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FLAVONOID PATTERNS IN THE *KOELERIA CRISTATA* SPECIES COMPLEX

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IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Kœleria cristata*; *K. vallesiana*; Poaceae; flavonoid; chemotaxonomy; intraspecific variation.

Abstract—Flavonoid patterns were determined in 172 individuals representative of five cytotypes and 67 populations of *Kœleria cristata*. Five chemotypes emerged from a factorial analysis of individual flavonoid patterns. Chemotype was strongly correlated with cytotype and geographical origin. A study of the closely related *Kœleria vallesiana* showed this species to be chemically very different from *Kœleria cristata*.

INTRODUCTION

Kœleria cristata (*L.*) Pers. is a grass species complex with three usually recognized taxa [1]. This collective species shows a noteworthy polymorphism [2], the main components of which are as follows. *Kœleria cristata* encompasses a polyploid series of five cytotypes, ($2X = 2n = 14$; $2n = 28$; $2n = 42$; $2n = 56$; $2n = 84$) [3]. This species, restricted to calcareous soils, grows in various niches such as littoral sand dunes for some diploid populations or high mountain meadows for the octoploid cytotype. In low altitude sites subject to drought *Kœleria cristata* cooccurs with hexaploid *Kœleria vallesiana* (Honck) Bertol. a morphologically very similar species [4]. The plant is widely distributed in: Europe, North America [5] and the U.S.S.R. In France, this species is present everywhere except in the granitic massifs. Such characteristics make *Kœleria cristata* a biological model, the complexity of which calls for a multidisciplinary approach to the taxonomy. Chemotaxonomic approaches based on phenolic metabolism, especially flavonoids, have, already furnished interesting results in several studies on Poaceae [6–10].

RESULTS

Taxonomic organisation of Kœleria cristata *and* Kœleria vallesiana *on a flavonoid basis*

A phenolic profile obtained from HPLC analysis of 172 individuals, representative of 67 populations, shows 32 stable and easily recognized peaks or groups of peaks. These variables correspond to *O*-glycosides and *C*-glycosides of flavones, mainly apigenin and tricin, two principal flavonoid classes in Poaceae [11]. From these 32 peaks, a data matrix is obtained for further mathematical treatment; each line or individual plant is so defined by the relative amounts of each phenolic compound, the sum of which is equal to 100. Indeed our approach is as complete as fingerprinting. The data matrix was therefore submitted to a factorial analysis of correspondances (F.A.C) [12]. The location of *Kœleria* individuals on the F1 × F2 plane of this analysis is shown in Fig. 1. Significant discontinuities within the flavonoid variability define four groups in these species: Group 1 individuals share a characteristic chemotype (Fig. 1) based on peaks 27, 28 and 29, 30. All the samples are dodecaploids and inhabit a very large area: 10 populations from France as a whole. Group 2 individuals, defined by another typical chemotype (Fig. 1) are distributed along the positive part of axis 1. The extreme points of this cluster correspond to tetraploid populations (on the left) with relatively abundant compound 9, and diploid populations (at the right) with abundant compound 21, respectively. Diploid and tetraploid populations of this group 2 were collected in plains and hills of western France. In the mean part of this cluster are situated six triploid plants obtained by controlled hybridization between a diploid and a tetraploid individual belonging to the left or right part of the cluster; These triploids accumulate equally both compounds 9 and 21. Group 3 is constituted by octoploid individuals, the chemotype of which presents a strong peak 30, (Fig. 1). The flavonoid content of group 4 is far more heterogeneous as are ploidy levels of *Kœleria cristata*; five diploids, 55 tetraploids, 14 hexaploids and three octoploids. Moreover a peculiar chemotype clearly characterizes the representatives of *Kœleria vallesiana*. Diploid and tetraploid populations of this group were collected respectively in Switzerland and eastern France. Hexaploid and octoploid populations have a larger geographical origin.

Detailed study of diploids and tetraploids of Kœleria cristata *according to flavonoid criteria*

Diploids and tetraploids are present in ordination groups 2 and 4, but those of group 2 come from western France, while these of group 4 were collected in eastern France and Switzerland (Valais). Apparently, as was previously described, diploids and tetraploids are chemically differentiated in group 2 and not in group 4 where their

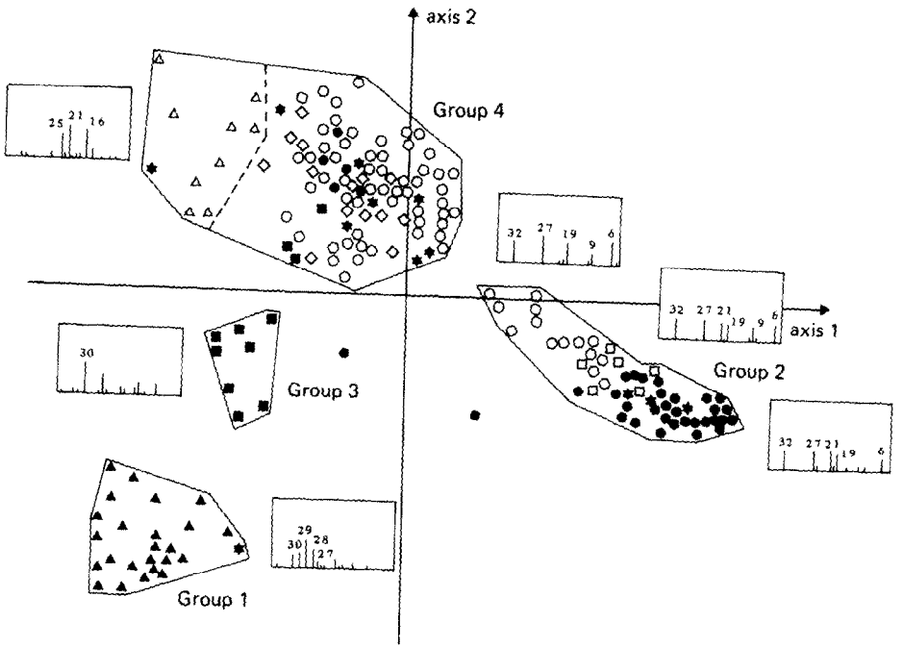

Fig. 1. ● Diploid cytotype; □ Triploid cytotype; ○ Tetraploid cytotype; ◇ Hexaploid cytotype; ■ Octoploid cytotype; ▲ Dodecaploid cytotype; ★ Unknown cytotype; Δ *Kœleria vallesiana*. Distribution on the F1 × F2 plane of a F.A.C. (45% of inerty) according to their flavonoid content of the 172 individuals of *Kœleria cristata* and 10 individuals of *Kœleria vallesiana*. For each group its characteristic chemotype is illustrated.

respective individuals are mixed together. Such observations suggest a strong structuration among the low ploidy levels.

Accordingly a special study of the diploid and tetraploid populations was carried out. In order to optimize our approach, we used a factorial analysis of correspondances on mean populational profiles, with subsequently projection of individual plants. This technique offers the advantage of a maximal stretching of inertia along the axes displaying at best the most subtle components of the phytochemical organisation.

The results appear in Fig. 2: two groups show up among diploids while the diploids and the tetraploids as a whole are divided into three significative clusters. Group A is identical to the previous group 2 corresponding to western diploids and tetraploids. Both groups B and C came from previous group 4; the division is based on geographical origin of plants. Therefore, populations originating from Franche Comté, Rhône Alpes and Provence (south eastern and eastern France) belong to group B, while samples from Alsace, Lorraine and Franche Comté (eastern and north eastern France) gave support to group C. This partition is chemically supported by the relative amounts of compounds 27, 28 (group B) and 32 (group C). Group C contains a diploid population collected in Switzerland (Valais). As pointed out in Fig. 3, a geographical demarcation divides the southeastern and northeastern *Kœleria cristata*.

DISCUSSION

Kœleria cristata displays a rich and complex flavonoid pattern. This phenolic diversity divides it into five chemotypes: the first two correspond to octoploid and dodecaploid cytotypes; two others are related to geographical origin: western diploids and tetraploids on one hand, eastern diploids and tetraploids (with addition of hexaploids and some octoploids) on the other. The fifth

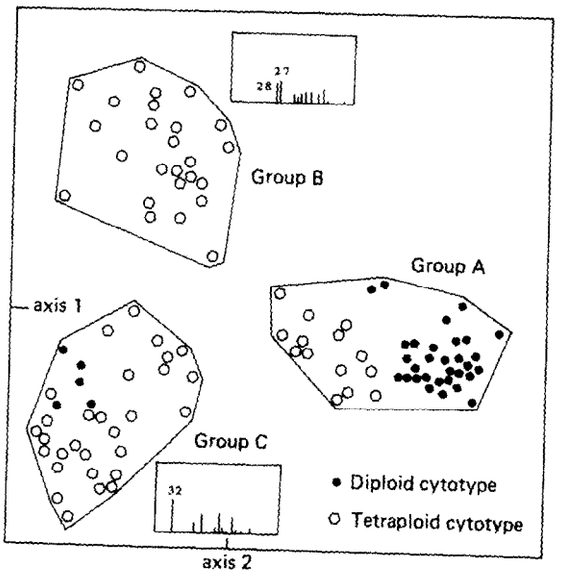

Fig. 2. ● Diploid cytotype; ○ Tetraploid cytotype. Distribution on the F1 × F2 plane of a F.A.C. (36% of inerty) according to their flavonoid content of the 36 diploid and the 70 tetraploid individuals of *Kœleria cristata*. F.A.C. is realised on mean populational profiles with subsequent projection of the individual plants.

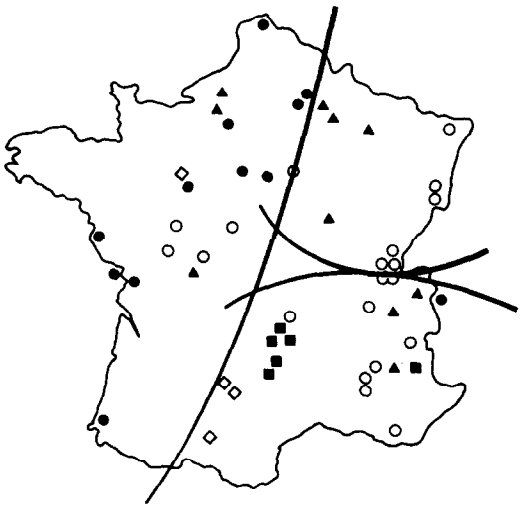

Fig. 3. ● Diploid cytotype; ○ Tetraploid cytotype; ◇ Hexaploid cytotype; ■ Octoploid cytotype; ▲ Dodecaploid cytotype. Geographical location of 52 French populations and one Swiss population of *Kœleria cristata*. The demarcation defined by the flavonoid pattern of the diploid and tetraploid cytotypes was drawn.

chemotype is restricted to the species *Kœleria vallesiana*, the phytochemical definition of which is very different from *Kœleria cristata*. Within the still rather complex cluster of *Kœleria cristata* low ploidy levels, the tetraploid originating from eastern regions could be divided between northern and southern origins.

Phytochemical analysis of this *Kœleria cristata* model affords results of considerable accuracy. This treatment is probably the first to reveal such a strong structure within the specific complex, based on a geographical component with four poles. This could support an investigation on the evolutionary origin of this species, coupled with the structural identification of biochemical markers.

EXPERIMENTAL

Plant material. 172 individuals of *Kœleria cristata* and 10 individuals of *Kœleria vallesiana* issued from various geographical origins (Fig 3 ; Table 1) were grown in the experimental garden at University Paris XI. Analyses were performed on young autumnal sprouts collected in Oct. 1986. The cytotypes were determined by R. Bajon [13].

Phytochemical analysis. Extraction of flavonoids has been previously described [14]. Visualization of flavonoid patterns was obtained using HPLC elution profiles (C18 reverse phase column, gradient of acetonitrile in H_2O with 2% HOAC as

Table 1. Description of the collection of *Kœleria cristata* and *Kœleria vallesiana* studied

Cytotype	nb. ind/pop.	Geographical location
14	13	F-78 Vetheuil
14	2	F-77 Fontainebleau
14	4	F-17 Châtelaillon
14	2	F-85 Saint Gilles sur Vie
14	1	F-02 Parfondru
14	2	F-02 Festieux
14	2	F-17 Sablanceau Ile de Ré
14	5	Les Follatères. Martigny Valais Switzerland
14	1	F-40 Le Penon. Hosségor
14	2	F-59 Zuydcoote
14	1	F-91 Etrechy
14	1	F-72 Saint Maixent
28	3	F-83 Sainte Baume
28	3	F-26 Saou
28	2	F-25 Montlebon
28	11	F-73 Aime
28	2	F-36 Pouligny St Pierre
28	1	F-68 Heiteren
28	2	F-84 Crestet
28	3	F-84/26 Ayguemarse
28	2	F-01 Col de Berthiand
28	1	F-63 Joze
28	1	F-25 Lac de l'entonnoir
28	1	F-79 Veluché
28	3	F-57 Bannstein
28	1	F-25 La Cessay
28	1	F-25 Mont d'Or
28	1	F-25 Labergement Ste Marie
28	13	F-37 Gizeux
28	3	F-18 Ennordres
28	12	F-68 Rothleiblen
28	4	F-10 Echemines
42	5	F-61 Appenai sous Bellème

Table 1. *Continued*

Cytotype	nb. ind/pop.	Geographical location
42	5	F-32 Bajon
42	2	F-58 Mesves
42	1	F-82 Montaigu du Quercy
42	1	F-47 Tournon d'Agenais
56	6	F-63 Beaune le Froid
56	1	F-15 Soulages
56	1	F-63 La Cassière
56	1	F-63 Chastres
56	1	F-15 Collanges
56	1	F-05 Ailefroide
84	5	F-76 Croixdalles
84	8	F-08 Ville sur Retourne
84	1	Botanical garden Brno (Czechoslovakia)
84	1	Botanical garden Brno (Czechoslovakia)
84	1	F-21 Corcelles les Monts
84	2	F-86 Lussac les Châteaux
84	1	F-76 Vallée du Héron
84	1	F-08 Leffincourt
84	1	F-55 Sivry la Perche
84	1	F-01 Col du Crozet
84	1	F-25 Col de la Joux Plane
84	1	F-05 Super Devoluy
21	6	Hybrids
?	1	Botanical garden Bâle (Switzerland)
?	1	Botanical garden Birmingham
?	1	Botanical garden Frankfurt am Main
?	2	Botanical garden Budapest University
14	1	Botanical garden I.N.A. Grignon (France)
?	1	Botanical garden I.N.A. Grignon (France)
42	2	Botanical garden Madison Wisconsin (U.S.A.)
14	1	Botanical garden Nantes (France)
?	1	Karaganda U.S.S.R
42	1	*K. vallesiana* F-83 Sainte Baume
42	1	*K. vallesiana* F-24 Sarliac
42	1	*K. vallesiana* F-30 Carsan
42	1	*K. vallesiana* F-30 Valbonne
42	1	*K. vallesiana* F-05 Super Devoluy
42	1	*K. vallesiana* F-83 Sainte Baume
42	1	*K. vallesiana* F-16 Angoulème
42	1	*K. vallesiana* F-86 Lussac les Châteaux
42	1	*K. vallesiana* F-30 Chartreuse de Valbonne
42	1	*K. vallesiana* F-30 Chartreuse de Valbonne
42	1	*K. vallesiana* F-05 Col du Noyer Devoluy

mobile phase). The data matrix had 182 lines (individual samples) and 32 columns (selected peaks were identified by the retention time and quantified by their height as a percentage of total height in the profile).

Mathematical treatment. The data matrix was submitted to factorial analysis of correspondances [12]. Graphic profiles of Figs 2 and 3 were obtained using Auda's graphical software [15].

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