

EVOLUTION OF LOTUS CORNICULATUS S.L. POPULATIONS
IN THE MERCANTOUR (FRENCH PREALPS).

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ABSTRACT :

On the top of Mont Ventoux and of Montagne de Lure (French Prealps), grow diploid Lotus with a common flavonoid pattern named chemotype D. In the Mercantour, diploids belonging to a very close chemotype named D1, meet another chemical race named chemotype D2 and probably issued from inner Alps. The encounter of these two chemical types unable of hybridization gave birth, by autotetraploidisation and introgression, to tetraploids with a large variety of chemical patterns.

INTRODUCTION :

A previous study on Lotus corniculatus collected in Massif du Ventoux and Montagne de Lure (French Prealps) has evidenced in both mountains, and above 1700m, a population of diploids with a common polyphenolic profile named chemotype D (REYNAUD et al.). We have then hypothesized that these diploids (as other species, FAVARGER 1975, OZENDA 1985) had survived from a residual population that covered the whole area before the last glaciations and is nowadays scattered over various sites. Autotetraploidisation of the original diploid population in the Mont Ventoux would have given birth to tetraploids of the chemotype TV, whereas by allotetraploidisation it would have led in the Montagne de Lure to tetraploids of the chemotype TL, each displaying distinct populational or microgeographical variations. We concluded that its transformation into tetraploid populations had permitted Lotus corniculatus to develop new biogenetic pathways different from one mountain to the other (REYNAUD J. and JAY M., 1989).

After the last glaciations, Lotus corniculatus settled again in the Alps, spreading most certainly from outer shelter areas towards inner mountains. We therefore chose to investigate the populations growing in the North-east neighbourhood of Mont Ventoux and Montagne de Lure to measure by chemical criterion their differentiation level versus that of more southern populations. In the present study were retained populations of the Massif du Mercantour where 131 samples have been collected between 1300m (near Colmars) and 2300m (Col des Champs and Col de la Cayolle).

METHODS :

* Plant material

131 samples were collected in 13 different sites (Table 1) in July 1985, and July 1986, with some samples dating back to 1984.

* Chromosome Examination

Ploidy was determined either by chromosome counting on root meristems colored by the Feulgen technique, or by biometric measures performed on six morphological characters significantly correlated with the degree of ploidy (This study has been done by S.BLAISE and D.CARTIER, Faculty of Sciences, Paris Orsay).

* Phytochemical Study

Samples were stored individually in methanol after collection, then polyphenolic compounds were extracted and analysed by High Performance Liquid Chromatography.

To each individual corresponds a chromatographic profile which peaks are characterized by their retention time (here coded alphabetically) and surface. Each individual is thus defined by its flavonoid pattern where the x peaks detected are summed to 100 (JAY M. et al., 1989).

* Statistical methods

Chromatographic plots allowed to define 23 characters (named A to W) used in the further mathematic treatment.

A Principal Component Analysis was performed on the whole lot of diploid and tetraploid samples (136).

RESULTS :

1. Geographical distribution of both cytotypes

* Diploids (43 samples in 131) are mainly found in screes, as in the Mont Ventoux or in the Montagne de Lure, but they only grow above 2200m, which makes them more alike Lotus of the Swiss Alps (URBANSKA 1979), whereas diploid Lotus of the Ventoux-Lure area begins at 1500m.

* Tetraploids (88 individuals in 131) grow between 1300 and 2300m in sites where competition is sharp (closed meadows), their behaviour is the same as that of tetraploids from Ventoux and Lure.

2. Diploid versus Tetraploid metabolic differentiation.

The Principal Components Analysis of the 131 samples evidenced, on the 1/2 axis ordination (Fig.1), a clear differentiation, between, and into, the two cytotypes, supported by differences in their polyphenolic metabolism.

* Diploids appear as two distinct and relatively homogenous groups. The first cluster (28 samples) belongs to a chemotype named D1 (Fig.2) in which the phenolic plot is characterized by four major compounds, H, L, P (and Q).

In the second cluster (14 samples), a new chemotype is defined and named D2 (Fig.2), which metabolism is characterized by at least three new molecules : F, J and M. It must be noted that samples of both chemotypes share the same area : for instance, in area n° XIIbis (Col des Champs, 2250m), 10 samples out of 16 belong to chemotype D1, whereas 6 samples belong to the chemotype D2. This phenomenon is of particular interest since this is the first evidence of metabolic differentiation among diploid samples otherwise indistinguishable, neither in their morphology nor in their cyanogenesis (BLAISE and CARTIER, person.comm.).

* The 88 tetraploid samples form a large but continuous cloud (Fig.1). This continuity reflects a subtle regulation of phenolic compounds J and L between all individuals, otherwise this tetraploid collection shows a rich and common phenolic background (Fig.2). Their graphical image (and consequently their phenolic metabolism) is half-way between the two diploid chemotypes.

DISCUSSION :

The biosynthesis of phenolic compounds is stable since individual samples collected in a given area at one year interval, or transplanted to an experimental garden, yielded phenolic profiles almost identical to those of samples treated immediately after having been harvested.

Original results obtained for diploid Lotus of the Mercantour raise the hypothesis of the existence, in this given area, of two diploid populations, perfectly distinct in their polyphenolic metabolism though totally alike in their morphological aspect. So, the Mercantour and surroundings seem to be the place where diploid populations of two distinct geographical origins meet : western diploids, as in Ventoux-Lure, which gave birth to chemotype D1, and eastern diploids which led to chemotype D2 and might have found a shelter in the inner Alps. The encounter of the two different chemotypes at the Col des Champs would be due to the fact that this particular area is supposed to have been the limit of the ice fields during the last glaciations.

In that respect, the behaviour of tetraploids seems of particular interest as they appear to be half-way between the two metabolic patterns of diploids. Indeed, their particular history could be traced in two different ways :

- the first hypothesis is that these tetraploids were originally identical to those of the Ventoux-Lure area; they would have sprung from the autotetraploidisation of chemotype D1 and, while colonizing new spots, they would have been altered by introgressions from the second diploid family, thus allowing tetraploids to widen their genetic background and to modify their regulation in favour of compounds such as J and M.

- The second hypothesis is that tetraploids in the Mercantour have derived from two populations of tetraploids : one issued from D1 diploids of the Prealps, the second one coming from D2 diploids of the inner Alps. The hybridization between the two types would account for the relative homogeneity of their basic polyphenolic metabolism ; the particular selective pressures encountered in the different settings would have enforced a secondary metabolic differentiation, causing tendencies to occur in the L-J balance.

It is important to notice that D1 and D2 diploids, though growing in the same places, cannot produce hybrids : that may reveal the existence of an incompatibility barrier.

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TABLE 1 : NUMBER AND LOCATION OF THE SAMPLES COLLECTED

N°of samples	Date and place of harvest	N°stations
122 to 132 11 tetraploids	under col des Champs (West face) 1300m 1985	stat. IX
133 to 142 10 tetraploids	id. but 1480m 1985	stat. X
143 to 152 10 tetraploids	id. but 1800m 1985	stat. XI
153 to 162 (not 159 plus 0'85.1 and 2) 5 diploids, 6 tetraploids	col des Champs 2220m 1985	stat. XII
250 to 269 16 diploids, 4 tetraploids	id. but 2250m	stat. XIIbis
270 to 279 (10 samples) 1 diploid, 9 tetraploids	id. but 2200m	stat. XIIter
163 to 172 10 tetraploids	towards Var (East face) 1800m 1985	stat. XIII
173 to 182 10 tetraploids	id. before col de la Cayolle (south face) 2000m 1985	stat. XIV
280 to 287 (not 283 and 284) 3 diploids, 3 tetraploids	just before col de la Cayolle (South face) 2250m 1986	stat. XV
288 to 292 plus A84.1 and 2 7 tetraploids	id. 2250m 1986 (and 1984)	stat. XVbis
183 to 192 10 diploids	Col de la Cayolle 2310m 1985	stat. XVI
T1.85 to T9.85 (not T6.85) 8 diploids	1 Km after col de la Cayolle (North face) 2200m 1985	stat. XVIbis
293 to 300 8 tetraploids	3 Km after col de la Cayolle 2150m 1986	stat. XVII

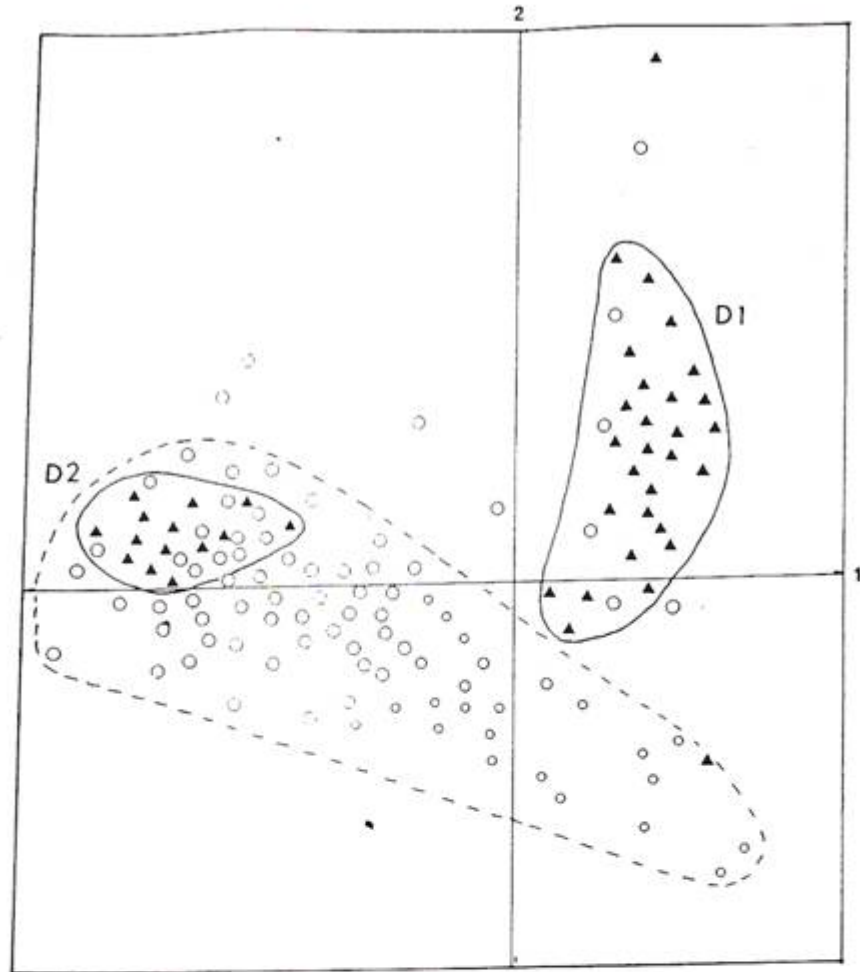


FIG.1. Principal Component Analysis (axes 1/2) of the 131 samples from Mercantour (French Alps).

▲ DIPLOIDS
○ TETRAPLOIDS



FIG.2.
Mean Phenolic Profiles