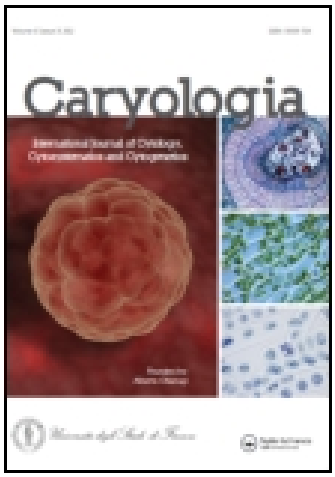

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Chromosome numbers of the Italian flora. From the Caryologia foundation to present

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Chromosome numbers of the Italian flora. From the *Caryologia* foundation to present

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After slightly more than four decades from the first chromosome counts ever, the very first chromosome count on Italian plants was published in 1925 by Alberto Chiarugi. From this starting point, fundamental cornerstones of Italian plant karyology are: 1) the foundation, in Pisa, of the international journal *Caryologia*, now edited in Florence and indexed by ISI Web of Science© under the category *Genetics and Heredity*. 2) the constitution, within the Italian Botanical Society, of the Working Group for *Plant Cytotaxonomy and Embryology* (currently *Plant Biosystematics*), which in 1970 (still in Pisa) fostered the publication of the column *Numeri Cromosomici per la Flora Italiana* on the journal *Informatore Botanico Italiano*. 3) the constitution, at the end of the '90s, of the online database *Chrobase.it – Chromosome numbers for the Italian flora*. The history and role of cytotaxonomic research is highlighted, by reconstructing life and science of four eminent scientists: Alberto Chiarugi (1901-1960), Giuseppe Martinoli (1911-1970), Francesco D'Amato (1916-1998) and Emilio Battaglia (1917-2011). Despite 86 years of more or less continuous research, the geographical and taxonomical coverage of the Italian territory is still incomplete.

Keywords: cytotaxonomy; history of science; Italy; karyology; plant chromosomes

Introduction

In the three issues of the first number of *Caryologia*, 25 papers dealing with plant cytology, cytosystematics and cytogenetics were published. The journal was founded and edited by Alberto Chiarugi (Fig. 1A) in Pisa during 1948. The first number hosts the first cytotaxonomic review for an Italian endemic species by A. Chiarugi himself (Chiarugi 1949), three contributions by Giuseppe Martinoli (Fig. 1B) coping with compared karyology of Mediterranean bulbous geophytes (Martinoli 1948, 1949a-b), twelve studies by Francesco D'Amato (Fig. 1C) – one of them in collaboration – about the mutagenic activity of some substances (D'Amato 1948a-c, 1949a-h; D'Amato and Avanzi 1949) and five papers by Emilio Battaglia (Fig. 1D), one of which deals with ploidy level variation within the same species (Battaglia 1948; Battaglia 1949a-d). All the above mentioned authors have passed away. The last one, E. Battaglia, left us on 4 September 2011 in Pisa, at the age of 94. A. Chiarugi died at the age of 59 in 1960, G. Martinoli in 1970, at the same age, and F. D'Amato in 1998, 82 years old. Those four eminent scientists shaped the history of Italian karyology, and the journal *Caryologia* was the main place for their publications.

It seems opportune to remember the scientific activities of those plant biologists during the twentieth century, since they inspired the work of many scholars (among them, the present senior author). Accordingly, a synthesis of their biographies and their main results on chromosome research of Italian plants is presented here.

Many other people have greatly studied the karyological aspects of Italian flora since the first contribution (Chiarugi 1925), and a database (Bedini *et al*. 2010 onwards) allows to verify all chromosome numbers published up to today (Bedini *et al*. 2011) in *Caryologia*, in the column "*Numeri Cromosomici per la Flora Italiana*" of the journal *Informatore Botanico Italiano* and in other contributions issued in further national and international journals.

Alberto Chiarugi

Alberto Chiarugi (1901-1960) was undoubtedly the first to apply karyological techniques to systematics, to taxonomy and to the study of speciation processes in Italian flora. Born in Florence, he became Full Professor of Botany at the University of Pisa when he was 29. In Pisa, he started a very prolific scientific period. Like his teacher Enrico Carano (1887-1943), A. Chiarugi excelled in plant embryology studies. Soon, however, he extended his interests to cytogenetics, mycology, paleobotany. Indeed, A. Chiarugi was also the first to introduce the palynological analysis for the phytoclimatic characterization of vegetation, demonstrating temporal continuity among the relic populations of the spruce (*Picea abies*

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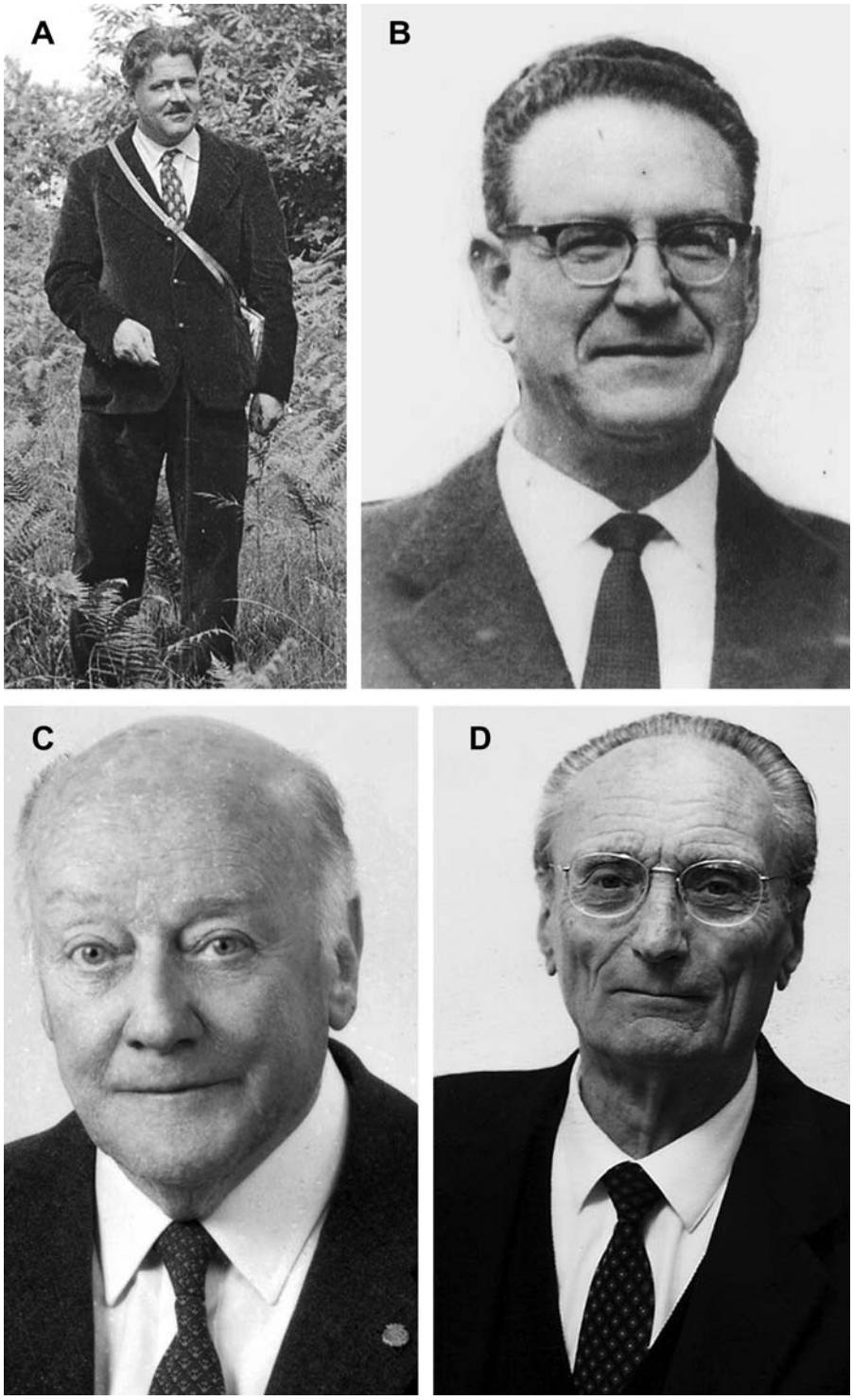

Figure 1. A: Alberto Chiarugi (1901-1960); B: Giuseppe Martinoli (1911-1970); C: Francesco D'Amato (1916-1998); and D: Emilio Battaglia (1917-2011).

(L.) H. Karst.) in N Apennine and the formerly occurring forests (Chiarugi 1936). After the foundation of *Caryologia*, A. Chiarugi created in Pisa a Centre for Plant Cytogenetics ("*Centro per la Citogenetica vegetale*"), within the Italian National Research Council (CNR). This Centre moved to Florence in 1950, after the transfer of A. Chiarugi to the university of this city. As already mentioned, also the first chromosome count on Italian plants is attributable to him (Chiarugi 1925). Among his numerous publications, we can mention a karyological, systematic and phytogeographical study on *Androsace vitaliana* (L.) Lapeyr. (Chiarugi, 1930); the essay, already cited above (Chiarugi 1949), on the origin of *Bellevalia webbiana* Parl. (Asparagaceae), a cental Italian

endemic which is still today a subject of biosystematic investigations (Borzatti von Loewenstern *et al*. 2012). He also studied another interesting southern Italian endemic, *Primula palinuri* Petagna (Chiarugi 1956), and a rare species occurring in Tuscany, Umbria and Latium: *Jonopsidium savianum* (Caruel) Arcang. (Chiarugi 1927b). His scientific activity was devoted also to the study of apomixis in *Artemisia nitida* Bertol. (Chiarugi 1926), in *Gymnadenia nigra* (L.) Rchb. f. (Chiarugi 1929) and in *Ochna serrulata* Walp. (Chiarugi and Francini 1930b), to the study of embryology in Cistaceae (Chiarugi 1925) and in Asteraceae (Chiarugi 1927a). A relevant review dealing with the evolution of genetic systems in plants was published in his last year (Chiarugi 1960).

A. Chiarugi was a man of great culture, a scrupolous naturalist, an attentive witness to the progress of botanical science but at the same time a scholar of its institutions and their didactic and cultural role (Garbari 2002). He was a member of prestigious academies and national and international scientific societies, and he was honorary President of the International Botanical Congresses held in Stockholm in 1950 and in Paris in 1954. A biography with a complete list of his publications was edited by E. Battaglia (Battaglia 1960), who shared the work with A. Chiarugi in Pisa between 1940 and 1950, being both a colleague and a friend for him. During the Congress of the Italian Botanical Society in Pisa, 20 October 1962, in the study now used by the senior author, a plaque was unveiled with the following words written by Giuseppe Martinoli: "*Ad Alberto Chiarugi (n. 1901 – m. 1960) che dal 1930 al 1950 con fervore di opera ed altezza d'ingegno diresse questo Istituto Botanico è dedicata questa stanza che custodisce la miscellanea da lui donata*". [To Alberto Chiarugi (b. 1901 – d. 1960) who from 1930 to 1950, with enthusiasm for his work and great intelligence directed this Botanical Institute, is dedicated this study which contains the miscellaneous papers donated by him]. Concerning cytotaxonomy, from the school of A. Chiarugi were bred as scientific offspring Giuseppe Martinoli, Francesco D'Amato and Emilio Battaglia when still in Pisa, and when at the University of Florence, Elena Maugini and Fernando Fabbri (who subsequently formed, directly or indirectly, new generations of cytotaxonomists in Florence and also in Sassari).

Giuseppe Martinoli

Commemorating 100 years after his birth, on November 25th 2011 a Congress was held in Rome, promoted by the "Comitato Provinciale romano dell'Associazione Nazionale Venezia Giulia e Dalmazia" and titled "*Giuseppe Martinoli, una vita dedicata alla botanica*" [Giuseppe Martinoli, a life dedicated to Botany] with talks by cultural personalities, professors, scholars and relatives. Giuseppe Martinoli (1911-1970) was born in Split (Dalmatia, currently Croatia). As a refugee, he moved to the University of Pisa, where he graduated in Natural Sciences in 1937. Alberto Chiarugi took him immediately as a volunteer assistant, but in 1938 Martinoli moved to the University of Cagliari, where he directed the Institute of Botany from 1946 to 1954. In 1955 he came back to Pisa, where he directed the Botanic Institute and Botanic Garden from 1955 to 1963. In 1963 he moved to the University of Rome.

In the commemoration made by professor Valerio Giacomini, after recalling his human qualities and personal vicissitudes, this colleague emphasized the lines of research that had characterized Martinoli's scientific works (Giacomini 1971). Initially, G. Martinoli oriented his research on cyto-embryology, but soon he focused on cytotaxonomy and cytogeography, with particular attention to Sardinian endemics. He incited his students towards a methodical karyological exploration of Italian flora, especially concerning its insular and Mediterranean elements. We can cite as an example his studies on *Bellevalia* (Martinoli 1948) and *Pancratium* (Martinoli 1949a), already mentioned in the Introduction, and the karyological contributions on taxonomically difficult genera such as *Ornithogalum* (Martinoli 1950a) and *Gagea* (Martinoli 1950b), continued by his scholars of first (Garbari *et al*. 2008 and literature cited therein) and second generation (Peruzzi 2003; Peruzzi 2008; Peruzzi and Aquaro 2005). G. Martinoli (Martinoli 1949) paid attention also to the bulbous Asparagaceae of the genera *Prospero* (*Scilla* p.p.) and *Charybdis* (*Urginea* p.p.); these plants, cultivated in the Botanic Garden of Pisa, were studied in detail later by E. Battaglia (see below). Some diploid and polyploid *Allium* representatives, collected in Sardinia by him (Martinoli 1955) were subsequently studied in the framework of a series of contributions devoted to this genus, studied for many years by F. Garbari and collaborators (cf. Bedini *et al*. 2010 onwards). Also *Hyoseris lucida* L. subsp. *taurina* (Martinoli) Peruzzi & Vangelisti (Martinoli 1953), *Morisia monanthos* (Viv.) Asch. (Martinoli 1940a), *Nananthea perpusilla* (Loisel.) DC. (Martinoli 1940b) and *Plagius flosculosus* (L.) Alavi & Heywood (Martinoli 1942), endemic or subendemic units of Sardinian-Corsican system, were investigated by G. Martinoli. He was the first Coordinator of the Working Group for Plant Cytotaxonomy and Embryology of the Italian Botanical Society (currently Working Group for Plant Biosystematics), created in 1967. This Group started the publication of the column "*Numeri Cromosomici per la Flora Italiana*", including chromosome counts of plants of certified spontaneous origin and linked to a voucher. These data were the basic elements for the constitution of *Chrobase.it* (Bedini *et al*. 2011). Thanks to G. Martinoli cytotaxonomy became one of the most widespread research lines of Italian botany, with the Universities of Pisa, Florence and Rome among the most active ones. For a synthesis of these aspects, see Garbari (1988). Concerning cytotaxonomy, from the school of G. Martin-

oli originated Fabio Garbari, Romano Capineri, Manlio Chiappini, Palmer David Marchi (and indirectly their subsequent scholars and collaborators in Cagliari, Catania, Pisa, Rome, Padua, Modena and Palermo). While the Universities of Padua and Modena called as Professors of Botany two early scholars of F. Garbari (Noemi Tornadore and Carlo Del Prete, respectively), few more words are deserved on the establishment of cytotaxonomy in the Universities of Catania and Palermo. While Francesco Maria Raimondo, from the latter, came for a period to Garbari's laboratory to learn the basic techniques in the mid '70s, from Catania Mario Cormaci came to the same lab. However, while Raimondo fruifully exploited those techniques in Palermo, Cormaci never applied them in Catania, since he soon changed his interest to phycology. Despite this, his written notes were found by Pietro Pavone, who learned (together with Salvatore Brullo) the basics in this way, originating there a further generation of Sicilian cytotaxonomists.

Francesco D'Amato

Another scholar of Alberto Chiarugi, destined to an outstanding scientific career, was Francesco D'Amato (1916-1998), who became assistant in 1940, just one year after he graduated in Natural Sciences at Pisa. Born in Grumo Appula (province of Bari), at the age of 30 he went to Svålov in Sweden, where he worked in the laboratories of Albert Levan and Ðke Gustafsson, studying the mutagenesis of barley seeds, radiobiology and radiogenetics, the cytotoxic effects of several substances. In 1951 he was at the John Innes Horticultural Institution in United Kingdom, in 1956 at the Brookhaven National Laboratory of Upton (U.S.A.). F. D'Amato is considered the founder of modern agricultural genetics in Italy. He published several works on chromosomal endoreduplication, on polyteny in embryo suspensor but also on cytotaxonomic research, dedicated to the critical genus *Colchicum* (D'Amato 1955; D'Amato 1957a-b). Other contributions were devoted to *Bellevalia* (D'Amato 1947) and *Euphorbia* (D'Amato 1939; D'Amato 1945; D'Amato 1946). The latter genus will be studied more in detail by Giuliano Cesca (Cesca 1963; Cesca 1966; Cesca 1967; Cesca 1969a-b; Cesca and Muzzi 1972), a scholar of G. Martinoli in Pisa and then of E. Battaglia in Bari. G. Cesca and F. Garbari, thanks to F. D'Amato (when he was director of the Botanical Institute of Pisa), obtained a grant for collecting plants in southern Italy and Sicily, to be cultivated in Pisa for cytotaxonomic studies, in the Spring of 1964. F. D'Amato was called to the University of Cagliari in 1956 as a Full Professor in Botany, and in 1959 came back in Pisa to occupy the first chair of Genetics ever instituted by a Faculty of Agricultural Sciences. His book "*Genetica vegetale*", published in 1971 and, in a second updated edition, in 1991, served Italian professors and scholars for dozens of years. His book "*Nuclear cytology in relation to development*", published in 1977 is a cornerstone for those studying this branch of plant biology. The school of D'Amato at Pisa considerably influenced cytological research. Several of his scholars reached brilliant results in the field of cell differentiation, in the role and development of embryo suspensor, of seed senescence and its mutagenesis, of environmental mutagenesis. An exhaustive picture of the life and science of F. D'Amato and his scholars can be found in Scarascia Mugnozza (2000). F. D'Amato formed many cytologists and plant geneticists, and some of them maintained some interest in comparative cytogenetics, such as for instance Roberto Cremonini (Pisa) and collaborators (i.e. the studies on *Vicia*: Frediani et al. 2005; Caputo et al. 2006; Ruffini Castiglione *et al*. 2007, 2009; Venora et al. 2000, 2008).

Emilio Battaglia

Born in Camaiore (province of Lucca), Emilio Battaglia (1917-2011) graduated in Natural Sciences at Pisa in 1940 and two years later in Pharmaceutical Sciences. After several years of attending the Institute of Botany of Pisa, a Canada-Unesco fellow position in Montreal, Canada (1950) and a Fullbright fellow in New York, USA (1954-1955), in 1961 he was called to the chair of Genetics at the University of Bari. In 1970, he moved to the University of Rome "La Sapienza", as a Full Professor of Botany and later of Plant Cytology and Embryology.

E. Battaglia was the cyto-embryologist by antonomasia. In 69 years of study, beginning with a contribution on *Ligularia* (Battaglia 1940), in more than 150 papers he described the development of male and female gametophytes in many plants and its phylogenetic implications (a synthesis in Battaglia 1951), apomixis (Battaglia 1963a), B-chromosomes (Battaglia 1964a), karyotypes in *Charybdis* (Battaglia 1957a-b, 1957d-e, 1958, 1964b, 1964e; Battaglia and Guanti 1966), *Dipcadi* (Battaglia 1954), *Nectaroscilla* (Battaglia 1959), *Oncostema* (Battaglia 1949a, 1949e, 1950; Battaglia et *al*. 1969), *Prospero* (Battaglia 1952a-b, 1953, 1955, 1956, 1957c, 1963b, 1964c-d) and *Sternbergia* (Battaglia 1949d). After stoppping his experimental work in 1971, beginning with 1980 he spent his efforts to solve historical, theoretical and terminological aspects of cytology and embryology, helped by his prodigious memory and the pleasure of finding the most minute and remote bibliographic facts. In the 80's he mainly devoted his attention to a series of publications called "*Embryological questions*", begun in 1980 (Battaglia 1980) and ended in 1991 (Battaglia 1991). Several of the later contributions concerning general terminological problems in eukaryotic cells were published in *Caryologia* (Battaglia 1993; Battaglia 1994; Battaglia 1995; Battaglia 1998; Battaglia 2000; Battaglia 2003; Battaglia 2005), including the most important and recent one (Battaglia 2009), which he used to define "my cytological testament". However, he paid attention also to terminologies in virology (Battaglia 2007). It is thanks to his late work that we know that the term chro-

mosome was coined by Heinrich Wilhelm Waldeyer in 1888 and the term chromatin by Walther Flemming in 1880 (Battaglia 1998); he forcefully rejected the use of these terms, which he proposed to substitute with caryoneme (see also Battaglia 1993) and caryonematin, respectively (Battaglia 2009). He was a Member of the Italian Botanical Society for 72 years, and Emeritus Life Member of the Botanical Society of America. E. Battaglia's publications constitute a valuable source of information for cytologists and plant embryologists; his book "*Lezioni di Citologia ed Embriologia vegetale. I. Embriologia*" (Battaglia E., 1969) is exemplary in this sense. Concerning cytotaxonomy, from the school of E. Battaglia originated Giuliano Cesca (see above for more details) and Fabio Maggini (and indirectly their subsequent scholars and collaborators in Bari, Cosenza, Siena and Viterbo). Also L. Peruzzi, graduated in Pisa under the supervision of F. Garbari, made his Ph.D. in the laboratory of G. Cesca at the University of Calabria (Cosenza).

Italian flora and chromosome counts

As already evidenced, the four figures discussed above substantially contributed to karyological research in Italy. The numerous data on chromosome numbers and eventual presence of B-chromosomes were collected and organized in an online database, namely *Chrobase.it* (Garbari and Bedini 2003; Garbari and Bedini 2004; Bedini *et al*. 2010 onwards), designed following the guidelines set by Berendsohn et al. (1997), which allows to carry out several interesting statistical considerations.

The database stems from a long-standing research project started in 1968 at the Botanical Garden of Pisa University. At that time, the number of chromosome counts available for Italian flora was growing rapidly: while only 490 counts had gradually accumulated from the late 1920s to the late 1960s (Garbari 1974), 1,538 were already available in 1976 (Garbari 1979). The database currently includes more than 7,000 records, pertaining to almost 3,000 taxa (about 35% of Italian vascular flora), derived from more than 1,300 different literature sources.

A graph (Fig. 2) summarizes the main contributors for the cytotaxonomical knowledge of Italian vascular flora: Pietro Pavone is followed by the present senior author, Salvatore Brullo and Claude Favarger (Fig. 3) from the University of Neuchâtel, who accordingly is the main foreign contributor. Lorenzo Peruzzi, Noemi Tornadore, Palmer D. Marchi, Askell Löve and Giovanni Federico D'Amato are following. Appropriate queries to *Chrobase.it* evidenced that 103 papers (8% of total) were published in the journal *Caryologia*, for a total of 659 counts (9% of total). The core of the chromosome counts (1578, 22% of the total) comes from the column "*Numeri Cromosomici per la Flora Italiana*", edited for many years by P. Marchi (Marchi and Pepe D'Amato 1995), substituted after his retirement by G. D'Amato, who prematurely passed away on 18 December 2008, and currently by L. Peruzzi. Our database stores chromosome numbers (*n* and/or 2*n*) and B-chromosomes quoted for Italian vascular flora, together with geographical data and bibliographic references. In a recent study (Bedini *et al*. 2011), we demonstrated how these karyological data in a digitized format can be of extreme interest for cytogeographic considerations. Indeed, concerning Italian flora, it was possible to evidence significant differences in the mean chromosome number among geographical groups (the mean increases according to a gradient Islands-South-North). Moreover, the frequency of the occurrence of B-chromosomes is about two-fold in N Italy compared with S Italy. In another study (Bedini *et al*. 2012a), we demonstrated that a quantitative analysis of the chromosome number variation can be a useful tool for taxonomical – and in some case phylogenetical – characterization of orders, families and genera. The work was focused just on Italian flora, but further studies conducted comparatively among Italy and New Zealand largely confirm these results (Peruzzi *et al*. 2011). According to the same authors, the mean chromosome number in plants from New Zealand is about two-fold that of Italian flora, and this suggests a dominant role of polyploidy in the origin of the New Zealand flora, as previously suggested by other authors. It was also possible to show that, contrary to what was expected given the comparable areas, latitude and altitudinal range of the two countries, they do not share the same pattern of chromosome number variation, suggesting that polyploidy increase among areas with increasing latitude might not be the same for the two hemispheres (Peruzzi *et al*. 2011). Other interesting observations concern B- chromosomes frequency differences between the two areas.

By narrowing the range of our interest to Italian endemic flora (currently 1,286 accepted species and subspecies), from the analysis of *Chrobase.it* it results that about 55% is covered by karyological knowledge, with a mean chromosome number almost identical to that of the whole Italian flora and a comparable frequency of B-chromosomes occurrence (Bedini *et al*., 2012b). This probably attests for similar evolutionary dynamics among endemic and non-endemic taxa. Another interesting observation deriving from the analysis of data in *Chrobase.it* is that the diploids dominate and are related to higher even ploidy levels by an exponential relationship (Bedini *et al*., 2012b). This finding certainly deserves further study. Despite greater efforts in the study of endemics by Italian plant biosystematists, Bedini *et al*. (2012b) outline a picture in which much work is still to be done.

Conclusions

After slightly more than four decades from the first chromosome counts ever (Strasburger 1882; see also Stace

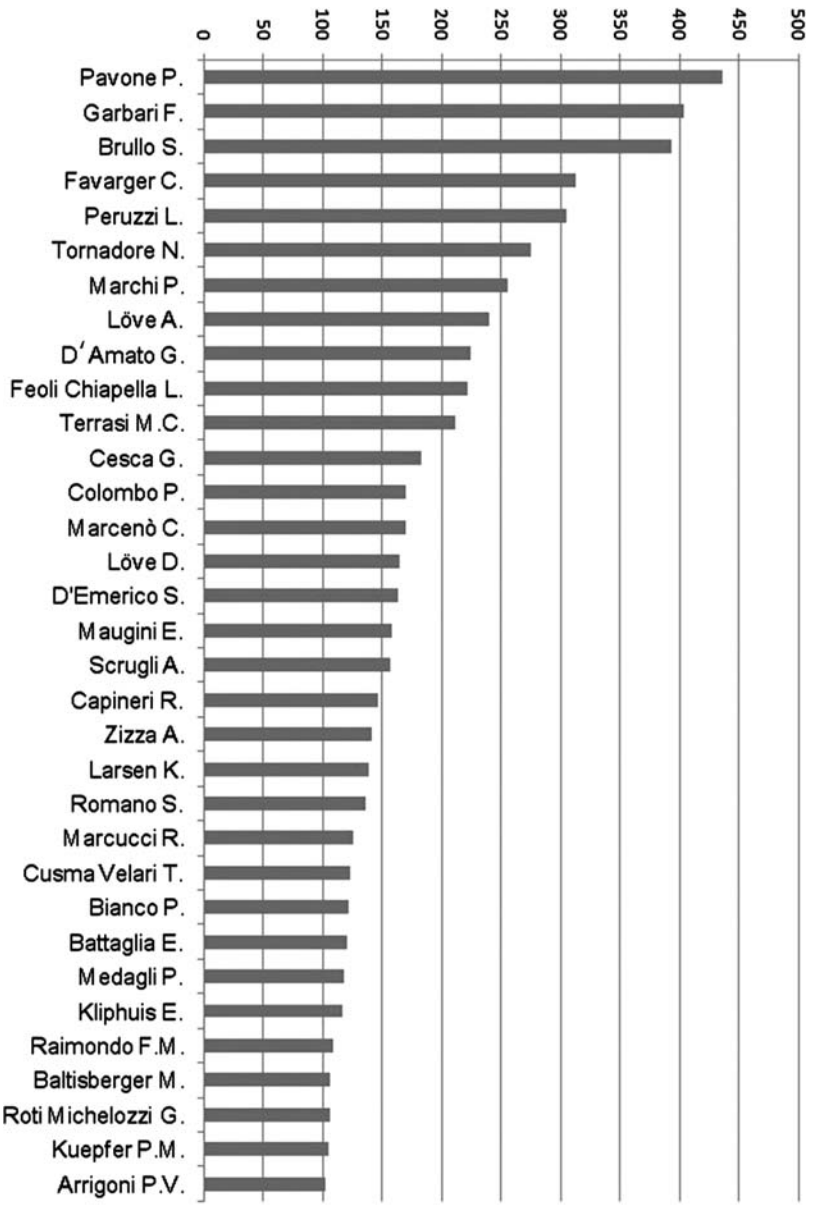

Figure 2. Main contributors for the cytotaxonomical knowledge of Italian vascular flora, in terms of number of counts.

2000), the very first chromosome count on Italian plants was published by Chiarugi (1925). From this starting point, cytotaxonomical research in Italy never stopped, even in critical historical periods (World War, etc.). Fundamental cornerstones of Italian plant karyology are: 1) the foundation, in Pisa, of the international journal *Caryologia*, now edited in Florence and indexed by ISI Web of Science© under the category *Genetics and Heredity*. 2) the constitution, within the Italian Botanical Society, of the Working Group for *Plant Cytotaxonomy and Embryology* (currently *Plant Biosystematics*), which in 1970 (still in Pisa) fostered the publication of the column *Numeri Cromosomici per la Flora Italiana* on the journal *Informatore Botanico Italiano*, soon emulated in other countries (i.e. *Números Cromosomáticos de Plantas Occidentales* on *Anales del Jardín Botánico de Madrid*; *Chromosome studies in the Southern African Flora* on *Journal of South Africa Botany*; *Mediterranean Chromosome Number Reports* on *Flora Mediterranea*). 3) the constitution, at the end of the '90s, of the online database *Chrobase.it – Chromosome numbers for the Italian flora*, which is continuously being updated (Bedini *et al*. 2010 onwards). In the last years the international scientific community agreed with the necessity for basic karyological data (see, for instance, the recent re-integration of the column *IOPB Chromosome Number Reports* in *Taxon*, official journal of the *International Association for Plant Taxonomy*). This is particularly crucial considering that for only 25% of angiosperms (the taxonomically richest group in the plant kingdom) at least one chromosome count is known (Bennett 1998; Stace 2000).

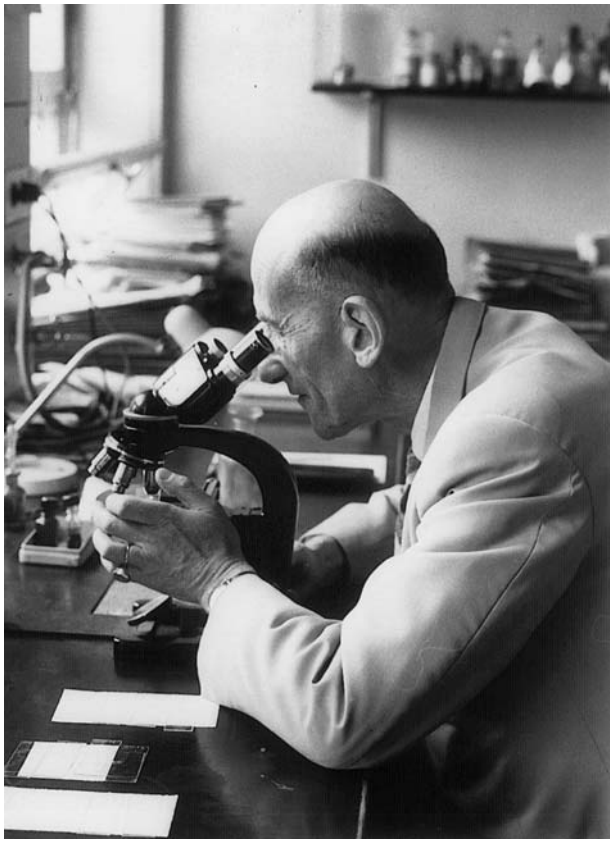

Figure 3. Claude Favarger (1913-2006).

Despite 86 years of more or less continuous research, the geographical and taxonomical coverage of the Italian territory is still incomplete (Bedini *et al*., 2011). We wish that, when 100 years of research is reached, this gap will be of much less extent. It will be possible only if the botanical scientific community maintains its interest in cytotaxonomy, a discipline already considered as passed away in the '90s, but now revealing an unsuspected vitality, also in support of more detailed cytogenetic and phylogenetic studies (i.e. Guerra 2008; Peruzzi *et al*. 2009; Wang *et al*. 2010).

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