

Asexual populations of the invasive weed *Oxalis pes-caprae* are genetically variable

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***Oxalis pes-caprae*, a native of South Africa is a very successful, globally widely distributed, aggressive colonizer weed. It spreads vegetatively (through bulbils and underground shoots) in most of its colonized areas. In Israel, it is characterized by the total lack of seed set. Using 121 amplified fragment length polymorphism (AFLP) putative loci we have analysed the genetic structure of five representative populations in Israel. Despite the complete lack of sexual reproduction and the total dependency on vegetative dispersal, populations were found to be genetically variable. Surprisingly, considerable genetic diversity was found within and between all of the populations tested. We speculate that this diversity could be the result of an accumulation of mutations or genome rearrangement.**

Keywords: vegetative reproduction; dispersal; tristylly; amplified fragment length polymorphism; pentaploidy

1. INTRODUCTION

One of the ideal characters for a weedy species is rapid and vigorous vegetative reproduction and dispersal (Baker 1965). However, if asexuality is its sole mode of reproduction it is expected that genetic diversity in the vegetative reproducing populations would be little or none (Fay *et al.* 1999; Rottenberg *et al.* 1999; Hollingsworth & Bailey 2000). This study reports on the considerable genetic diversity found in ostensibly clonal populations of an asexual vegetatively spreading weed.

Oxalis pes-caprae L. (syn. *O. cernua* Thunb.) is a native of the Cape, South Africa, where wild populations thrive but weedy races are also found. It is tristylous (Ornduff 1974, 1987), that is, three different flower morphs occur (figure 1): (i) long style (with mid-length and short anthers); (ii) mid style (with long and short anthers); and (iii) short style (with long and mid-length anthers; see Dulberger 1970; Barrett 1993). Legitimate pollination occurs between individuals of different floral morphs, hence ensuring cross-pollination.

Oxalis pes-caprae has spread and colonized large parts of the Mediterranean basin and many other areas worldwide, especially southern Australia, California and South America. In its native habitat in South Africa all three flower morphs usually coexist resulting in seed set (Ornduff

1974, 1987); these sexual forms are either diploids $2n = 2x = 14$ or tetraploid $2n = 4x = 28$. Aggressive, invasive weedy populations consist of individuals of a single type that is pentaploid $2n = 5x = 35$ and have a short style (Baker 1965; Ornduff 1987). For an unknown reason, in most of the invaded areas only the pentaploid short-style morph occurs (Baker 1965; Ornduff 1987). In these areas this invasive species is remarkably successful, manifesting high adaptability to a wide range of different habitats and is quite often a noxious weed (Baker 1965). Indeed, most of the literature regarding this species deals with the large efforts to eliminate it from cultivated fields and gardens (reviewed by Peirce 1998). *Oxalis pes-caprae* arrived in the central Mediterranean Basin towards the end of the eighteenth century (Henslow 1891; Clarke 1934) spreading eastwards and reached Israel ca. 1906 (Galil 1968; Dafni & Heller 1990). Populations in Israel (as in most of its colonized areas) are pentaploid, short-style only and characterized by strong vegetative reproduction through bulbils as there is no seed set (Galil 1968; Ornduff 1987; Dafni & Heller 1990; Danin 1990; R. Dulberger, personal communication). This inability to produce seed is related to its odd ploidy, which is furthermore strengthened by the presence of only the short-style morph (which implies illegitimate pollination only). As a result of these two components, sterility of this species is full here, and it has never been seen to set a single fruit or seed.

The question then arises whether populations of this asexual colonizer would be found to be genetically uniform. This cannot be taken for granted, as there are few, but established cases of vegetatively reproducing (functionally asexual) populations of other species that were reported to be genetically variable (Ford & Richards 1985; Tuskan *et al.* 1996; Rottenberg *et al.* 2000). Thus, the aim of this study was to test the genetic diversity in populations of this functionally sterile noxious weed.

2. MATERIAL AND METHODS

(a) Populations examined

Five representative populations in Israel were examined (table 1). These populations comprise pentaploid, short-style individuals only and have never been seen to contain other style morphs, neither to set a single seed. To confirm these sterility observations, 10 plants of each population were manually cross-pollinated, but again, no fruits or seeds were observed (it is worth mentioning that legitimate pollinations of tetraploid, all-styles plants from South Africa do set viable seeds; A. Rottenberg, unpublished data; Ornduff 1987). In all five populations, plants were sampled along transects, with 2–3 m between samples. A few leaves from each plant were dried in silica gel and then frozen at $-20\text{ }^{\circ}\text{C}$ until DNA extraction.

(b) Amplified fragment length polymorphism procedure

DNA was extracted using a Nucleon Phytopure extraction kit. Amplified fragment length polymorphism (AFLP) analysis was performed as described by Van Der Merwe *et al.* (2000). An overnight *MseI*-*PstI* restriction and, in addition, adapter-ligation in the presence of restriction enzymes were performed to ensure complete restriction to avoid possible artefactual products (Mueller & Wolfenbarger 1999). Following a screening of 12 primer sets, three primer pairs were chosen for the analysis: *MseI*.2/*PstI*.1, *MseI*.3/*PstI*.1 and *MseI*.3/*PstI*.2 (*MseI*.2, 5'-GATGAGTCCTGAGATAACA-3'; *MseI*.3 5'-GATGAGTCCTGAGTAAAGC-3'; *PstI*.1 5'-GACTGCGTACATGCAGAC-3' and *PstI*.2 5'-GACTGCGTACATGCACCA-3'). In a reproducibility test, duplicates of four different individuals were sampled. Three of them were identical in all bands to their standards and one differed in a single band (98.4% identity).

(c) Statistical analysis

Bands were scored as present or absent. Only strong reproducible bands ranging between 70 and 260 base pairs were scored. The

Table 1. Genetic variability in five *Oxalis pes-caprae* populations from Israel. (*N*, number of individuals tested; *H*, Nei's (1973) genetic diversity; *P*, percentage of polymorphic loci.)

locality	<i>N</i>	<i>H</i> (\pm s.e.)	<i>P</i>	remarks
Qiryat Tivon	12	0.31 \pm 0.18	80.9	small stands in different domestic gardens of a rural village
Oranim	31	0.31 \pm 0.17	87.6	a huge population in a botanical garden
Nahal Oren	27	0.27 \pm 0.20	71.0	slightly disturbed natural habitat (stream banks)
Miqve Yisrael	24	0.26 \pm 0.19	70.2	one of the oldest populations in Israel (<i>ca.</i> 100 years), in a grove
Jerusalem	14	0.07 \pm 0.16	17.3	a very young urban population in a colder climatic zone

Table 2. Genetic identity between the five tested populations of *Oxalis pes-caprae*.

	Jerusalem	Miqve Yisrael	Nahal Oren	Oranim
Qiryat Tivon	0.75	0.88	0.86	0.88
Oranim	0.73	0.90	0.85	
Nahal Oren	0.73	0.85		
Miqve Yisrael	0.77			

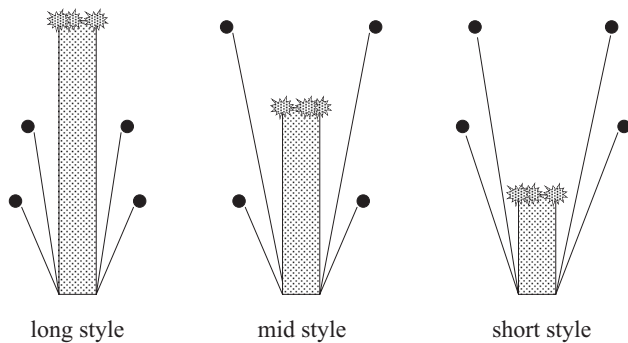


Figure 1. The three floral types of the tristylus *Oxalis pes-caprae* (styles and stigmas are dotted, anthers are black). Legitimate pollination is between different floral types.

resultant data matrix was analysed using the popular POPGENE software (Yeh *et al.* 1999) for population genetic diversity and genetic distances between populations (Nei 1973). An AMOVA statistical program (based on Excoffier *et al.* 1992) was used for analysis of molecular variance.

3. RESULTS AND DISCUSSION

The most prominent finding in this study is the existence of genetic variability in the functionally sterile, vegetative reproducing populations of *O. pes-caprae* in Israel (table 1). A total of 121 strong reproducible bands was revealed from the three primer combinations, of which 107 were polymorphic. No bands were found to be uniquely associated with any single population. Considerable genetic diversity was found within and between all populations tested (table 1). The possibility that this observed genetic diversity is artefactual must be ruled out as AFLP, a highly reliable technique (Mueller & Wolfenbarger 1999), has already been successfully applied to other species of *Oxalis* (Tosto & Hopp 2000). In addition, in this study, the recommended overnight DNA digestion (Mueller & Wolfenbarger 1999) was performed, to eliminate the possibility of producing artefacts resulting from incomplete restriction, and reproducibility was as high as 98.4%.

Genetic diversity in four populations ranged between $H=0.26$ and 0.31 (table 1) with the percentage of polymorphic loci between $P=70.2\%$ and 87.6% . The Jerusalem population by contrast manifested much lower values of $H=0.07$ and $P=17.3\%$ and accordingly it was found to be genetically distant from the other populations (table 2). Indeed, the Jerusalem population deserves closer examination; this population is very young. It was established in the Nayot neighbourhood 5–6 years ago and since then it has been spreading. Regarding this fact, the existence of any genetic diversity at all is surprising. It is worth mentioning that this low variation also rules out the possibility of artefactual results in this study; if the variation was an artefact of the technique then one should see just as much variation in the Jerusalem population as in the other populations.

Inter-population genetic identity was high (table 2), typical if within-population diversity is also high. In addition, AMOVA (Excoffier *et al.* 1992) showed that 77% of the diversity is attributed to within populations and 23% to between populations. These values are close to those reported in the tristylous, sexual, seeding species *Oxalis corniculata* L. (Shibaike *et al.* 1997).

A functionally sterile population that manifests genetic diversity is a very rare phenomenon but not unknown. Given the absence of sexual reproduction one might generally assume that population would be clonal and hence genetically uniform. This could be true but not necessarily so. Studies involving vegetatively reproducing populations in different species manifested various results; some of them were found to be genetically uniform (Fay *et al.* 1999; Rottenberg *et al.* 1999; Hollingsworth & Bailey 2000). By contrast, other ostensibly clonal populations were found to be genetically diverse (Ford & Richards 1985; Rottenberg *et al.* 1999, 2000). In these last cases (as well as the present case study), the inevitable question arises as to the sources of this observed genetic diversity. Indeed, if genetic diversity in a given asexual population is low and comprised only very few identified genotypes and their duplicates, there is always the possibility of

multiple introductory events of different genotypes. This should be disregarded, however, in cases where considerable genetic diversity was found. Therefore, it is speculated that at least two major evolutionary forces could be involved in this unexpected diversity: mutations and genome rearrangement. One may assume a single ancestor reproducing vegetatively for long periods. Neutral mutations will thus spontaneously occur and in the absence of sexual reproduction and selection will not be eliminated. Thus, the actual genetic variability is a reflection of somatic mutations accumulated for a very long time (Whitham & Slobodchikoff 1981; Tuskan *et al.* 1996). In addition, genome rearrangement is believed to be a common source for genetic diversity in polyploids (Soltis & Soltis 2000). This could be achieved, for example, by somatic recombination (mitotic homologous cross-over; reviewed by Puchta *et al.* 1994). If it happens in a meristematic cell that will later develop into a dispersing organ, such as a bulb, then 'progenies' will segregate. However, we emphasize that these are still mere speculations. If confirmed, it would point to the presence of an additional way to recombine and mould genetic variation in plants. Generally speaking, one also has to consider very rare sexual reproduction events as a source of variation. However, in the case of *O. pes-caprae* in Israel this must be strictly ruled out as not a single seed was ever observed here, both in controlled experiments (see § 2) and in nature (A. Rottenberg, personal observation; D. Zohary and R. Dulberger, personal communication; Galil 1968; Dafni & Heller 1990; Danin 1990).

The tremendous success of the asexual weed *O. pes-caprae* is a great mystery. This ambiguity is further increased, as only its pentaploid short-style type plays a role as the aggressive colonizer. It raises questions regarding the evolution, adaptability and dispersal, as well as to the control of invasive weeds. Thus, further studies of native and worldwide-invasive populations are necessary to shed more light on the mechanisms that bring about and maintain genetic diversity in this enigmatic species, and how these are related to its remarkable capacity for invasion, colonization and becoming such a successful weed.

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