Studies on the Breeding System of *Oxalis tuberosa* MOL.

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Summary

A study has been made of the morphological basis and the compatibility relations of the tristylous flowers of *Oxalis tuberosa* MOL., a cultivated tuber-bearing species from South America. Despite its long history of asexual propagation *O. tuberosa* has been found to retain the full complement of characters associated with tristyly, i.e. three corresponding style and stamen lengths and three pollen grain sizes which accord with three types of stigmatic papillae. Pollination experiments have shown that the physiological pollen — stigma self and own-type incompatibility reaction is also still functional so as to promote outcrossing and inhibit selfing.

Introduction

Oxalis tuberosa MOL. (oca in quechua) is a tuber-bearing crop species of the Andes which has been cultivated since pre-incaic times and which still forms part of the staple diet of many peasant communities along the Cordillera from Venezuela to Bolivia. The cultivation of *O. tuberosa* exactly parallels that of the potato and also of ulluco (*Ullucus tuberosus* LOZ.) and it is commonly grown in the Andes of Peru in rotation with these species in small fields and terrace systems at altitudes between 2.500—4.250 m.s.m. Again, as with the potato many indigenous cultivar varieties of oca now exist as a result of its long history of cultivation by Man. HODGE (1951) and LEON (1964) give details of some of these varieties and the methods and extent of cultivation of this species.

HILL (1939) and LEON (1964) have described the tristylous flowers of *Oxalis tuberosa*. DARWIN (1877) clearly established the nature of tristyly with pollination experiments which showed that it is an outbreeding mechanism which is analogous in several features to the more widely distributed distylous system of *Primula* and other genera. However, tristyly appears to be restricted to the families Lythraceae, Oxalidaceae and Pontederiaceae (VUILLEUMIER 1967). In tristylous species the population consists of three kinds of individuals each of which produces flowers of one of three types which differ in style length, thus: long-style flowers, with mid- and short-length stamens; mid-style flowers, with long- and short-length stamens; and short-style flowers with long- and mid-length stamens. The style/stigma and stamen/anther levels correspond with each other between the three flower types. HILDE-BRAND (1871) and DARWIN (1877) showed that in the tristylous species *Lythrum salicaria*, *Oxalis valdiviensis*, *O. „Bowii"* and *O. speciosa*[1]) the "legitimate" pollinations

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1) DARWIN *(loc. cit)* expressed some doubt as to the identity of his *Oxalis Bowii*. ORNDUFF (1974) has suggested that this taxon may have been *O. bowiei* LINDL. *(Oxalis* sect. *Cornuae)* and that DARWIN's *O. speciosa* may have been *O. purpurea* L. *(Oxalis* sect. *Stictophyllae)*.

(i. e. those which are successful in terms of seed set) are those between flowers with anthers and stigmas at corresponding levels.

ORNDUFF (1964, 1972, 1974) has shown that in a number of species of *Oxalis* the tristylous system is susceptible to evolutionary breakdown leading to virtual distyly, homostyly or regular self-fertilisation.

EITEN (1963) has emphasised the distinction which should be made between species with "morphological tristyly" and those which have been demonstrated to possess "functional tristyly". There do not appear to be any published accounts of detailed studies on either the morphological basis of tristyly or its mode of functioning in *Oxalis tuberosa* although this species presents an interesting situation as a cultivated plant which has been propagated asexually for generations and so for which there has been no selection to maintain seed fertility or genic heterozygosity. Under such circumstances the breeding system in *O. tuberosa* could either have remained "fossilised" in a functioning state, or alternatively accidental selection by the cultivators could have led the flowers to evolve towards homostyly or self-fertility or sterility. The latter possibility is raised by HODGE (1951) who commented that oca does not appear to set seed and by LEON (1964) who noted that capsules are only rarely formed and that the flowers usually fall soon after opening. An understanding of the tristylous system in *Oxalis tuberosa* is of more than theoretical interest since any programme of tuber improvement by breeding experiments must begin with a study of the reproductive biology of the species.

Methods

Mass gatherings of flowers of *Oxalis tuberosa* were made of various varieties in cultivation at Cuyo Cuyo (Department of Puno) and Huancayo in Peru. The flowers were sorted into the long-, mid- and short-styled forms and a random sample of each type was measured for style length and the two anther levels. The results are set out in Table 1. Pollen grain diameters were also scored for these samples of flowers from Cuyo Cuyo and Huancayo and these pollen measurements for *O. tuberosa* are set out in Table 2. With facilities at Huancayo which were made available by the International Potato Centre a programme of controlled pollinations was carried out in which all of the 18 legiti-

Table 1. Mean length and standard error of mean (in mm) of the floral parts of trimorphic *Oxalis tuberosa* in cultivation at Cuyo Cuyo and Huancayo

Floral form		Number of flowers	Style length	Long-stamen length	Mid-stamen length	Short-stamen length
LONG	(1)	50	9.3 (0.06)	—	6.7 (0.03)	4.9 (0.03)
	(2)	25	9.3 (1.2)	—	5.4 (1.0)	4.0 (0.9)
MID	(1)	50	6.3 (0.06)	8.8 (0.06)	—	4.8 (0.04)
	(2)	25	5.8 (0.8)	8.3 (0.6)	—	3.8 (0.7)
SHORT	(1)	50	4.8 (0.08)	8.9 (0.07)	6.4 (0.06)	—
	(2)	25	4.0 (0.3)	7.9 (0.4)	5.6 (0.8)	—

(1) Cuyo Cuyo   (2) Huancayo

Table 2. Mean diameter and standard error of mean (in μ) for pollen grains from the two anther levels in trimorphic flowers of *Oxalis tuberosa* in cultivation at Cuyo Cuyo and Huancayo*

Floral form		Long-level anther	Mid-level anther	Short-level anther
LONG	(1)	—	45.3 (2.84)	39.7 (2.53)
	(2)	—	43.8 (3.45)	39.2 (3.43)
MID	(1)	53.2 (3.33)	—	41.2 (2.64)
	(2)	53.3 (0.88)	—	40.8 (0.42)
SHORT	(1)	55.4 (7.99)	44.3 (6.77)	—
	(2)	51.6 (4.46)	44.2 (1.96)	—
(1) Cuyo Cuyo	(2) Huancayo			

* Each figure represents a mean of means derived from counts of 100 pollen grains per anther from ten flowers of each type at each locality.

Differences between mean pollen diameters for different anther levels in the same flower type are significant; differences between mean pollen diameters for the same anther level in different flower types are not significant (p = 0.05).

mate, illegitimate and own-form pollinations which are possible with a tristylous breeding system were included.

It was found that the "cut-stem" technique commonly employed in experimental pollinations in potato studies could be readily used with *Oxalis tuberosa*. With this technique flowering stems are cut and kept in jars of water so as to facilitate pollination manipulations and the maintenance of the material until fruits are mature. Flowering stems were gathered in the early morning and subsequently arranged in jars according to flower type. Suitable young flowers were emasculated and pollinated with an appropriate donor by rubbing the stigmas directly with a dehiscing anther. Pollinated flowers, together with a number of emasculated but unpollinated flowers as controls were enclosed in light-weight bags of surgical gauze and maintained in screen frames until fruiting. The water in the jars was flushed out and topped up daily to minimise stem decay. The results of the experimental pollinations are set out in Table 3.

In addition a quantity of cut flowering stems was taken to Lima and a further series of 150 pollinations were carried out in the screen house at the International Potato Centre, La Molina. Unfortunately, probably as a consequence of the warm temperature prevailing at the coast, all the capsules of this series dropped within $5-8$ days following pollination.

Observations of pollen and stigma morphology were made by means of a Cambridge Scientific Instrument Co. stereoscan 600. Pollen grains from flowers preserved in alcohol were dried on to fragments of cover glass glued on a stub, and coated with approximately 150 Å layers of carbon and gold in a conventional vacuum coating unit. Styles and stigmas were first prepared by critical point drying (ANDERSON 1951) before coating.

Discussion

1. Trimorphy in style and stamen length

The data presented in Table 1 indicates that the short-, mid- and long-stamen lengths accord with a high degree of precision to the three style lengths in the three flower forms of *Oxalis tuberosa*. EITEN (1963) devised an index to estimate the degree of morphological heterostyly in species of *Oxalis* sect. *Corniculatae* which consists of computing the ratio of the distance of the stigma level from the nearest anther

Table 3. Results of controlled pollination experiments with *Oxalis tuberosa*

cross	total flowers	number of flowers with capsules	total seeds	% flowers with capsules	average seeds per capsule	average seeds per flower pollinated	% seed germination
LONG × long/SHORT*	28	28	220	100	7.9	7.9	13
LONG × mid/SHORT	28	10	22	35.7	2.2	0.8	0
LONG × long/MID	20	18	86	90	4.8	4.3	14.2
LONG × short/MID	20	4	16	20	4.0	0.8	0
MID × mid/SHORT	26	26	252	100	9.7	9.7	28.8
MID × long/SHORT	18	8	26	44.4	3.3	1.4	0
MID × mid/LONG	22	22	210	100	9.5	9.5	3.5
MID × short/LONG	16	6	16	37.5	2.7	1	0
SHORT × short/LONG	20	20	114	100	5.7	5.7	3
SHORT × mid/LONG	22	6	24	30	4.0	1.1	0
SHORT × short/MID	22	22	122	100	5.5	5.5	6.9
SHORT × long/MID	14	—	—	—	—	—	—
LONG × mid/LONG	18	8	16	44.4	2	0.9	0
LONG × short/LONG	24	4	28	16.7	7	1.2	7
MID × long/MID	16	8	20	50	2.5	1.3	0
MID × short/MID	18	4	12	22.2	3.0	0.7	0
SHORT × long/SHORT	20	4	6	20	1.5	0.3	0
SHORT × mid/SHORT	24	14	46	58.3	3.3	1.9	0

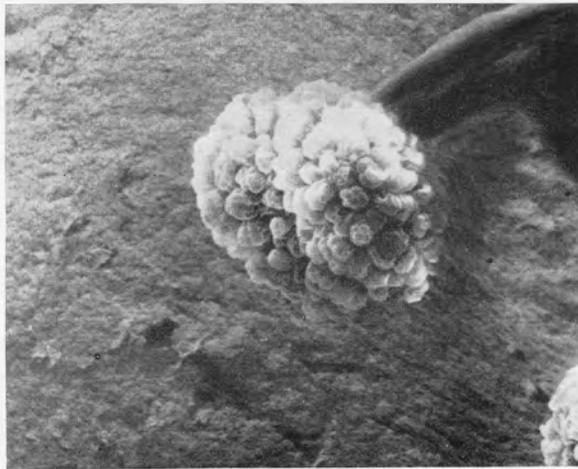* LONG × long/SHORT = long-style pollinated with pollen from the long anther of a short-styled flower etc.
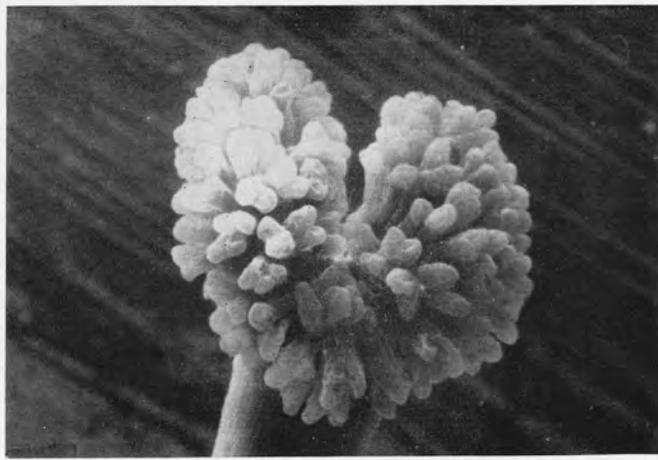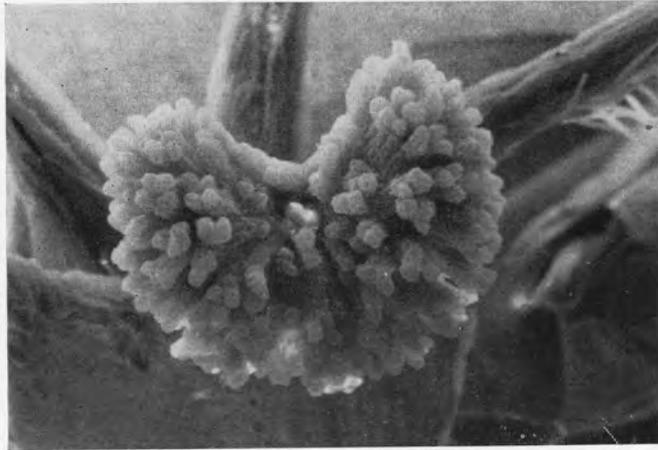
Fig. 1—3. stigmatic heads in *Oxalis tuberosa*. Fig. 1. long-style; Fig. 2. mid-style; Fig. 3. short-style (×60).

level to the length of the longest internal part of the flower (i.e. long anther or long style according to the flower type).

The index of heterostyly for *Oxalis tuberosa* at Cuyo Cuyo (i.e. the mean value for the 150 flowers measured) is 0.24. This compares with values for species of *Oxalis* sect. *Corniculatae* of 0 (for homostylous species) to 0.30 for "strongly heterostylous species" with an exceptional value of 0.60 for the species *O. stricta* (EITEN 1963). *O. tuberosa* can be regarded, therefore, as a distinctly heterostylous species and there appears to have been no erosion of trimorphy in flower form.

2. Pollen

Pollen grain measurements for *O. tuberosa* are summarised in Table 2. They agree with data available for a number of species of *Oxalis* in that there is trimorphy in pollen grain size with the largest grains produced by the long-level anthers, intermediate sized grains by the mid-level anthers, and the smallest grains by the short-level anthers. The differences in pollen grain diameters correspond to the three types of stigmatic head and papillae size found in the three style lengths (HILL 1939) such that the long-style has a rather spreading, deeply cleft stigma of long, loosely packed papillae, the mid-style has a bilobed head of shorter papillae, whilst the short-style terminates in a globose head of densely packed papillae (Figs. 1—6).

The heteromorphy in pollen grain size represents an interesting example of parallel evolution since it is also found in the other tristylous genera *Lythrum* and *Pontederia* as well as in some distylous genera such as *Primula, Linum, Jepsonia* etc. Presumably this character has evolved independantly in these diverse groups in response to a common selection pressure to minimise contamination with self pollen. The most likely source of such self pollen will originate in a downward direction from high level anthers as the insect vector thrusts into the flower. With the arrangement found in many heteromorphic flowers this will bring "large" pollen grains down onto "small" stigmatic papillae with consequent diminished chances of adherence.

Overall, the mean diameters of pollen grains from the same anther level in different flower types in *Oxalis tuberosa* are very similar, whilst the mean diameters of grains from different anther levels in the same flower type are significantly different. These clear differences in pollen grain size are similar to those found by HILDEBRAND (1871) and DARWIN (1877) for several species of *Oxalis* including *O. valdiviensis* but they contrast with the situation reported by ORNDUFF (1964) for *O. suksdorfii* in which there is little difference in size between pollen from the mid- and short-level anthers.
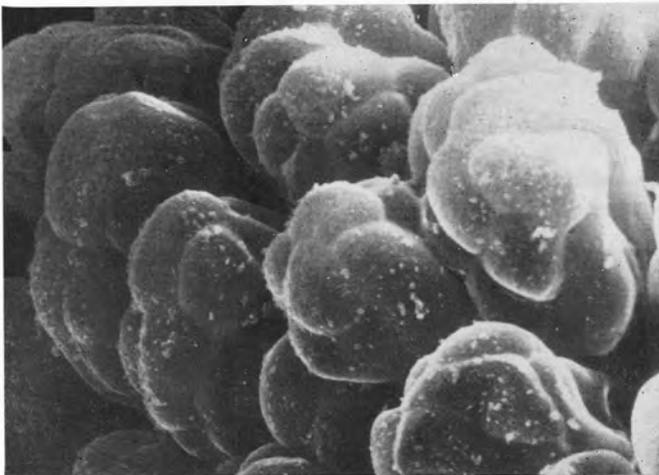
Considerable variation in the percentage of sterile or abhorted grains was encountered ranging from 2—79 %. There appear to be no clear cut correlations between anther level or flower type and pollen sterility and until meiotic studies can be carried out the cause for such sterility is not known.
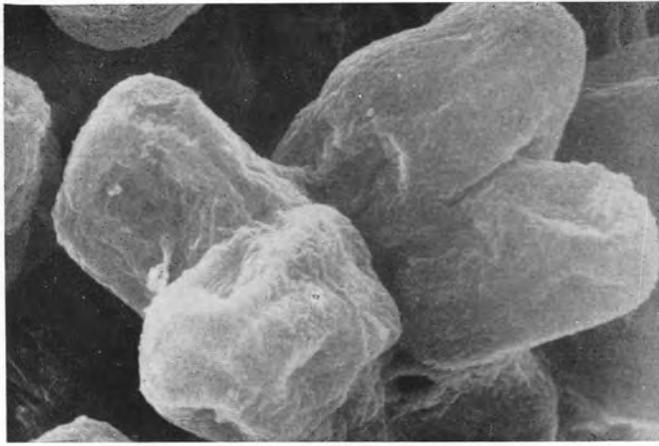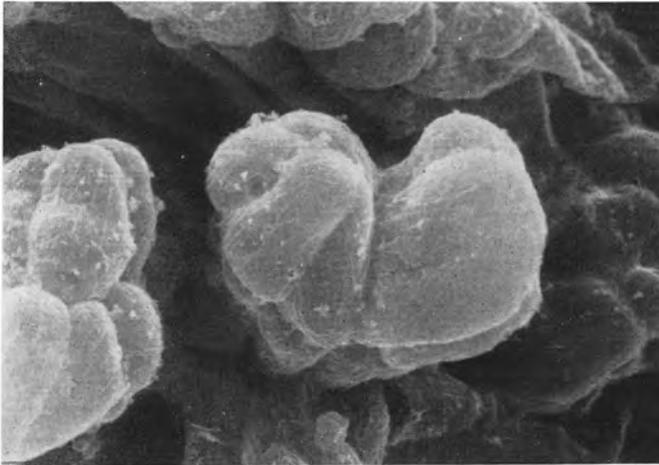
Fig. 4—6. stigmatic papillae in *Oxalis tuberosa*. Fig. 4. long-style; Fig. 5. mid-style; Fig. 6. short-style (×750).

3. Compatibility reactions

The results of the pollination experiments set out in Table 3 clearly suggest that in addition to the morphological components of the tristylous breeding system *Oxalis tuberosa* also retains a physiological pollen-stigma reaction which favours outcrossing. Thus, 100 % capsule formation in pollinated flowers together with relatively high seed set only occurred in the six legitimate pollinations, Viz. LONG×long/SHORT and LONG×long/MID, MID×mid/LONG and MID×mid/SHORT, SHORT×short/ LONG and SHORT×short/MID. The seed yields in these legitimate crosses are certainly not high with an average of 7.4 seeds/capsule[1]) but this compares with an average seed set of only 2.9 seeds/capsule from all the other types of pollinations. Furthermore, although the quantity of seed available for sowing was limited so that the results achieved for percentage germinations must be interpreted with caution (seeds of *Oxalis* species can show poor germination) it is probably not entirely without significance that, with one exception, germinations were only achieved with seed resulting from legitimate pollinations.

No differences were observed in the results for seed set from self-v e r s u s own-form pollinations and these results were combined in Table 3. The seed set following illegitimate and self or own-form pollinations are fairly uniform averaging 2.7 and 3.2 seeds/capsule respectively. Clearly, there is some breakdown in both self-compatibility and also following cross illegitimate pollinations but the level of breakdown which emerges from this series of pollination with *O. tuberosa* is similar to that reported by DARWIN (1877) for *Oxalis valdiviensis* and *O. speciosa* and much less than that noted by ORNDUFF (1964) in *O. suksdorfii*.

It is interesting to note that the pollinations which a p r i o r i one might expect to result in poor set on the basis of the greatest discrepancy in pollen-stigma sizes, i.e. SHORT×long/MID and SHORT×long/SHORT, do in fact have low yields of 0 and 1.5 seeds/capsule respectively. In these cases it is possible that the physical problems encountered by the largest pollen grains being deposited on the smallest stigmatic heads may reinforce any physiological incompatibility mechanism. However, although the converse pollinations LONG×short/LONG and LONG×short/MID resulted in above average seeds set (i.e. for non-legitimate pollinations) there is overall no correlation between pollen grain size, stigmatic papillae size and seed yield, but perhaps it would have been surprising if this had proved to be the case when pollination was effected artifically by rubbing pollen-laden anthers directly onto the stigmas.

Conclusions

It is not possible to give any precise datings for the origins of *Oxalis tuberosa* as a cultivated plant in the highlands of Peru or Bolivia. The earliest recorded evidence

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1) *Oxalis tuberosa* has a 5-loculed ovary with c. 5 ovules/locule so that there is a potential seed set of c. 25 seeds. However, LEON (1964) reports that oca capsules usually have 5—15 seeds and these observations were presumably made on open pollinated flowers.

seems to be the decorative patterns on pottery of the Tiahuanaco — Nazca style from the Central Peruvian highlands near Ayacucho. These ceramic works date from c. 1000 AD and they clearly depict oca as well as other tuber-bearing plants (HAWKES 1967). However, the cultivated origin of *Oxalis tuberosa* is clearly very much more ancient as is evinced by the widespread geographical distribution of this species in South America, the apparent loss of the wild-type ancestral species, and the diversity of tuber cultivars.

When the early inhabitants of the Andean uplands began to cultivate *Oxalis tuberosa* they would have had an outbreeding species and hence one with a high genic heterozygosity from which the rich variety of tuber types could be culled and fixed by preferential selection. With continued asexual propagation by means of seed tubers the rate of cultivar diversification could be expected to slow down. However, despite its long history of cultivation *O. tuberosa* seems to have retained its tristylous outbreeding system virtually intact. The conspicuous yellow flowers are freely visited by insects and it has been observed that the plants in cultivation at Cuyo Cuyo and Huancayo do set capsules. Whether seedlings from such presumably out-crossed seed can occasionally survive with the primitive methods of soil preparation employed in the Andean terrace systems is unknown, but if so, such "rogue" plants could contribute sporadically to the continued heterozygosity of the germplasm in cultivated *Oxalis tuberosa*.
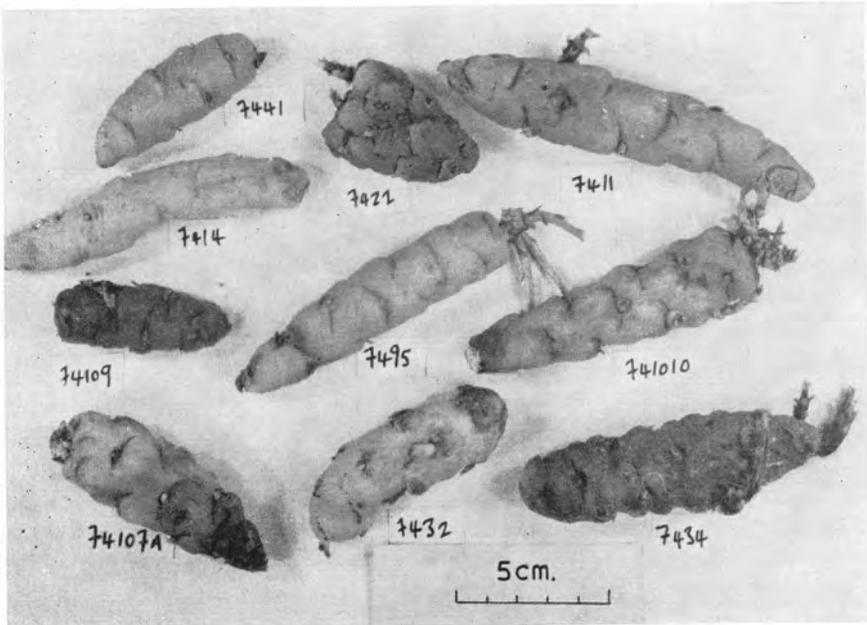

Fig. 7. A selection of cultivar varieties of *Oxalis* tubers.

Acknowledgements

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