.

Some of the tubers did not seem to belong definitely with any of the other morphotypes (hereafter called mismatch tubers). In these cases the color and other characteristics of the tuber were noted, and they were either designated as of uncertain identification or tentatively identified as the cultivars they most resembled. The first samples for AFLP included only tubers for which the farmers and I agreed on the cultivar group to which
the tuber belonged, whereas later sampling included some of the questionable matches.

AFLP data were generated for 95 tubers collected in the three communities in Pisac district. In addition to *O. tuberosa* accessions, one plant each of three wild tuber-bearing taxa was sampled to compare with the cultivated oca samples. Two of these, *O. pichensis* and the unnamed taxon of Bolivia, were identified by previous results as possible progenitors of octoploid oca (Emshwiller and Doyle, 2002). The third wild taxon, *O. chicliformis* of northwestern Argentina, is another candidate as a putative progenitor, based on unpublished *ncpG* sequence data. An additional 30 oca samples from other areas in Peru and Bolivia were included in the assessment of AFLP polymorphism but were not part of the ethnotaxonomic comparison.

**Data Isolates and Fluorescent AFLP Procedure**

DNA was isolated from silica gel dried leaves using DNeasy Plant Kits (Qiagen, Carlsbad, CA, USA). DNA template was prepared by restriction with *Eco*RI and *Mse*I and ligation with T4 DNA ligase (from New England Biolabs, Beverly, MA, USA) of adapters supplied with the Applied Biosystems AFLP Plant Mapping Kit (for Small Plant Genomes) according to the manufacturer’s instructions (except that templates were diluted by only 1/5, not 1/20, at each step). The labeled amplification products were separated by electrophoresis through Long Ranger acrylamide gels in an ABI Prism 377 automated DNA sequencer and visualized using GeneScan software. GeneScan-500 (ROX) size standards permitted automatic sizing of fragments. Data were scored using GeneScan and GenoTyper software (PE Applied Biosystems, Foster City, CA) to create the binary matrix, which was then edited by hand. Repetitability was assessed by including some replicate samples, including separate DNA isolations from the same plant prepared for AFLP and run either on the same gel or on separate gels, different restriction-ligation reactions prepared from the same DNA sample, template from one preselective amplification that was amplified twice with the same selective primer combination but on separate dates and run on separate gels, and the same selective amplification product run on more than one gel. Here I report results with a single AFLP primer combination, *Eco*RI-AC/*Mse*I-CAC, which was chosen based on good amplification and polymorphism detection. The primer pair is designated here in abbreviated form as “ac/cac” (based on the two and three selective bases of the *Eco*RI and *Mse*I primers, respectively).

**Results and Discussion**

**AFLP Polymorphism and Reproducibility**

The data matrix for primer combination ac/cac included 116 peaks of 95–505 bp (smaller fragments were excluded as being mostly monomorphic or not unambiguously scorable). Polymorphism was assessed not only among the oca accessions from Pisac and the three wild *Oxalis* taxa but also the 30 oca samples from other areas. Among this larger sample, data from ac/cac included 86 peaks that were polymorphic in oca, 7 monomorphic in all samples, 13 monomorphic in oca but absent in at least one wild tuber-bearing taxon, and 10 absent in oca but present in at least one wild tuber-bearing taxon.

Replicate samples run on the same gels had profiles that were remarkably similar, not only in identical presence of bands, but even in their shapes and relative sizes. Duplicate samples run on different gels were less similar in shapes of profiles and were not necessarily identical in band presence (up to 4.3% difference, especially if reaction strength varied; see table 14.2). Unreliable bands were detected and eliminated from the data matrix based on the replicate samples, which to date have been run for about 10% of accessions. Additional replicates are a high priority for very divergent samples because their differences might possibly result from weak reactions or degraded or contaminated DNA templates (see Dyer and Leonard,
As has been observed by others and is discussed later, replicate AFLP profiles are not always identical (Douhovnikoff and Dodd, 2003).

Correspondence of AFLP Data with Use Categories

The AFLP data agree with the classification of oca by Quechua farmers in Pisac District into two use categories. That is, the different oca use categories were particularly distinct from each other in their preliminary AFLP data, as revealed in both ordination (PCOA and PCA) and clustering (UPGMA and NJ) analyses. P’osqo, the cultivar usually used in Pisac for processing into khaya,
Table 14.2 Maximum pairwise distances between replicate samples or between samples within the same cluster. The simple cultivars listed are those that had at least seven samples.

<table>
<thead>
<tr>
<th>Groups Compared</th>
<th>Standard Distance</th>
<th>Nei and Li Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple Cultivars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puka posgo</td>
<td>0.087</td>
<td>0.0146</td>
</tr>
<tr>
<td>Yuraq panti</td>
<td>0.094</td>
<td>0.0185</td>
</tr>
<tr>
<td>Puka panti</td>
<td>0.060</td>
<td>0.0131</td>
</tr>
<tr>
<td>Kusipata</td>
<td>0.035</td>
<td>0.0065</td>
</tr>
<tr>
<td>Posgo</td>
<td>0.181</td>
<td>0.0450</td>
</tr>
<tr>
<td>Complex Cultivars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Udpa</td>
<td>0.077</td>
<td>0.0150</td>
</tr>
<tr>
<td>Yellow group</td>
<td>0.112</td>
<td>0.0245</td>
</tr>
<tr>
<td>Misitu within A</td>
<td>0.068</td>
<td>0.0117</td>
</tr>
<tr>
<td>Misitu A to B</td>
<td>0.137</td>
<td>0.0243</td>
</tr>
<tr>
<td>Misitu C to A or B</td>
<td>0.224</td>
<td>0.0424</td>
</tr>
</tbody>
</table>

is separated from all the wayk' u cultivars in the results of NJ analyses, and the three wild tuber-bearing taxa are found between the two clusters in unrooted NJ networks when using Nei and Li distances (figure 14.3). Results with different distance measures (e.g., standard distance or simple matching) or different algorithms (e.g., UPGMA) differ somewhat in the branch lengths and in the arrangements between the wild species and the two use categories, but they are consistent in the distinct separation of the two use categories from each other. These results suggest the interesting hypothesis that these use categories may have different evolutionary histories.

Correspondence of AFLP Data with Simple Folk Cultivars

Overall there was a good correspondence between the AFLP data and the morphological groups recognized by Quechua farmers in the three Pisac communities. With only a few exceptions, each morphotype that is generally recognized by farmers forms a separate cluster in the results of NJ analysis (figure 14.4). Tubers of the same morphotype clustered together regardless of the distance measure used, although there were differences in branch lengths and some rearrangements in relationships between the clusters, as well as within them, in different analyses. Thus, data from a single AFLP primer pair were able to distinguish all simple folk cultivars recognized by most farmers. In some cases, the AFLP data could clearly distinguish between genotypes that were so similar in color that they are easily confused when distinguished on visually observable tuber traits alone (e.g., the pink tubers of kusipata and puka panti or the white tubers of yuraq kishwar and yuraq usha). In most cases tubers of the same folk cultivar (e.g., puka panti, kusipata, puka p'osgo, yuraq kishwar) had similar but not necessarily identical AFLP profiles (figure 14.4 and table 14.2), indicating that they probably are members of the same clonal lineage (genet). Different tubers of the same cultivar often had a few differences, which might reflect either real differences between them (somatic mutations) or experimental error (AFLP artifacts or scoring ambiguities). Replicate AFLP profiles often are not exactly identical, and Douhovnikoff and Dodd (2003) determined that real differences between samples from different ramets or even different leaves from the same stem may be more numerous than those from experimental error. Evidence of somatic mutation in clonal lineages has also been documented for other marker types (e.g., variable number of tandem repeats; Rogstad et al., 2002). Data from additional primer combinations may distinguish more genotypes from within these clusters and may possibly show that some of these clusters do not represent a single genet (i.e., that some of the tubers in the group are separated by at least one sexual generation). Nonetheless, even if they are not all of the same clonal lineage, their close similarities suggest that they are probably closely related genotypes (e.g., siblings or parent—offspring).

A few rare morphotypes were encountered very infrequently in the household stores of oca tubers. Some of these were among cultivars that farmers had mentioned, in response to inquiries, as cultivars that were disappearing or had disappeared. These cultivars could be distinguished from the others on the basis of AFLP data but do not form a cluster in the NJ network because only one or two of each has been included in this sample (e.g., Damaso, machasqa, and puka chilitu). The AFLPs were also helpful in the case of the mismatch tubers that could not be identified unambiguously with any of the other morphotypes. Although the AFLP data indicated that a few of these tubers did belong with one of the known cultivar groups, in most cases the AFLP data confirmed these tubers as being distinct from any of the cultivar clusters. Thus at least some of these mismatch tubers are indeed different clonal genotypes than the predominant ones in the named cultivars. The presence of both the low-frequency named cultivars and the
mismatch tubers indicate that the genotypic diversity in these communities would be underestimated by a cursory survey of morphotypes.

Correspondence of AFLP Data with Complex Folk Cultivars

Each of the complex cultivars (heterogeneous cultivar groups or cultivars that include subcultivars) is discussed separately because they differ with respect to their correspondence with the AFLP data.

The Ushpa Group

The AFLP data confirmed the farmers' classification of the ushpa group despite their wide range of tuber pigmentation. All ushpa tubers had very similar AFLP profiles, diverging no more than samples within simple cultivars (table 14.2). The color variants were scattered within the ushpa cluster, so the data from primer combination ac/cac do not clearly distinguish between them. However, additional AFLP data may distinguish between the different color shades. On the other hand, the color differences might not be reflected in differences in AFLP at all, as might be the case if they are the result of somatic mutations or especially if the differences are developmental. Therefore this group represents a case in which the farmers' classification based on the prized floury texture is a better clue to the genetic similarity of these tubers than is their color variation (although the mortled or splotched patterning of the pigmentation is an important similarity). This underscores the importance of the farmers' close familiarity with their cultivars, through not only growing but also eating them.

The Misitu Group

The situation in the ushpa group contrasts with the misitu group, in which the tubers have enough AFLP differences that they do not appear to be a single clone. Some of the tubers had very similar (or even identical) AFLP profiles with ac/cac, and these are probably clone mates (see cluster A in figure 14.4; table 14.2). However, other misitu tubers differ in several markers. The divergence between misitu clusters A and B (up to 0.137 standard distance, 0.0243 Nei and Li distance; see table 14.2) is in the same range as divergence between samples of different morphotypes (e.g., between puka panti and y uma kishwar), suggesting that these different misitu subgroups probably are separated by at least one cycle of sexual recombination. Misitu group C tubers were still more divergent (table 14.2), but their differences must be confirmed by additional replicate samples. These AFLP differences validate the discrimination, by at least a few farmers, of different subtypes of misitu on the basis of tuber shape and secondarily by shade of coloration. These latter distinctions reflect real genetic differences, as the misitu group appears, on the basis of this single primer combination, to be polyclonal. In the results of NJ analysis of this single AFLP primer combination (figure 14.4), these putative separate clonal genotypes all join a single cluster, so they appear to be closely related rather than having converged independently on the same streaked color pattern.

The Yellow Group

Finally, the cluster designated as the yellow group (figure 14.4) is heterogeneous both molecularly and morphologically. Their tubers have yellow as the primary color, with varied patterns of secondary red pigment in some cultivars. I initially saw them as comprising at least three different morphotypes, and the farmers also gave them different names. Q'ello k'aytu tubers are yellow with red eyes, whereas the morphotype called either q'ello panti or señorita is evenly pale yellow, without markings. The morphotype called han'go q'ello or wagankillay grades from a yellow base to variable degrees of red at the apex. Although I expected that some of these morphotypes might comprise multiple genotypes, I did not anticipate that morphotypes would be intermingled within a single heterogeneous cluster (figure 14.4). Divergences in this cluster overall are greater than within simple wayku cultivars (see table 14.2), suggesting they are not a single clone. The four sampled q'ello panti or señorita tubers separate from each other and group in several places in the network, some among the han'go q'ello tubers and others outside the yellow cluster. Increased sampling and data from additional primer combinations will be necessary to determine how many different genotypes make up this complex yellow group.

Insights from AFLP About Posqo, the Single Cultivar for Making Khaya

Ethnobotanically and morphologically there seemed to be a single homogeneous cultivar, known by a single Quechua name, posqo (meaning sour, tart, bitter, fermented; Morató Peña and Morató Lara 1995), that was exclusively used for processing into khaya (although some farmers also used the firm cultivar kusipata, or indeed any undersized tubers, for processing into khaya as well). Despite their morphological similarity, however, the AFLP data of the six posqo tubers had surprisingly high divergence (figures 14.3 and 14.4). Indeed, variation within posqo (up to 0.181 standard
distance, 0.0450 Nei and Li distance; table 14.2) is greater than within any wayku cultivars except misitu and greater than many comparisons between different wayku cultivars.

In addition to the divergence between the two use categories discussed earlier, the AFLP data also provide a hint that the posgo tubers might differ in ploidy level from the wayku cultivars. Like the three wild tuber-bearing taxa, the six posgo accessions amplified a smaller number of bands than most of the wayku cultivars. The number of peaks scored per plant in the ac/cac profiles ranged from 32 to 62 in the samples overall. Most wayku samples amplified 48–62 peaks, but the wild taxa and the posgo accessions amplified only 32–47 peaks. Whereas most studies have found cultivated O. tuberosa to be octoploid, O. pichensis is tetraploid (Emshwiller, 2002b). The other wild tuber-bearing taxa probably are polyploid as well because they consistently have multiple sequence types for ncpGs (i.e., they show fixed hetrozygosity, one of the criteria of allopolyploidy; Emshwiller and Doyle, 2002, and unpublished data). The divergence of AFLP data and the smaller number of peaks amplified by the posgo tubers both led to the speculation that the posgo genotypes might have a lower ploidy level than the dominant octoploid level in most oca studied to date. A similar situation has been found in potato, in which species of lower ploidy level amplify a smaller number of peaks for most primer pairs than species with higher ploidy levels (Kardoulis et al., 1998). Thus it may be that oca is similar to the situation in Andean native potatoes, in which the several use categories comprise different species of Solanum of several ploidy levels (Brush et al., 1981; Zimmerer, 1991b). This would also be consistent with the clustering analyses in that the posgo accessions grouped with two of the wild tuber-bearing taxa (figure 14.3) or in other analyses were more distant from wayku oca cultivars than were those two wild taxa.

More molecular, morphological, and cytological data are clearly needed to confirm this difference in ploidy level and to investigate the relationship between use categories. If posgo has a lower ploidy level, then the question arises as to its relationship to the more common octoploid cultivars of oca. Some possibilities include that posgo might represent a surviving line from the progenitor of octoploid oca (meaning that oca was initially domesticated at a lower ploidy level) or, alternatively, that wayku oca derives from a different progenitor and different origin of polyploidy, and perhaps that the two use categories have entirely separate origins of domestication. In either scenario, it is likely that there has been little or no gene flow between the two use categories for a long time.

Conclusions and Research Needs

These preliminary data from a single AFLP primer pair indicate that the named folk cultivars, at least when applied by knowledgeable farmers, usually designate either individual clonal genotypes or groups of genetically similar genotypes. The AFLP data also indicate that the classification of oca into two use categories by farmers in Pisac reflects a fundamental biological difference. Nonetheless, several aspects of these results indicate that the genotypic diversity of oca in Pisac is underestimated by the number of named cultivars. Many mismatch tubers were confirmed as genotypes that did not belong to any of the primary clusters. A few cultivars were found at very low frequency (and probably would be missed in a brief germplasm collecting visit). Complex cultivars such as misitu apparently include more than a single clonal genotype, but only a few knowledgeable farmers distinguished them with separate names. Additional AFLP data may uncover other differences within these clusters. Thus a cursory look at the number of morphologically different tuber types would substantially underestimate the genetic diversity present in these communities.

Others studies have also found that folk taxonomy corresponds well overall but provides a net underestimate of genotypic diversity compared with molecular data. Such was the case in the pioneering research in native Andean potatoes by Quiros et al. (1990) and other studies in potato and cassava that found that individual cultivar names are applied to more than one genotype (e.g., Zimmerer and Douches, 1991; Elias et al., 2000a, 2001b). Turnover in the composition of clones cultivated over time has been noted in temporal studies of oca (Ramírez, 2002). It is still unknown whether the infrequent genotypes sampled herein reflect such genotypic turnover and, if so, whether these genotypes are coming or going (i.e., whether they represent new recombinant genotypes that are not yet at high frequency and are new introductions to the community or, alternatively, whether some of them are in decline). The possibility that some of the uncommon cultivars such as machasga and Damaso are disappearing was suggested by the recollections of Older farmers that they had been more abundant in the past. Interestingly, the few tubers found of these and some other rare cultivars usually were small, suggesting that they may be declining because of increasing viral load, mutational load, or perhaps clonal senescence. There is a need for more temporal studies of oca and of other clonal crops (e.g., Hamlin and Salick, 2003) to elucidate the causes of genotypic turnover. Understanding of spatial structure of genetic diversity
at various scales is also crucial for vegetatively propagated crops. Although there is agreement that the relationship between in situ and ex situ conservation should be complementary, there is little understanding of how this can be accomplished. Data on the evolution of clonal crops in traditional agricultural systems are paramount for this goal.

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References


Crop Genetics on Modern Farms
Gene Flow Between Crop Populations

The Green Revolution and other modern farming practices dramatically changed the composition of farmers’ fields. In early assessments, a few modern varieties bred to produce high yields in very specific conditions were found to be rapidly replacing traditional varieties, which were bred and selected by farmers over millennia (Frankel and Hawkes, 1975; Frankel et al., 1995). This apparent abandonment of traditional varieties was cause for concern because crop breeders often used these cultivars as a source for resistance traits to combat devastating crop epidemics (Frankel and Hawkes, 1975; Frankel et al., 1995). However, careful fieldwork later demonstrated that traditional crops were not doomed, especially in marginal farming conditions. Several studies in different regions of the world showed that farmers often maintained traditional varieties even while adopting modern cultivars (Brush, 1992, 1995, 2000; Bellon and Brush, 1994; Maxted et al., 1997). Thus, a tenuous coexistence appears to have developed decades after the Green Revolution. In this chapter I focus on the genetic implications of that coexistence, paying particularly close attention to gene flow between modern and traditional crops.

The central issue is that modern crop populations are typically large and genetically homogeneous. They can swamp out a smaller population when interbreeding occurs, causing rapid losses of genetic diversity in traditional