THE PATHWAY OF CHEMICAL EVOLUTION IN BEARDED *IRIS* SPECIES BASED ON FLAVONOID AND XANTHONE PATTERNS

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Abstract - Four diploid and seven allopolyploid bearded Iris were surveyed for their leaf flavonoid, isoflavonoid and xanthone constituents in order to investigate their phylogeny. Eighteen flavone *C*-glycosides and eight *C*-glucoxanthones were identified and proved to be useful evolutionary markers. The phenolic data showed that *Iris pseudopumila* was a strong candidate as a diploid parent of some allopolyploid taxa e.g. *I. marsica* and *I. lutescens* but rules out the participation of *I. pallida*. Amongst the 2n = 44 allopolyploids the results suggested that *I. germanica* and *I. albicans* are very closely related and that they have evolved from the 2n = 40 taxa e.g. *I. bicapitata*.

Key words - Bearded irises, flavonoid, xanthone, chemical evolution

Introduction

In Italy, bearded irises are widely distributed (Fig.1) and a knowledge of their phylogeny is important to interpret their evolution. In central Italy, there are a number of *Iris* species of allopolyploid origin (Fig.2) belonging to subgenus *Iris*, section *Iris*, the so-called bearded irises. These either have a chromosome number of 2n = 40: *I. lutescens* Lam., *I. marsica* I. Ricci and Colas. (Ricci and Colasante, 1973), *I. revoluta* Colas. (Colasante, 1976-77), *I. bicapitata* Colas. (Colasante, 1996) and *I. setina* Colas. (Colasante, 1989; 1992) or 2n = 44: *I. germanica* L. and *I. albicans* Lange. It is still uncertain which diploid species were the parents e.g. *I. marsica* is thought to have originated from *I. pseudopumila* Tineo (2n= 16) x *I. variegata* L. (2n = 24) but other possible parents are *I. pallida* Lam., *I. cengialti* Kern. (Terpin *et al*. 1996), *I. illyrica* Tomm. (2n = 24) and *I. reichenbachii* Heuff. (2n = 24). Attempts to determine the evolutionary origin of these allopolyploid *Iris* by morphological means has been frustrated by the paucity of suitable characters and the highly polymorphic nature of the plants a situation where phytochemistry might help in providing additional independent characters. A previous survey of the *Iridaceae* has revealed useful phenolic variation in *Iris* species and a number of glycoflavones and xanthones e.g. mangiferin **(20)** and iso-mangiferin **(21)** have been identified. The main flower anthocyanin is

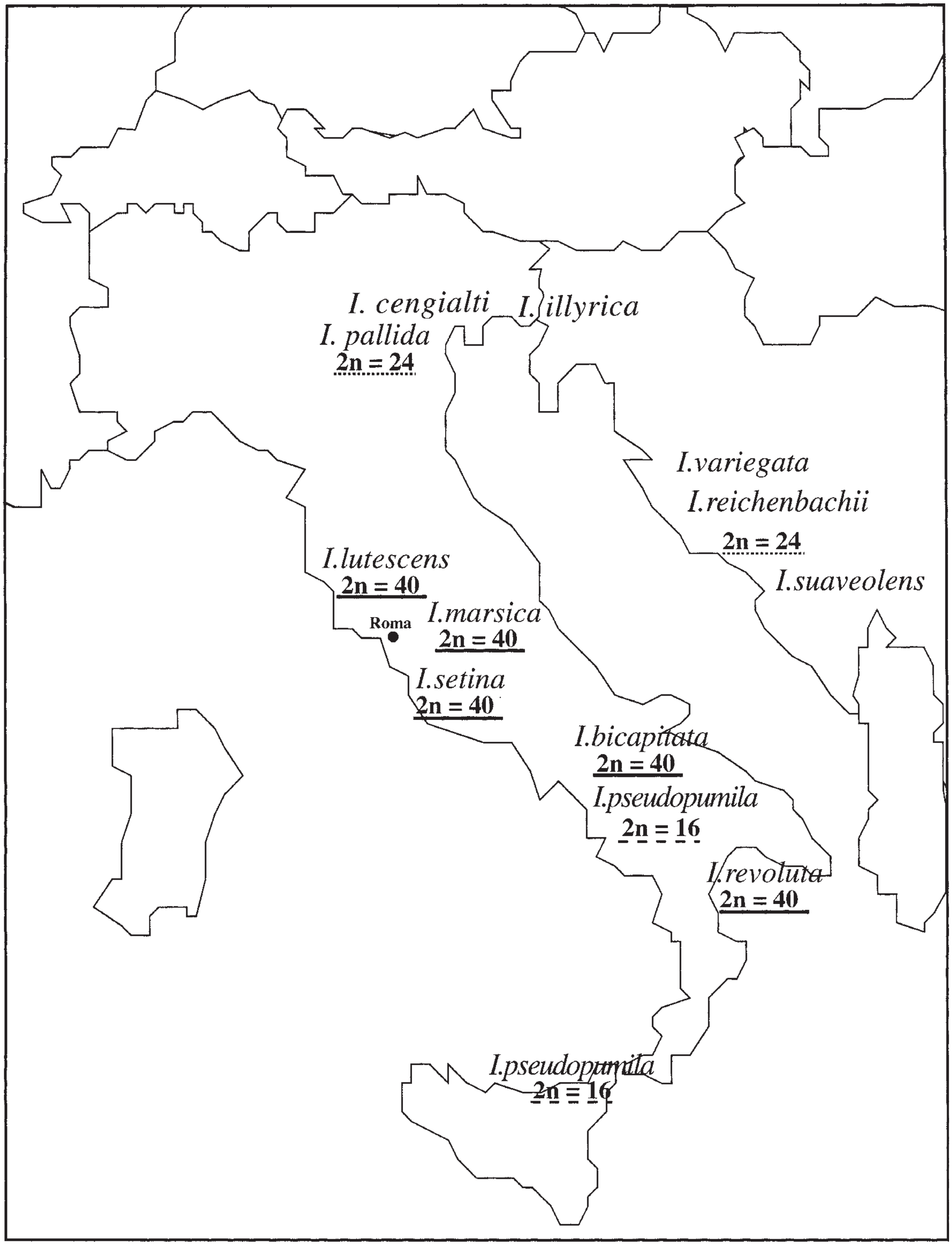

Figure 1 - Distribution map of bearded irises in Italy. *I. pallida* is spread in the rest of Italy as cultivated plant.

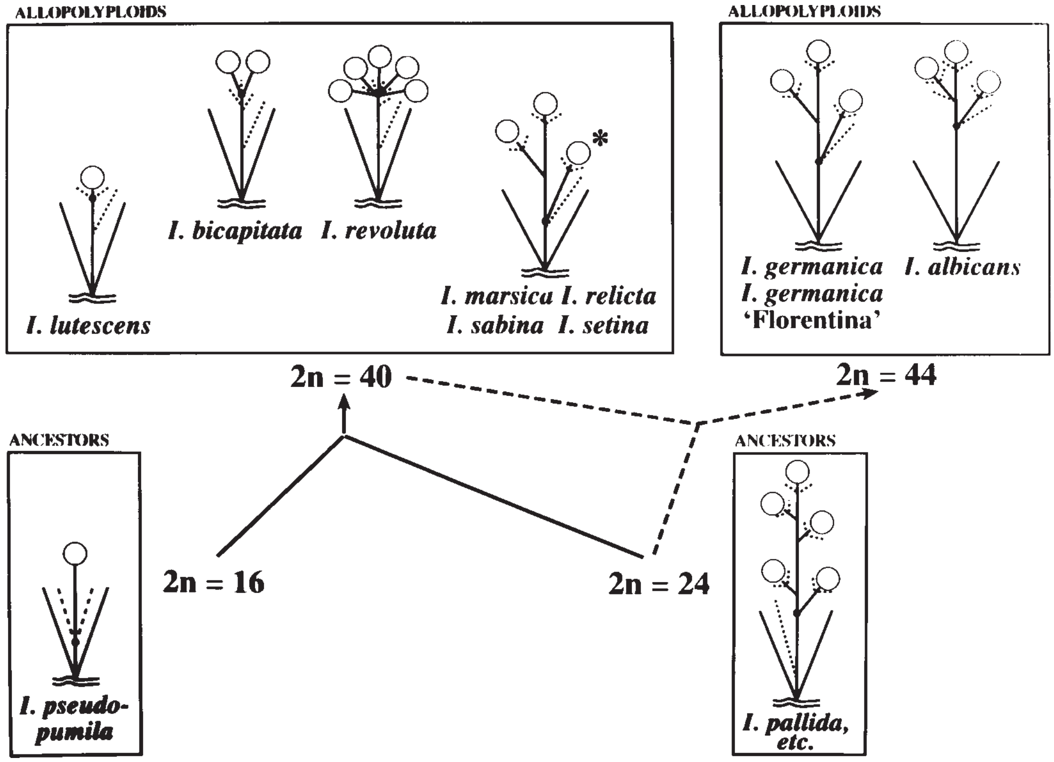

Figure 2 - Scheme of some bearded *Irises* present in Italy and their possible allopolyploid origin
* - Branch present or missing in the different species mentioned

delphanin (24) and isoflavonoids are frequent rhizome constituents. The purpose of the present study was to add new information to the previous data (Williams *et al*., 1997) screening bearded iris species for flavonoids and xanthones and to determine whether any of these constituents might indicate which diploids are involved in allopolyploid production.

Material and methods

Plants were collected from wild and naturalised populations in the Italian countryside as well as Giardino Botanico di Roma (RBG), Orto Botanico near the Vatican and the Botanic Garden of the University of Reading and two herbaria specimens from RNG and RO. Full details are given in Williams *et al*. (1997). Particularly we updated *I. biflora sensu* Petagna in *I. bicapitata* Colas. (Colasante, 1996).

Leaf flavonoids and xanthones were extracted in hot 80%MeOH and analysed by 2DPC in BAW and 15%HOAc and HPLC after partial purification. The major constituents were separated by mutiple 2DPPC in BAW and 15%HOAc and identified by Rf, co-TLC, UV analysis and comparative HPLC Rts with authentic markers. A more detailed account is given in Williams *et al*. (1997).

Results

A summary of the leaf flavonoid and xanthone pigments in four diploid and ten allopolyploid bearded iris taxa is presented in Table 1 and a composite 2D chromatogram of the pigments in Figure 3. Eighteen glycoflavones and eight glycoxanthones were identified. Both diploid and allopolyploid taxa showed stable profiles and anthocyanin patterns in the flowers and isoflavonoids in the rhizomes were generally uniform throughout the group.

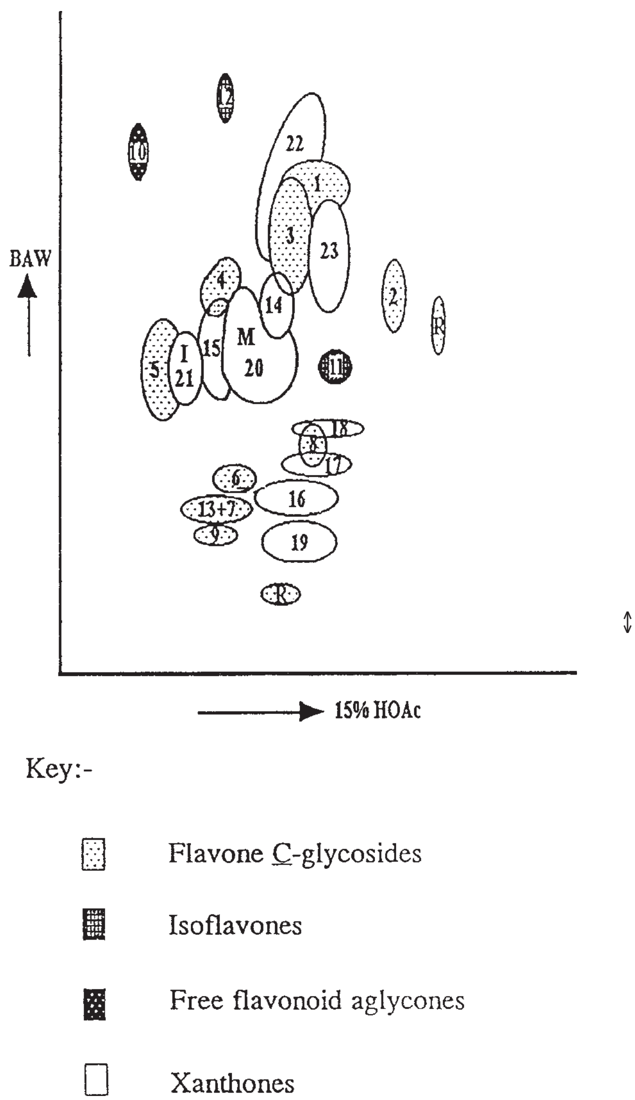

Figure 3 - A composite 2D chromatogram of the pigments. M = mangiferin, I = isomangiferin and R = rutin. For key to spot numbers and the structures identified within them see Tables1-3.

TABLE 1 - A SUMMARY OF THE LEAF FLAVONOID AND XANTHONE PIGMENTS IN *IRIS* TAXA FROM SECTION *IRIS* (WILLIAMS *ET AL.*, 1997)

	a																			*b*	*c*			*d*					
Taxon	**1**	**2a**	**2b**	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**	**4d**	**4e**	**5a**	**5b**	**6a**	**6b**	**7a**	**7b**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**18**	**19**	**22**	**23**
I. reichenbachii							++									++	++			+								(++)	
I. pseudopumila						+	+++							+	+	+	+	(+)	(+)	+	+	+							++
I. variegata							+++	++++																					
I. pallida		++++	(+)																					+	+				
I. pallida			++++		(+)		(+)	(+)																+	+	+			
Iris sp. Ienne	+++						+	+																		+	+		
Iris sp. Vallepietra	++						+	++																		+	+		
I. marsica	+++						(+)	++																		+	↑	↕	‖
I. albicans	+			+	++		+	++	+	+													(+)					(+)	§
I. bicapitata	+++					++	++	+				(+)	(+)					(+)											§
I. setina	+++					+	+									+	+	+										+	
I. germanica	+			+	++		+	++	+	+		(+)	(+)	(+)	(+)	(+)	(+)												
I. revoluta	+++					++	+++			++	+																		
Iris sp. Mt. Lauzo	+++						++	+++																				(+)	
I. lutescens	+++						+	++						¶	¶	‖	‖	↑										++	↕

a: Flavone C-glycosides
b: Flavones
c: Isoflavones
d: Xanthones
Xanthone pigments of universal occurence are not included in this table.
≠ Present in 14% of the sample. ↕ Present in 28% of the sample. § Present in one of two samples. ‖ Present in 43 % of the sample.
¶ Present in at least one taxon but obscured by xanthones in remainder.

TABLE 2 - A KEY TO FLAVONOIDS AND XANTHONES IN FIGURE 1

Compound No	Compound
1	Apigenin 7-methyl ether-6-*C*-glucoside (swertisin)
2a	Apigenin 7,4’-dimethyl ether-6-*C*-glucoside 2”-O- rhamnoside
2b	Apigenin 7-methyl ether-6-*C*-glucoside 2”-O- rhamnoside (swertisin 2” rhamnoside)
3a	Luteolin 7-methyl ether-6-*C*-glycoside (swertiajaponin)?
3b	Isovitexin
3c	An apigenin-based *C*-glycoside R_t 16.30
4a	Iso-orientin
4b	A luteolin *C*-glycoside R_t 14.53
4c	An apigenin-based *C*-glycoside R_t 17.11
4d	Vitexin
4e	An apigenin-based *C*-glycoside R_t 17.58
5a	Orientin
5b	A luteolin *C*-glycosides R_t 14.89
6a and **b**	Apigenin di-*C*-glycosides
7a and **b**	Luteolin di-*C*-glycosides
8	Apigenin-based di-*C*-glycosides R_t 7.92
9	An apigenin-based di-*C*-glycosides R_t 16.37
10a	Apigenin
10b	Chrysoeriol
10c	Acacetin
11	An isoflavone *O*-glucoside
12a	Tectorigenin
12b	Isoflavone R_t 24.82
12c	Isoflavone R_t 29.60
13	Apigenin di-*C*-glycosides R_t 15.12
14	Xanthone
15	Xanthone
16	Isomangiferin *O*-glucoside R_t 5.21
17	Mangiferin *O*-glucoside R_t 5.56
18	Xanthone
19	Xanthone
20	Mangiferin
21	Isomangiferin
22a and b	Possibly acylated mangiferin derivatives
23	A methylated mangiferin derivative

TABLE 3 - FLAVONE C-GLYCOSIDES AND XANTHONES OF POSSIBLE TAXONOMIC SIGNIFICANCE IN THE EVOLUTION OF *IRIS* TAXA FROM SECTION *IRIS*

Compound	Occurence in Diploids	Occurence in Allopolyploids
Apigenin di-*C*-glycoside (6a & b)	*I. pseudopumila*	*I. germanica*
Luteolin di-*C*-glycoside (7a & b)	*I pseudopumila*	*I. germanica*
	I. reichenbachii	*I lutescens*
		I. setina
An apigenin-based di-*C*-glycoside (3c)	*I. pseudopumila*	*I revoluta*
		I. setina
		I. bicapitata
A methylated mangiferin derivative (23)	*I. pseudopumila*	*I lutescens*
		I. marsica
		I. bicapitata
An acylated mangiferin *O*-glucoside (22)	*I. reichenbachii*	*I. lutescens*
		I. marsica
		I. albicans
		Iris sp. Mt. Lauzo

DISCUSSION

In extending our earlier findings on the chemosystematics of *Iris* (Williams, Harborne and Colasante, 1997), we now discuss the following points.

The distribution of five marker compounds, which appear to be important in tracing the evolution of the allopolyploid bearded *Iris*, are listed in Table 3. The presence of marker xanthones **23** of *I. pseudopumila* and **22** of *I. reichenbachii* in most of the allopolyploids suggests a role for both these diploids in the origin of the hybrids. However, *I. pallida* is eliminated as a putative parent by the presence of four major constituents (**2**a + b, **14** and **15**), unique to this species, which are unlikely to have been lost on hybridisation. The glycoflavone, swertisin (**1**) is noticeably absent from all diploids but is a major constituent of all allopolyploids with 2n = 40, apparently confirming the origin of 2n = 44 *Iris* from 2n = 40 taxa, probably by introgressive hybridisation with the 2n = 24 parent.

In conclusion, the phenolic data (1) strongly implicate *I. pseudopumila* as a possible diploid parent of some allopolyploid taxa e.g. *I. lutescens* and *I. marsica*; (2) implicate *I. reichenbachii* as a possible second diploid parent in the origin of *I. lutescens* and probably *I. marsica* but the investigation of other *Irises* with 2n=24 is still need (see 7a, 7b, 22, pags 55-56); (3) rule out the participation of *I. pallida* as the diploid parent of any of the allopolyploid taxa surveyed; (4) support the suggestion that *I. pseudopumila* 2n=16 is an ancestor of the sympatric *I. bicapitata* 2n=40 (Fig. 4) and (5) confirm the origin of the 2n = 44 Irises from the 2n = 40 taxa.

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Figure 4 - *I. bicapitata* Colas. (dark flower) and *I. pseudopumila* L. (pale flower): sympatric in wild area.

References

Arasawa M., Morita N., Kondo Y. and Takemoto T., 1973 - *Costituents of the rhizome of* Iris florentina *and the constituents of the petals of* Iris japonica. Yakugaku Zasshi **93**: 1655 -1659.

Colasante M., 1973 - Iris marsica. *n.sp*. Ann. Bot. (Roma) **32**:217-23.

Colasante M., 1976-77 - *Un nuovo endemismo italiano:* Iris revoluta *n.sp. e relativa analisi citotassonomica*. Ann. Bot. (Roma) **35**:155-168.

Colasante M., 1986 - *Genus* Iris *subgenus* Iris *section* Iris: *taxonomic observations on* Iris *with 2n=40 chromosomes*. Giorn. Bot. Ital. **120** (Suppl. 2):172.

Colasante M., 1989 - *On the three misunderstood wild bearded* Irises: I. bicapitata *nom. nov. and* I. relicta *nom. nov.,* I. setina *sp. nov.* Suppl.1. Giorn. Bot. Ital. **123**, n.1, 2:112.

Colasante M., 1992 - *Considerations sur les espèces "critique" d' Iris barbus italiens et individualisation de quelques espèces*. Iris et Bulbeus Rev. **107**:3-8.

Colasante M., 1996 - *Taxonomic note on some italian bearded* Irises: Iris relicta *Colas. nom. nov. and* Iris bicapitata *Colas. sp. nov*. Flora Mediterranea. **6**:213-217.

Kawase A. and Yagishita K. (1968). *A new C-glycosylflavone, embinin, from the petals of* Iris germanica. Agri. Biol. Chem. **32**:537 - 538.

Harborne J.B., 1967 - Comparative Biochemistry of the Flavonoids. Academic Press, London.

Ingham J.L., 1983 - *Naturally occurring isoflavonoids (1955-1981).* Fortschritte d. Chem. org. Naturst. **43**:1.

Terpin K., Nardi E. and Garbari F., 1996 - *Author and type of* I. cengialti (Iridacee). Giorn. Bot. Ital. **130**:575-578.

Williams C.A., Harborne J.B. and Goldblatt P., 1986 - *Correlation between phenolic patterns and tribal classification in the family* Iridaceae. Phytochemistry **25**:2135-2154.

Williams C.A., Harborne J.B. and Colasante M., 1997 - *Flavonoid and xanthone patterns in bearded* Iris *species and the pathway of chemical evolution in the Genus*. Biochem. System. Ecol. **25:** 309 - 325.