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Do all existing *Sophora toromiro* descend from one individual?

MARCIA RICCI
Jardín Botánico Nacional, CORPORACION NACIONAL FORESTAL, Casilla 317,
Viña del Mar, Chile

LAFAYETTE EATON
Dept. Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile,
Casilla 653, Santiago, Chile

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Sophora toromiro is now extinct in its natural habitat, Rapa Nui (Easter Island or Isla de Pascua). The only living individuals of the species are in a few Botanical Gardens and private gardens. Efforts are being made to reintroduce this small leguminous tree to the island. In this contribution, we report several adult trees not previously noted, trace the origin of each, and examine the genetic variability among the adult Chilean individuals and their offspring. It is probable that all living trees are self pollinated offspring of the last individual which grew in Rapa Nui. Genetic variability is limited and is likely to be concentrated in homozygotes, although we observed non-random segregation in one variable isozyme system. Efforts must be made to cross among surviving individuals, in order to recombine the remaining variability in the trees which are reintroduced to the island.

Keywords: *Sophora toromiro*; Rapa Nui; Easter Island; isozymes; conservation.

Introduction

Rapa Nui (Easter Island or Isla de Pascua), of volcanic origin, is located in the Pacific Ocean (27°09′S and 109°26′W); it lies approximately 2300 miles (3760 km) W of mainland Chile and 1260 miles (2000 km) SE of the closest other inhabited island, tiny Pitcairn. It is more than 2515 miles (4000 km) from Tahiti and 4300 miles (7000 km) SE of Hawaii. The island has a triangular area of 166 km^2; the landscape is dominated by the three major extinct volcanoes (Rano Kao, Rano Aroi and Poike), and mount Maunga Terevaka (523 m). The climate is subtropical-oceanic with a mean temperature of 20°C and an average annual rainfall of 1370 mm (Hajeck and Espinoza, 1987).

Rapa Nui was settled around AD 300 by Polynesians, probably from the Marquesas Islands (Lee, 1989). The island was rediscovered in 1722 by Jacob Roggeveen and in 1770 Felipe González arrived and claimed the island for Spain. After 1800, whalers began stopping on the island and left behind venereal diseases. In 1860, slave raids carried off to Peru some 2000 islanders. By 1877, only 111 inhabitants remained from a postulated original population of 10 000 (Lee, 1989).

Rapa Nui has an impoverished flora; the island is predominantly grassland. There are five endemic species of angiosperms, one of them known only from the fossil register (Zizka, 1991) and five species of fern (Rodríguez, 1990). After Skottsberg (1922), Zizka (1991) has provided the most recent compilation of the flora of the island, in which he lists 171 flowering plants including five endemic species, 25 indigenous species, 67 species

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inadvertently brought by man and established, and 74 species cultivated by the islanders.

One of the principal characteristics of the flora of Rapa Nui is the degree to which man has influenced its composition. The majority of the land has at one time been cleared for agriculture. Introduced herbs compete with native species, while the introduction of cattle and other domestic animals has produced considerable grazing pressure which introduced species resist better than indigenous ones. The endemic species of herbs are all endangered (Zizka, 1991).

Of the two indigenous woody species, *Paschalococos disperta* (Arecaceae = Palmae) is long extinct while *Sophora toromiro* (Leguminosae), whose wood was used for handicraft, disappeared from the island about 1960 and was thought to be found only in botanical gardens (Orliac, 1990).

Since 1965 there have been 13 attempts to reintroduce *Sophora toromiro* to Rapa Nui, seven of them using Chilean material and four using European material, plus two that were later found to belong to another species (Bordeu, 1994; Maunder, 1995). Since this tree has mythical significance for the Pascuense people these efforts are of particular interest. The gene pool of this species has been drastically reduced. It is therefore of prime importance to utilize all possible sources of genetic variability in the individuals in a reintroduction programme, and to insure that all individuals really belong to the taxon *S. toromiro*.

In this contribution, we examine the genetic variability of the existing adult individuals of *S. toromiro* in Chile and demonstrate that all living individuals most probably descend from the last tree which grew on the island.

Existing individuals of *Sophora toromiro*

Earlier papers indicated that the only remaining individuals of *S. toromiro* were in the Botanical Gardens of Göteburg, Bonn and Viña del Mar, Chile (Alden, 1982; Lobin and Barthott, 1988; Lobin, 1990). However, we have located four other individuals in private gardens in Chile. We shall identify these trees by the names of their owners, Mr Behn, Ms Schick, Ms Sudzuki and Mr Titze, and that of the Chilean National Botanical Garden by its acronym, VINAD.

Materials and methods

We collected leaves of trees identified as *S. toromiro* from the private gardens of Mr Titze, Ms Schick, Mr Behn and Ms Sudzuki, from the 'mother tree' of VINAD in Viña del Mar, plus 44 offspring of the VINAD tree and 13 from Titze. We used leaves from one individual of *S. macrocarpa* and from one *S. microphylla* as controls and for comparison. We later made a separate run with the VINAD mother tree and 33 of her offspring, with a few modifications in the methods as noted below.

Leaves were macerated in buffer Tris HCL 0.05 M, pH 6.7 with the following additives (to 25 ml of buffer): 1.75 g of soluble PVP-40, 250 mg of soluble PVP-360 (second run); 2.56 g sucrose; 4 mg sodium EDTA; 4 mg dithiothreotol; 5 mg sodium ascorbate; 25 mg bovine albumin and one drop of 2-mercaptoethanol. We performed starch gel electrophoresis, using the techniques described in Conkle *et al.* (1982), staining for 15 enzyme systems (listed in Results). In the second run we added 1 mg of methylumbelliferyl butyrate to the fluorescent esterase stain and 40 mg of Fast Black K to the acid phosphotase stain. Chemical products were obtained from SIGMA. Banding patterns of variable enzymes were given a presumptive Mendelian interpretation.

The proportions of enzymes which were variable in progenies were compared by G tests (Sokal and Rohlf, 1995) to frequencies expected with self-pollination for the offspring of VINAD and Titze.

Results

We obtained more or less adequate staining in all 15 systems tested. The great majority were invariant in *S. toromiro*. No variation was expressed for the following systems (number of isozymes in parentheses): CAT (1); Fl-EST (2); GDH (1); IDH (2); LAP (1); MNR (3); 6-PGD (3); SKDH (2); TPI (1).

Possibly variable systems include AAP (1), which exhibited very little separation and AAT [GOT] (2), which exhibited irregular staining and little separation.

MDH expressed five bands below in almost all individuals, and two more above. 'Schick', 'Titze' and 2 of the 13 'Titze offspring' are different, possibly heterozygous for a second allele which runs more slowly. PER presented three isozymes, one anodal and one just on the cathodal side of the origin (both invariant), while the third, more cathodal, segregated with two alleles (monomeric). PGI showed two isozymes, of which the slower was dimeric with two alleles. ACP stained with Fast Garnet GBC showed one isozyme (ACP_2). The addition of Fast Black K revealed a second isozyme; the faster ACP_1 monomeric and variable with 2 alleles, the slower ACP_2 streaked and probably invariant. Table 1 gives the putative genotypes for PER_3 and PGI_2 from the second run.

The segregation observed in Table 2 for PER_3 in VINAD is clearly not compatible with the 1:2:1 expected from self-pollination. The second run confirmed the notable lack of

Table 1. Electromorphs (genotypes) of individuals of *Sophora toromiro*

	Adult trees	
	PER_3	PGI_2
VINAD	12	22
Titze	12	12
Behn	12	11
Sudzuki	12	12
Schick	22	22

Table 2. Segregation of PER in offspring

	Electromorphs			
Mother tree	11	12	22	
VINAD	4	19	21	$P < 0.01$
Titze	0	8	5	$P > 0.05$
VINAD-2°	2	18	13	$P < 0.02$

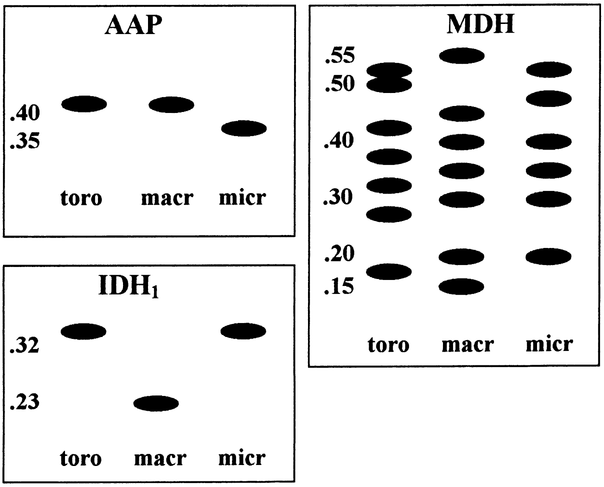

Figure 1. Schematic zymograms of three enzymes from three species of *Sophora*. **toro** is *S. toromiro*, **macr** is *S. acrocarp* and **micr** is *S. microphylla*. Numbers represent R_F values.

presumptive genotype 11. The same distortion is apparent in the offspring of Titze, although not significant because of the small sample size. We have no explanation for these results, some strong selection against allele 1 in each case must have acted to produce such distorted proportions. This presumed selection must have acted early in the reproductive cycle, since 95% of the VINAD seeds germinated and 100% established (Ricci, unpublished data).

There were clear and notable differences between *S. macrocarpa* and *S. toromiro* in nine systems (6-PGD, ACP, PGI, MNR, IDH, LAP, PGM, MDH and AAT). *S. microphylla* showed greater similarity to *S. toromiro*, but presented clear differences in MDH and LAP (Fig. 1). The banding patterns among the different individuals of *S. toromiro* were very similar in all systems, which is strong evidence that they all belong to the same taxon and that none of them is a hybrid with another species of *Sophora*.

Discussion

Human activity has largely been the determinant factor in the destruction of the flora of Rapa Nui (Zizka, 1991), including the extinction of *S. toromiro* in its native habitat.

The initiatives to reintroduce the species culminated in 1995 with the arrival of 65 plants from the Botanical Gardens of Bonn and Göteborg (Bordeu, 1994) and with the experimental introduction of nine offspring of the tree from the Chilean National Botanical Garden, thanks to a co-operative agreement between the Municipalities of Maipu (Santiago) and Rapa Nui (Fig. 2).

The similarities among the individuals of *S. toromiro* at isozyme loci contrast with the differences of the 'controls' *S. macrocarpa* and *S. microphylla*. This provides strong evidence that all the *S. toromiro* individuals studied are indeed *S. toromiro* and none are hybrids with the other species. Mr Titze gave seven seedlings of his tree to Rapa Nui in 1994, of which five are currently living in the nursery of the Isla de Pascua National Park.

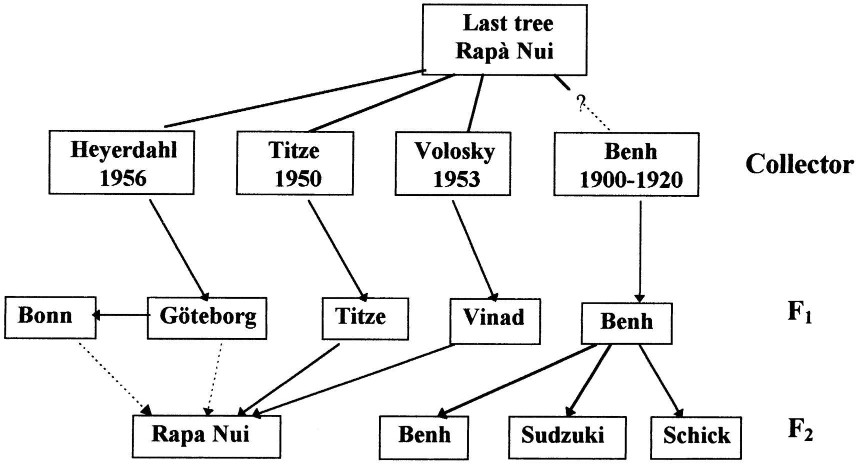

Figure 2. Genealogy of the known adult individuals of *Sophora toromiro*. The dotted lines represent proposed reintroductions to Rapa Nui. F_1 and F_2 represent generations (of self-pollination) beginning with the last tree of the island.

However, his *S. toromiro* grows next to individuals of *S. microphylla* and *S. fernandeziana* (personal observation), this had raised some doubt that the seedlings might be hybrids, however, our results make it very unlikely that these seedlings are hybrid.

Our results do not provide any evidence that the original collection of Mr Benh (grandfather) came from a different individual than the other collections. The variability in the three offspring of this tree is consistent with self-pollination of the last native tree, thus it is probable that the last tree was also the source of these individuals. The results presented here suggest that all existing *S. toromiro* do descend from one individual. However, the limited electrophoretic variability detected leaves doubts that could be clarified by DNA studies.

The extinction of *S. toromiro* has been avoided for the moment because of its ability to self-pollinate. But the strange segregations we observed argue that the offspring produced are not a random sample of the genome of the mother. All living adult individuals must be the products of one or two generations of selfing, the seedlings which are being reintroduced have gone through at least two generations of selfing. Allelic variation was observed, thus it is important to cross among them to produce offspring which recombine the limited remaining variability. Simply planting the selfed offspring of different trees together would postpone recombination until the next generation, it would be much better to make artificial crosses among the living trees and introduce these offspring to the island.

The plans of the *Sophora toromiro* Management Group (Alden, 1994; Maunder, 1995) include sending material from Europe to VINAD in order to have as many as possible of the remaining individuals together. The Chilean Forestry Service (CONAF) will sponsor a controlled cross pollination programme between the trees found in Chile, with the aim of maximizing the genetic variation of their offspring. These offspring will then be used in the reintroduction programme on Rapa Nui. *S. toromiro* is attacked by a large number of pathogens (Alden, 1994), maximizing potential variation may be critical to the continued survival of the species.

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