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FLAVONOID VARIABILITY WITHIN *PINUS SYLVESTRIS*

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IN MEMORY OF TONY SWAIN, 1922 1987

Key Word Index—*Pinus sylvestris*; Pinaceae; Scots Pine; flavonoids; polymorphism.

Abstract—In a given population of Scots Pine, two flavonoid chemomorphs coexist. One is rich in prodelphinidin and lacks taxifolin ($=T^-$ morph); the other exhibits more *o*-dihydroxylated flavonoids, including quercetin and taxifolin ($=T^+$ morph). The latter situation occurs only at low elevations, whereas the high altitude or latitude populations are almost entirely made up of the T^- morph. These results shed light on the chemical biogenesis, history and systematics of the Scots Pine in Europe.

INTRODUCTION

Gymnosperms are a very old living group (they cohabited with dinosaurs), but their present distribution remains large (conifers constitute half of the world's standing timber). For these fundamental (systematic and phylogenetic) and practical (ecological and economic) reasons, our laboratory undertook the chemotaxonomic study of most of these species many years ago. Following phytochemical screenings [1, 2] and preliminary results [3–5], we offer here a comprehensive report on the flavonoid polymorphism of the Scots Pine, *Pinus sylvestris* L.; the present work is devoted to the memory of our friend Tony Swain.

The Scots Pine taxon is doubtless heterogeneous, from both morphological and physiological points of view; this loosely reflects its large geographical and ecological distribution (in Europe, from the south of Spain to north of the Polar circle). In this situation, two opposing systematic positions are possible: following Carlisle [6], one can recognize no less than 144 different 'types' within this species; following Jalas and Suominen [7], in the Atlas Florae Europeae, one can consider it 'as a collective taxon' only.

Our results, obtained with flavonoid markers in 10 [12] distinct populations, allow us to propose a structural and dynamic picture which clears up part of the above mentioned biological complexity.

RESULTS AND DISCUSSION

Detection of a flavonoid polymorphism at the population level

A preliminary sample (30 individual trees from the same forest of Basses-Vosges, France, cultivated in controlled conditions by I.N.R.A.) was thoroughly analysed for proanthocyanidin, flavanonol and flavonol contents. The more striking result is the coexistence of two distinct flavonoid chemomorphs: the first ($n=17$ individuals) is characterized by the predominance of prodelphinidin, in both relative and absolute value ($90\pm2\%$ of the total amount of the two proanthocyanidins; 2.03 ± 0.29 mg/g of dried needle weight). In the second group ($n=13$), the phenyl-trihydroxylation decreases in favour of dihydroxylation of the lateral ring. Indeed, not only prodelphinidin is rivalled by procyanidin (prodelphinidin: $56\pm5\%$ and 0.95 ± 0.19 mg/g, whereas procyanidin raised from 0.23 to 0.75 mg/g), but the quercetin content doubles (from 0.29 ± 0.08 to 0.62 ± 0.14 mg/g; from 23 ± 5 to $30\pm4\%$ of the total amount of the flavonols).* Moreover, taxifolin (=-2,3-dihydroquercetin) appears, which is completely absent from the first chemomorph (Fig. 1).

The correlation of these different chemical characters is obvious from a biosynthetic point of view (Fig. 2). That of taxifolin is the more striking, and the most useful for routine analysis (see below): we chose it to characterize the chemomorphs, T^- and T^+ (taxifolin absent and present, respectively). The second chemomorph is more diversified and more oxidized, therefore more 'evolved'; the first is the more primitive, character classically attributed to prodelphinidin.

Biogeographical distribution of the two chemomorphs

As within the Vosges population we studied above, there is dualism at the biogeographical level: in two populations (southern Sweden: $15\,T^+/9\,T^-$; southern France: $10\,T^+/21\,T^-$), the two chemomorphs co-occur in comparable proportions†; but in seven others (Alps: Valais, CH; Tarentaise, F. Jura, CH. Massif central: Velay, F. Pyrenees: Cerdagne, F. Sierra Nevada, SP. Sweden: north of the Polar circle), the chemomorph T^- is

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*Differences are significant at the risk $p\leqslant0.001$; Student t test.

†Southern Sweden (T^+: 62%) is significantly different from France (T^+: 32%) ($p<0.05$, chi square test); but the two populations do not differ from Vosges (T^+: 43%). Moreover, none of these three populations differs statistically from a T^+/T^- 50/50 distribution.

largely predominant (92–100%). The map (Fig. 3) summarizes the results of Table 1.

By adding 28 other individual trees from central and southern Sweden to the former sample, T^+ frequency decreases to 44%; for Eastern Europe (23 individuals from Russia, Poland, Czekoslovakia, etc, cultivated by I. N. R. A.), the T^+ frequency is equal to 35%. On the other hand, 12 individuals obtained from Scotland, not only are all T^- but are particularly poor in quercetin ($7\pm3\%$ of total flavonol content; 0.06 ± 0.02 mg/g); prodelphinidin rises here to $95\pm4\%$ of the total proanthocyanidins. For all individuals ($n = 317$), prodelphinidin richness, quercetin paucity and taxifolin absence are correlated.

Determinism of the flavonoid dimorphism

The localization of the populations studied here gives a first indication; the seven T-populations are indeed growing at high altitudes (> 800 m) or latitude (> 67°N), but the three others are not: Vosges (270 m), Provence (420 m), Southern Sweden (170 m, 60°N). Moreover, there is a highly significant correlation (($r = +0.688$; $p < 0.001$) between the altitude of origin of the trees and the absolute prodelphinidin content for 48 French samples cultivated in controlled conditions [3]. The first ecological parameter which can be related to altitude is temperature, which gives a strong (negative) correlation with prodelphinidin content ($r = -0.628$; $p \leqslant 0.001$, for the thermal annual mean); the correlation is better for June than for January ($r = -0.682$ and -0.472 respectively). A positive correlation exists with annual solar insolation (hr/year), but it is less significant ($r = +0.413$). No correlation exists with annual rainfall ($r = +0.079$).

In other words, the T^- morph can be considered as thermo(helio)philic; the T^- morph (more sparing in biochemical energy: is there a causal relationship?) would be characteristic for the 'cold populations' of the species *Pinus sylvestris*. However, the case of the Spanish Sierra Nevada population (1700 m; T^-: 100%; prodelphinidin: 3.2 mg/g) is enough to prevent a direct acceptance of the T^+/T^- dimorphism to a *present* thermal determinism. Although high in elevation, this Mediterranean population inhabits in fact in a hot environment (at the level of

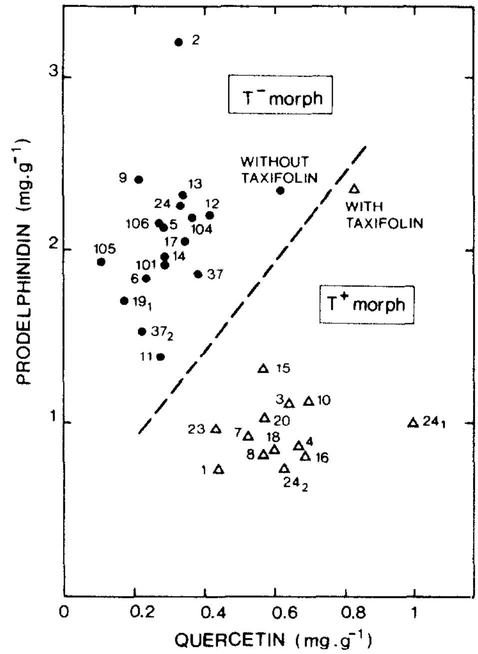

Fig. 1. Flavonoid composition of the 30 individual trees issued from Basses-Vosges (population no. 3).

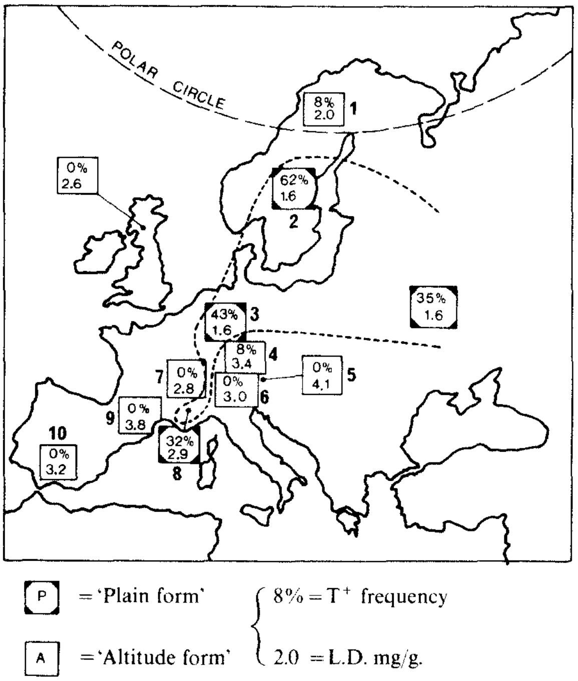

Fig. 3. Flavonoid map of the 10 (12) populations of Scots Pine studied here.

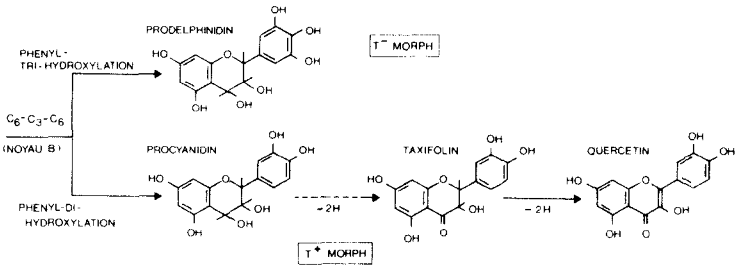

Fig. 2. Biogenetic interrelations of the flavonoids in the two chemomorphs of Scots Pine.

Table 1. Flavonoid analysis of 10 European populations of Scots Pine

Population (*n* individ.)	Altitude (m)	Frequency taxifolin%	L. A.$_{tot}$ mg/g	L.D.%	L. D. (mg/g)	Aglyc. (mg/g)	Q%
1. Sweden (North) (*n* = 24)	370	08	2.18 ± 0.46	T+ : 68 T− : 91 ± 1	1.96 ± 0.48	—	—
2. Sweden (South) (*n* = 24)	170	62	1.96 ± 0.64	T+ : 70 ± 3 T− : 92 ± 2	1.58 ± 0.73	(1.08 ± 0.26	31 ± 6)
3. Vosges (F) (*n* = 30)	270	43	2.04 ± 0.39	T+ : 56 ± 5 T− : 90 ± 2	1.58 ± 0.65	1.58 ± 0.44	27 ± 6
4. Jura (CH) (*n* = 24)	870	08	3.86 ± 0.87	T+ : 61 T− : 89 ± 2	3.39 ± 0.89	1.53 ± 0.31	38 ± 7
5. Valais (CH) (*n* = 25)	820	0	4.49 ± 0.63	91 ± 2	4.10 ± 0.59	1.59 ± 0.18	38 ± 7
6. Tarentaise (F) (*n* = 23)	900	0	3.18 ± 0.79	93 ± 1	2.96 ± 0.77	1.11 ± 0.22	35 ± 6
7. Velay (F) (*n* = 24)	950	0	2.95 ± 0.66	93 ± 1	2.75 ± 0.62	1.28 ± 0.28	39 ± 5
8. Provence (F) (*n* = 31)	420	32	3.44 ± 1.21	T+ : 59 ± 4 T− : 89 ± 3	2.85 ± 1.32	—	—
9. Cerdagne (F) (*n* = 30)	1530	0	4.11 ± 1.00	93 ± 1	3.84 ± 0.94	1.48 ± 0.20	37 ± 6
10. Sierra Nevada (*n* = 19)	1700	0	3.46 ± 0.81	92 ± 2	3.17 ± 0.75	—	—

L.A.$_{tot}$ = total of proanthocyanidins; L.D. = prodelphinidin; Aglyc = total of flavonol aglycones; Q = quercetin.

the Green Oak community). It is more convenient to consider the problem in an *historical* context: above 800 m, the present area of the Scots Pine (Alps, Jura, Massif central, Pyrenees, Sierra Nevada) probably corresponds to a downward re-extension from the relictual stations localized at higher elevations during times of thermal maxima, as above the Polar circle.

We can therefore accept that the T^- morph is the 'boreoalpine' strain of the species, and that it was the one present in Mean Europe during the ice age. Between cold and warm periods, the flat open countries have permitted the (re)conquest (from the East? or South ??) by the thermophilic T^+ morph, which constitutes here nearly the half of the individual trees, in equilibrated competition with the T^- chemomorph. The latitudinal gap between the North and South of Spain (Pyrenees and Sierra Nevada, respectively) does not permit such a migration as in France: the T^- morph remains on its own in a hot environment, but without competition. The study (in progress) of the genetic transmittance of the two characters T^- and T^+ should provide better frequency comparison for the two morphs.

Systematic propositions

A single (chemical) character is insufficient for producing a classification; nevertheless, the discovery of the T^+/T^- dimorphism and the knowledge of its geographical extension throw new light on the former systematic propositions concerning the Scots Pine. The very low quercetin content validates the *scotica* Schott. subspecies; but the frequencies of the chemomorphs do not help the numerous 'varieties' here and there described! With the present chemical basis, it is not possible to discriminate the variety *nevadensis* Christ. from the other mountain taxa, or the variety *rigensis* Loud. from the 'pin des Vosges' var. *haguenensis* Loud.

We propose here to recognize for the European part of the Scots Pine, two sub-groups, respectively called 'A' (altitude) and 'P' (plain). At present, our attitude is prudent until we identify other criteria, biochemical or otherwise. Nevertheless, one can hope that the individual definition of each tree as a T^+ or T^- morph can help the practice of forest management.

EXPERIMENTAL

Botanical sampling. Populations nos 4–10 (Table 1) and trees in Scotland were collected *in natura*, with vouchers on their origin. The populations from Sweden and the samples furnished by I. N. R. A. (Vosges, other parts of France, Eastern Europe) come from controlled origins and plantations. A particular study (3) has demonstrated that the sum of the uncertainties (both biological and analytical) was inferior or equal to ± 10% at the individual level. There is intraclonal stability. Except for the young needles (from May to August), the flavonoid content remains constant during both months and years; this is also true for variation in place of cultivation.

Flavonoid analysis. The chemical analysis concerns mature and dried needles (drying at room conditions). 2 g of ground material was submitted to hot acidic treatment (2 N HCl, 160 ml, with aeration) during 45 min; by this treatment, cyanidin and delphinidin are generated from homologous proanthocyanidins, whereas kaempferol, quercetin and isorhamnetin glycosides give the corresponding aglycones. Total anthocyanidin is measured at 530 nm in acidic medium, flavonol at 425 nm after ether extraction, re-dissolving in MeOH and chelation with Al^{3+}; the percentages of each flavonoid are determinated by

HPLC. (C 18 MicroBondapak. Solvent MeOH–H_2O–HOAC: 3/6/1 for anthocyanidins, 8:11:1 for flavonols). Although taxifolin can be detected by HPLC (elution before flavonols), we preferred TLC (Merck 11 F_{254} polyamide. Solvent Bz–MeOH–MEK: 4/3/4; R_f 0.57, brown-black spot under UV light).

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