

## ORIGINS OF DOMESTICATION AND POLYPLOIDY IN OCA (*Oxalis tuberosa*: OXALIDACEAE): nrDNA ITS DATA<sup>1</sup>

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As part of a study aimed at elucidating the origins of the octoploid tuber crop “oca,” *Oxalis tuberosa*, DNA sequences of the internal transcribed spacer of nuclear ribosomal DNA (nrDNA ITS) were determined for oca and several wild *Oxalis* species, mostly from Bolivia. Phylogenetic analysis of these data supports a group of these species as being close relatives of oca, in agreement with morphology and cytology, but at odds with traditional infrageneric taxonomy. Variation in ITS sequences within this group is quite low (0–7 substitutions in the entire ITS region), contrasting with the highly divergent (unalignable in some cases) sequences within the genus overall. Some groups of morphologically differentiated species were found to have identical sequences, notably a group that includes oca, wild populations of *Oxalis* that bear small tubers, and several other clearly distinct species. The presence of a second, minor sequence type in at least some oca accessions suggests a possible contribution from a second genome donor, also from within this same species group. ITS data lack sufficient variation to elucidate the origins of oca precisely, but have identified a pool of candidate species and so can be used as a tool to screen yet unsampled species for possible progenitors.

**Key words:** crop evolution; domestication; nrDNA ITS; oca; Oxalidaceae; *Oxalis tuberosa*; polyploidy.

*Oxalis tuberosa* (Molina), commonly known as “oca,” is one of over two dozen crops first domesticated in the Andes (National Research Council, 1989). It is cultivated at high altitude (2800–4100 m) in the central Andes, primarily in small plots by traditional agriculturists, along with other Andean tuber crops of unrelated families: *Tropaeolum tuberosum* R. & P. (Tropaeolaceae), *Ullucus tuberosus* Lozano (Basellaceae), and several tuber-bearing *Solanum* species (Solanaceae). It is grown in greatest abundance in the highlands of Ecuador, Peru, and Bolivia, although it is found as far north as Venezuela and as far south as Chiloe Island in Chile (National Research Council, 1989). In recent decades it has become a commercial crop in New Zealand (National Research Council, 1989) and is also a common minor crop in the Transverse Neovolcanic Axis of Mexico, but the diversity of cultivars there is very low, and several sources of evidence indicate that it arrived in Mexico after the Spanish conquest (King and Bastien, 1990). The crop has always been considered to be undoubtedly of Andean origin (León, 1967; Brücher, 1969, 1989). Although published reports have consistently denied the existence of wild oca, or any related species bearing tubers (León, 1967; Hermann,

1992), some wild populations of *Oxalis* bearing small tubers have been found recently in Bolivia (see below).

The wild progenitor of domesticated oca is unknown, as is the origin(s) of polyploidy (most reports [see below] consider oca to be octoploid with 64 chromosomes). It is unknown whether parental genomes were contributed in single or multiple events and whether one or more ecotypes of a single progenitor species or more than one well-differentiated species were involved. The occurrence of meiotic abnormalities and frequent high levels of pollen sterility (Gibbs, Marshall, and Brunton, 1978) has been cited as evidence of autopolyploidy, but cannot be considered conclusive because no controlled crosses demonstrating whether inheritance is polysomic or disomic (e.g., Jackson and Casey, 1982) have been reported.

**Taxonomic background**—*Oxalis* is a large genus of over 800 species, variable in habit and ecology, with greatest diversity of species in South America and southern Africa. The most recent monographic treatment of the entire genus is that of Knuth (1930). However, it is generally acknowledged that the 38 sections defined by Knuth are quite artificial, being based primarily on a few vegetative characters, and his keys are considered nearly unusable (Macbride, 1943; Salter, 1944; Brücher, 1969; Denton, 1973; A. Lourteig, Museum national d’Histoire naturelle, Paris, personal communication), so this monograph has not been very useful as a guide to which species may be most closely related to oca. Knuth’s treatment (e.g., 1930, and addenda 1931, 1935, 1936, 1940) is the most comprehensive work on the genus, however. Earlier monographs did not cover many South American species, and their infrageneric classifications were clearly artificial (e.g., Jacquin, 1794; DeCandolle, 1824; Zuccarini, 1825, 1831). Subsequent published work has considered particular sections or geographical regions, but not the whole genus (e.g., Salter, 1944; Eiten, 1963; Denton, 1973; Lourteig, 1975, 1979, 1981a, b, 1983, 1994). No published revision has considered a group including *O.*

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TABLE 1. Accessions of *Oxalis* (including *Xanthoxalis*) sampled for ITS sequence. Collections of E. Emshwiller et al. except as noted below.

| Collection no.      | Voucher no. | Country, department, province, locality                      | Species ID                                    | Section <sup>b</sup>             | 2n =                                   | Sequence type  |
|---------------------|-------------|--|---|----------------------------------|--|----------------|
| MHG861 <sup>c</sup> | EE133       | Bolivia, Oruro, Avaroa, Challapata                           | <i>O. tuberosa</i> Molina (cultivated)        | <i>Ortgieseae</i> Knuth          | 64 <sup>i</sup>                        | A              |
| MHG884 <sup>c</sup> | EE136       | Bolivia, Potosí, Tomas Frias, Chuitara                       | <i>O. tuberosa</i> Molina (cultivated)        | <i>Ortgieseae</i> Knuth          | 64 <sup>i</sup>                        | A              |
| MHG913 <sup>c</sup> | EE140       | Bolivia, Cochabamba, Carrasco, Totora                        | <i>O. tuberosa</i> Molina (cultivated)        | <i>Ortgieseae</i> Knuth          | 64 <sup>i</sup>                        | A (&E?)        |
| EE260               | EE260       | Bolivia, Cochabamba, Ayopaya, near Independencia             | aff. <i>O. tuberosa</i> Molina (wild)         | <i>Ortgieseae</i> Knuth          | —                                      | A              |
| EE284               | EE284       | Bolivia, Cochabamba, Chapare, Candelaria                     | aff. <i>O. tuberosa</i> Molina (wild)         | <i>Ortgieseae</i> Knuth          | —                                      | A              |
| EE168               | EE168       | Bolivia, La Paz, Nor Yungas, Unduavi                         | <i>O. unduavensis</i> (Rusby) Knuth           | <i>Ortgieseae</i> Knuth          | —                                      | A              |
| EE291               | EE291       | Bolivia, La Paz, Nor Yungas, below Chuspipata                | <i>O. unduavensis</i> (Rusby) Knuth           | <i>Ortgieseae</i> Knuth          | —                                      | A              |
| EE351               | EE351       | Bolivia, La Paz, Nor Yungas, Unduavi                         | <i>O. unduavensis</i> (Rusby) Knuth           | <i>Ortgieseae</i> Knuth          | —                                      | A              |
| EE308               | EE308       | Bolivia, La Paz, Nor Yungas, between Chuspipata and Yolosa   | <i>O. sp.</i> "O" <sup>g</sup>                | —                                | —                                      | A              |
| EE294               | EE294       | Bolivia, La Paz, Nor Yungas, between Chuspipata and Yolosa   | aff. <i>O. distincta</i> Knuth (hybrid?)      | <i>Clematodes</i> Knuth          | —                                      | A (&B?)        |
| EE289               | EB289       | Bolivia, La Paz, Nor Yungas, between Chuspipata and Yolosa   | aff. <i>O. distincta</i> Knuth                | <i>Clematodes</i> Knuth          | —                                      | B              |
| EE321               | EE321       | Bolivia, La Paz, Nor Yungas, between Chuspipata and Yolosa   | aff. <i>O. distincta</i> Knuth                | <i>Clematodes</i> Knuth          | —                                      | B              |
| EE447               | EE447       | Bolivia, La Paz, Nor Yungas, between Chuspipata and Yolosa   | aff. <i>O. distincta</i> Knuth                | <i>Clematodes</i> Knuth          | —                                      | B              |
| EE187               | EE187       | Bolivia, La Paz, Larecaja, Sorata                            | <i>O. spiralis</i> R & P                      | <i>Ortgieseae</i> Knuth          | 16 <sup>ik,l</sup> or 48 <sup>lm</sup> | B              |
| EE64                | EE64        | Bolivia, Cochabamba, Chapare, Locotal                        | <i>O. longissima</i> (Kuntze) Schumann        | <i>Ortgieseae</i> Knuth          | —                                      | B              |
| EE247               | EE247       | Bolivia, Cochabamba, Chapare, above Locotal                  | <i>O. longissima</i> (Kuntze) Schumann        | <i>Ortgieseae</i> Knuth          | —                                      | B              |
| EE418               | EE418       | Bolivia, La Paz, Murillo, Mecapaca                           | <i>O. peduncularis</i> HBK                    | <i>Carnosae</i> (Reiche) Knuth   | 16 <sup>i</sup>                        | C              |
| EE184               | EE184       | Bolivia, La Paz, Larecaja, Sorata                            | aff. <i>O. spiralis</i> R & P (not in flower) | <i>Ortgieseae</i> Knuth          | 16 <sup>ik,l</sup> or 48 <sup>lm</sup> | C              |
| EE249               | EE249       | Bolivia, Cochabamba, Chapare, above Locotal                  | <i>O. spiralis</i> R & P                      | <i>Ortgieseae</i> Knuth          | 16 <sup>ik,l</sup> or 48 <sup>lm</sup> | C              |
| EE250               | EE250       | Bolivia, Cochabamba, Chapare, above Locotal                  | <i>O. spiralis</i> R & P                      | <i>Ortgieseae</i> Knuth          | 16 <sup>ik,l</sup> or 48 <sup>lm</sup> | C              |
| EE190               | EE190       | Bolivia, La Paz, Larecaja, Sorata                            | <i>O. melilotoides</i> Zuccarini              | <i>Clematodes</i> Knuth          | 16 <sup>i</sup>                        | C              |
| EE359               | EE359       | Bolivia, La Paz, Sud Yungas, between Unduavi and Chulumani   | <i>O. mollissima</i> (Rusby) Knuth            | <i>Clematodes</i> Knuth          | 16 <sup>i</sup>                        | C              |
| EEx415              | Eex415      | Bolivia, La Paz, Murillo, Río Zongo                          | <i>O. mollissima</i> (Rusby) Knuth            | <i>Clematodes</i> Knuth          | 16 <sup>i</sup>                        | C              |
| VULC1 <sup>d</sup>  | EE124       | unknown (El Salvador, Costa Rica, Panama <sup>e</sup> )      | <i>O. vulcanicola</i> Donn. Smith             | <i>Ortgieseae</i> Knuth          | —                                      | D              |
| HERR1 <sup>d</sup>  | EE459       | unknown ("Peru" <sup>f</sup> )                               | <i>O. herrerae</i> Knuth                      | <i>Herrerea</i> Knuth            | 16 <sup>i</sup>                        | E              |
| PED1 <sup>d</sup>   | EE466       | unknown ("Ecuador, Peru" <sup>f</sup> )                      | <i>O. peduncularis</i> HBK                    | <i>Carnosae</i> (Reiche) Knuth   | 16 <sup>i</sup>                        | F              |
| EE246               | EE246       | Bolivia, Cochabamba, Chapare, below Colomi                   | <i>X. flagellata</i> Rusby <sup>h</sup>       | <i>Clematodes</i> Knuth          | —                                      | G              |
| EE331               | EE331       | Bolivia, La Paz, Sud Yungas, Ikiko                           | <i>X. flagellata</i> Rusby <sup>h</sup>       | <i>Clematodes</i> Knuth          | —                                      | G              |
| EE345               | EE345       | Bolivia, La Paz, Murillo, Rinconada                          | <i>O. nubigena</i> Walpers                    | <i>Capillares</i> (Reiche) Knuth | 48–50 <sup>n</sup>                     | — <sup>o</sup> |
| EE315               | EE315       | Bolivia, La Paz, Nor Yungas, between Chuspipata and Yolosa   | <i>O. andina</i> Britton                      | <i>Clematodes</i> Knuth          | —                                      | H              |
| EE437               | EE437       | Bolivia, La Paz, Murillo, Río Zongo                          | <i>O. yungasensis</i> Rusby                   | <i>Corniculatae</i> DC           | —                                      | H              |
| EE350               | EE350       | Bolivia, La Paz, Sud Yungas, between Unduavi and Chulumani   | <i>O. yungasensis</i> Rusby                   | <i>Corniculatae</i> DC           | —                                      | H              |
| EE292               | EE292       | Bolivia, La Paz, Nor Yungas, between Chuspipata and Yolosa   | <i>O. dolichopoda</i> Diels                   | <i>Myriophyllum</i> Knuth        | —                                      | I              |
| EE219               | EE219       | Bolivia, La Paz, Nor Yungas, between Cotapata and Chuspipata | <i>O. sp.</i> "R" <sup>g</sup>                | —                                | —                                      | I              |
| EE295               | EE295       | Bolivia, La Paz, Nor Yungas, below Chuspipata                | <i>O. sp.</i> "Z" (new species?)              | —                                | —                                      | J              |

TABLE 1. Continued.

| Collection no.      | Voucher <sup>a</sup> no. | Country, department, province, locality                              | Species ID                    | Section <sup>b</sup>           | 2n =  | Sequence type  |
|---------------------|--------------------------|--|-------------------------------|--------------------------------|---|----------------|
| EE310               | EE310                    | Bolivia, La Paz, Nor Yungas, between Chuspipata and Yolosa           | <i>O. boliviana</i> Britton   | <i>Ortgieeseae</i> Knuth       | —   | K              |
| ORT1 <sup>d</sup>   | EE458                    | unknown (“Andes of Peru” <sup>f</sup> )                              | <i>O. ortgiesii</i> Regel     | <i>Ortgieeseae</i> Knuth       | 14 <sup>p,q,r</sup>                                 | L              |
| EE387               | EE387                    | Bolivia, La Paz, Murillo, near La Paz                                | <i>O. pachyrrhiza</i> Weddell | <i>Acetosellae</i> DC          | —   | M              |
| REG1 <sup>d</sup>   | EE456                    | unknown (“Peru, Brazil, Bolivia, Paraguay, Argentina” <sup>f</sup> ) | <i>O. regnellii</i> Miquel    | <i>Articulatae</i> Knuth       | 28 <sup>s</sup>                                     | N              |
| EE235               | EE235                    | Bolivia, Cochabamba, Tiraque, Toralapa                               | <i>O. latifolia</i> HBK       | <i>Ionoxalis</i> (Small) Knuth | 14 <sup>u</sup> , 28 <sup>u</sup> , 42 <sup>u</sup> | O              |
| EE445               | EE445                    | Bolivia, Cochabamba, Chapare, between Colomi and Locotal             | <i>O. martiana</i> Zuccarini  | <i>Ionoxalis</i> (Small) Knuth | 56 <sup>v</sup>                                     | P              |
| EE366               | EE366                    | Bolivia, La Paz, Ingavi, near Viacha                                 | <i>O. bisfracta</i> Turcz     | <i>Clematodes</i> Knuth        | —   | Q              |
| PES1 <sup>d</sup>   | EE125                    | unknown (“S. Africa” <sup>f</sup> )                                  | <i>O. pes-caprae</i> L.       | <i>Cernuae</i> Knuth           | 28 <sup>m,r</sup> or 35 <sup>t,q</sup>              | R <sup>o</sup> |
| CRASS1 <sup>d</sup> | EE463                    | unknown (“perhaps South America” <sup>f</sup> )                      | <i>O. crassipes</i> Urb.      | <i>Articulatae</i> Knuth       | —   | S <sup>o</sup> |

<sup>a</sup> Vouchers deposited at BH, with duplicates of wild Bolivian *Oxalis* deposited at LPB.

<sup>b</sup> Sections of Knuth (1930, 1935, 1936).

<sup>c</sup> Accessions of cultivated oca from Programa de la Investigación de la Papa (PROINPA), Cochabamba, Bolivia.

<sup>d</sup> Plants purchased from Merry Gardens, Camden, ME.

<sup>e</sup> Native area as inferred from Smith, 1897; Lourteig, 1970, 1981b, and herbarium specimens at NY.

<sup>f</sup> Native areas as reported for these species in *Hortus Third* (Liberty Hyde Bailey Hortorium, 1976).

<sup>g</sup> New species proposed by A. Lourteig on specimen annotations, but not yet published (see Materials and Methods: Sampling).

<sup>h</sup> Combination as a species of *Oxalis* proposed by both Lourteig and Eiten on specimen annotations, but unpublished.

<sup>i</sup> de Azkue and Martínez, 1990. Other members of the “*Oxalis tuberosa* alliance” studied by de Azkue and Martínez but not sampled here (but see Tosto and Hopp, 1996) are *O. medicaginea*, *O. oblongiformis*, *O. subintegra*, *O. tabaconasensis*, *O. aff. villosula*, and *O. sp.* (these with  $2n = 16$ ), and *O. lotoides* ( $2n = 32$ ).

<sup>j</sup> Brücher, 1969 (as *O. pubescens* H. B. K.).

<sup>k</sup> Favarger and Huynh, 1965.

<sup>l</sup> Huynh, 1965.

<sup>m</sup> Mathew, 1958 (as *O. pubescens*).

<sup>n</sup> Diers, 1961.

<sup>o</sup> Only partial sequences were determined for these plants (*O. nubigena*, ~470 bp; *O. pes-caprae*, ~300 bp; *O. crassipes*, ~210 bp).

<sup>p</sup> Heitz, 1927.

<sup>q</sup> Warburg, 1938.

<sup>r</sup> Marks, 1956.

<sup>s</sup> Naranjo et al., 1982.

<sup>t</sup> Franke, 1975

<sup>u</sup> Weller and Denton, 1975

<sup>v</sup> Hill, 1984.

*tuberosa*, although such work is in progress by Lourteig (personal communication). The many conflicting determinations of specimens in herbaria (E. Emshwiller, personal observations) indicate the need for basic work on species delimitation. This lack makes identification of specimens difficult, makes the identities of plants for which there are published chromosome counts uncertain, and has also complicated both sampling and interpretation of results of this study (see below). Although progress has been made in parts of the genus, Brücher’s (1969) comment that the systematics of genus *Oxalis* is still at its beginning remains very much true today.

**Cytological background**—Base chromosome numbers in *Oxalis* vary from  $x = 5$  to  $x = 12$ , with  $x = 7$  most frequent (Cronquist, 1981), and polyploidy is common in the genus. Cytological work by de Azkue and Martínez (1990) found a group of a dozen morphologically similar Andean species that share a base chromosome number of  $x = 8$ , which is rare in *Oxalis*. This group includes octoploid ( $2n = 8x = 64$ ) *O. tuberosa*. There have been conflicting reports of chromosome numbers in cultivated oca (two reports of  $2n = 14$ , one of various euploid  $x = 8$  cytotypes [i.e.,  $2n = 16, 24, 32, 48, 64$ ], and others from  $2n = 57$ – $70$ : Heitz, 1927; Kostoff, Dogadkina, and

Tichonowa, 1935; Cárdenas and Hawkes, 1948; Gibbs, Marshall, and Brunton, 1978; Talledo and Escobar, 1995; Guamán, 1997). However, over 100 accessions of cultivated oca have been found to have  $2n = 64$  (Medina, 1994; Valladolid, Arbizu, and Talledo, 1994; Valladolid, 1996).

The species of the “*O. tuberosa* alliance” described by de Azkue and Martínez (1990) (footnote i, Table 1) belong to four of Knuth’s sections: *Ortgieeseae*, *Carnosae*, *Clematodes*, and *Herrerea* (Knuth, 1930, 1935, 1936). With the exception of *Herrerea* these sections all include not only the species with  $x = 8$ , but other species with published base numbers of  $x = 5, 7$ , and  $9$  (Heitz, 1927; Marks, 1956; Naranjo et al., 1982; de Azkue and Martínez, 1984). A few other species have published chromosome counts with  $x = 8$  (Mathew, 1958; Brücher, 1969; Federov, 1969). Most of these are also Andean species that share some morphological similarities with the alliance species, and all but one were placed by Knuth into one of the same four sections (the very tiny *O. nubigena* [ $2n = 48$ – $50$ : Diers, 1961] is a member of section *Capillares*).

Earlier speculations by other workers concerning the wild relatives of oca also have pointed to species of the  $x = 8$  alliance. Brücher (1969) wrote that oca’s progenitor

was probably "a species similar to *O. melilotoides*," an  $x = 8$  species not included in the study of de Azkue and Martínez (1990). Annotations by Eiten on specimens at NY refer the  $x = 8$  species and other morphologically similar species to either an "*O. tuberosa* complex" or an "*O. scandens* complex" (of unknown chromosome number). His only published reference to these species is a brief mention of "a group of South American species related to *O. scandens*," distinguishing them from section *Corniculatae* (Eiten, 1963, p. 290), and listing some of the species (including *Xanthoxalis flagellata*, sampled in this study; see below).

The  $x = 8$  alliance probably includes other species for which cytological information is not yet available. Macbride (1943) described several Peruvian named species as being similar to (or perhaps conspecific with) some of the alliance species. In addition to the morphologically similar species mentioned by Macbride (1943) and Eiten (1963), yet other taxa that resemble the  $x = 8$  species were encountered in observations of herbarium specimens and field collections (E. Emshwiller, unpublished data), but these other species were not included in the study by de Azkue and Martínez (1990), and do not yet have published chromosome counts.

As part of a larger project investigating the systematics, genetic diversity, and ethnobotany of oca, we have initiated molecular and morphological studies aimed at establishing the identities of oca's progenitor(s) and clarifying the origins of polyploidy in the species. In this paper we report on the application of nucleotide sequence data from the internal transcribed spacer of nuclear ribosomal DNA (nrDNA ITS; reviewed in Baldwin et al., 1995) to the question of the relationships and origins of *Oxalis tuberosa*.

## MATERIALS AND METHODS

**Sampling**—DNA was isolated (Doyle and Doyle, 1990) from either fresh leaves of greenhouse-grown plants, or leaves dried in silica gel in the field (Chase and Hills, 1991) and kept at 4°C after return to the laboratory. DNA isolations were made from single individual plants in all cases except EE345, *O. nubigena*. The plants sampled for ITS sequence (Table 1) included *Oxalis* from the following categories.

- 1) Cultivated *O. tuberosa* accessions kindly provided by the germplasm bank of PROINPA (Cochabamba, Bolivia). Individuals of three genotypes were selected that differed in tuber pigmentation and collection locality.
- 2) Members of the  $x = 8$  alliance and species of unknown chromosome number but resembling  $x = 8$  species. Most of these occur in the cloud forest regions of the eastern slopes of the Andes, although some are found in drier habitats at higher elevations.
- 3) *Oxalis* species that were found in similar Andean habitats (e.g., cloud forests) as the alliance species, but differed from that group morphologically and lacked chromosome number data.
- 4) Species that belonged to the same four sections as alliance species.
- 5) *Oxalis* species that were found growing in areas where oca is cultivated, even if they were not thought to be particularly closely related to oca (e.g., acaulescent bulbous species of section *Ionoxalis*, weedy species of section *Corniculatae*).
- 6) Some divergent *Oxalis* species, from the Andes or elsewhere, included to assess the levels of diversity in ITS sequence in the genus and to serve as outgroups.

Determinations of some of the collections (Table 1) must still be

considered tentative, due to the taxonomic uncertainties in the genus and the lack of good keys that cover these species. Identifications were made using a combination of keys and descriptive information in Knuth (e.g., 1930, 1935, 1936), Macbride (1943), and Eiten (1963), as well as the original species descriptions, and plants were compared with herbarium specimens, including types of some of the species.

In two cases the plants collected matched specimens at NY annotated by Lourteig with yet-unpublished names (Table 1). Isotypes (Bang 315) were examined of one of these new species, here designated as *O. sp.* "O." The second species, here designated as *O. sp.* "R," matches Buchtien 620 p.p. (right-hand side; left side is type of *Xanthoxalis unduavensis* Rusby), annotated by Lourteig with a new specific epithet honoring Rusby.

**DNA amplification and sequencing**—Amplifications were performed in either 100  $\mu$ L or 25  $\mu$ L volumes. Amplification reactions contained 20 mmol/L Tris-HCl, 50 mmol/L KCl, 2.5 mmol/L MgCl<sub>2</sub>, 0.2  $\mu$ mol/L each dNTP, 0.6 mmol/L each of primers ITS4 and ITS5 (White et al., 1990), and 2.5 units *Taq* polymerase (Gibco BRL, Bethesda, MD, or Promega, Madison, WI) per 100  $\mu$ L reaction. Thermocycling profiles were set at an initial 1 min at 97°C, followed by 39 cycles of 1 min at 97°C, 1 min at 48°C, 3 min at 72°C, with a final incubation of 7 min at 72°C. Amplification products from one or more reactions were then purified by cutting bands out of a 1% agarose gel, with excess primers and dNTPs removed by Wizard<sup>®</sup> PCR Preps (Promega, Madison, WI) or QIAquick<sup>®</sup> Gel Extraction Kit (QIAGEN, Basel, Switzerland) columns.

Direct sequencing of double-stranded products followed the standard Sequenase<sup>®</sup> version 2.0 (US Biochemical Corp., Cleveland, OH) protocols and labeling with <sup>35</sup>S-dATP, with the following modifications as recommended by Nickrent (1994): (1) a higher concentration of primer (20  $\mu$ mol/L); (2) dilution of the label mix by only 3.75 $\times$  instead of 5 $\times$ ; and (3) modification of the annealing step to include subjecting the primer/template mixture to 2 min at 99°–100°C, followed by quick chilling in an ethanol bath at –80°C for 2 min, adding the reaction buffer, and incubating for 20 min at 37°C. Sequencing reactions usually were separated in two different acrylimide gels to maximize readable sequence: the first (6% acrylimide) for ~2 h, the second (4.5 to 6% acrylimide) for ~6 h. Fuji x-ray film was exposed to the dried gels for a minimum of 4 d.

Nucleotide sequences were determined for both strands, by use of primers ITS2, ITS3, ITS4, and either ITS5 or ITS1 (White et al., 1990; Fig. 1). In addition, another primer ("ITS3b") was designed near the 3' end of the 5.8S coding region to facilitate reading the sequence of the ITS-2 region. This primer anneals at a similar but not identical position to primer "ITS3a" published by Downie and Katz-Downie (1996) and has the sequence: 5'-AGGGCACGYCTGCCTGGGTGTC-3'. Areas of compressions or other ambiguities presumably caused by secondary structure could usually be resolved by comparison with the complementary strand. The middle portion of the 5.8S gene was not read in all taxa, and was invariant in all that were read, so it was not included in the analysis. Downstream of this portion was a region that was only read in the forward direction in most cases (the 3' end of the 5.8S and 5' end of the ITS-2), but this segment was free of ambiguities and was the most conserved part of the ITS region, with few characters contributed to the main analysis. Boundaries of ITS regions with the coding sequences were determined by comparison with sequences in Yokota et al. (1989).

**Alignment and analysis**—Sequences were aligned using Clustal V (Higgins and Sharp, 1989) as implemented in the DNASTAR package, using several different multiple sequence gap and gap length penalties. Because of problems in alignment of the more divergent sequences (see below), two separate sets of analyses were performed: (1) the primary analyses of sequences that were readily alignable with those of the  $x = 8$  group ("main analyses"), using characters from the ITS-1, 5.8S, and

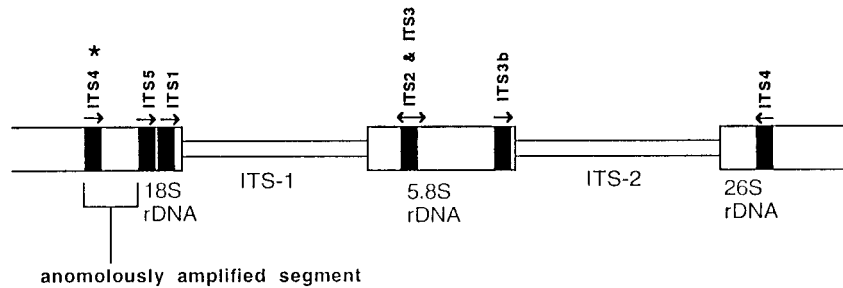


Fig. 1. Internal transcribed spacer (ITS) region of nuclear ribosomal DNA, showing the anomalously amplified segment which appeared in some amplification reactions of *Oxalis* DNA and indicating its position relative to the primers ITS1–5 (White et al., 1990) and the additional primer used in this study. Asterisk indicates ITS4 primer in an unusual position and orientation.

ITS-2 regions; and (2) analyses of all sequences (“outgroup analyses”), using only characters from the ITS-2 and 3’ end of the 5.8S, the results of which were used only for rooting the main analyses.

Each alignment was used in equally weighted parsimony analyses as implemented in PAUP version 3.1.1 (Swofford, 1991). Branch and Bound searches were conducted with MULPARS on and TBR branch swapping, and included only one or two representatives of identical sequences. Gaps were treated as missing data, and later mapped onto the resulting most parsimonious trees.

## RESULTS

**Amplification products and sizes of ITS region**—Amplifications of the ITS/5.8S region from *Oxalis* species normally resulted in a single product of ~700 bp (base pairs), typical in size for angiosperms (Baldwin et al., 1995). In some amplifications a second product appeared as well, ~60 bp larger than the first. When this second amplification product was sequenced, it was found to be identical to the smaller product but included an additional segment at its 5’ end (Fig. 1). Comparison of the longer sequence with published sequences of the 18S nrRNA genes of *Daucus carota* and *Vicia faba* (Yokota et al., 1989) showed similarity in the first 40 bp upstream of the ITS5 primer, with the 5’-most 20 bp of the *Oxalis* product being complementary to the ITS4 primer. Apparently this larger amplification product is produced when the ITS4 primer anneals in the forward direction, upstream of the position of the ITS5 primer. Thus this product is not, apparently, due to the presence of a divergent paralogous locus in some taxa or to a contaminant in some samples.

The ITS-1 region was 200 bp in *Oxalis tuberosa* and close relatives and varied from 198 to 202 bp in the species included in the main analyses. Sizes of ITS-1 in outgroup taxa were more variable, up to 226 bp in *O. martiana* (exact sizes unknown for some species due to unsequenced segments). The ITS-2 region was 225 bp in oca, varying from 213 to 226 bp in the other *Oxalis* sampled. Sequences are deposited in GenBank as accessions U74260–U74290.

In alignments that included sequences from all sampled *Oxalis* species (the outgroup analyses), a core group of very similar sequences (Table 1, sequence types A through M) aligned nearly identically and with relatively few gaps, regardless of alignment parameters used. This core group was then used in the main analyses. Sequences from other species were difficult to align with those

of this core group (i.e., those of *O. regnellii*, *O. latifolia*, *O. martiana*, *O. bisfracta*, and partial sequences from *O. pes-caprae* and *O. crassipes* [Table 1, sequence types N through S]). They were found to differ from the core group in the position of a conserved sequence in ITS-1 (Liu and Schardl, 1994). Large (or many) insertion/deletion (indel) differences between the ITS-1 sequences of these taxa and those of the  $x = 8$  species were suggested by the differences in the length of the segments 5’ and 3’ of that conserved sequence. Length differences also occurred for these same taxa in the region at the 3’ end of the ITS-2 designated “variable region 6” by Hershkovitz and Zimmer (1996). Some sequence ambiguities existed in other parts of the ITS-2 as well for these divergent sequences, leading to differences among the results under different alignment parameters, especially with respect to the most divergent sequence, that of *O. bisfracta*. Sequences of these divergent species were not included in the main analyses, and only portions of them were used for the outgroup analyses, for the purpose of rooting the closely related taxa (see “Phylogenetic analysis,” below).

**ITS sequence types**—When possible, sampling within the  $x = 8$  group included several populations thought to belong to the same species (Table 1). In some of these cases accessions that were identified as the same species had different sequence types (usually differing at a single nucleotide position). If not artifactual, this could be due either to intraspecific polymorphism or in some cases, given the taxonomic uncertainties in *Oxalis*, to these accessions actually belonging to different species (see Discussion).

In other cases, plants identified as different species, some of these quite distinct morphologically, had identical ITS sequences. A notable example is a group of species that share the same ITS sequence with oca (Table 1). Among species identified as members of the  $x = 8$  alliance, sequences had no or very few differences across the entire ITS region. Within the “ $x = 8$  clade” (see below), sequences differed by a maximum of seven substitutions and no indels, while pairwise divergence between taxa included in the main analyses reached 35 substitutions and seven indels of from one to seven base pairs.

All three cultivated oca accessions appeared at first to have a single sequence type (that designated as “A” in

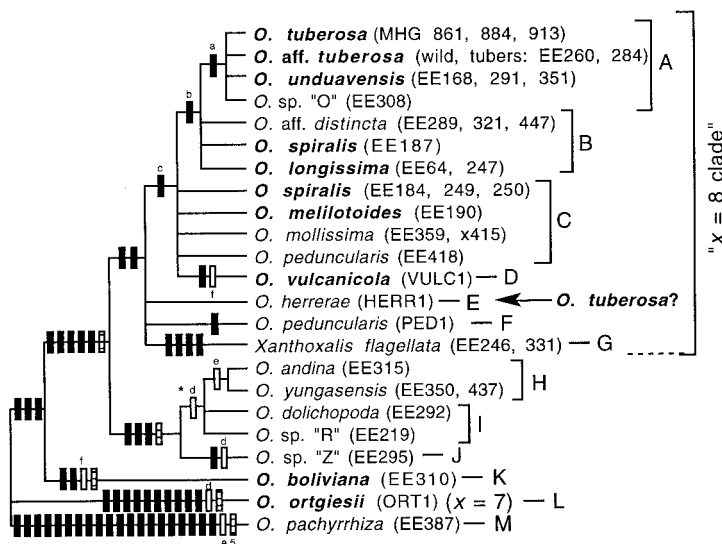


Fig. 2. Phylogeny of *Oxalis* nrDNA ITS sequences. One of two equally most parsimonious trees found in the “main analyses,” which differs from the alternative tree only by the optimization of character “d” (asterisk indicates the node that collapses in the alternative tree). Species in boldface are classified as members of sect. *Ortgieae* by Knuth (1930). Numbers in parentheses are collection numbers (see Table 1). Rectangles are characters: black = nonhomoplasious changes; white = homoplasious characters; horizontal lines = insertions or deletions, not used as characters in this analysis. Lowercase letters refer to particular characters (see text); uppercase letters denote ITS types. The more divergent ITS sequences of *O. bisfracta*, *O. crassipes*, *O. latifolia*, *O. martiana*, *O. pes-caprae*, and *O. regnellii* were not unambiguously alignable with those of the taxa shown above, so they were not included in the main analyses. EE294, a suspected hybrid that had both sequence types “A” and “B,” is not included in the figure, nor is the incomplete sequence of EE345 (*O. nubigena*). The individual characters shown on the branch to *O. pachyrrhiza* may be either synapomorphies of the ingroup or autapomorphies of *O. pachyrrhiza*.

Fig. 2), but on closer examination one of the accessions showed faint secondary bands at the three positions whose transformations are designated as characters “a,” “b,” and “c” in Fig. 2. Assuming that only two sequence types exist, (i.e., that no recombination has occurred), this would indicate that oca has not only sequence type “A,” but also sequence type “E,” with the plesiomorphic nucleotides at these three positions (see Sang, Crawford, and Stuessy, 1995, for a similar case of ITS additivity in *Paeonia*). Other than *O. tuberosa*, the only plant among those included in the main analyses that had more than one sequence type in an individual (EE294, Table 1) was one suspected of being a hybrid a priori, on the basis of its intermediate morphology. Among the more divergent taxa, two polyploid species (*O. pes-caprae* and *O. martiana*) also had multiple sequence types within an individual.

As indicated in Table 1, only a partial sequence was obtained for *Oxalis nubigena*, so its sequence type is not indicated. However, the regions that have been sequenced are consistent with the placement of this species with the other members of the *x* = 8 alliance.

**Phylogenetic analysis**—Parsimony analyses of ITS-2 sequences (the outgroup analyses) nearly always identified the same topology for the subset of the sequences that were of primary interest here and that were used subsequently in the main analyses (see below) regardless of alignment. Although the placements of more divergent sequences varied, the *O. pachyrrhiza* sequence appeared as sister to this core group of sequences in all but one analysis (one among the several in which the highly divergent and very difficult to align *O. bisfracta* sequence

was included). Therefore, *O. pachyrrhiza* was used as the outgroup for the main analysis. This rooting also seems to be in agreement with morphology, although no formal cladistic analysis of morphological data yet exists for these species.

Analyses of alignments produced using different parameters all resulted in the same two topologies for the main analysis (Fig. 2). These differed only due to two possible optimizations for one character (character “d” in Fig. 2). The Consistency Index (CI) and Retention Index (RI) were calculated with all duplicate sequences removed; the CI excluding uninformative characters was 0.870, and the RI was 0.914. Two gaps were potentially informative in the main analyses, but were not used in tree searches; neither these nor other gaps showed homoplasy when mapped onto the most parsimonious trees (Fig. 2).

DISCUSSION

**Levels of ITS divergence and utility in *Oxalis***—Levels of nucleotide substitution and length variation in ITS sequence in the genus as a whole appear to be quite high, such that it proved impossible to produce reliable alignments of sequences of some of the more divergent *Oxalis* species with members of the *x* = 8 group that were the focus of this study. A comparison of the more divergent sequences indicated that areas of similarity had many small indel differences. In addition, the presence of other, larger indels were suggested by (1) segments of sequence that did not appear to have homologues in sequences of other taxa, (2) the shifted position of a conserved sequence in ITS-1 (Liu and Schardl, 1994), and (3) differ-

ences in length as well as sequence in the 3' portion of ITS-2 designated as "variable region 6" by Hershkovitz and Zimmer (1996). Characters from the more conserved areas (the 5.8S and 5' region of ITS-2) could be aligned and analyzed, suggesting that these portions of the sequence could be used in an analysis across the genus, or perhaps even throughout the family Oxalidaceae. However, the level of variation in sequences of the complete ITS region seems too high for practical use at these higher taxonomic levels (but see Hershkovitz and Lewis [1996] for discussion of "deep-level" utility of ITS sequences).

Although these alignment problems precluded the use of several divergent ITS sequences in the main analysis, some of these sequences were quite similar to each other, suggesting that ITS data could be used in future studies to investigate phylogenetic relationships in other species groups of *Oxalis*. For example, *Oxalis* native to southern Africa have tunicate bulbs (e.g., *O. pes-caprae*), whereas some species of the Americas have scaly bulbs (e.g., *O. martiana*, *O. latifolia*), and some others have scaly rhizomes (e.g., *O. regnellii*). The similarity and alignability of ITS sequences of the few examples of these habits included in this study suggest that further sampling could test the speculations of Denton (1973) on the possibly polyphyletic origin of the bulbous habit in *Oxalis*.

*Xanthoxalis* Small is one of several segregate genera proposed by Small (1903, 1907) and adopted by some other workers (e.g., Rose, 1906; Rusby, 1920; Holub, 1973). Some members of the  $x = 8$  alliance are included in this segregate genus along with creeping weedy species that are placed by others in *Oxalis* section *Corniculatae* (e.g., Knuth, 1930; Eiten, 1963; Lourteig, 1979). Although they share some overall morphological similarities, the very high levels of divergence in ITS sequence between the members of the  $x = 8$  alliance and the weedy species sampled so far (the Andean *O. bisfracta* and a partial sequence of *O. stricta* L. for which data are not presented here) suggest that they are not closely related. Further sampling of ITS data could test these hypothesized relationships.

**ITS support of  $x = 8$  "*Oxalis tuberosa*" alliance vs. traditional taxonomy**—Phylogenetic analysis of ITS sequences supports the monophyly of the  $x = 8$  "*Oxalis tuberosa* alliance" of de Azkue and Martínez (1990), in that all plants identified as members of the alliance were found within a single clade on the ITS tree (Fig. 2 and Table 1, footnote i). Chromosome numbers of other species whose ITS sequences fall within this clade have not yet been determined, but all of these species are morphologically similar to the  $x = 8$  group (see below), so it seems likely that they also will be found to be based on  $x = 8$ , or at least to have been derived from ancestors with this base number. On the other hand, the results of the analysis are not consistent with Knuth's (1930, 1936) sectional classification, because sequences from members of different sections are scattered across very different parts of the tree, and the clade that includes the  $x = 8$  alliance also includes members of several other sections (Fig. 2; Table 1). One notable example is that *O. ortgiesii*, the type species of the section (*Ortgieseae*) into which Knuth placed *oca*, not only has  $x = 7$  instead of  $x = 8$

(Heitz, 1927; Warburg, 1938; Marks, 1956), but its ITS sequence is much less closely related to that of *oca* than are those of several  $x = 8$  species (e.g., *O. peduncularis*, *O. herrerae*, *O. mollissima*) that are placed by him in different sections. However, with the exception of *O. boliviana* (of unknown chromosome number), other members of the section *Ortgieseae* that were sampled are supported by the ITS tree as closely related to *oca*.

Within the  $x = 8$  clade there is very little variation in ITS sequence, yet the species are diverse morphologically. Although they do share some features of floral and inflorescence morphology, and are succulent, with large, adnate stipules, the species vary in habit, indument, and leaflet shape. They are especially variable in size, ranging from a few centimeters tall (e.g., *O. nubigena*), to thick sprawling vines of several meters (e.g., aff. *O. distincta*). The morphological variation is partially due to high levels of phenotypic plasticity, yet preliminary observations from "common garden" greenhouse experiments indicate that genetic polymorphism among different populations is also important (E. Emshwiller, unpublished data). The alliance species are ecologically diverse as well, being found from tropical cloud forest levels in the eastern Andean foothills, through higher and drier environments, up to almost the limits of vegetation at high altitudes.

Sister to the clade that includes sequences from the species known to be based on  $x = 8$  is a clade of ITS sequences of several other species collected in cloud forest regions on the eastern slopes of the Andes in Bolivia (e.g., *O. andina*, *O. dolichopoda*). These species do not have published chromosome counts, so it is unknown whether they share a base number of  $x = 8$  with *O. tuberosa* and its allies. They share some morphological similarities with the known alliance species (e.g., corolla yellow with red veins, relatively large stipules), but differ in lacking succulence, instead bearing leathery leaves on thin, somewhat woody stems. Whether or not they share the same base chromosome number, there is no evidence from ITS data that they were involved in the origins of *oca* (see below).

**Origins of *Oxalis tuberosa***—Investigations of origins of polyploidy and domestication are often complicated by the number of different processes that may be at work to one extent or another. In addition to the questions about whether a polyploid arose from a single progenitor or more than one species or population and whether it had a single or multiple origin (reviewed in Soltis and Soltis, 1993) is the question of whether domestication occurred before or after formation of the polyploid. The area of domestication may have been localized or diffuse (Harlan, 1971), and predominantly vegetatively propagated crops may still have some sexual recombination. Feral escapes may be indistinguishable from possible progenitors, and crop-wild gene flow may add additional complexity. Duplicated and diverged paralogous loci, which in the case of ITS might even remain within a single ribosomal DNA array, can confound the identification of homeologous loci.

The study of the origins of domesticated *O. tuberosa* is particularly challenging because of the rudimentary state of *Oxalis* systematics. The unresolved problems of species delimitation make it uncertain whether some local

variants belong to separate species or ecotypes of a highly polymorphic species. For example, *O. spiralis* (sometimes called *O. pubescens* H. B. K., an illegitimate name) is a very widespread and morphologically variable species complex for which both diploid and hexaploid cytotypes have been described (Mathew, 1958; Favarger and Huynh, 1965; Huynh, 1965; Brücher, 1969; de Azkue and Martínez, 1990), and it is uncertain whether all the plants identified here as *O. spiralis* are truly conspecific. Species delimitation is also a problem with *O. peduncularis*, in which case there have been various species recognized by Knuth and other taxonomists that may or may not be distinct from this species. Regardless of the species concept used, these problems may not be resolved for many years, if ever. Our emphasis at this time is on identifying populations that may have contributed to the origins of oca, rather than on determining which populations are or are not conspecific.

The morphological diversity within the  $x = 8$  group discussed above contrasts with the low levels of ITS sequence variability and the sharing of the same sequence by different species. This sequence similarity may be due to low levels of divergence or could indicate that interspecific gene flow is widespread. The latter possibility is suggested by the existence of some individuals with morphologies intermediate between species, which could be hybrids (e.g., EE294, as well as seedlings that have appeared in greenhouse collections). Crossing relationships among species of the  $x = 8$  group are currently under investigation (A. Valladolid, International Potato Center, personal communication), yet it is well known that it can be difficult to distinguish the extent to which hybridization as opposed to primary divergence has affected the pattern of variation among natural populations (reviews in Heiser, 1973; Rieseberg and Wendel, 1993). Despite the challenging situation, the ITS data have been able to contribute to the understanding of oca's origins.

The congruence of the  $x = 8$  alliance with a monophyletic group identified by the phylogenetic analysis of ITS sequences confirms the cytologically defined group as a natural assemblage. The ITS data, therefore, can be used to predict species likely to share this base chromosome number. Both sources of data support this group of species as being the closest relatives of cultivated oca. However, the ITS variation among species in this clade is low, with only three informative characters, so that it provides minimal resolution of relationships among the sequence types found within the group. The primary sequence type "A" from oca is found within this group (Fig. 2), as is the second sequence type "E" that appeared faintly in one individual of oca. This indicates that one, and possibly two, genome donors of cultivated *O. tuberosa* may be found within this  $x = 8$  group. Alternatively, given only the ITS data, this faint sequence type might be interpreted as gene flow from wild *Oxalis*, or duplicated and diverged paralogous repeat types, also from within the  $x = 8$  clade. In any case, there is no evidence of origins of oca genomes from outside of this group.

The two sampled populations of wild *Oxalis* with tubers had the same sequence type as *O. tuberosa* ("A," the primary sequence in oca). Although neither population showed any signs of the second sequence type, this

second type was not always observed in oca either, so its absence cannot be taken as definitive. Wild populations with tubers could be truly wild or could merely be escapes from cultivation. Both situations appear to occur in our sample, based on information from stelar morphology. Oca is cultivated as a clonal crop, and each cultivar includes only one of the three possible floral morphs of this tristylous species. Although oca can produce viable seed (Alandia, 1967; Gibbs, 1976; Vallenás, 1992; Carrión, 1995), seedlings are rarely observed in field conditions (Hill, 1939; E. Emshwiller, personal observations). A population formed by an escape from cultivation might be founded by a single tuber, and if so would be expected to include only one floral morph. In some populations (e.g., EE284), plants bore relatively large tubers and the population was nearly monomorphic for the mid-styled morph. This suggests that plants of this population are propagating clonally and probably were escapes from cultivation. In contrast, all three floral morphs were present in plants of the wild population with small tubers (e.g., EE260), indicating that these were probably reproducing by seed, and thus were possibly truly wild.

Sequence type "A" was also shared by other wild *Oxalis*: three plants (EE168, 291, and 351) identified as *O. unduavensis*, as well as EE308, which matches specimens annotated by Lourteig with a yet-unpublished name (here designated *O. sp. "O"*). *O. unduavensis* is similar enough to oca morphologically that it is considered by Lourteig (unpublished annotations of herbarium specimens, e.g., Buchtien 621 and 3250) to be a variety of *Oxalis tuberosa*, although it lacks tubers and differs in the form of the inflorescence. *O. sp. "O,"* however, is clearly a distinct species from oca, differing in height, indument, inflorescence morphology, and flowering phenology. The fact that the same ITS sequence is shared not only by cultivated oca and the wild populations with tubers, but also by *O. unduavensis* and especially *O. sp. "O,"* suggests that the nrDNA ITS is not variable enough to fulfill the goals of identifying with exactitude the progenitor or progenitors of oca and of indicating how many times the polyploid arose. The ITS data do, however, support some of the  $x = 8$  species as being more likely candidates than others to have contributed to the polyploid genome of *Oxalis tuberosa*.

The second sequence type "E" in oca was inferred by assuming that no recombination had occurred among the three sites that showed faint signs of a second nucleotide. No recombinational types were observed among the three characters ("a," "b," and "c," Fig. 2) in the sequences sampled in this study. However, the ITS sequence for *O. tuberosa* submitted to Genbank by D. S. Tosto and E. H. Hopp (accession Z66546; Tosto and Hopp, 1996) differs from sequence type "A" at three sites, one of which is character "b," where they report the plesiomorphic state. Thus this sequence could represent a recombined type; alternatively, it could be a case of simple homoplasy. When this sequence is included in the cladistic analysis, it causes the collapse of the branches supported by characters "a" and "b" (Fig. 2).

The sequence type "E" in unrecombined form was found in *O. herrerae* in this study and is also identical to the single ITS sequence type published by Tosto and Hopp (1996; Genbank accession Z66547) for *O. villosula*

Knuth, *O. tabaconasensis* Knuth, *O. oblongiformis* Knuth, and *O. peduncularis*. This sequence type also appears on the ITS tree within the  $x = 8$  group, indicating that a second progenitor of cultivated oca might be found in a different part of the “ $x = 8$  clade” (Fig. 2). This placement is consistent with our preliminary results from sequences of the chloroplast-expressed isozyme of glutamine synthetase (ncp-GS), a single-copy nuclear-encoded locus. More than one sequence type of ncp-GS has been found within individual plants of oca, perhaps representing homeologous loci of an allopolyploid, each contributed by a different member of the  $x = 8$  group (E. Emshwiller and J. J. Doyle, unpublished data).

The demonstration of interlocus concerted evolution in allopolyploid *Gossypium* (Wendel, Schnabel, and Seelman, 1995) suggests one possible explanation for the inconsistent and faint appearance of the second sequence type in *O. tuberosa*. This process has the potential to “erase” one of the parental sequence types in an allopolyploid. If concerted evolution has acted to homogenize sequence types across loci, but has not yet done so completely, there may still be some ITS repeats remaining of the alternative type. In this case the parental repeat types would no longer be present in numbers reflecting the proportion of genomes contributed by each parent. Alternatively, repeat number in the highly variable and dynamic nrDNA may differ among sequence types even when concerted evolution does not act across loci. The fact that only one sequence type was detected in some oca sequencing reactions suggests that the number of repeats of the second type, if this repeat type is present in these individuals at all, may be much lower than that of the primary type. It is possible that “PCR drift” (Wagner et al., 1993) may be favoring the repeat type that is already present in greater numbers, further enhancing its levels over the second type. This could be true whether the original difference in numbers of the two sequence types were due to interlocus concerted evolution or simply to fewer repeats at the second locus. Baldwin et al. (1995) also report instances in which sequence types found in cloned DNA were absent in direct sequencing of PCR products from the same individual. With the current data it is not possible to distinguish the extent to which the weak presence of the second sequence type represents its actual proportion or is an artifact of PCR.

Thus, within a genus with high levels of divergence in ITS sequence overall, ITS data support a group of species, including *Oxalis tuberosa*, as a natural lineage, within which the levels of divergence are quite low. This group is congruent with the  $x = 8$  “*Oxalis tuberosa* alliance” of de Azkue and Martínez (1990) and with morphology, but includes additional species not yet characterized cytologically. The sectional classification of Knuth (e.g., 1930, 1935, 1936), however, is not supported by these data. More importantly, ITS sequence data identify a pool of species closely related to oca, from any of which one or more of its genomes may have been derived. Other sources of data will be necessary to determine more precisely the progenitor(s) of octoploid *O. tuberosa* and the number of times it arose, both because of the low variability of ITS sequences in this group, and because of the possibility that some parental ITS types may not be observed. However, the ITS data have already

eliminated some taxa from consideration as progenitor candidates in the absence of cytological data, and they have distinguished among some members of the  $x = 8$  alliance those more likely to have been genome donors in the formation of the octoploid. Thus, these data can continue to be used to screen yet unsampled *Oxalis* species from other areas of the Andes for other possible progenitors of oca.

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