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Ploidy Levels among Species in the '*Oxalis tuberosa* Alliance' as Inferred by Flow Cytometry

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The '*Oxalis tuberosa* alliance' is a group of Andean *Oxalis* species allied to the Andean tuber crop *O. tuberosa* Molina (Oxalidaceae), commonly known as 'oca'. As part of a larger project studying the origins of polyploidy and domestication of cultivated oca, flow cytometry was used to survey DNA ploidy levels among Bolivian and Peruvian accessions of alliance members. In addition, this study provided a first assessment of C-values in the alliance by estimating nuclear DNA contents of these accessions using chicken erythrocytes as internal standard. Ten Bolivian accessions of cultivated *O. tuberosa* were confirmed to be octoploid, with a mean nuclear DNA content of approx. 3·6 pg/2C. Two Peruvian wild *Oxalis* species, *O. phaeotricha* and *O. picchensis*, were inferred to be tetraploid (both with approx. 1·67 pg/2C), the latter being one of the putative progenitors of *O. tuberosa* identified by chloroplast-expressed glutamine synthetase data in prior work. The remaining accessions (from 78 populations provisionally identified as 35 species) were DNA diploid, with nuclear DNA contents varying from 0·79 to 1·34 pg/2C.

Key words: Andean crops, C-value, DNA ploidy, flow cytometry, nuclear DNA content, oca, Oxalidaceae, *Oxalis tuberosa*, octoploid, ploidy levels, polyploidy.

INTRODUCTION

The '*Oxalis tuberosa* alliance' was first described on cytological grounds by de Azkue and Martínez (1990) as a group of morphologically similar *Oxalis* species related to the Andean tuber crop oca [*O. tuberosa* Molina (Oxalidaceae)] that shares $x = 8$, a base chromosome number that is rare in *Oxalis*. The genus overall has base chromosome numbers varying from five to 12, with seven being most frequent (Marks, 1956; Talledo and Escobar, 1995). In addition to the dozen diploid and polyploid species studied by de Azkue and Martínez (1990), a few other *Oxalis* species have been reported with $x = 8$ (Table 1). Additional *Oxalis* species for which cytological information is as yet lacking also appear to belong to the group, based on the morphological similarities they share with members of the alliance (Emshwiller, 1999*a*, *b*). The members of the alliance are primarily Andean, being found in highlands from Central America and northern Venezuela to northwestern Argentina (Emshwiller, 2002). Although the species of the alliance were classified by Knuth (e.g. 1930) in several sections, the more recent treatment by Lourteig (2000) places almost all members of the alliance in two sections, *Lotoideae* Lourteig and *Herrerae* R. Knuth, with a small number in section *Ortgieseae* R. Knuth. De Azkue and Martínez (1990) found domesticated *O. tuberosa* to be the only octoploid in the alliance, with $2n = 8x = 64$. This chromosome number for oca has since been confirmed by several workers, but different chromosome counts have been reported in both older and more recent reports, some of which differ markedly (see citations in Table 2).

Phylogenetic analysis of DNA sequences of two nuclear loci, the internal transcribed spacer region (ITS) of nuclear ribosomal DNA and the chloroplast-expressed isozyme of glutamine synthetase (ncpGS), has confirmed the monophyly of the *O. tuberosa* alliance and the inclusion in the group of octoploid oca as well as many of the morphologically similar species mentioned above that still lack cytological data. That is, sequences of all these species appear in a single clade on each of the gene trees of ITS or ncpGS data, whether analysed separately or simultaneously (Emshwiller and Doyle, 1998, 1999, 2002; Emshwiller, 2002). The *O. tuberosa* alliance, as recognized here, includes these other morphologically similar species that join the same clade as the original alliance species. However, although *O. andina* Britton has also been reported to have $x = 8$ (de Azkue, 2000), I exclude it from the alliance because there is no evidence from either gene that this species or its allies (a clade that forms the sister group of the alliance) were involved in the origins of oca. Data from ncpGS indicated that oca is allopolyploid, derived from hybridization of multiple species, and two wild tuber-bearing taxa were identified as progenitor candidates, *O. picchensis* R. Knuth from southern Peru and an as yet unnamed taxon from Bolivia (Emshwiller and Doyle, 2002). However, several alternative hypotheses remained about oca's origins that were all congruent with the ncpGS data (Emshwiller, 1999*a*), and distinguishing the most likely of these hypotheses required additional data, including information on ploidy levels of the putative progenitors. Some

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Table 1. *Chromosome numbers reported for* x = *8* Oxalis *other than* O. tuberosa

Species*	2*n*	Reference
O. herrerae R. Knuth	16	de Azkue and Martínez (1990)
		Valladolid (1996)
O. medicaginea Kunth	16	de Azkue and Martínez (1990)
O. mollissima (Rusby) R. Knuth	16	de Azkue and Martínez (1990)
O. oblongiformis R. Knuth	16	de Azkue and Martínez (1990)
O. peduncularis H.B.K	16	Diers (1961)
		de Azkue and Martínez (1990)
		Valladolid (1996)
O. picchensis R. Knuth†	16	Valladolid (1996)
O. ptychoclada Diels	16	Favarger and Huynh (1965)
		Huynh (1965)
O. subintegra R. Knuth	16	de Azkue and Martínez (1990)
O. tabaconasensis R. Knuth	16	de Azkue and Martínez (1990)
O. aff. *villosula* R. Knuth‡	16	de Azkue and Martínez (1990)
O. sp.§	16	de Azkue and Martínez (1990)
O. melilotoides Zuccarini	16	Valladolid (1996)
	32	Brücher (1969)
O. lotoides Kunth	32	de Azkue and Martínez (1990)
O. spiralis R. & P. ex G. Don	16	Favarger and Huynh (1965)
	48	Huynh (1965)
		Brücher (1969)
		(as *O. pubescens* Kunth)
		de Azkue and Martínez (1990)
		Mathew (1958)
		(as *O. pubescens* Kunth)
O. nubigena Walpers	48–50	Diers (1961)
O. spp. (others unidentified)	16	Valladolid (1996)
	48	Diers (1961)
O. andina Britton¶	16	de Azkue (2000)

* Because of the difficulties of identifying *Oxalis* species, it is possible that some determinations may be incorrect.

† My examination of this plant, in CIP glasshouses, lead me to question this identification. It may be *O. rigidicaulis* R. Knuth, a taxon that I believe, contrary to Lourteig (2000), may be distinct from *O. boliviana* Britton.

‡ *O. villosula* is probably a synonym of *O. san-miguellii* or *O. urubambensis*, which are both endemic in southern Peru. The plant studied by de Azkue and Martínez was collected in Churuja, the same area as one of the accessions sampled here (EE807), of a species that I designate as *Oxalis* sp. 'V' (see Table 3).

§ This plant was collected in the vicinity of Machu Picchu, suggesting that it might be *O. san-miguellii* R. Knuth or *O. urubambensis* R. Knuth, both of which are found in that area.

¶ See discussion in text about relationships of *O. andina* and its allies to the *O. tuberosa* alliance.

TABLE 2. *Chromosome numbers reported for* Oxalis tuberosa *Molina*

2*n*	Reference
14	Heitz (1927) (as *O. crenata* Jacquin)
63–64, 68–70	Kostoff *et al.* (1935)
66	Cárdenas and Hawkes (1948)
58–66	Gibbs *et al.* (1978)
64	de Azkue and Martínez (1990)
64	Valladolid *et al.* (1994)
64	Medina Hinostroza (1994)
14	Talledo and Escobar (1995)
64	Valladolid Cavero (1996)
24, 32, 40, 48, 64	Guamán Calderón (1997)
64	Vinueza Vela (1997)
49	Hayano Kanashiro (1998)

individuals of wild *Oxalis* species were heterozygous for ncpGS sequences, and ploidy information was necessary to interpret this heterozygosity as either normal allelic polymorphism or as homologous loci (Emshwiller, 1999*a*; Emshwiller and Doyle, 2002).

Cultivated oca has an important place in the diets and farming systems of rural communities in the Andean highlands. Knowledge of the origins of the crop, its relationships with wild allies and the ploidy levels of its close relatives is crucial for the potential use of wild relatives in future breeding programmes for oca. Information about genome size can also have practical relevance for molecular characterization by techniques such as RAPDs or AFLPs, in which the choice of primers and/or restriction enzymes is affected by the nuclear DNA content (Bennett *et al*., 2000). In addition, because the evolution of higher-level polyploids is less well understood than that of tetraploids, the study of the *O. tuberosa* alliance and the origins of oca provides an important case study.

As part of a larger project studying the origins of cultivated oca and evolutionary relationships of members of the *O. tuberosa* alliance, a survey was conducted of ploidy levels among Peruvian and Bolivian populations of alliance members. The primary objectives of this survey were to use flow cytometry as an independent data source to confirm the octoploidy of cultivated oca, to determine the ploidy level of *O. picchensis*, one of the putative progenitors of cultivated oca identified by ncpGS data (Emshwiller, 1999*a*; Emshwiller and Doyle, 2002), and to survey ploidy levels in other members of the alliance as an initial assessment of frequency of polyploidy in the alliance. As a secondary objective, this flow cytometry survey provides the first exploration of the patterns of variation of DNA content within and among diploid species in the *O. tuberosa* alliance. Some observations of repeatable variation among species, populations or individuals are reported here to suggest avenues for further study.

Flow cytometry has frequently been used in ploidy analysis (e.g. Costich *et al*., 1993; Braeutigam and Braeutigam, 1996; Lysák and Doležel, 1998; Brummer *et al*., 1999; Gamiette *et al*., 1999; Dansi *et al*., 2001). Some of the advantages of flow cytometry for this purpose, as summarized by Doležel (1997), include its ease, rapidity, ability to detect mixoploidy and the use of a small amount of leaf tissue rather than the need for dividing cells. However, the use of flow cytometry to infer ploidy level is only appropriate when comparing accessions from the same or closely related species, where large differences in chromosome size are not expected. Although divergent members of the large genus *Oxalis* have chromosomes of diverse sizes (Heitz, 1927; Warburg, 1938; Marks, 1956; Brücher, 1969; Naranjo *et al*., 1982; de Azkue and Martínez, 1983, 1984, 1988; de Azkue, 2000), the members of the *O. tuberosa* alliance that have been studied are consistently reported to have small chromosomes, of roughly similar size (Brücher, 1969; Gibbs *et al*., 1978; de Azkue and Martínez, 1990;

Valladolid *et al*., 1994; Valladolid, 1996). This observation parallels the low ITS sequence variation found within the alliance, contrasting with high levels of variation among divergent members of the genus overall (Tosto and Hopp, 1996; Emshwiller and Doyle, 1998). Thus, although I would be hesitant to extend this application of estimates of DNA content from flow cytometry to infer ploidy levels across the entire genus, where chromosome sizes vary greatly, the use of this technique to infer ploidy levels among members of the *Oxalis tuberosa* alliance is justified because of the relative uniformity of chromosome size reported among these species.

MATERIALS AND METHODS

Sampling

Most of the accessions of wild *Oxalis* used in this study were collected in Bolivia and Peru (Table 3), while cultivated oca accessions were kindly provided by the Programa de Investigación de la Papa (PROINPA), Cochabamba, Bolivia. Also included were a few purchased horticultural *Oxalis* plants that had been sampled in prior molecular studies (Emshwiller and Doyle, 1998) as well as some 'volunteer' seedlings that appeared in glasshouse collections. Some of the species determinations should be considered provisional (see Discussion). Although the recent treatment by Lourteig (2000) has greatly improved the taxonomy of the genus, some problems of species delimitation still remain. Pending further study, I provisionally retain the use of some names that Lourteig (2000) has reduced to synonymy, because the names reflect differences among the plants, such as molecular or ploidy differences, or morphological differences that are stable in common garden conditions. In a few cases, alternative names that have been reduced to synonymy by Lourteig (2000) are listed alongside the accepted name.

Flow cytometry

Initial attempts to prepare samples using $MgSO_4$ buffer (Arumuganathan and Earle, 1991) or Lysis Buffer LB01 (Doležel *et al*., 1989; Galbraith *et al*., 1997) did not produce satisfactory results, presumably due to the acidity of the *Oxalis* leaves (pH approx. 1·7), which may have exceeded the buffering capacity of the solutions. Successful sample preparation followed a two-step protocol (Otto, 1990; Doležel and Göhde, 1995; Galbraith *et al*., 1997; see also Dansi *et al*., 2001) with simplifying modifications as posted at http://www.ueb.cas.cz/olomouc1/lcgcm/Flow/protocol/andc3.htm. Typically, a small amount of young leaves was chopped with a new razor blade in 0·5 ml of ice-cold 'Otto I' solution [0·1 M citric acid monohydrate, 0·5 % (v/v) Tween 20] in a Petri dish (also over ice). In some cases about 0·5 ml of ice-cold Otto I solution was added and mixed well with a pipette, whereas in later preparations less additional solution was added to avoid diluting the nuclei. The suspension was filtered through 50-μm nylon mesh, and incubated at room temperature from 1 h to overnight. Centrifugation was not effective in concentrating the nuclei, so it was not continued. Two volumes of 'Otto II' solution (0·4 M $Na_2HPO_4 \cdot 12H_2O$ containing 2 μl ml^{-1} β-mercaptoethanol) containing propidium iodide and RNase (each at a final concentration of 50 μg ml^{-1}) were added to the samples just before they were analysed in a cytometer (within 5 min).

Samples were run on a Becton-Dickinson FACSCalibur flow cytometer operated by the Cornell School of Veterinary Medicine, with an argon laser exciting at 488 nm. Pulse area was detected using FL2-A (585 mean/42 bandwidth) with a threshold at FLS 35. CellQuest software (Apple version 3·1·f 3·1·3) was used to visualize and measure histogram peaks. Initial samples used co-chopped rice (*Oryza sativa*) leaves as an internal standard, but the fluorescence peak for this standard was superimposed on those of some of the diploid *Oxalis,* so this standard was not continued. Subsequent samples used erythrocytes from a young Babcock B300 (egg-laying breed) chicken (CRBCs) as internal standard. Although plant standards are preferable for determination of plant nuclear DNA content in absolute units (Johnston *et al*., 1999; R. Price, pers. comm.; J. Doležel, pers. comm.), CRBCs are an adequate standard for ploidy determinations, and offer convenience because a single blood sample can be used for all runs. The blood was used within 2 weeks of collection, kept in a heparinized tube and diluted for use with 'CRBC buffer II' [140 mM NaCl, 5 % (v/v) Triton X-100; see recipes at: http://www.ueb.cas.cz/olomouc1/lcgcm/Flow/protocol/recept.htm]. The fluorescence of CRBCs declined relative to that of the sample within 1 or 2 h (data not shown), which might indicate that the propidium iodide concentration was not optimal. Therefore all results reported here are from samples that were run as quickly as possible after addition of 'Otto II' solution and CRBCs. The genome size of each sample was calculated using 2·5 pg as the DNA content of CRBCs, which is an approximate mean of several reported estimates (Tiersch and Wachtel, 1991; Johnston *et al*., 1999; R. Price, pers. comm.; J. Doležel, pers. comm.). Although the use of animal standards for plant samples may cast some doubt upon these estimates as absolute values, the use of a consistent methodology for all samples and the repeatability of measurements of the same accessions allows for comparison of similarities and differences among species and populations.

RESULTS

Quality and reliability of the flow cytometric data

In addition to the linearity tests conducted daily by the flow cytometry facility, the instrument linearity was also monitored by comparing the proportionality of peaks of different ploidy in the same sample to the ideal ratio (see Vilhar *et al*., 2001). In general, the proportionality over most runs was 1–1·5 % below the expected ratio of two. Specifically, the ratio of 4C to 2C peaks over all runs averaged 1·979, and that of 8C to 4C peaks averaged 1·972. Most coefficient of variation (CV) values of the sample 2C peaks ranged between 2·3 and 7·5 (mean 5·44), with a few higher values

TABLE 3. *Accessions of* Oxalis *sampled for nuclear DNA content*

Accession number*	Taxon	Latitude (°S)	Longitude (°W)	Altitude (m asl)	Provenance	Ploidy	DNA content pg/2C†	s.d.	Number of measurements (number of plants‡)
MHG847	*O. tuberosa* Molina	16°09′	69°04′	3850	Bolivia, La Paz, Manco Kapac, Copacabana	8*x*	3·553		1(1)
MHG861	*O. tuberosa* Molina	18°54′	66°46′	3750	Bolivia, Oruro, Avaroa, Challapata	8*x*	3·693		1(1)
MHG864	*O. tuberosa* Molina	19°19′	65°55′	3800	Bolivia, Potosí, Tomás Frías, Yocalla	8*x*	3·664	0·184	2(1)
MHG865	*O. tuberosa* Molina	19°19′	65°55′	3800	Bolivia, Potosí, Tomás Frías, Huancarani, Yocalla	8*x*	3·578		1(1)
MHG884	*O. tuberosa* Molina	19°25′	65°44′	3800	Bolivia, Potosí, Tomás Frías, Chiutara, Potosí	8*x*	3·543	0·119	2(1)
MHG891	*O. tuberosa* Molina	19°25′	65°44′	3600	Bolivia, Potosí, Tomás Frías, Manquiri, Potosí	8*x*	3·551		1(1)
MHG894	*O. tuberosa* Molina	19°47′	65°30′	4100	Bolivia, Potosí, Linares, Potosí	8*x*	3·562		1(1)
MHG897	*O. tuberosa* Molina	19°36′	65°24′	3650	Bolivia, Potosí, Saavedra, Lagunillas, Betanzos	8*x*	3·678		1(1)
07	*O. tuberosa* Molina	–	–	–	Bolivia	8*x*	3·623		1(1)
Tiraque 10	*O. tuberosa* Molina	–	–	–	Bolivia	8*x*	3·670		1(1)
EE561	*O. phaeotricha* Diels	13°13′	72°18′	3300	Peru, Cusco, Urubamba, Quebrada Tajcac above Ollantaytambo	4*x*	1·673	0·022	3(1)
EE500	*O. picchensis* R. Knuth	13°34′	71°53′	3400	Peru, Cusco, Cusco, San Jerónimo, Rd toward Paruro	4*x*	1·676	0·059	3(1)
EE541	*O. spiralis* R. & P. ex G. Don	13°08′	72°31′	2400	Peru, Cusco, Urubamba, Machu Picchu, Puente Ruinas	2*x*	1·280	0·115	2(2)
EE588	*O. spiralis* R. & P. ex G. Don	11°43′	75°04′		Peru, Junín, Concepción, Comas	2*x*	1·216	0·039	3(1)
EE602	*O. spiralis* R. & P. ex G. Don	12°00′	74°54′		Peru, Junín, Huancayo, Road to Pariahuanca	2*x*	1·311	0·017	3(2)
EE607	*O. spiralis* R. & P. ex G. Don	11°20′	75°39′		Peru, Junín, Tarma, Acobamba—Chipián	2*x*	1·062		1(1)
EE618	*O. spiralis* R. & P. ex G. Don	11°15′	75°33′	2400	Peru, Junín, Tarma, Rd to San Ramón, Carpapata	2*x*	1·106		1(1)
EE620b	*O. spiralis* R. & P. ex G. Don	11°10′	75°25′	1500	Peru, Junín, Tarma, Rd to San Ramón, San Luis	2*x*	1·081		1(1)
EE914	*O. spiralis* R. & P. ex G. Don	13°02′	72°02′	2800	Peru, Cusco, Calca, Valley of Río Lares	2*x*	1·246	0·051	4(2)
EE927	*O. spiralis* R. & P. ex G. Don	13°08′	72°31′	2400	Peru, Cusco, Urubamba, Aguas Calientes	2*x*	1·339	0·034	2(1)
Seedlings	*O. spiralis* R. & P. ex G. Don	–	–	–	seedlings appeared in greenhouse collections	2*x*	1·115	0·022	6(6)
EE532	*O. cuzcensis* R. Knuth	13°29′	71°52′	3750	Peru, Cusco, Calca, C.C. Picol	2*x*	1·173	0·026	4(2)
EE291	*O. unduavensis* (Rusby) R. Knuth	16°17′	67°48′	2940	Bolivia, La Paz, Nor Yungas, Unduavi—Coroico Rd	2*x*	1·011		1(1)
EE438	*O. unduavensis* (Rusby) R. Knuth	16°09′	68°07′	3050	Bolivia, La Paz, Murillo, Zongo Valley	2*x*	1·031	0·052	3(3)
EE868	*O.* sp. cfr. *melilotoides* Zuccarini	13°05′	72°23′	2450	Peru, Cusco, Urubamba, Lucumayu Valley, San Luis	2*x*	0·930		1(1)
EE578	*O. petrophila* R. Knuth	11°49′	75°19′		Peru, Junín, Concepción, La Esperanza, Ch'iqchi	2*x*	0·983	0·031	2(1)
EE655	*O.* sp. S	14°27′	69°32′	3400	Peru, Puno, Sandia, Cuyo Cuyo	2*x*	1·028	0·022	2(2)
EE706	*O.* sp. S	14°25′	69°29′	3000	Peru, Puno, Sandia, below Cuyo Cuyo	2*x*	0·972		1(1)
EE485	*O. lucumayensis* R. Knuth ssp. *lucumayensis*	13°06′	71°41′	2700	Peru, Cusco, Paucartambo, below Challabamba	2*x*	0·929		1(1)
EE289	*O. lucumayensis* R. Knuth ssp. *subiens* Lourteig	16°17′	67°48′	2940	Bolivia, La Paz, Nor Yungas, Unduavi—Coroico Rd	2*x*	0·919		1(1)
EEx359	*O. lucumayensis* R. Knuth ssp. *subiens* Lourteig	16°17′	67°50′	2700	Bolivia, Cochabamba, Sud Yungas, Unduavi—Chulumani Rd	2*x*	0·986		1(1)
EE447	*O. lucumayensis* R. Knuth ssp. *subiens* Lourteig	16°16′	67°47′	2610	Bolivia, La Paz, Nor Yungas, Unduavi—Coroico Rd	2*x*	0·890		1(1)

TABLE 3. *Continued*

Accession number*	Taxon	Latitude (°S)	Longitude (°W)	Altitude (m asl)	Provenance	Ploidy	DNA content pg/2C†	s.d.	Number of measurements (number of plants‡)
EE294	*O. lucumayensis* (hybrid?)	16°17′	67°48′	2940	Bolivia, La Paz, Nor Yungas, Unduavi—Coroico Rd	2*x*	0·998		1(1)
MV s/n	*O.* sp. aff. *lucumayensis*	–	–	–	Peru, Puno, Sandia province	2*x*	0·907		1(1)
VULC1§	*O. vulcanicola* Donn. Sm.	–	–	–	'Central America' purchased from Merry Gardens	2*x*	0·886		1(1)
EE604	*O. humbertii* R. Knuth	12°00′	74°54′	3300	Peru, Junín, Huancayo, Chilifruta	2*x*	1·040		1(1)
EE592	*O.* sp. C	11°43′	75°05′		Peru, Junín, Concepción, Comas	2*x*	1·037		1(1)
EE777	*O. medicaginea* Kunth	06°56′	78°11′	3150	Peru, Cajamarca, Celendín, Quilimbash	2*x*	0·836		1(1)
EE809	*O. medicaginea* Kunth	06°14′	77°53′	2230	Peru, Amazonas, Chachapoyas, Quebrada Molina	2*x*	0·803		1(1)
EE810	*O. medicaginea* Kunth	06°15′	77°51′	2750	Peru, Amazonas, Chachapoyas, Pumaurqu	2*x*	0·851	0·040	3(2)
EE783	*O. lotoides* Kunth	06°48′	77°56′	2720	Peru, Amazonas, Chachapoyas, Marañón valley above Balsas	2*x*	0·881		1(1)
EE789	*O. lotoides* Kunth	06°47′	77°55′	2930	Peru, Amazonas, Chachapoyas, Marañón valley above Balsas	2*x*	0·864	0·035	2(2)
EE851	*O. lotoides* Kunth	07°24′	78°47′	3850	Peru, Cajamarca, Contumazá, near Bosque Cachil	2*x*	0·826	0·014	2(1)
EE798	*O. mollis* Kunth	05°51′	77°58′	2070	Peru, Amazonas, Bongará, Pedro Ruiz Gallo—La Florida Rd	2*x*	0·892	0·016	2(1)
EE356	*O. oulophora* Lourteig	16°18′	67°52′	2700	Bolivia, La Paz, Sud Yungas, Unduavi—Chulumani Rd	2*x*	0·872		1(1)
EE415	*O. oulophora* Lourteig	16°09′	68°07′	3160	Bolivia, La Paz, Murillo, Valley of Río Zongo	2*x*	0·883		1(1)
EE705	*O. marcapatensis* R. Knuth	14°25′	69°29′	3000	Peru, Puno, Sandia, Ñakonki, below Cuyo Cuyo	2*x*	0·897	0·043	2(1)
EE910	*O. peduncularis* Kunth	13°12′	72°23′	2800	Peru, Cusco, Urubamba, Misk'i Puquio, Pisqacucho	2*x*	0·985	0·055	4(2)
EE507	*O. peduncularis* Kunth	13°16′	72°11′	2900	Peru, Cusco, Urubamba, Urubamba Valley, Yanahuara	2*x*	1·033	0·032	2(2)
EE512	*O. peduncularis* Kunth	13°14′	72°10′	3300	Peru, Cusco, Urubamba, Quebrada Mant'anáy above Yanahuara	2*x*	0·959	0·025	2(2)
EE514	*O. peduncularis* Kunth	13°14′	72°09′	3450	Peru, Cusco, Urubamba, Quebrada Mant'anáy above Yanahuara	2*x*	1·019		1(1)
EE912	*O. peduncularis* Kunth	13°11′	72°23′	3490	Peru, Cusco, Urubamba, Misk'i Puquio	2*x*	0·983		1(1)
EE595	*O. peduncularis* Kunth (*O. ledigii* R. Knuth)	12°00′	75°09′		Peru, Junín, Huancayo, Porbolín, km 13 from Huancayo	2*x*	0·980	0·024	2(1)
EE746	*O. peduncularis* Kunth (*O. weberbaueri* R. Knuth)	09°00′	77°44′	3700	Peru, Ancash, Huaylas, west side of Cordillera Blanca, Parón	2*x*	1·107	0·072	3(2)
EE752	*O. peduncularis* Kunth (*O. weberbaueri* R. Knuth)	09°00′	77°31′	3800	Peru, Ancash, Yungáy, east side of Cordillera Blanca, Quebrada Ranicuráy	2*x*	1·055		1(1)
EE753	*O. peduncularis* Kunth (*O. weberbaueri* R. Knuth)	09°05′	77°39′	3700	Peru, Ancash, Yungáy, Llanganuco Lakes region	2*x*	1·157		1(1)
EE850	*O.* cfr. *peduncularis* Kunth	07°24′	78°47′	2600	Peru, Cajamarca, Contumazá, near Bosque Cachíl	2*x*	1·139	0·018	2(2)
EE853	*O.* cfr. *peduncularis* Kunth	07°23′	78°49′	2700	Peru, Cajamarca, Contumazá, near Contumazá	2*x*	1·085	0·053	2(2)
EE778	*O. peduncularis* Kunth var. *pilosa*	06°53′	78°07′	3140	Peru, Cajamarca, Celendín, Jelig	2*x*	1·128		1(1)
EE780	*O. peduncularis* Kunth var. *pilosa*	07°06′	78°19′	3040	Peru, Cajamarca, Cajamarca—Celedín Rd	2*x*	1·070		1(1)
EE787	*O. peduncularis* Kunth var. *pilosa*	06°50′	77°57′	1900	Peru, Amazonas, Chachapoyas, Marañón valley above Balsas	2*x*	1·087		1(1)
EE791	*O. peduncularis* Kunth var. *pilosa*	06°48′	77°56′	2660	Peru, Amazonas, Chachapoyas, Marañón valley above Balsas	2*x*	1·112	0·036	2(2)
EE792	*O. peduncularis* Kunth var. *pilosa*	06°50′	77°57′	2030	Peru, Amazonas, Chachapoyas, Marañón valley above Balsas	2*x*	1·053		1(1)

TABLE 3. *Continued*

Accession number*	Taxon	Latitude (°S)	Longitude (°W)	Altitude (m asl)	Provenance	Ploidy	DNA content pg/2C†	s.d.	Number of measurements (number of plants‡)
EE804	*O. peduncularis* Kunth var. *pilosa*	06°53′	78°07′	3150	Peru, Cajamarca, Celendín, Jelig	2*x*	1·163	0·019	2(1)
EE807	*O.* sp. V	06°00′	77°57′	1520	Peru, Amazonas, Bongará, Churuja	2*x*	1·043	0·037	2(2)
EE813	*O.* sp. V	06°13′	77°49′	2040	Peru, Amazonas, Chachapoyas—Molinopampa Rd	2*x*	1·010		1(1)
EE548	*O. paucartambensis* R. Knuth	13°54′	71°29′	3400	Peru, Cusco, Quispicanchi, Paucarpata, near Cusipata	2*x*	0·894	0·020	2(2)
EE961	*O. paucartambensis* R. Knuth	13°24′	71°51′	3700	Peru, Cusco, Calca, C.C. Viacha	2*x*	0·873	0·017	2(1)
EE962	*O. paucartambensis* R. Knuth	13°24′	71°51′	3700	Peru, Cusco, Calca, C.C. Viacha	2*x*	0·864		1(1)
EE865	*O. paucartambensis* R. Knuth	13°33′	71°52′	3250	Peru, Cusco, Cusco, San Jerónimo	2*x*	0·959		1(1)
EE553	*O. herrerae* R. Knuth	13°31′	71°58′	3400	Peru, Cusco, Cusco, Cusco, Barrio San Blas	2*x*	0·867		1(1)
EE560	*O. herrerae* R. Knuth	13°13′	72°18′	3300	Peru, Cusco, Urubamba, Quebrada Tajcac, above Ollantaytambo	2*x*	0·852	0·017	2(2)
EE911	*O. herrerae* R. Knuth	13°12′	72°23′	3400	Peru, Cusco, Urubamba, Misk'i Puquio, Piscacucho	2*x*	0·815	0·033	2(1)
EE913	*O. herrerae* R. Knuth	13°03′	72°02′	3230	Peru, Cusco, Calca, Velley of Río Lares, Choquecancha	2*x*	0·926	0·027	2(2)
EE600	*O. ptychoclada* Diels var. *trichocarpa* Lourteig	12°00′	74°54′	3700	Peru, Junín, Huancayo—Pariahuanca Rd, km 63, above Chilifruta	2*x*	0·950	0·020	2(1)
EE610	*O. ptychoclada* Diels	11°15′	75°35′	2600	Peru, Junín, Tarma, Rd. to Huasahuasi	2*x*	0·837	0·008	2(2)
EE613	*O. ptychoclada* Diels	11°16′	75°37′	2400	Peru, Junín, Tarma, Tarma—San Ramón Rd	2*x*	0·886		1(1)
EE916	*O. ptychoclada* Diels	12°58′	72°04′	2500	Peru, Cusco, Calca, Valley of Río Lares	2*x*	0·790	0·014	3(2)
EE545	*O. san-miguelii* R. Knuth	13°08′	72°31′	2400	Peru, Cusco, Urubamba, Machu Picchu, Puente Ruinas	2*x*	0·830	0·001	2(1)
EE920	*O. san-miguelii* R. Knuth	13°08′	72°31′	2600	Peru, Cusco, Urubamba, Machu Picchu	2*x*	0·874		1(1)
EE926	*O. urubambensis* R. Knuth	13°08′	72°31′	2400	Peru, Cusco, Urubamba, Aguas Calientes	2*x*	0·880	0·029	2(2)
EE511	*O.* sp. cfr. *teneriensis* R. Knuth	13°14′	72°10′	3200	Peru, Cusco, Urubamba, Quebrada Mantanáy above Yanahuara	2*x*	0·966	0·034	2(2)
EE504	*O.* sp. A	13°33′	72°34′	2100	Peru, Cusco, Anta, near Puente Cunyac at Río Apurímac	2*x*	1·097	0·018	2(2)
EE786	*O. tabaconasensis* R. Knuth	06°42′	77°55′	2800	Peru, Amazonas, Chachapoyas, above Leimebamba	2*x*	0·993		1(1)
EE790	*O. tabaconasensis* R. Knuth	06°48′	77°55′	2700	Peru, Amazonas, Chachapoyas, above Leimebamba	2*x*	1·051	0·009	2(1)
EE797	*O. tabaconasensis* (*O. oblongiformis*) R. Knuth	05°56′	77°59′	1460	Peru, Amazonas, Bongará, Pedro Ruiz Gallo—La Florida Rd	2*x*	0·983	0·023	2(2)
EE605	*O. coralleoides* R. Knuth	11°48′	75°37′		Peru, Junín, La Oroya—Jauja Rd., Km 55	2*x*	1·064		1(1)
HERR1§	*O. herrerae* R. Knuth	–	–	–	'Peru' purchased from Merry Gardens	2*x*	0·794		1(1)
PED1§	*O. peduncularis* Kunth	–	–	–	'Peru, Ecuador' purchased from Merry Gardens	2*x*	0·927		1(1)
Hybrid?	*O. peduncularis* × *O. herrerae?*	–	–	–	Seedling that appeared in glasshouse collections when only purchased plants were present	2*x*	0·873	0·014	2(1)
EE921¶	*O. boliviana* Britton	13°08′	72°33′	2400	Peru, Cusco, Urubamba, Machu Picchu	2*x*	0·957		1(1)
EE773¶	*O. megalorrhiza* Jacquin	09°12′	77°42′	2700	Peru, Ancash, Yungáy, below Guitarrero Cave	2*x*	0·814		1(1)

(up to 10·8). The higher CV values were usually in samples with high background. High background noise is not unusual in the lower channel numbers (e.g. Kudo and Kimura, 2001*a*, *b*), and has been alternatively ascribed to broken cells damaged during the extraction procedure (Lagunes-Espinoza *et al*., 2000) or to autofluorescence of chloroplasts in the cytosol (Galbraith *et al*., 1997). In addition to this background signal, anomalous additional smaller peaks were observed in profiles of several samples of older leaves of oca, but these were later confirmed to derive from contamination by glasshouse whitefly larvae (2C approx. 0·89 pg, 4C approx. 1·78 pg). All data described below, unless otherwise indicated, are based on samples that included chicken erythrocytes as internal standards.

Multiple preparations from the same plant run on different days sometimes varied at least as much as the samples from different plants of the population, possibly due to instrumental fluctuations (see Kudo and Kimura, 2001*a*). With the exception of cultivated *O. tuberosa* accessions, this fluctuation was usually less than 0·1 pg. In the case of cultivated oca, most of the ten accessions were sampled on more than 1 d, using either CRBCs or rice leaves as internal standards. The day-to-day variation in DNA content estimates exceeded both the variation among oca accessions and that between values estimated with the two different standards (Fig. 1). Because averaging the measurements from different days into a separate mean for each accession would distort and exaggerate the differences among them, the values shown in Table 3 are those from the

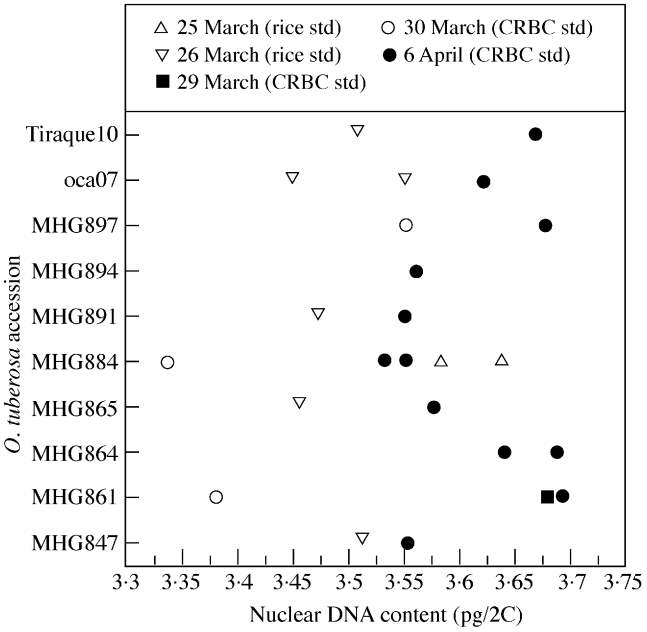

FIG. 1. Estimated values of 2C nuclear DNA content for the ten accessions of cultivated oca measured on different days, using either CRBCs or rice leaves as internal standard (0·87 pg/2C used as value for rice standard). The day-to-day variation (e.g. between samples run on 6 April and those run on 30 March) was greater for the cultivated *O. tuberosa* accessions than for wild *Oxalis* samples, for unknown reasons, but did not depend on the standard used. Only the values from 6 Apr. 1999 are shown in Table 3, because only on that day were all ten samples measured. The mean value of all oca accessions measured on that day is 3·610 pg/2C (s.d. = 0·061), whereas the mean for all days is 3·514 pg/2C (s.d. = 0·116) and the mean of those using rice leaves as internal standard was 3·524 pg/2C (s.d. = 0·066).

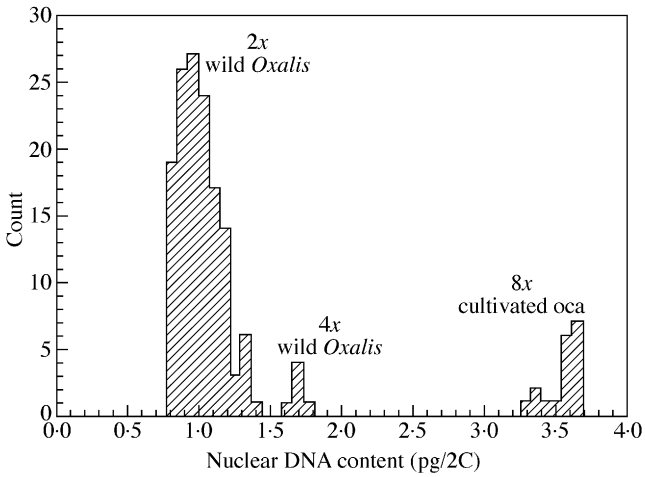

FIG. 2. Distribution of all estimates of 2C values in samples of members of the *Oxalis tuberosa* alliance that were tested with CRBCs as internal standard in this study. The discontinuities between the ranges of diploid, tetraploid and octoploid values make them easily distinguishable. The day-to-day variation among the octoploid cultivated samples (see text and Fig. 1) contributes to the spread of values among those samples.

* Vouchers are deposited at F and BH with duplicates of subsets of herbarium vouchers at CUZ, CPUN, LPB, BOLV, USM, and/or duplicates of living material at CIP, CICA (UNSAAC), INDEA (UNC) or PROINPA. All cultivated oca accessions were kindly provided by PROINPA's germplasm bank under the management of Ing. Maria Luisa Ugarte. Collectors according to prefix of accession numbers are: MHG, David Morales, Michael Hermann, Willman García; MV, Mauro Vallenas Ramirez; EE, E. Emshwiller with others: Bente Eriksen, Ulf Molau, Gregorio Meza, Pedro Cruz, Alfredo Tupayachi, Percy Núñez V., Andrés Castelo, Rosa Urrunaga, Francisco Vivanco López, Lucio Torres T., Isidoro Sánchez Vega, Michael Hermann, Bill and David Mutch.

† Measurements of 2C DNA content (in picograms) include all measurements for a particular accession number that included CRBCs as internal standard, with the exception of the cultivated *O. tuberosa* accessions, for which only the measurements taken on 6 Apr. 1999 are shown (see discussion of octoploid *O. tuberosa* in the text).

‡ Although the number of plants and number of samples per accession are indicated, the measurements from all plants sampled of each wild population are considered together. The number of plants does not necessarily indicate the number of different genetic individuals, because the plants in the glasshouse were grown from vegetative cuttings collected in the field (in some cases from plants that spread clonally), so there may be some uncertainty about whether they were truly collected from separate plants.

§ Three plants, accessions PED1, HERR1 and VULC1, were purchased from Merry Gardens, Camden, Maine, USA. Voucher information for these is available in Emshwiller and Doyle (1998).

¶ The last two species are outgroup species that are not members of the alliance, see text.

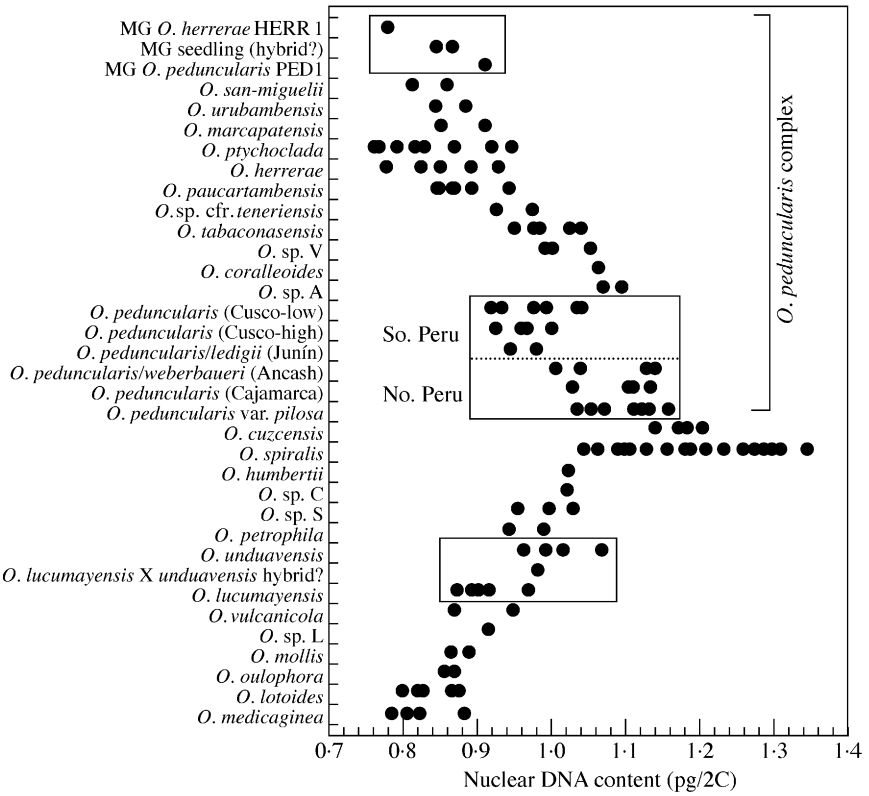

FIG. 3. Estimates of 2C values of diploid species in the *O. tuberosa* alliance, arranged to compare variation among and within species. The middle box illustrates one of several examples in which 'lumping' populations into a single species, *O. peduncularis*, may contribute to artificially high 'intraspecific' variation in genome size. The other two boxes illustrate cases of possible hybrids that have DNA contents intermediate between their putative parents. The '*O. peduncularis* complex' indicates a group of morphologically similar taxa in which species boundaries are problematic. In prior analyses of ncpGS data (Emshwiller and Doyle, 2002), all of the accessions in this complex that were sampled for ncpGS joined the subclade designated '*O. peduncularis* clade', which also included the sequence of *O. cuzcensis*. The members of the '*O. peduncularis* complex' do not differ in their range of nuclear DNA contents from the other alliance members sampled, most of which joined the other subclade within the alliance, designated the '*O. lotoides* clade' (other than *O. petrophila*, which joined neither subclade; see Emshwiller and Doyle, 2002).

only day on which all oca accessions were sampled (6 Apr. 1999).

Utility of flow cytometry to infer ploidy levels of members of the O. tuberosa *alliance*

DNA ploidy levels of samples were determined by comparing their DNA contents with those of taxa of known ploidy level in various ways. The distribution of DNA content estimates for species in the alliance is discontinuous (Fig. 2), so that although there is some variation in the contents of diploid species (see below), ploidy levels can be easily inferred. The range of values observed for cultivated oca in Fig. 2 is mostly due to day-to-day variation, as discussed above. All estimates for cultivated *O. tuberosa* accessions fell in the octoploid range, with approx. four-times the DNA content of the diploids. Specifically, cultivated *O. tuberosa* accessions measured on 6 Apr. 1999 varied from 3·543 to 3·693 pg (Table 3), with a mean value of 3·610 (mean for all days = 3·514, see Fig. 1). A quarter of these 2C values for oca would be 0·903 for 6 April (or 0·879 for all measurements), very close to the modal value for the diploid species (Fig. 2). Two of the wild *Oxalis* accessions, *O. phaeotricha* Diels (EE561) and *O. picchensis* R. Knuth (EE500), had similar 2C values of approx. 1·67 pg, considered to be in the tetraploid range. The remainder of the species in the *O. tuberosa* alliance had DNA contents that varied from 0·790 to 1·339 pg. These included species reported to have diploid chromosome counts (see Table 1). None of the accessions sampled appeared to be hexaploid.

The polyploid status of *O. tuberosa* and *O. picchensis* was also confirmed by comparing the ratio of their fluorescence peaks with that of a diploid accession run together in the same tube (albeit not co-chopped). Polyploids frequently have DNA contents that are somewhat below the ratio 'expected' from closely related diploids (Lysák and Doležel, 1998; Bennett *et al*., 2000). For instance, a tetraploid may have a DNA content somewhat less than twice that of closely related diploids. Not surprisingly, this situation seems to be the case in the *O. tuberosa* alliance. The ratio of the fluorescence peaks of two oca accessions, 07 and MHG847, to that of co-run diploid *O. peduncularis* Kunth (accession PED1) was 3·830 and 3·941, respectively, i.e. close to four-times the diploid, confirming their octoploid status. Tetraploidy of accession EE500 of *O. picchensis* was corroborated by running simultaneously

with accession EE916, tentatively identified as the diploid *O. ptychoclada* Diels, resulting in a ratio of fluorescence peaks of 2·079. This ratio is very close to two, rather than below two, but this particular diploid species had one of the smallest genome sizes among the alliance members sampled in this study. These co-run samples offered an additional opportunity to confirm the internal consistency of measurements by calculating the DNA contents of the polyploids based on the co-run diploids as calibration standards (the diploids having been previously estimated using CRBC or rice internal standards). These tests resulted in estimates very similar to the direct measurements of the same polyploids (i.e. 2C estimates of 3·65 pg and 3·55 pg for two accessions of O. *tuberosa* based on PED1 as standard, and 1·65 pg for *O. picchensis* based on EE916 as standard).

In a few cases the DNA ploidy found in this study differs from ploidy levels reported previously for the same species. These cases may reflect either differences in species identifications or real variation in cytotypes. Although both are inferred in this study to be diploid, different cytotypes have been reported (see citations in Table 1) for *O. lotoides* Kunth (4*x*) and *O. spiralis* R. & P. ex G. Don (both 2*x* and 6*x*). Both of these are widespread species, and *O. spiralis* has a wide ecological amplitude as well (see below), so the finding of diploid levels in the accessions sampled here would not preclude the possibility of other cytotypes in these species. On the other hand, differing species delimitations are relevant in the case of accession EE532, identified as *O. cuzcensis*, which is here inferred to be DNA diploid (Table 3). I tentatively retain the name *O. cuzcensis*, which is reduced by Lourteig (2000) to synonymy with *O. nubigena* Walpers, a species that would be hexaploid according to the chromosome count of Diers (1961).

Endopolyploidy

In some of the *Oxalis* samples a cluster of nuclei formed a visible 8C peak. This suggests that these leaf tissues exhibit some endopolyploidy, an increase in DNA content due to endomitosis (or endoreduplication; i.e. somatic duplication of chromosomes without nuclear division). In most cases in which such a peak was discernible, the 8C peak was small relative to the 2C and 4C peaks [i.e. the number of 'events' (i.e. nuclei) was usually only 2–5 % of the number in the 2C peak, although in a very few samples it reached nearly 8 %). Scatter plots of FL2-A *vs*. FL2-W (pulse area *vs*. pulse width) of a few representative samples with relatively large 8C peaks were examined and gated ('doublet discrimination') to eliminate the possibility that these larger 8C peaks represented aggregated nuclei. The latter possibility is also unlikely as there was no peak in a position that would correspond to 6C (see De Rocher *et al*., 1990).

Endopolyploidy has been reported in many plant taxa (see Lagunes-Espinoza *et al*., 2000; Kudo and Kimura, 2001*a*, *b*, and references therein), and is especially associated with large epidermal cells (Melaragno *et al*., 1993) and succulents with small genomes (De Rocher *et al*., 1990). Members of the *O. tuberosa* alliance all have somewhat succulent leaves with very large epidermal cells, and are often succulent throughout the plant, so the occurrence of endopolyploidy in the alliance is concordant with observations in other succulents. Endopolyploidy may explain the giant nuclei reported in the carpel mesophyll of *O. ptychoclada* and *O. spiralis* as well as in the different types of glandular hairs on the anther filaments, sepals and pedicels of the latter species (Huynh, 1965). Recent reports of endoreduplication in the pod wall of legumes (Lagunes-Espinoza *et al*., 2000) may refer to a similar phenomenon to the giant nuclei observed by Huynh (1965) in carpel mesophyll in these two *Oxalis* species. Although *O. spiralis* rarely showed an 8C peak in flow cytometric profiles, this is not surprising because only vegetative tissues were sampled for this study, rather than the inflorescence organs in which glandular hairs occur in this species.

DISCUSSION

Octoploid Oxalis tuberosa

The finding of octoploid-level DNA contents in these ten cultivated *O. tuberosa* accessions is consistent with the reports of $2n = 8x = 64$ in well over 100 samples of cultivated oca from diverse areas of the Andes (de Azkue, 1990; Medina Hinostroza, 1994; Valladolid *et al*., 1994; Valladolid, 1996; Vinueza Vela, 1997). The finding of octoploid levels in cultivated oca is also consistent with previous evidence from ncpGS data, in which multiple sequence types (putative homologues) found within individual plants show fixed heterozygosity and join separate parts of the gene tree, suggesting that oca is allopolyploid and perhaps autoallopolyploid (Emshwiller, 1999*a*; Emshwiller and Doyle, 2002). The flow cytometric data for the ten oca accessions sampled here showed no evidence of euploid variation as reported by Guamán Calderón (1997), nor lower diploid levels consistent with the reported counts of $2n = 14$ (e.g. Heitz, 1927; Talledo and Escobar, 1995). On the other hand, these flow cytometric data alone would not provide strong evidence against the report by Hayano Kanashiro (1998) that oca is $2n = 7x = 49$, because heptaploid and octoploid DNA content levels might be difficult to distinguish. However, molecular, cytological and morphological data confirm oca's close relationship with a group of species that are all based on $x = 8$ (de Azkue and Martínez, 1990; Tosto and Hopp, 1996; Emshwiller and Doyle, 1998, 2002; Emshwiller, 1999*b*). In addition, although some genotypes show a high proportion of inviable pollen (Gibbs, 1976; Trognitz *et al*., 2000), oca is somewhat fertile, producing copious seed in at least some circumstances (Alandia Borda, 1967; Panti Pacheco, 1972; Vallenas Ramírez, 1992; Pallares Ponce, 1998; Trognitz *et al*., 1998; Trognitz and Hermann, 2001), whereas odd polyploids are usually sterile (Allard, 1960). The genome size for oca reported here does not negate the possibility of aneuploidy in oca, as reflected in the slight variation in chromosome numbers in some reports (Kostoff *et al*., 1935; Cárdenas and Hawkes, 1948; Gibbs *et al*., 1978). Vegetative propagation and dispersal by humans mean that oca may not be under the same selection pressures to regain fertility by reducing meiotic abnormalities that operate in seed-propa-

gated species, and so some aneuploid genotypes might be preserved. However, the observation by Vinueza Vela (1997) of chromosome breakage at the nucleolus organizer region (NOR; often observed as a secondary constriction in metaphase chromosomes) might explain some of the reports of numbers above 64. Because of the small number of cultivated oca accessions sampled here, it is also possible that different accessions might have other ploidy levels. The report by Guamán Calderón (1997) of other euploid numbers is particularly interesting because the accessions she studied were from areas of Bolivia where wild tuber-bearing *Oxalis* grows in close proximity to cultivated oca (pers. obs.). The existence of different ploidy levels in cultivated oca in this region would be significant because it might indicate possibilities such as crop/wild hybridization across ploidy levels, multiple domestications or the persistence of earlier domesticated forms of oca. Thus these reports should be confirmed through measurement of genome size and/or independent chromosome counts.

Tetraploid O. picchensis *and* O. phaeotricha

Oxalis picchensis, here inferred to be tetraploid, is one of the wild tuber-bearing taxa identified by ncpGS data as a possible genome donor of octoploid oca (Emshwiller, 1999*a*; Emshwiller and Doyle, 2002). *Oxalis picchensis* might be autotetraploid because there was little variation in the ncpGS sequence in the two plants sampled. *Oxalis phaeotricha*, on the other hand, had multiple sequences of ncpGS within an individual plant (E. Emshwiller, unpubl. res.), so it might be allopolyploid. The latter species was not included in the study of oca's origins cited above because of technical difficulties in determining its DNA sequences for ncpGS, and because enough of the sequence was available to exclude it as one of the progenitors of oca.

Information regarding the ploidy level of *O. picchensis* has proved useful in the study of the origins of octoploidy of *O. tuberosa*, by helping to eliminate some of the competing hypotheses about oca's origins that were consistent with ncpGS data (Emshwiller, 1999*a*; Emshwiller and Doyle, 2002). For instance, hypotheses of the origins of polyploidy in *O. tuberosa* (e.g. Fig. 5·7 in Emshwiller, 1999*a*) included the possibility that oca arose through hybridization of a diploid *O. picchensis* with a hexaploid Bolivian wild tuber-bearing taxon (i.e. $2x + 6x = 8x$), a scenario that could involve polyploidization through either asexual or bilateral sexual means (*sensu* Mendiburu and Peloquin, 1976). This hypothesis is now eliminated by the finding that *O. picchensis* is tetraploid. However, no living plants were available to determine ploidy of the other putative genome donor of octoploid oca, namely Bolivian populations of wild tuber-bearing *Oxalis* of a yet unnamed taxon. Thus, several alternative scenarios still remain (because various modes of polyploidization are possible, as reviewed by Mendiburu and Peloquin, 1976).

Comparison of DNA content with phylogenetic relationships

The variation in nuclear DNA content among *Oxalis* species sampled in this study did not form a pattern according to phylogenetic relationships, either in terms of comparisons of the alliance members with outgroup taxa or among groups within the alliance. Sampling included two Andean species, *O. megalorrhiza* Jacquin and *O. boliviana* Britton, which are not members of the *O. tuberosa* alliance, as indicated by their placement outside of the alliance clade in the results of phylogenetic analyses of ITS and ncpGS data (Emshwiller and Doyle, 1998, 2002). Both of these taxa were found to have DNA contents similar to those of diploid members of the *O. tuberosa* alliance. Chromosome numbers for *Oxalis megalorrhiza* (under the name *O. carnosa* Molina, a name frequently misapplied to *O. megalorrhiza*) are reported to be either 14 (Heitz, 1926, cited in Federov, 1974) or 18 (de Azkue, 2000). *Oxalis boliviana* has an unknown chromosome number.

There is also no pattern with respect to the subclades within the *Oxalis tuberosa* alliance clade that were found in phylogenetic analyses of ncpGS data (Emshwiller and Doyle, 2002). There is nearly complete overlap in the ranges of DNA contents of diploid species that joined each of the subclades within the alliance in that study. In some cases species that were sisters on the ncpGS gene tree have quite divergent DNA contents (e.g. accessions EE916 and EE504).

Intraspecific variation within diploid members of the alliance

With a few exceptions to be discussed below, the estimates for different accessions that were identified as the same species usually varied by no more than approx. 0·1 pg, i.e. no more than the day-to-day variation in measurements of the same plant (see Fig. 3, in which taxa are arranged in groups for purposes of the following discussion). Most cases of greater intraspecific variation are those in which there is variation in the tristylous breeding system (see below) or considerable morphological and/or ecological variation among the plants or populations. Thus, the intraspecific variation would be considered 'orthodox' in the sense of Greilhuber (1998), who discusses genome size differentiation among populations that are separated by geographical barriers into distinct reproductive communities. Andean environments are extremely heterogeneous in altitude, rainfall and other environmental variables over very short geographic distances (Frère *et al*., 1975). Gene flow among populations may be limited by the distances between appropriate habitats, or else it may have been intermittent in the past, as vegetation zones shifted during Pleistocene cycles of glaciation (Vuilleumier, 1971). Here, intraspecific sampling was not designed to test for correlation of nuclear DNA content with any environmental parameters (see review in Poggio *et al*., 1998), and the interaction of multiple factors might make interpretation of such patterns complex.

Assessment of intraspecific variation in species of the *O. tuberosa* alliance is complicated by the aforementioned problems of species delimitation that still remain despite the clarification of *Oxalis* taxonomy by Lourteig (2000). The situation in some species complexes in the alliance seems to reflect the problem described by Greilhuber (1998), who asserts that intraspecific variation in genome size 'can be a

taxonomical artefact' and provides the example of *Scilla biflora* L. *s.l.*, which if 'treated as one species, shows a two-fold genome size variation, but if split up taxonomically, intraspecific variation diminishes to hardly more than methodological error'. The group designated here as the *O. peduncularis* complex is one in which these problems are especially evident. This group differs morphologically and ecologically from the rest of the alliance (Emshwiller, 1999*a*, *b*, 2002), being found in drier conditions (such as inter-Andean valleys) than the moist forest habitats of the other alliance members. All populations in this complex join the same subclade in the ncpGS gene tree (designated '*O. peduncularis* clade' in Emshwiller, 1999*a*, 2002; Emshwiller and Doyle, 2002). However, this clade also includes sequences of species that do not share the same morphological characteristics, i.e. *O. picchensis* and *O. cuzcensis*, so these species are not included here in the *O. peduncularis* complex.

The *O. peduncularis* complex corresponds roughly, but not exactly, with the taxa classified by Lourteig (2000) in section *Herrerae*, in which she included four species, with one subspecies and two varieties (plus autonyms), and reduced several previously published species to synonymy. However, my study of morphology of species in the complex led me to the opinion that this group includes more than four species, and that not only should some of the species names that were reduced by Lourteig be retained, but additional names may need to be proposed. Recognizing only four species in this complex would result in even higher 'intraspecific' variation in DNA content than that seen within the species as provisionally determined here (Fig. 3). Thus, the situation in the *O. peduncularis* complex/section *Herrerae sensu* Lourteig (2000) is similar to the case of *Scilla* cited by Greilhuber (1998). As an illustration, populations of *O. peduncularis* from different parts of Peru are separated in Fig. 3 by region of origin and variety. Populations of *O. peduncularis* in northern Peru have higher values (albeit overlapping) than those in southern Peru ($P < 0{\cdot}001$), suggesting that these populations are separated by distance into at least separate gene pools, if not separate species. Genome sizes of *O. paucartambensis* R. Knuth and *O. peduncularis* var. *pilosa* are non-overlapping (Fig. 3), supporting the retention of the former as a distinct species (*contra* Lourteig, 2000). On the other hand, morphologically different populations from high and low elevations in Cusco Department have similar DNA contents (Fig. 3). With sampling directed specifically toward these questions, estimates of nuclear genome size may be able to complement other data in helping to resolve some of the problems of species delimitation in the alliance.

The *O. spiralis* complex also has high intraspecific variation in genome size, which may have taxonomic implications. The plants identified here as *O. spiralis* not only had the largest genome sizes among the diploid species sampled, but also the widest range of apparently intraspecific variation (Fig. 3). Yet the plants sampled include little of this taxon's broad range of morphological, geographical and ecological variation (Emshwiller, 2002), and none of the hexaploid cytotypes that have been reported for *O. spiralis* (see citations in Table 1). More thorough sampling in the *O. spiralis* complex may elucidate the relationship of DNA content to variation in environmental factors or species and/or population boundaries.

This variation in genome size in *O. spiralis* might be related to the tristylous breeding system. Some populations are tristylous (the norm in *Oxalis*), with individuals of short-styled (S), mid-styled (M) and long-styled (L) forms, whereas others are semi-homostylous and apparently self-compatible (e.g. accession EE607 and the seedling plants that appeared in glasshouse collections). Semi-homostyled plants also occur in other members of the *O. tuberosa* alliance, including cultivated oca (pers. obs., see also Carrión *et al*., 1995). The semi-homostylous plants had the smaller DNA contents among the sampled *O. spiralis*. In some other alliance species, individuals of different style morph differed in DNA content, but results were equivocal and sampling was insufficient to test significance. Although flow-cytometry has elucidated dimorphism in genome size in dioecious taxa and has been used in plant sex-determination (Costich *et al*., 1991; Doležel and Göhde, 1995), I am not aware of any studies assessing genome size differences in heterostylous taxa.

The possibility of hybridization, suggested by the observation of plants of intermediate morphology, complicates the species delimitation problems in some parts of the alliance. Two suspected hybrid *Oxalis* included in this study (see boxes in Fig. 3) each had estimated 2C-values intermediate between those of their putative parents, similarly to the interspecific hybrids in *Alstroemeria* L. studied by Buitendijk *et al*. (1997). One was a seedling that was suspected to be a hybrid between the purchased plants of *O. peduncularis* (PED1) and *O. herrerae* (HERR1), which had a DNA content intermediate between that of those accessions. The second plant, EE294, had both an intermediate morphology and an intermediate DNA content between *O. lucumayensis* R. Knuth ssp. *subiens* Lourteig and *O. unduavensis* R. Knuth, two species present in the area in which EE294 was collected.

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